



57th BRAZILIAN CONGRESS OF PHARMACOLOGY AND EXPERIMENTAL THERAPEUTICS

Pharmacology in the New Era of One Health

October 21-24, 2025 | CDI / INOVA - USP | São Paulo - Brazil

**1st SYMPOSIUM ON INNOVATION AND NATURAL PRODUCTS
TOWARDS NEW MEDICINES**

ESIB Butantan - October 20

PROGRAM AND ABSTRACTS



**October 21-24, 2025
São Paulo/SP**

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Welcome Letter

Dear Colleagues,

On behalf of the Brazilian Society of Pharmacology and Experimental Therapeutics (SBFTE) and the Organizing Committees, we are pleased to invite you to the **57th Brazilian Congress of Pharmacology and Experimental Therapeutics**, to be held from 21 - 24 October, 2025, at the Centro de Difusão Internacional (CDI) and INOVA Complex, Universidade de São Paulo.

We are also delighted to host the **1st Symposium on Innovation and Natural Products Towards New Medicines**, on 20 October, 2025, at the Escola Superior do Instituto Butantan (ESIB), as well as joint sessions with the *Società Italiana di Farmacologia* and the *Society of Pharmacology and Experimental Therapeutics of Hungary*, under the initiative “Pharmacology Without Borders.”

This year’s meeting, themed “**Pharmacology in the New Era of One Health**” will bring together scientists, students and leaders from Brazil and abroad to share discoveries, exchange ideas and promote collaboration across human, animal and environmental health.

The scientific program will include the **Sérgio Ferreira Opening Lecture, Keynote Lectures, JRV Young Scientist Award Session, Symposia, Round Tables** with funding agencies and **Oral Communications** by undergraduate and graduate students.

With the valued support of the University of São Paulo (CDI, INOVA, and ICB), ESIB, and our public (CNPq, CAPES, and FAPESP) and private sponsors, we look forward to welcoming you to an inspiring and memorable scientific event in São Paulo, a vibrant centre of culture, innovation and gastronomy.

We can’t wait to see you in São Paulo!

Warm regards,



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SBFTE Jovem is a committee composed of young pharmacologists within the Brazilian Society of Pharmacology and Experimental Therapeutics (SBFTE), founded in 2013.

We work closely with the SBFTE Board of Directors, with the mission of creating a permanent political-scientific forum dedicated to undergraduate and graduate students, postdoctoral fellows, early-career researchers, and junior faculty. Our goal is to foster the discussion of pharmacology-related scientific topics, support the development of early-career researchers, and encourage the participation, integration, and collaboration of our members in SBFTE activities.

This year, during the 57th Brazilian Congress of Pharmacology and Experimental Therapeutics in São Paulo, we are organizing the following activities: **Scientific Dissemination for Local School Students** – A science outreach initiative held on October 21st, 2025, from 08:00 am to 12:00 pm at *EMEF Dama Entre Rios Verdes*, and on October 22nd, 2025, from 09:00 am to 12:00 pm and from 2:00 to 5:00 pm at the congress venue, where students from *EMEF Dama Entre Rios Verdes* and *CE 240 – SESI Ferraz de Vasconcelos* will participate. In this project, we bring pharmacology and neuroscience to high-school students through accessible language and interactive activities. Students will also be invited to visit our stand and poster sessions during the congress to experience the scientific environment and witness knowledge exchange first-hand. **Meet the Expert:** A welcoming space for undergraduate and graduate students to interact with established professors, share experiences, and exchange ideas on career challenges, scientific projects, and academic development. This event will take place on October 22nd, 2025, from 12:10 pm to 1:40 pm. **Beyond the Academy Roundtable:** An open discussion on innovation, emerging scientific challenges, and opportunities beyond academic careers, including engagement with the pharmaceutical industry and other professional pathways. This session is scheduled for October 23rd, 2025, from 09:00 to 10:30 am.

In addition, we are pleased to announce the **5th edition of the SBFTE Jovem Cultural Contest for Scientific Dissemination**, an initiative that promotes the popularization of science. In this contest, students are encouraged to produce creative and informative videos on pharmacology using audiovisual resources, lay terms, and metaphors. Submissions are first evaluated by pharmacology professors and experts, and the finalists are then selected by public vote on social media. The winners will be announced during the Closing Session of the 2025 Congress.

Finally, we warmly invite young pharmacologists to visit our stand this year, where we will launch a new activity designed to listen to you – your expectations, questions, plans, and prospects for the future.

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Daniel Dias Rufino Arcanjo (UFPI)	Lucas Cezar Pinheiro (UFSC)
Daniel Fernandes (UFSC)	Luciano Augusto de Araújo Ribeiro (Univasf)
Daniela de Almeida Cabrini (UFPR)	Luciene Bruno Vieira (UFMG)
Daniele Maria Ferreira (IPP)	Lucienne da Silva Lara Morcillo (UFRJ)
Darizy Flavia Silva A. de Vasconcelos (UFBA)	Lucindo José Quintans Junior (UFS)
Deysi Viviana Tenazoa Wong (UFC)	Luisa Mota da Silva (UFSC)
Diego Veras Wilke (UFC)	Marco Aurélio Martins (Fiocruz)
Dirson João Stein (UFRGS)	Maria Aparecida Barbato Frazao Vital (UFPR)
Djane Braz Duarte (UnB)	Maria Christina W. de Avellar (Unifesp-EPM)
Edilson Dantas da Silva Junior (UFRN)	Maria Elena Crespo López (UFPA)
Edla de Azevedo Herculano (UFF)	Maria Fernanda de Paula Werner (UFPR)
Edson Antunes (Unicamp)	Maria Martha Campos (UnB)
Eduardo Koji Tamura (UESC)	Mariana Lima Vale (UFC)
Elaine Cruz Rosas (Fiocruz)	Maurício Schüller Nin (FURG)
Elisa Mitiko Kawamoto (USP-SP)	Michele Mazzaron de Castro (USP-RP)
Elizabeth Soares Fernandes (IPP)	Nara Lins Meira Quintão (Univali)
Emer Suavinho Ferro (USP-SP)	Nilberto Robson F. do Nascimento (UECE)
Enilton Aparecido Camargo (UFS)	Patrícia Machado Rodrigues e Silva (Fiocruz)
Erick José Ramo da Silva (Unesp-Botucatu)	Paulo Caleb J. de Lima Santos (Unifesp-EPM)
Fabio Cardoso Cruz (Unifesp-EPM)	Paulo de Assis Melo (UFRJ)
Fernanda Regina de Castro Almeida (UFPI)	Quintino Moura Dias Júnior
Flávia Almeida Santos (UFC)	Priscila de Souza (Univali)
Francisco Isaac Fernandes Gomes (USP-RP)	Regina de Sordi (UFSC)
Francisco Silveira Guimarães (USP-SP)	Regina Pekelmann Markus (USP-SP)
Francisney Pinto do Nascimento (Unila)	Richardt Gama Landgraf (Unifesp-Diadema)
Fúlvio Rieli Mendes (UFABC)	Roberto César Pereira Lima Júnior (UFC)
Geane Antiques Lourenço (UFAM)	Rosane Gomez (UFRGS)
Gilda Angela Neves (UFRJ)	Rosely Oliveira Godinho (Unifesp-EPM)
Giles Alexander Rae (UFSC)	Rosimeire Ferreira dos Santos (UFPI)
Guilherme Carneiro Montes (UERJ)	Sandra Elisa Haas (Unipampa)
Helena Maria Tannhauser Barros (UFSCPA)	Stephen Fernandes de P. Rodrigues (USP-SP)
Ingrid Ferreira Metzger (UnB)	Tatiana Paula Teixeira Ferreira (Fiocruz)
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Isis Nem de Oliveira Souza (UFRJ)	Valber Frutuoso (Fiocruz)
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Juliana Elaine Perobelli (Unifesp-BS Santista)	
Leandro José Bertoglio (UFSC)	

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Akila Lara de Oliveira (Unicamp)	Alexandra Acco (UFPR)
Aldeídia Pereira de Oliveira (UFPI)	Alexandre D Souza (KUiversiteit Leuven)

Amanda da Silva Chaves (Fiocruz)	Priscila Rezeck Nunes (Unesp-Botucatu)
Ana Carolina de Carvalho Correia (UPE)	Rafael Matos Ximenes (UFPE)
Ana Jersia Araujo (UFPI)	Regina Pekelmann Markus (USP-SP)
Anderson Fellyp Avelino Diniz (UFPB)	Regina Souza Aires (UFPE)
Andre Luiz Franco Sampaio (Fiocruz)	Renato Sampaio Carvalho (UFRJ)
Armando Genazzani (University of Torino)	Rita de Cassia Meneses Oliveira (UFPI)
Arquimedes Gasparotto Junior (UFGD)	Robson da Costa (UFRJ)
Bianca Portugal Tavares de Moraes (Unirio)	Simone Aparecida Teixeira (USP-SP)
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Claudia Bincoletto Trindade (Unifesp-EPM)	Thadeu Estevam M. Maramaldo Costa (Fiocruz)
Claudia Lucia Martins da Silva (UFRJ)	Vanessa Moreira (Unifesp-EPM)
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Dalton Dittz (UFPI)	
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Débora B V Costa (Michigan State University)	
Dielly Catrina Favacho Lopes Rêgo (UFPA)	
Elisa Mitiko Kawamoto (USP-SP)	
Emanuel Kennedy Feitosa Lima (UFERSA)	
Emer Suavinho Ferro (USP-SP)	
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Fabiana Figueiredo (UFMT)	
Fabio Cardoso da Cruz (Unifesp-EPM)	
Fabiola Taufic Mónica Iglesias (Unicamp)	
Fernanda Regina Giachini Vitorino (UFMT)	
Gabrielle Delfrate (UFSC)	
Gardenia Carmen Gadelha Militão (UFPE)	
Gilda Angela Neves (UFRJ)	
Gisele Facholi Bomfim (UFMT)	
Hyago da Silva Gomes (Fiocruz)	
Ingrid Ferreira Metzger (UnB)	
Isis Nem de Oliveira Souza (UFRJ)	
Jhohann Richard de Lima Benzi (USP-SP)	
João Agostinho Machado Neto (USP-SP)	
Joelmir Lucena Veiga da Silva (UFRJ)	
Joilson de Oliveira Martins (USP-RP)	
Karen de Moraes-Zani (IBu)	
Karina Carla de Paula Medeiros (UFRN)	
Lucas Antonio Duarte Nicolau (UFDPAr)	
Luciene Bruno Vieira (UFMG)	
Luis Eduardo Menezes Quintas (UFRJ)	
Marcelo Abrahão Strauch (UFRRJ)	
Maria Aparecida Barbato Frazão Vital (IBu)	
Maria Cristina Breno (IBu)	
Maria das Graças M. O Henriques (Fiocruz)	
Maria Fernanda de Paula Werner (UFPR)	
Mariana Renovato Martins (UFRJ)	
Maurício Schüller Nin (UFCSPA)	
Nara Lins Meira Quintão (Univali)	
Patrícia Ferreira da Silva Castro (UFG)	
Patricia Martins Bock (UFRGS)	
Patrícia Rodrigues (UFMS)	
Paulo de Assis Melo (UFRJ)	
Paulo Michel Pinheiro Ferreira (UFPI)	
Paulo Sérgio de Almeida Augusto (USP-RP)	
Pedro Azalim Neto (USP-SP)	

Useful Information

Secretariat

Congress Secretariat will be open from 8:00 am to 6:00 pm

E-Posters

All e-posters are available at the Totens and will be presented according to the schedule. Check below for schedule.

- **Session 1:** October 21st, 2:00 pm-3:30 pm
- **Session 2:** October 22nd, 3:50 pm-5:20 pm
- **Session 3:** October 23rd, 5:20 pm-6:50 pm
- **Session 4:** October 24th, 9:30 am-11:00 am

Certificates

The Certificates will be available online in the system (<https://www.nuieventos.com.br/sbfte/>) up to 10 days after the end of the Congress. You can download the PDF file in the Certificates area.

Courses

The course certificate will be given for the participants with at least 2 classes attendance.

Media Desk

Media desk (online) will be open from 8:00 am to 6:00 pm. All speakers are requested to leave the material at Media Desk (online) at least two hours before presentations. All rooms have data show. If you need any other equipment, please inform Media Desk as soon as possible.

Badges

The use of badge is mandatory for all activities and circulation areas in the Meeting Areas (CDI and Inova).

Abstracts

Abstracts presented at the Poster Sessions will be available at SBFTE website (<https://sbfte.org.br/congressos-anteriores>).



Gilberto De Nucci

Gilberto De Nucci is a noted Brazilian physician, scientist and university professor in the field of pharmacology. He was born in Campinas, São Paulo. Gilberto De Nucci studied Medicine at Ribeirão Preto Medical School, University of São Paulo (USP), from where he graduated in 1981. Soon after graduation, Prof. De Nucci moved to London to begin his post-graduate studies at the Royal College of surgeons, where he obtained his PhD in 1986 under the supervision of Prof. Salvador Moncada and YS Bahkle. Prof. De Nucci then did post-doctoral training at the William Harvey Research Institute of St. Bartholomew's Hospital Medical College under the supervision of Sir John Vane, Nobel Laureate in Medicine or Physiology in 1982. From 1988 to 1989, Prof. De Nucci was a visiting professor in the Department of Pharmacology at the University of Naples, Italy. In 1989, Prof. De Nucci created the Miguel Servet Clinical Pharmacology Unit in the Department of Pharmacology and University Teaching Hospital at Unicamp. Prof. De Nucci became part-time professor of Pharmacology in the Department of Pharmacology in the Biomedical Institute at the University of São Paulo (USP), São Paulo. In 1997, he established the Cartesius Analytic Unit in the Department of Pharmacology at USP. At the time, the Cartesius Unit was responsible for ~60% of all bioavailability/bioequivalence studies in Brazil. In 2013, Prof. De Nucci obtained a degree in Medicinal Chemistry from the Faculty of Pharmacy, University Federico II, Naples. Through his work at the Miguel Servet Clinical Pharmacology Unit at Unicamp and the Cartesius Analytic Unit at USP, Prof. De Nucci was a pioneer in the implementation of bioavailability and bioequivalence studies in Brazil that subsequently lead to creation of the Brazilian Generic Medicines Law (9787/99). Many of the professionals who work in Clinical Pharmacology in Brazil did part of their academic training under Prof. De Nucci's supervision at Unicamp and USP. In recognition of his pioneering work in bioavailability/bioequivalence studies, Prof. De Nucci is cited in the Good Clinical Practice Guidelines for Bioavailability and Bioequivalence Studies issued by the Brazilian Regulatory Agency (ANVISA). Over the years, Prof. De Nucci has supervised 66 MSc and 66 PhD students, as well as 23 post-doctoral students, many of whom occupy positions in Brazilian universities and the national pharmaceutical industry. Prof. De Nucci is a member of the Brazilian National Academy of Medicine (since 2003), the Brazilian National Academy of Pharmacy (since 2009) and the Brazilian National Academy of Sciences (since 2011). Prof. De Nucci has published more than 370 papers indexed in Pubmed with 17,551 citations; *h*-factor of 60 and 31 patents registered in the National Industrial Property Institute (INPI) and 35 patents registered in the World Intellectual Property Organization (WO).



Emilio Giuseppe Ignazio Clementi

Full Professor of Pharmacology, University of Milano, School of Medicine, Milan, Italy. Director, Pharmacovigilance, Clinical Research and International centre for pesticides safety, L. Sacco Hospital (NHS), Milan, Italy. Coordinator, Pharmacogenetics section, Italian Society of Pharmacology. Over 30 years of experience of research in basic, translational and clinical pharmacology. Editor in Chief, Pharmacological Research, Elsevier. Secretary General, the International Union of Basic and Clinical Pharmacology, IUPHAR. Member in the National Paediatric Ethics Committee Member in the Pharmacovigilance Risk Assessment Committee- European Medicine Agency. I started by working on calcium and sphingolipid metabolism signalling, helping to identify the type 3 ryanodine receptor, to then introduce nitric oxide as regulator of these pathways; this led me to investigate the role of nitric oxide in mitochondrial bioenergetics, a work done at UCL, and then contribute to identify nitric oxide role in mitochondrial biogenesis and the control of cell metabolism. The relevance of this in therapeutic perspective led me to investigate the role of nitric oxide in the

pathophysiology of skeletal muscle and as a therapeutic in Duchenne Muscular Dystrophy with studies moving from basic to translational research and finally to a phase I and a phase I/II clinical study. The advantage of being director since 2005 of a Clinical Pharmacology Unit allowed me to deepen my study on metabolism by addressing metabolic adverse drug reactions, in particular the ones associated with the use of antipsychotic drugs in paediatric patients, through approaches that combined basic research with pharmacokinetic, pharmacogenetic and pharmacoepidemiological analyses.

- 2017 First Senior Pharmacologist Award Recipient: João B. Calixto (UFSC, Cienp)
- 2019 Second Senior Pharmacologist Award Recipient: Fernando de Queiroz Cunha (USP-RP)
- 2021 Third Senior Pharmacologist Award Recipient: Mauro Martins Teixeira (UFMG)
- 2023 Fourth Senior Pharmacologist Award Recipient: Bernardo Boris Vargaftig (USP-SP)
- 2025 Fifth Senior Pharmacologist Award Recipient: Gilberto De Nucci (Unicamp)

First Place Winner History



- 1998: Maria Martha Campos (UFSC; Adviser: João Batista Calixto)
1999: José Eduardo da Silva Santos (UFSC; Adviser: Jamil Assreuy)
2000: Ana Paula V. Dantas (USP-SP; Adviser: Maria Helena Catelli de Carvalho)
2001: Liliam Fernandes (USP-SP; Adviser: Maria Helena Catelli de Carvalho)
2002: Isaías Gleizer (USP-SP; Adviser: Cristoforo Scavone)
2003: Juliano Ferreira (UFSC; Adviser: João Batista Calixto)
2004: João Alfredo de Moraes (UERJ; Adviser: Thereza Christina Barja-Fidalgo)
2005: Tiago Chiavegatti (Unifesp-EPM; Adviser: Rosely O. Godinho)
2006: Ana Letícia G. Cabral Maragno (USP-RP; Adviser: Marcelo Damário Gomes)
2007: Maria Fernanda de Paula Werner (UFSC; Adviser: Giles A. Rae)
2008: Ana Luiza Andrade de Paula Lopes (Unifesp-EPM; Adviser: Rosely O. Godinho)
2009: Sílvio Manfredo Vieira (USP-RP; Adviser: Fernando de Q. Cunha)
2010: Vanessa Olzon Zambelli (Instituto Butantan; Adviser: Yara Cury)
2011: Tatiana Paula Teixeira Ferreira (Fiocruz; Adviser: Patrícia Machado Rodrigues e Silva)
2012: Maíra Assunção Bicca (UFSC; Adviser: João Batista Calixto)
2013: Jaqueline Raymondi Silva (USP-RP; Adviser: Fernando de Q. Cunha)
2014: Jhimmy Talbot (USP-RP; Adviser: Fernando de Q. Cunha)
2015: Daniele Maria Ferreira (UFPR; Adviser: Maria Fernanda de Paula Werner)
2016: Gabriela S Kinker (USP, Adviser: Pedro Augusto Carlos Magno Fernandes)
2017: Fernando Olinto Carreño (UFRGS, Adviser: Teresa C. Dalla Costa)
2018: Bruna da Silva Soley (UFPR, Adviser: Daniela de Almeida Cabrini)
2019: Douglas da Silva Prado (USP-RP) Adviser: José Carlos Alves Filho
2021: Rianne Remus Pulcinelli (UFRGS) Adviser: Rosane Gomez
2022: Fabio Bonifacio de Andrade (USP-RP) Adviser: Thiago M. Cunha
2023: Nathália Ferreira de Oliveira(UFRJ) Adviser: Claudia Lucia Martins Silva
2024: Larissa Benvenuti (Univali) Adviser: José Roberto Santin

José Ribeiro do Valle Award – 2025 Finalists

	Julia Sapienza Passos BSc in Pharmacy and Biochemistry (USP-SP) (2015 – 2019) PhD in Biological Sciences (USP-SP) Adviser: Luciana Biagini Lopes
	Brena Freire de Oliveira Claudino BSc in Pharmacy (UFPB) (2018-2023) MSc in Bioactive Natural and Synthetic Products Adviser: Bagnólia Araújo Costa
	Luiz Adriano Damasceno de Queiroz BSc in Biomedicine (UEPA) (2014-2018) MsC in Pharmacy (Physiopathology and Toxicology) (USP-SP) (2018-2020) PhD in Pharmacy (Physiopathology and Toxicology) (USP-SP) Adviser: Joilson de Oliveira Martins
	Mariana Burille Moretti BSc in Biomedical Sciences (Unesp-Botucatu) (2018 – 2023) MsC in Pharmacology (Unicamp) Adviser: Fabíola Taufic Zakia Monica
	Carolina Medina Coeli da Cunha BsC in Biomedicine (Unirio) (2017-2022) MsC in Neuroimmunology (2022-2024) (UFF) PhD in Cellular and Molecular Biology (Fiocruz). Adviser: Cassiano Felipe Gonçalves de Albuquerque

Finalists Abstracts

Julia Sapienza Passos

- ◆ 10.014 **Multifunctional Thermosensitive Nanocarriers for Local Treatment of Non-Invasive Breast Cancer: Tissue Localization, Cytotoxic and Molecular Effects.** Passos JS^{1,2}, Machado-Neto JA¹, Panitch A¹, Lopes LB¹ ¹ICB-USP, Department of Pharmacology, Functional and Molecular Biology Program, Brazil; ²Georgia Institute of Technology, Department of Biomedical Engineering, USA

Brena Freire de Oliveira Claudino

- ◆ 08.002 **Prevention of Gut Microbiota Imbalance: The Role of *Arthrospira platensis* in Hypercaloric Diet-Induced Dysbiosis.** Claudino BFO^{1,2}, Arruda RRA^{1,2}, Diniz AFA^{1,2}, Melo MB¹, Sousa-Filho JEC¹, Colunga AAG⁴, Nobre CSN⁴, Silva BA^{1,2,3} ¹UFPB, Lab of Functional Pharmacology Prof. George Thomas, Institute of Research in Drugs and Medicines, Brazil ²UFPB, Postgraduate Program in Bioactive Natural and Synthetic Products, Health Sciences Center, Brazil ³UFPB, Department of Pharmaceutical Sciences, Health Sciences Center, Brazil ⁴University of Minho, Fermentations Lab, Centre of Biological Engineering, Portugal

Luiz Adriano Damasceno de Queiroz

- ◆ 04.030 **Aging, Immunity and Metabolism: Divergent Effects of Rapamycin in Senescence-Prone and -Resistant Mice.** Queiroz LAD¹, Barros RS¹, Assis JB², Pantoja KC¹, Bustia SX¹, Sá-Nunes A², Rodrigues SFP³, Turato WM⁴, Silva CCC⁵, Migliorini S¹, Martins JO¹ ¹USP, FCF, Dept. of Clinical and Toxicological Analyses, Lab. of Immunoendocrinology, Brazil; ²USP, ICB, Dept. of Immunology, Lab. of Experimental Immunology, Brazil; ³USP, ICB, Dept. of Pharmacology, Lab.

of Vascular Nanopharmacology, Brazil; ⁴USP, IQ, Dept. of Biochemistry, Energy Metabolism Lab., Brazil; ⁵USP, FCF, Dept. of Clinical and Toxicological Analyses, Multi-user Preclinical Imaging Center, Brazil

Mariana Burille Moretti

- ◆ 07.010 **Probenecid Potentiates the Relaxant Effect of β -Adrenergic Agonists in the Prostate of Rats.** Moretti MB, Leonardi GR, Barros JVC, Lima JM, Monica FZ Unicamp, PPG Pharmacology, Brazil

Carolina Medina Coeli da Cunha

- ◆ 04.068 **Tyrosine Kinase Inhibitor Reduces Systemic and Cerebral Inflammation in Experimental Sepsis.** Cunha CMCD^{1,2,3}, Moraes BPT^{1,3,4}, Abreu VHP^{1,3}, Soares GVM^{1,3}, Moraes-de-Souza IM^{1,2,3}, Almeida MAP¹, Estado V¹, Sayão PGF^{1,3,4}, Souto HA^{1,3}, Bozza PT^{1,2}, Castro-Faria-Neto HC^{1,2}, Silva AR^{1,2}, Gonçalves-de-Albuquerque CF^{1,2,3,4} ¹IOC-Fiocruz, Immunopharmacology Lab, Rio de Janeiro, Brazil. ²IOC-Fiocruz Postgraduate Program in Cellular and Molecular Biology, Rio de Janeiro, Brazil. ³Unirio, Immunopharmacology Lab, Rio de Janeiro, Brazil. ⁴Unirio Postgraduate Program in Molecular and Cellular Biology, Rio de Janeiro, Brazil

1º Simpósio de Inovação e Produtos Naturais Rumo a Novos Medicamentos (1st Symposium on Innovation and Natural Products Towards New Medicines)

Oct 20th, 2025 (Monday)

	Sala Amarelo/Azul	Sala Branco/Verde
08:00 am	Credenciamento	
08:30 am	Abertura	
09:00 am	Conferência de Abertura	
09:30 am	Coffee Break	
10:00 am Simpósios	SS1 – Novas Abordagens em Bioprospecção	SS2 – Pesquisa Pré-Clínica para o Desenvolvimento de Fármacos
12:00 pm	Almoço	
01:30 pm Simpósios	SS3 – Farmacologia Geral, Celular, Molecular e Bioquímica	SS4 – Farmacologia Clínica e os Desafios Translacionais
03:30 pm	Coffee Break	
04:00 pm	Apresentação de Pitches	
05:15 pm	Conferência de Encerramento	
05:45 pm	Encerramento	

Oct 22nd, 2025 (Wednesday)

12:10 pm	Reunião dos membros da Rede de Cooperação em Farmacologia do Centro-Oeste, Norte e Nordeste Brasileiro dentro da programação do Congresso Anual da SBFTE

Oct 24th, 2025 (Friday)

13:20 pm	Divulgação das menções honrosas para as melhores apresentações orais dos alunos de pós-graduação a ser anunciado na sessão de encerramento da SBFTE.

57th Brazilian Congress of Pharmacology and Experimental Therapeutics

Oct 21st, 2025 (Tuesday)

	Sé Room (CDI – Support Room)	Butantan Room (CDI – Audience)	Iris Room (Inova)	Liberdade Room (CDI - Mezzanine 1)	Auditorium (Inova)	Bixiga Room (CDI – Mezzanine 2)
08:00 am	SBFTE e Divulgação de Farmacologia na Escola Pública em São Paulo (Promoting Pharmacology in Primary Public Schools in São Paulo)					
08:30 am	Venue Secretariat					
09:00 am	Meeting of SBFTE Board of Directors and Deliberative Council					
11:00 am	Interval					
11:20 am			Meeting of SBFTE Permanent Forum of Graduate Courses in Pharmacology			
12:10 pm	Interval					
01:00 pm Courses	Cr1 – Principles of Systematic Review (Princípios de Revisão Sistemática) (Presented in Portuguese)		Cr2 – Applications of Physiology-Based Pharmacokinetics in the Drug Development (Aplicações de Farmacocinética Embasada em Fisiologia na Área de Desenvolvimento de Novos Fármacos) (Presented in Portuguese)	Cr3 – Neuropsychopharmacology of Reinforcement and Reward Behaviors (Neuropsicofarmacologia de Comportamentos de Reforço e Recompensa) (Presented in Portuguese)		Cr4 – Experimental Models in Cancer and Their Interface with Environmental Pollutants (Modelos Experimentais em Câncer e sua Interface com Poluentes Ambientais) (Presented in Portuguese)
02:00 pm	E-Poster Session 1 (with Coffee Break)					
03:40 pm Roundtable			RT1 – Research Development Policies in Brazil: How to Ensure Sustainability and Impact? (Políticas de Fomento à Pesquisa no Brasil: Como Garantir Sustentabilidade e Impacto?) (Presented in Portuguese)			
05:00 pm		Opening Session Cultural Presentation				
06:00 pm Opening Lecture		L1 – <i>Sergio Ferreira Lecture</i>				
06:50 pm	Cocktail (Cultural presentation)					

Oct 22nd, 2025 (Wednesday)

	Sé Room (CDI – Support Room)	Butantan Room (CDI – Audience)	Iris Room (Inova)	Liberdade Room (CDI - Mezzanine 1)	Auditorium (Inova)	Bixiga Room (CDI – Mezzanine 2)
08:30 am	Venue Secretariat and SBFTE Secretariat Opening					
08:30 am Courses	Cr1 – Principles of Systematic Review (Princípios de Revisão Sistemática) (Presented in Portuguese)		Cr2 – Applications of Physiology-Based Pharmacokinetics in the Drug Development (Aplicações de Farmacocinética Embasada em Fisiologia na Área de Desenvolvimento de Novos Fármacos) (Presented in Portuguese)	Cr3 – Neuropsychopharmacology of Reinforcement and Reward Behaviors (Neuropsicofarmacologia de Comportamentos de Reforço e Recompensa) (Presented in Portuguese)		Cr4 – Experimental Models in Cancer and their Interface with Environmental Pollutants (Modelos Experimentais em Câncer e sua Interface com Poluentes Ambientais) (Presented in Portuguese)
09:30 am Symposia / Oral Communication		S1 – Novel Approaches to Cardio- and Neuroprotection	RT2 – 55 Years of the Post-graduate Course in Pharmacology at the Escola Paulista de Medicina - Unifesp: Training Researchers Who are Up to Date with Science and Innovation in Brazil	S2 – Research and Innovation in Natural Product: From Biodiversity to Therapeutic Application		S3 – Understanding Psychedelics and Non-Hallucinogenic Psychedelic Analogs
11:00 am	Coffee-break					
11h20 am Lectures		L2 – Skeletal Muscle and Transulfuration Pathway	L3 – Leveraging Physiology-based Models in Perinatal Pharmacology and in Drug-Induced Liver Injury			
12:10 am	Lunch					
12:10 pm	Meeting of the N-NE-CW Region Pharmacology Network (with Lunch Box)		Meet the Expert (with Lunch Box)			
02:00 pm Symposia / Oral Communication		S4 – Translational Approach to the Treatment of Parkinson's Disease	S7 – Basic Pulmonary Pharmacology and Clinical Practice for Respiratory Diseases	S5 – Sex, Hormones and Age as Determinants for Disease Severity and its Implications on Therapeutics		S6 – Impact of Sex Hormones on Vascular Biology: New Perspectives and Insights
03:30 pm	Coffee-Break					
03:50 pm	E-Poster Session 2					
05:20 pm	Cultural Presentation					

06:00 pm	Get Together Party					
Oct 23 rd , 2025 (Thursday)						
	Sé Room (CDI – Support Room)	Butantan Room (CDI – Audience)	Liberdade Room (CDI - Mezzanine 1)	Iris Room (Inova)	Auditorium (Inova)	Bixiga Room (CDI – Mezzanine 2)
08:30 am	Venue Secretariat and SBFTE Secretariat Opening					
09:00 am Symposia/Oral Communication		S8 – Role and Modulation of Cardiac and Neural Ion Channels	S9 – Therapeutic Potential of Natural Products: From Ancient uses to Modern Practices	RT3 – Beyond the Academy (Além da Academia) (Presented in Portuguese)	S10 – Placenta, Preeclampsia, and Neuroprotection: Emerging Therapies and Future Horizons	
10:30 am Symposium		José Ribeiro do Valle Award				
12:30 pm	Lunch					
12:30 pm				SBFTE Young Assembly		
02:00 pm				SBFTE Assembly		
04:10 pm Lectures		L4 – Nicotinamide Phosphoribosyl-Transferase (NAMPT) and nicotinic acid phosphoribosyl transferase (NAPRT) are novel cytokines involved in inflammation		L5 – Microbiota-derived polyphenol metabolites modulate the T helper 17 to regulatory T cell (Th17/Treg) imbalance, promoting resilience to stress-induced anxiety- and depressive-like behavior		
05:00 pm	Coffee-Break					
05:20 pm	E-Poster Session 3					

Oct 24th, 2025 (Friday)

	Sé Room (CDI – Support Room)	Butantan Room (CDI – Audience)	Liberdade Room (CDI - Mezzanine 1)	Iris Room (Inova)	Auditorium (Inova)	Bixiga Room (CDI – Mezzanine 2)
08:30 am	Venue Secretariat and SBFTE Secretariat Opening					
08:30 am Courses	Cr1 – Principles of Systematic Review (Princípios de Revisão Sistemática) (Presented in Portuguese)		Cr3 – Neuropsychopharmacology of Reinforcement and Reward Behaviors (Neuropsicofarmacologia de Comportamentos de Reforço e Recompensa) (Presented in Portuguese)	Cr2 – Applications of Physiology-Based Pharmacokinetics in the Drug Development (Aplicações de Farmacocinética Embasada em Fisiologia na Área de Desenvolvimento de Novos Fármacos) (Presented in Portuguese)		Cr4 – Experimental Models in Cancer and Their Interface with Environmental Pollutants (Modelos Experimentais em Câncer e sua Interface com Poluentes Ambientais) (Presented in Portuguese)
09:30 am	E-Poster Session 4					
11:00 am	Coffee-break (brunch)					
11:30 am Lectures		L6 – Preclinical and Phase I Clinical Development of a Novel Multi-Target Drug Candidate for Chronic Neuropathic Pain		L7 – Transforming Molecular Pharmacology into Innovative Drug Discovery		
12:30 pm Closing Lecture		L8 – Molecular Therapeutics Targets for Muscular Dystrophy- the Role of Translational Pharmacology				
01:20 pm		Closing Ceremony Cultural presentation Awards Announcement				

1º Simpósio de Inovação e Produtos Naturais Rumo a Novos Medicamentos (1st Symposium on Innovation and Natural Products Towards New Medicines)

Oct. 20th, 2025 (Monday)

08h00-08h30	Credenciamento
Sala Amarelo/Azul	
08h30-09h00	Abertura Coordenação da REDE, Diretoria do Instituto Butantan e Diretoria da SBFTE
09h00-09h30	Conferência de abertura Instituto Butantan no Cenário de Desenvolvimento de Imunobiológicos no Brasil e no Mundo Esper Georges Kallás (Diretor, IBu) Coordenadora: Vanessa Olzon Zambelli (IBu)
09h30-10h00	Coffee Break
10h00-12h00	Simpósios
Sala Amarelo/Azul	SS1 – Novas Abordagens em Bioprospecção Coordenadora: Djane Braz Duarte (UNB) • <i>Modelo Zebrafish: importância na descoberta de Moléculas Bioativas</i> Monica Valdyrce dos Anjos Lopes Ferreira (IBu) • <i>Desenvolvimento de Fármacos a partir da Biodiversidade Microbiana</i> Spartaco Astolfi Filho (UFAM) • <i>Avaliação não-clínica do extrato de planta medicinal da relação do SUS: Base científica de eficácia e segurança para um novo tratamento das doenças inflamatórias intestinais</i> João Batista Calixto (UFSC/CIEnP) • <i>Apresentação Oral 1: 12.006 Pharmacological Potential of Small Molecules from Marine Bacteria and Extremophiles: A Search for Antitrypanosomal Candidates.</i> Albuquerque V¹, Lago JHG², Santos C³, Pimenta DC⁴, Tempone AG¹ ¹IB, Lab. of Pathophysiology, PPG Toxinology, Brazil, ²UFABC, Center of Natural Sciences and Humanities, Brazil, ³Universidad de La Frontera, Dept Chemical Science and Natural Resources, Chile, ⁴IB, Lab. of Ecology & Evolution, PPG Toxinology, Brazil • <i>Apresentação Oral 2: 02.072 Esterification of Geraniol to Geranyl Acetate Enhances its Therapeutic Potential in Adult Zebrafish.</i> Bastos LM¹, Gallas-Lopes M¹, Rosa GO¹, Patelli-Alves A¹, Portela SM¹, Costa PM³, Herrmann AP¹, Piato A^{1,2} ¹UFRGS, Porto Alegre, PPG Farmacologia e Terapêutica, Brazil; ²UFRGS, Porto Alegre, PPG Neurociências, Brazil; ³UFCSPA, Porto Alegre, Brazil

Sala Branco/Verde	SS2 Pesquisa Pré-Clínica para o Desenvolvimento de Fármacos Coordenadora: Ana Maria Moura da Silva (IBu) <i>Os desafios da prova de conceito em bancada ao escalonamento e produção</i> Elizabeth Angelica Leme Martins (IBu) <i>Avanços no Estabelecimento de uma Rede Pré-clínica no Instituto Butantan</i> Durvanei Augusto Maria (IBu) <i>Os desafios de desenvolver um projeto da bancada ao estudo clínico</i> Lucindo José Quintans Júnior (UFS) <i>Apresentação Oral 3: 09.023 Galactomannan- and Silver Nanoparticle-Based Dressing Accelerates Wound Healing in vitro and in vivo Experimental Models.</i> Silva FA¹, Silva ELES¹, Lucena LCP¹, Flor JF¹, Almeida JH¹, Silva EC¹, Nunes DM², Albuquerque PBS², Lima LRA², Rodrigues NER², Correia ACC², Ferro JNS¹ ¹UFAL, Lab Biologia Celular, ²UPE, Lab Bioprospecção e Etnofarmacotoxicologia Aplicada e de Biologia Celular. <i>Apresentação Oral 4: 05.029 Investigation of the Antinociceptive Potential of Geranyl Acetate in an Animal Model of Non-Inflammatory Diffuse Pain.</i> Pereira SAP¹, Martins RYC¹, Castro JLS¹, Ferraz SLNS¹, Martins DFA¹, Acha BT¹, Sousa DP², Oliveira, FA¹, MLS¹, Almeida, FRC¹ ¹UFPI, Lab Pain Pharmacology, Medicinal Plants Research Center ²UFPB, Dept Pharmaceutical Sciences, Health Sciences Center
12h00-13h30	Almoço
13h30-15h30	Simpósios
Sala Amarelo/Azul	SS3 – Farmacologia Geral, Celular, Molecular e Bioquímica Coordenadora: Alice Valença Araújo (UFPE) <i>Das toxinas animais aos compostos vegetais: ação antitumoral e seu potencial translacional</i> Paulo Michel Pinheiro Ferreira (UFPI) <i>Óleo da gordura visceral do Jacaré-do-Pantanal (Caiman yacare) como protetor cardiorrenal: uma fonte alternativa aos ácidos graxos poliinsaturados de peixes marinhos de águas frias?</i> Arquimedes Gasparotto Junior (UFGD) <i>Utilização de resíduos da indústria vinífera no tratamento da hipertensão arterial</i> Fernanda Regina Casagrande Giachini Vitorino (UFMT) <i>Apresentação Oral 5: 09.037 Methyl Cinnamate Down-Regulates Epithelial Mesenchymal Transition Through Modulating NF-κB/p65 Pathway in Alveolar Epithelial Cell.</i> Ferreira EGA, Barros ABB, Fidelix MSP, Silva GRS, Silva JP, Barreto E UFAL, Lab Biologia Celular <i>Apresentação Oral 6: 09.043 Luteolin Ameliorates Valproic-Acid Autism-Like Behaviors in Rats through the Attenuation of Neuroinflammation, Oxidative Stress, and Neural Damage.</i> Longo B¹, Nunes RKS¹, De Siqueira MCB², Ferreira PC², Cazarin CA², De Queiroz e Silva TF², Dos Santos AC², Venzon L², Da Silva LM², Pagliochi AC², Sievers J², De Souza MM², Da Silva LM¹. ¹UFSC, Dept Pharmacology, ²Univali, Graduate Program in Pharmaceutical Sciences
Sala Branco/Verde	SS4 Farmacologia Clínica e os Desafios Translacionais Coordenador: José Wilson do Nascimento Corrêa (UFAM)

	• <i>Perspectivas da aplicação de bioativos oriundos da biodiversidade amazônica no desenvolvimento de novos medicamentos</i> Emerson Silva Lima (UFAM) • <i>Os desafios na pesquisa translacional no Brasil: da ideia ao produto</i> Adriano Antunes de Souza Araújo (UFS) • <i>Desenvolvimento da Onco BCG recombinante contra câncer de bexiga</i> Luciana Cezar de Cerqueira Leite (IBu) • <i>Apresentação Oral 7: 07.003 6-Nitrodopamine as a Mediator of Prostate Contractility in Mouse and Man.</i> Pereira LGS¹, Dias DRM¹, Olivotti AB¹, Oliveira DL², Schenka AA², Souza VB², De Nucci², de Oliveira MG¹ ¹USF, Lab of Pharmacology, PPG Health Sciences, ²UNICAMP, Dept Translational Medicine, PPG Pharmacology • <i>Apresentação Oral 8: 10.028: IL-33 Expression and M1 Macrophage Polarization in Triple-Negative Breast Cancer: A Translational Study.</i> Isabelle de Fátima Vieira Camelo Maia
16h00-17h15 Sala Amarelo/Azul	Apresentação de Pitches ao painel de especialistas Indústria-Academia Coordenador: João Batista Calixto (UFSC/CIEnP) • <i>Uso Terapêutico do Nitrito na Hipertensão Gestacional: Investigação em Modelos in vitro e Potencial na Aplicação Clínica.</i> Gabriela Morelli Zampieri, Karoliny dos Santos Nunes, Priscila Oliveira Barbosa, Vinícius Aniceto; Ricardo de Carvalho Cavalli e Valéria Cristina Sandrim. Universidade Estadual Paulista, Instituto de Biociências de Botucatu • <i>FK-1000: Fração Natural com Potencial Antimicrobiano Multifuncional em Saúde e Agropecuária.</i> Marta de Oliveira Domingos, Geovana Ferreira dos Anjos, Kaique Ferrari Miranda, Ronaldo Maciel Marques, Hugo Vigerelli, Paola dos Santos da Silva, Marcia Regina Franzolin, Roxane Maria Fontes Piazza, Suzete Aparecida Lanza Destefano, Paula Maria Alejandra Lucchesi, Alejandra Kruger, Ana Elisa Juarez, Christine Laganá, Rosa Maria Chura Chambi, Luis Fernando dos Santos e Daniel Carvalho Pimenta. Instituto Butantan • <i>Prevenção da Dor Antes que Ela Exista: Uma Inovação Terapêutica Através do Veneno de Abelha.</i> Ana Maria Boaventura de Oliveira, Leonor MGR, Silva DF, Brazil MS, Almeida TC, Assis da Silva GS, Marques-Porto R e Pico G. Instituto Butantan • <i>PolissAg Derm: Curativo dérmico biodegradável e sustentável à base de Galactomanana e Nanopartículas de Prata para cicatrização de Feridas.</i> Fernanda Alexandre da Silva, Everlaine Leite Estevam dos Santos, Luana Cristina Pincho, Jonata Felix Flor, James Henrique Almeida, Emilly Cristina, Daniel Medeiro Nunes, Priscilla Barbosa Sales de Albuquerque, Luiza Rayanna Amorim de Lima, Natalie Emanuelle Ribeiro Rodrigues, Ana Carolina de Carvalho Correia, Janylle Nunes de Souza Ferro. Universidade Federal de Alagoas • <i>Desenvolvimento Pré-Clínico de Formulação Veterinária à Base de Silexan® para o Tratamento da Ansiedade Canina.</i> Raquel Rodrigues Soares Santos e Renata Cristina Mendes Ferreira. Universidade Federal de Minas Gerais • <i>Plant SUS: Rede de Pesquisa em Plantas Medicinais.</i> Ionara Rodrigues Siqueira e Valquiria Linck Bassani. Universidade Federal do Rio Grande do Sul

15h30-16h00	Coffee Break
17h15-17h45 Sala Amarelo/Azul	Conferência de Encerramento Pesquisa e desenvolvimento de novos fármacos do bioma amazônico José Carlos Tavares Carvalho (UNIFAP) Coordenadora: Soraia Katia Pereira Costa (USP-SP)
17h45-18h00	Encerramento Comissão Organizadora

Oct 22nd, 2025 (Wednesday)

12h10-13h40	Reunião dos membros da Rede de Cooperação em Farmacologia do Centro-Oeste, Norte e Nordeste Brasileiro dentro da programação do Congresso Anual da SBFTE

Oct 24th, 2025 (Friday)

13h20-14h00	Divulgação das menções honrosas para as melhores apresentações orais dos alunos de pós-graduação a ser anunciado na sessão de encerramento da SBFTE.

Oct 21st, 2025 (Tuesday)

08:00 am-12:30 pm	SBFTE e Divulgação de Farmacologia na Escola Pública em São Paulo <i>EMEF Dama entre Rios Verdes</i> (Promoting Pharmacology in Primary Public School in São Paulo) Chairs: Soraia K. P. Costa (SBFTE President) / Weverton Castro Coelho-Silva (Coordinator SBFTE Jovem Committee, USP-RP)
08:30 am-18:00 pm	Venue Secretariat
09:00-11:00 am	Meeting of SBFTE Board of Directors and Deliberative Council
Sé Room (CDI – Support Room)	
11h00-11h20 am	Interval
11:20 am -12:10 am	Meeting of SBFTE Permanent Forum of Graduate Courses in Pharmacology
Iris Room (Inova)	
12:10-13:00 pm	Interval
01:00-01:50 pm	Courses
Sé Room (CDI – Support Room)	Cr1 – Principles of Systematic Review (Princípios de Revisão Sistemática) (Presented in Portuguese) Chair: Adriane Ribeiro Rosa (UFRGS) ♦ Class 1: <i>Overall concepts of systematic review</i> (<i>Conceitos gerais de revisão sistemática</i>) Adriane Ribeiro Rosa (UFRGS)
Iris Room (Inova)	Cr2 – Applications of Physiology-Based Pharmacokinetics in the Drug Development (Aplicações de Farmacocinética Embasada em Fisiologia na Área de Desenvolvimento de Novos Fármacos) (Presented in Portuguese) Chair: Bibiana Verlindo Araujo (UFRGS) ♦ Class 1: <i>What is PBPK, model building and translation of in vitro/in vivo data</i> (<i>O que é PBPK, construção de modelos e translação de dados in vitro/in vivo</i>) Bibiana Verlindo de Araujo (UFRGS)
Liberdade Room (CDI - Mezzanine 1)	Cr3 – Neuropsychopharmacology of Reinforcement and Reward Behaviors (Neuropsicofarmacologia de Comportamentos de Reforço e Recompensa) (Presented in Portuguese) Chair: Helena Maria Tannhauser Barros (UFCSPA) ♦ Class 1: <i>Understanding the factors related to drug addiction using animal models</i> (<i>Fatores relacionados à dependência de drogas usando modelos animais</i>) Cristina Aparecida Jark Stern (UFPR) (Participation of Luana Freese, Northwestern University, USA – by video)
Bixiga Room (CDI – Mezzanine 2)	Cr4 – Experimental Models in Cancer and their Interface with Environmental Pollutants (Modelos Experimentais em Câncer e sua Interface com Poluentes Ambientais) (Presented in Portuguese) Chair: Juliana Elaine Perobelli (Unifesp-Baixada Santista)

	♦ Class 1: <i>Components of the Tumor Microenvironment and Their Modulation by Environmental Pollutants (Componentes do Microambiente Tumoral e sua Modulação por Poluentes Ambientais)</i> Débora Barbosa Vendramini Costa (Michigan State University, USA)
02:00-03:30 pm	E-Poster Session 1 (with coffee break)
	01. Cellular and Molecular Pharmacology Totem 09 (01.001-01.004; 01.027) Totem 15 (01.006-01.008, 01.031) 02. Neuropharmacology Totem 01 (02.001-02.004) Totem 02 (02.005-02.008, 02.061) Totem 10 (02.009-02.012) Totem 11 (02.013-02.016) Totem 23 (02.017-02.021) 03. Psychopharmacology Totem 16 (03.001-03.005) Totem 23 (03.006-03.007) 04. Inflammation and Immunopharmacology Totem 03 (04.001-04.004, 04.019) Totem 17 (04.005-04.010) Totem 24 (04.011-04.015, 04.068) 05. Pain and Nociception Pharmacology Totem 04 (05.001-05.004) Totem 12 (05.005-05.008) Totem 18 (05.009-05.012) 06. Cardiovascular and Renal Pharmacology Totem 05 (06.001-06.005) Totem 19 (06.006-06.009) 07. Endocrine, Reproductive and Urinary Pharmacology Totem 06 (07.001-07.004, 07.008) Totem 25 (07.005-07.006) 08. Respiratory and Gastrointestinal Pharmacology Totem 25 (08.001-08.003) 09. Natural Products and Toxinology Totem 07 (09.001-09.005) Totem 13 (09.006-09.009) Totem 20 (09.010-09.014, 09.060) 10. Cancer Pharmacology Totem 08 (10.001-10.005) Totem 14 (10.006-10.010) Totem 21 (10.011-10.014) Totem 26 (10.015-10.019) 11. Clinical Pharmacology, Pharmacokinetics, Pharmacogenomics and Toxicology Totem 22 (11.001-11.005)
03:40-05:00 pm	Roundtable

Iris Room (Inova)	RT1 – Research Development Policies in Brazil: How to Ensure Sustainability and Impact? (Políticas de Fomento à Pesquisa no Brasil: Como Garantir Sustentabilidade e Impacto?) (Presented in Portuguese) Chair: Rosane Gomez (Coordinator Permanent Forum of Graduate Courses in Pharmacology, UFRGS) ◆ <i>Capes' challenges in financing and coordinating Brazilian postgraduate courses (Os desafios da Capes no financiamento e coordenação da pós-graduação Brasileira)</i> Antonio Gomes de Souza Filho (UFC, CAPES Evaluation Director) ◆ <i>The future of science in Brazil: Funding strategies and the role of CNPq (O futuro da ciência no Brasil: Estratégias de financiamento e o papel do CNPq)</i> Ricardo Magnus Osorio Galvão (USP-SP, President CNPq) ◆ <i>The FAPESP Model: Sustainability and efficiency in funding scientific research (O Modelo da Fapesp: Sustentabilidade e eficiência no financiamento da pesquisa científica)</i> Alexandre Alarcón Steiner (USP-SP, Fapesp)
Butantan Room (CDI – Audience)	
05:00-06:00 pm	Opening Session / Cultural Presentation
	Orquestra de Viola e Violão do Instituto de Biociências (OVVIBB-Unesp-Botucatu)
06:00-06:40 pm Opening Lecture	<i>Sergio Ferreira Lecture</i> L1 – Serendipity at its Best: The Identification of Novel Endogenous Catecholamines Gilberto de Nucci (Unicamp / USP-SP) Fifth Senior Pharmacologist Award Recipient (Sponsor Ache) Presented by Soraia K. P. Costa (USP-SP, President SBFTE)
06:40-07:40 pm	Cocktail
	Coral da Universidade de São Paulo (CoralUSP)

Oct. 22 nd , 2025 (Wednesday)	
08:30 am-18:00 pm	Venue Secretariat and SBFTE Secretariat Opening
08:30-09:20 am	Courses
Sé Room (CDI – Support Room)	Cr1 – Principles of Systematic Review (Princípios de Revisão Sistemática) (Presented in Portuguese) Chair: Adriane Ribeiro Rosa (UFRGS) ♦ Class 2: <i>Research question, eligibility and data extraction</i> (<i>Questão de pesquisa, elegibilidade dos estudos e extração de dados</i>) Patrícia Martins Bock (UFRGS)
Iris Room (Inova)	Cr2 – Applications of Physiology-Based Pharmacokinetics in the Drug Development (Aplicações de Farmacocinética Embasada em Fisiologia na Área de Desenvolvimento de Novos Fármacos) (Presented in Portuguese) Chair: Bibiana Verlindo Araujo (UFRGS) ♦ Class 2: <i>Key-parameters in PBPK model building: volume of distribution and clearance</i> (<i>Parâmetros chave em PBPK: volume de distribuição e clearance</i>) Natalia Valadares de Moraes (University of Florida, USA)
Liberdade Room (CDI- Mezzanine 1)	Cr3 – Neuropsychopharmacology of Reinforcement and Reward Behaviors (Neuropsicofarmacologia de Comportamentos de Reforço e recompensa) (Presented in Portuguese) Chair: Helena Maria Tannhauser Barros (UFCSPA) ♦ Class 2: <i>Neurobiology of addictive-like behavior</i> (<i>Neurobiologia de comportamentos aditivos</i>) Helena Maria Tannhauser Barros (UFCSPA) (Participation of Carine Lampert (Northwestern University, USA – by vídeo))
Bixiga Room (CDI – Mezzanine 2)	Cr4 – Experimental Models in Cancer and their Interface with Environmental Pollutants (Modelos Experimentais em Câncer e sua Interface com Poluentes Ambientais) (Presented in Portuguese) Chair: Juliana Elaine Perobelli (Unifesp-Baixada Santista) ♦ Class 2: <i>In Vitro Models for Cancer Research</i> (<i>Modelos in vitro para o Estudo do Câncer</i>) Débora Barbosa Vendramini Costa (Michigan State University, USA)
09:30-11:00 am	Symposia/Oral Communication
Butantan Room (CDI – Audience)	S1 – Novel Approaches to Cardio- and Neuroprotection Chair: Peter Ferdinandy (Semmelweis University / Pharmahungary Group, Hungary) & Alexandre Denadai Souza (Katholieke Universiteit Leuven, Belgium) ♦ <i>Development of miRNA therapeutics for cardioprotection and other indications</i> Peter Ferdinandy (Semmelweis University / Pharmahungary Group, Hungary) ♦ <i>Pharmacological modulation of endothelial precursor cell-mediated brain capillary repair mechanisms</i> István A. Krizbai (HUN-REN Biological Research Centre, Hungary / Western University of Arad, Romania)

	◆ <i>Harnessing single-cell and spatial omics to unveil novel therapeutic targets</i> Alexandre Denadai Souza (Katholieke Universiteit Leuven, Belgium)
Liberdade Room (CDI - Mezzanine 1)	S2 – Research and Innovation in Natural Product: From Biodiversity to Therapeutic Application Chair: Bagnólia Araujo Costa (UFPB) ◆ <i>Pharmacological potential of food compounds in the management of immuno-mediated inflammatory diseases</i> Marina Sanchez Hidalgo (University of Seville, Spain) ◆ <i>Therapeutic potential of <i>Spirulina platensis</i> for obesity-induced disorders</i> Bagnólia Araújo Costa (UFPB) ◆ <i>Oral presentation 1: 09.026 Impact of Marine Animal Extracts on Neuronal Viability and Inflammation in Alzheimer's Disease Models.</i> Alves MM¹, Vigerelli H², Kitahara MV³, Kerkis I⁴, Sciani JM⁵ ¹IBu, PPG Sciences – Toxinology, Lab. of Biochemistry, Brazil, ²IBu, Center of Excellence in Target Discovery, Brazil, ³USP, Center for Marine Biology, Brazil, ⁴IBu, Lab. of Genetics, Brazil, ⁵USF, Lab. of Natural Products, Brazil ◆ <i>Oral presentation 2: 09.047 Cockroaches (<i>Nauphoeta cinerea</i>) as an Experimental Model for Screening Coralsnake Venoms.</i> Oliveira IN¹, Oliveira RS¹, Dias DRC², Guimarães MP³, Vinadé LHC⁴, Dal Belo CA^{1,3}, Silva NJ Jr⁵, Hyslop S¹ ¹FCM-UNICAMP, Depto de Farmacologia, PPG Farmacologia, Brazil; ²UFES, Depto de Estatística; ³EPPEN-Unifesp-Osasco, Depto Multidisciplinar, ⁴Unipampa-São Gabriel, ⁵PUC-GO, PPG em Ciências Ambientais e Saúde
Bixiga Room (CDI – Mezzanine 2)	S3 – Understanding Psychedelics and Non-Hallucinogenic Psychedelic Analogs Chair: Isis Nem de Oliveira Souza (UFRJ) ◆ <i>Beyond the Trip: The Molecular Basis of Lasting Behavioral Effects of 2-Br-LSD and its Comparison to Classical Psychedelics</i> Argel Aguilar Valles (Carleton University, Canadá) (via streaming) ◆ <i>The antidepressant potential of ayahuasca: from preclinical studies to clinical trials</i> Rafael Guimarães dos Santos (USP-RP) ◆ <i>Oral presentation 1: 02.038 Cannabidiol Attenuates General Neurotoxicity and Paclitaxel-Induced Cognitive Impairment (Chemobrain) in a Sex- and Treatment Regimen-Dependent Manner.</i> Pereira ACMP¹, Queiroz ABWV², Vatanabe IP¹, Barros ASM², Meyer E¹, Lisboa SF¹ ¹FCFRP-USP, Brazil, ²FMRP-USP, Brazil ◆ <i>Oral presentation 2: 02.039 Antidyskinetic Effects of Cannabidiol and Cannabigerol in Parkinsonian Rats: Role of 5-HT1A Receptors.</i> Mattos BA¹, Nascimento GC², Crippa JA³, Hallak J³, Raisman-Vozari R⁴, Michel PP⁴, Guimaraes FS⁵, Del Bel EA² ¹FMRP-USP, Department of Physiology, Ribeirao Preto, Brazil; ²FORP-USP, Dept Basic and Oral Biology, Ribeirao Preto, Brazil; ³FMRP-USP, Department of Neuroscience and Behavior, ⁴ICM-CNRS-Inserm, Paris Brain Institute, Sorbonne Université, Hôpital Pitié Salpêtrière, Paris, France; ⁵FMRP-USP, Dept of Pharmacology, Brazil.

Iris Room (Inova)	RT2 – 55 Years of the Postgraduate Course in Pharmacology at the Escola Paulista de Medicina - Unifesp: Training Researchers Who are Up to Date with Science and Innovation in Brazil (55 anos da Pós-Graduação em Farmacologia da Escola Paulista de Medicina – Unifesp: Formando Pesquisadores Atualizados com Ciência e Inovação no Brasil) (Presented in Portuguese) Chair: Paulo Caleb J. L. Santos (Unifesp-EPM) ◆ <i>History of the Postgraduate Course in Pharmacology</i> (Histórico da Pós-Graduação em Farmacologia) Fábio C. Cruz (Unifesp-EPM) ◆ <i>Current results, Technologies and Innovation</i> (Resultados Atuais, Tecnologias e Inovação) Mirian Akemi Furuie Hayashi (Unifesp-EPM) ◆ <i>Perspectives and Aspects of the Program</i> (Perspectivas e Aspectos do Programa) Miriam Galvonas Jasiulionis (Unifesp-EPM)
11:00-11:20 am	Coffee-break
11:20 am-12:10 pm	Lectures
Butantan Room (CDI – Audience)	L2 – Skeletal Muscle and Transulfuration Pathway Giuseppe Cirino (University of Naples Federico II, Italy) Presented by
Iris Room (Inova)	L3 – Leveraging Physiology-based Models in Perinatal Pharmacology and in Drug-Induced Liver Injury Pieter Anaert (Katholieke Universiteit Leuven, Belgium) Presented by Bibiana Verlindo Araújo (UFRGS)
12:10-01:40 pm	Lunch
12:10-01:40 pm Iris Room (Inova)	Meet the Expert (with Lunch Box) Chair: SBFTE Jovem Committee ◆ Alexandre Denadai Souza (Katholieke Universiteit Leuven, Belgium) ◆ Alicia Ermelinda Damiano (University of Buenos Aires, Argentina) ◆ Armando Genazzani (President Italian Society of Pharmacology, University of Torino, Italy) ◆ Emilio Clementi (Iuphar Secretary General, University of Milano, Italy) ◆ Joyce T. da Silva (University of Maryland, USA) ◆ Marina Sanchez Hidalgo (University of Seville, Spain) ◆ Patrick Pierre Michel (Paris Brain Institute, France) ◆ Paulo Caleb J. L. Santos (Unifesp-EPM)
12:10-01:40 pm Sé Room (CDI – Support Room)	Meeting of the North-Northeast and Central West Region Pharmacology Network (with Lunch Box)
02:00-03:30 pm	Symposia
Butantan Room (CDI – Audience)	S4 – Translational Approach to the Treatment of Parkinson's Disease Chair: Elaine Del-Bel (USP-RP) ◆ <i>Translational approach for a pharmacological modulation of neuropsychiatric symptoms due to disorders of the striatal pathway</i> Louise-Laure Mariani (Sorbonne University, France) ◆ <i>Unraveling the anti-inflammatory mechanisms of the two synthetic cannabinoids 4'F-CBD and HU-910 on brain glial cells</i> Patrick Pierre Michel (Paris Brain Institute, France) ◆ <i>Dyskinesia and protection in preclinical models of Parkinson's disease</i>

	Elaine Del-Bel (USP-RP)
Liberdade Room (CDI - Mezzanine 1)	S5 – Sex, Hormones and Age as Determinants for Disease Severity and its Implications on Therapeutics Chair: Aleksander Roberto Zampronio (UFPR) ◆ <i>Using functional MRI to assess sex and age differences in pain processing at the whole-brain level</i> Joyce T. da Silva (University of Maryland, USA) ◆ <i>Sex differences and influence of female-sex hormones on systemic inflammation</i> Aleksander Roberto Zampronio (UFPR) ◆ <i>Oral presentation 1: 05.010 Sex Differences in Behavioral Responses Across Rat Models of Migraine.</i> Baggio DF, Da Luz FMR, Lejeune VBP, Chichorro JG UFPR – Lab of Craniofacial Pain, PPG Pharmacology, Brazil ◆ <i>Oral presentation 2: 04.031 Evaluation Of Different Estradiol Treatment Strategies In Lung Inflammation of Brain Death Donors After Lung Transplantation.</i> Wellichan G¹, Sousa PS, Vidal-dos-Santos M, Correia CJ¹, Breithaupt-Faloppa AC, Moreira LFP, Ricardo-da-Silva FY InCor-FM-USP Lab Cirurgia Cardiovascular e Fisiopatologia da Circulação, São Paulo, Brasil
Bixiga Room (CDI – Mezzanine 2)	S6 – Impact of Sex Hormones on Vascular Biology: New Perspectives and Insights Chair: Rita C Tostes (USP-RP) ◆ <i>Estrogen signaling impacting perivascular adipose tissue function in postmenopausal model</i> Tiago Januário da Costa (USP-SP) ◆ <i>Beyond genders: unraveling testosterone role in the vasculature</i> Jeimison Duarte Santos (USP-RP) ◆ <i>Oral presentation 1: 06.030 Aging Differentially Affects Male and Female Thoracic Aorta Perivascular Adipose Tissue: A Study Using a Murine Model of Accelerated Aging.</i> Marques BVD, Akamine EH USP, Department of Pharmacology ◆ <i>Oral presentation 2: 06.027 Renal Impact of Subacute Exposure to Endocrine Disruptors in Female Rats: Evidence of Preserved Function.</i> Esteves GAB^{1,2}, Accioli DSG^{1,2}, Alves LM¹, Morcillo LSL^{1,2} ¹ICB-UFRJ, ²IBCCF-UFRJ, Centro de Pesquisa em Medicina de Precisão
Iris Room (Inova)	S7 – Basic Pulmonary Pharmacology and Clinical Practice for Respiratory Diseases Chair: Patrícia Machado Rodrigues e Silva Martins (FIOCRUZ) ◆ <i>CT Imaging for Respiratory Diseases: Bridging Experimental Models and Clinical Practice</i> Lorenzo Ball (University of Genoa, Italy) ◆ <i>Nicotinic receptors in respiratory diseases: Insight from experimental models</i> Carla Máximo Prado (Unifesp-Baixada Santista) ◆ <i>Development and validation of methodology for preclinical evaluation of candidate compounds for the treatment of chronic pulmonary disease through microCT</i> Patrícia Machado Rodrigues e Silva Martins (Fiocruz)
03:30-03:50 pm	Coffee-Break
03:50-05:20 pm	E-Poster Session 2

	01. Cellular and Molecular Pharmacology
	Totem 01 (01.009-01.013)
	02. Neuropharmacology
	Totem 02 (02.022-02.025)
	Totem 03 (02.026-02.029)
	Totem 12 (02.030-02.032, 02.048)
	Totem 13 (02.034-02.037)
	Totem 20 (02.038-02.041, 02.075)
	03. Psychopharmacology
	Totem 14 (03.008-03.011)
	04. Inflammation and Immunopharmacology
	Totem 04 (04.016-04.018)
	Totem 15 (04.020-04.024)
	Totem 21 (04.025-04.030)
	05. Pain and Nociception Pharmacology
	Totem 05 (05.013-05.016)
	Totem 16 (05.017-05.020)
	06. Cardiovascular and Renal Pharmacology
	Totem 06 (06.010-06.014)
	Totem 17 (06.015-06.018)
	Totem 22 (06.019-06.022, 06.030)
	07. Endocrine, Reproductive and Urinary Pharmacology
	Totem 04 (07.007, 07.009)
	08. Respiratory and Gastrointestinal Pharmacology
	Totem 04 (08.004)
	Totem 23 (08.005-08.009)
	09. Natural Products and Toxinology
	Totem 08 (09.015-09.019, 09.051)
	Totem 18 (09.020-09.024)
	Totem 24 (09.025-09.030)
	10. Cancer Pharmacology
	Totem 09 (10.020-10.024)
	Totem 25 (10.025-10.029)
	11. Clinical Pharmacology, Pharmacokinetics, Pharmacogenomics and Toxicology
	Totem 10 (11.006-11.011)
	12. Drug Discovery and Development
	Totem 11 (12.001-12.007)
	14. Pharmacology: Other
	Totem 19 (14.001-14.005)
	Totem 26 (14.006-14.010)
5:20 pm	Cultural Presentation Tubatuque (IQ-USP) Farmatuque (FCF-USP)

	Manda Chuva (FFLCH-USP)
6:00 pm	Get together Party Bonfa's Beer Av. Comendador Alberto Bonfiglioli, 341 Jardim Bonfiglioli São Paulo, SP

Oct 23 rd , 2024 (Thursday)	
08:30 am-18:00 pm	Venue Secretariat and SBFTE Secretariat Opening
09:00-10:30 am	Symposia/Oral Communication / Roundtable
Butantan Room (CDI – Audience)	S8 – Role and Modulation of Cardiac and Neural Ion Channels Chair: Erika Pinter (University of Pécs, Medical School, Hungary) ♦ <i>Decreased repolarization reserve as a possible mechanism behind drug induced proarrhythmia</i> Andras Varro (University of Szeged, Hungary) ♦ <i>Lipid raft disruption around nociceptive TRP channels provides novel analgesic opportunity</i> Eva Szoke (University of Pécs, Medical School, Hungary) ♦ <i>Regulatory role of TRPA1 receptors in neurodegenerative disorders associated with dementia</i> Erika Pinter (University of Pécs, Medical School, Hungary)
Liberdade Room (CDI - Mezzanine 1)	S9 – Therapeutic Potential of Natural Products: From Ancient uses to Modern Practices Chair: Fúlvio Rielí Mendes (UFABC) ♦ <i>Natural products for neurodegenerative diseases: potential candidates, current studies and perspectives</i> Fúlvio Rielí Mendes (UFABC) ♦ <i>Effects of Natural Polysaccharides in Cancer Models</i> Alexandra Acco (UFPR) ♦ <i>Oral presentation 1: 02.053 Effects of the Prebiotic Inulin on Behavioral Changes in a Model of Autism Spectrum Disorder (ASD) In Adolescent Rats.</i> Amorin BM¹, Bertotti G², Haddad NM², Longo B¹, Silva LM¹ ¹LAFATI-UFSC, Lab of Gastrointestinal Tract Pharmacology and Interactions ²Univali, Medical School
Auditorium (Inova)	S10 – Placenta, Preeclampsia, and Neuroprotection: Emerging Therapies and Future Horizons Chair: Fernanda Regina Casagrande Giachini Vitorino (UFMT) ♦ <i>AQP3: More than a water channel – A potential therapeutic target of aspirin in preeclampsia</i> Alicia Ermelinda Damiano (University of Buenos Aires, Argentina) ♦ <i>Placental and amnion derivatives: From preclinical research to therapeutic innovation</i> Sebastian San Martín (University of Valparaíso, Chile) ♦ <i>Oral presentation 1: 06.019 Impact of Esomeprazole on Nitric Oxide Bioavailability in a Rat Model of Gestational Hypertension.</i> Da Silva MLS, Da Silva AAF, Toghi CJ, Dragoneti BJ, Da Costa LC, Dias-Junior CA IB-UNESP-Botucatu, Department of Biophysics and Pharmacology ♦ <i>Oral presentation 2: 06.039 Sodium Nitrite Attenuates NLRP3 Associated Genes in a Hypoxia in vitro Model of Early-Onset Preeclampsia.</i> Santos KN, Nunes PR, Pereira DA, Zampieri GM, Bueno-Pereira TO, Passeti LFP, Matheus MB, Sandrim VC IBB-Unesp- Botucatu, Department of Biophysics and Pharmacology - Brazil
Iris Room (Inova)	RT3 – Beyond the Academy (Além da Academia) (Presented in Portuguese) Chair: Weverton Castro Coelho-Silva (USP-RP, SBFTE Jovem) ♦ <i>If my career doesn't fit within the University, what then? Moving from academia to pharma without losing scientific identity (E se a minha</i>

	<i>carreira não couber na Universidade? Migrando da academia para a indústria farmacêutica sem perder a identidade científica)</i> Lorena C. Corrêa Fehlberg (Pfizer) ♦ <i>Anatomy of an Entrepreneur Scientist (Anatomia de um Cientista Empreendedor)</i> Fabrício Alano Pamplona (Vital Green Holding) ♦ <i>Is the Entrepreneurial Spirit an Innate or Adaptive Force? (O espírito empreendedor é uma força inata ou adaptativa?)</i> Andreas Kaasi (Eva Scientific)
10:30-10:50 am	Coffee-break
11:00 am-12:40 pm	Symposium
Butantan Room (CDI – Audience)	José Ribeiro do Valle Award Chair: Emiliano O. Barreto (UFAL) <i>Julia Sapienza Passos</i> ♦ 10.014 Multifunctional Thermosensitive Nanocarriers for Local Treatment of Non-Invasive Breast Cancer: Tissue Localization, Cytotoxic and Molecular Effects. Passos JS^{1,2}, Machado-Neto JA¹, Panitch A¹, Lopes LB¹ ¹ICB-USP, Department of Pharmacology, Functional and Molecular Biology Program, Brazil; ²Georgia Institute of Technology, Department of Biomedical Engineering, USA <i>Brena Freire de Oliveira Claudino</i> ♦ 08.002 Prevention of Gut Microbiota Imbalance: The Role of <i>Arthrosira platensis</i> in Hypercaloric Diet-Induced Dysbiosis. Claudino BFO^{1,2}, Arruda RRA^{1,2}, Diniz AFA^{1,2}, Melo MB¹, Sousa-Filho JEC¹, Colunga AAG⁴, Nobre CSN⁴, Silva BA^{1,2,3} ¹UFPB, Lab of Functional Pharmacology Prof. George Thomas, Institute of Research in Drugs and Medicines, Brazil ²UFPB, Postgraduate Program in Bioactive Natural and Synthetic Products, Health Sciences Center, Brazil ³UFPB, Department of Pharmaceutical Sciences, Health Sciences Center, Brazil ⁴University of Minho, Fermentations Lab, Centre of Biological Engineering, Portugal <i>Luiz Adriano Damasceno de Queiroz</i> ♦ 04.030 Aging, Immunity and Metabolism: Divergent Effects of Rapamycin in Senescence-Prone and -Resistant Mice. Queiroz LAD¹, Barros RS¹, Assis JB², Pantoja KC¹, Bustia SX¹, Sá-Nunes A², Rodrigues SFP³, Turato WM⁴, Silva CCC⁵, Migliorini S¹, Martins JO¹ ¹USP, FCF, Dept. of Clinical and Toxicological Analyses, Lab. of Immunoendocrinology, Brazil; ²USP, ICB, Dept. of Immunology, Lab. of Experimental Immunology, Brazil; ³USP, ICB, Dept. of Pharmacology, Lab. of Vascular Nanopharmacology, Brazil; ⁴USP, IQ, Dept. of Biochemistry, Energy Metabolism Lab., Brazil; ⁵USP, FCF, Dept. of Clinical and Toxicological Analyses, Multi-user Preclinical Imaging Center, Brazil <i>Mariana Burille Moretti</i> ♦ 07.010 Probenecid Potentiates the Relaxant Effect of β-Adrenergic Agonists in the Prostate of Rats. Moretti MB, Leonardi GR, Barros JVC, Lima JM, Monica FZ Unicamp, PPG Pharmacology, Brazil <i>Carolina Medina Coeli da Cunha</i>

	♦ 04.068 Tyrosine Kinase Inhibitor Reduces Systemic and Cerebral Inflammation in Experimental Sepsis. Cunha CMCD^{1,2,3}, Moraes BPT^{1,3,4}, Abreu VHP^{1,3}, Soares GVM^{1,3}, Moraes-de-Souza IM^{1,2,3}, Almeida MAP¹, Estado V¹, Sayão PGF^{1,3,4}, Souto HA^{1,3}, Bozza PT^{1,2}, Castro-Faria-Neto HC^{1,2}, Silva AR^{1,2}, Gonçalves-de-Albuquerque CF^{1,2,3,4} ¹IOC-Fiocruz, Immunopharmacology Lab, Rio de Janeiro, Brazil. ²IOC-Fiocruz Postgraduate Program in Cellular and Molecular Biology, Rio de Janeiro, Brazil. ³Unirio, Immunopharmacology Lab, Rio de Janeiro, Brazil. ⁴Unirio Postgraduate Program in Molecular and Cellular Biology, Rio de Janeiro, Brazil
12:40-02:00 pm	Lunch
12:40-02:00 pm Iris Room (Inova)	SBFTE Young Assembly
02:00-04:00 pm Iris Room (Inova)	SBFTE Assembly
04:10-05h00	Lectures
Butantan Room (CDI – Audience)	L4 – Nicotinamide Phosphoribosyl-Transferase (NAMPT) and nicotinic acid phosphoribosyl transferase (NAPRT) are novel cytokines involved in inflammation Armando Genazzani (President Italian Society of Pharmacology, University of Torino, Italy) Presented by Zsuzsanna Helyes (University of Pécs, Medical School, Hungary) / Soraia K. P. Costa (USP-SP)
Iris Room (Inova)	L5 – Microbiota-Derived polyphenol metabolites modulate the T helper 17 to regulatory T cell (Th17/Treg) imbalance, promoting resilience to stress-induced anxiety- and depressive-like behavior Giulio Maria Pasinetti (Mount Sinai School of Medicine, USA) Presented by Fulvio Rieli Mendes (UFABC)
05:00-05:20 pm	Coffee-Break
05:20-06:50 pm	E-Poster Session 3
	01. Cellular and Molecular Pharmacology Totem 01 (01.014-01.018) Totem 26 (01.019-01.025) 02. Neuropharmacology Totem 02 (02.042)-02.045) Totem 10 (02.033, 02.046-02.049) Totem 11 (02.050-02.053) Totem 18 (02.054-02.057) 03. Psychopharmacology Totem 03 (03.012-03.018, 03.031) 04. Inflammation and Immunopharmacology Totem 04 (04.031-04.034) Totem 12 (04.035-04.039) Totem 19 (04.040-04.046)

	05. Pain and Nociception Pharmacology
	Totem 05 (05.021-05.024)
	Totem 13 (05.025-05.028)
	Totem 20 (05.029-05.032)
	06. Cardiovascular and Renal Pharmacology
	Totem 14 (06.023-06.026)
	Totem 21 (06.027-06.031)
	07. Endocrine, Reproductive and Urinary Pharmacology
	Totem 15 (07.010-07.013)
	08. Respiratory and Gastrointestinal Pharmacology
	Totem 22 (08.010-08.014)
	09. Natural Products and Toxinology
	Totem 06 (09.031-09.036)
	Totem 16 (09.037-09.041)
	Totem 23 (09.042-09.047)
	10. Cancer Pharmacology
	Totem 17 (10.030-10.034)
	Totem 24 (10.035-10.039)
	11. Clinical Pharmacology, Pharmacokinetics, Pharmacogenomics and Toxicology
	Totem 07 (11.012-11.017)
	Totem 25 (11.018-11.023)
	12. Drug Discovery and Development
	Totem 08 (12.008-12.013)
	13. Pharmacology Education and Technology
	Totem 09 (13.001-13.004)

Oct 24 th , 2025(Friday)	
08:30 am	Venue Secretariat and SBFTE Secretariat Opening
08:30-09:20 am	Courses
Sé Room (CDI)	Cr1 – Principles of Systematic Review (Princípios de Revisão Sistemática) Chair: Adriane Ribeiro Rosa (UFRGS) ♦ Class 3: <i>Bias Risk, concepts and interpretation of meta-analysis (Risco de Viés, conceitos e interpretação de de metanálise)</i> (Presented in Portuguese) Patricia Martins Bock (UFRGS)
Iris Room (Inova)	Cr2 – Applications of Physiology-Based Pharmacokinetics in the Drug Development (Aplicações de Farmacocinética Embasada em Fisiologia na Área de Desenvolvimento de Novos Fármacos) (Presented in Portuguese) Chair: Bibiana Verlindo Araujo (UFRGS) ♦ Class 3: <i>Real-world impact of PBPK: applications across drug development (Impacto da PBPK no mundo real: aplicações no desenvolvimento de fármacos)</i> Valva Vozmediano (Clinical Trial and Consulting Services, USA)
Liberdade Room (CDI - Mezzanine 1)	Cr3 – Neuropsychopharmacology of Reinforcement and Reward Behaviors (Neuropsicofarmacologia de comportamentos de reforço e recompensa) (Presented in Portuguese) Chair: Helena Maria Tannhauser Barros (UFCSPA) ♦ Class 3: <i>Animal models of reinforcement and reward in drug dependence (Modelos animais de reforço e recompensa em dependência a drogas)</i> Helena Maria Tannhauser Barros (UFCSPA) / Cristina Aparecida Jark Stern (UFPR)
Bixiga Room (CDI - Mezzanine 2)	Cr4 – Experimental Models in Cancer and their Interface with Environmental Pollutants (Modelos Experimentais em Câncer e sua Interface com Poluentes Ambientais) (Presented in Portuguese) Chair: Juliana Elaine Perobelli (Unifesp-Baixada Santista) ♦ Class 3: <i>In Vivo Cancer Models: Assessing the Impact of Environmental Pollutants on Tumor Development (Modelos in vivo de Câncer: Avaliação dos Impactos de Poluentes Ambientais no Desenvolvimento Tumoral)</i> Débora Barbosa Vendramini Costa (Michigan State University)/Juliana Elaine Perobelli (Unifesp-Baixada Santista)
09:30-11:00 am	E-Poster Session 4
	01. Cellular and Molecular Pharmacology Totem 07 (01.026, 01.028-01.030) Totem 15 (01.005; 01.032-01.034) 02. Neuropharmacology Totem 01 (02.007, 02.058-02.060) Totem 08 (02.062-02.065) Totem 09 (02.066-02.069) Totem 16 (02.070-02.074) 03. Psychopharmacology Totem 02 (03.015; 03.019-03.024)

	Totem 17 (03.025-03.030) 04. Inflammation and Immunopharmacology Totem 03 (04.047-04.051) Totem 10 (04.052-04.056) Totem 18 (04.057-04.063) Totem 24 (04.013; 04.064-04.067) 05. Pain and Nociception Pharmacology Totem 04 (05.033-05.036) Totem 19 (05.037-05.042) 06. Cardiovascular and Renal Pharmacology Totem 05 (06.032-06.035) Totem 11 (06.036-06.039) Totem 20 (06.040-06.044) Totem 25 (06.045-06.051) 07. Endocrine, Reproductive and Urinary Pharmacology Totem 12 (07.014-07.018) 09. Natural Products and Toxinology Totem 06 (09.048-09.050, 09.052-09.053, 09.068) Totem 13 (09.054-09.058) Totem 21 (09.059-09.063) Totem 26 (09.065-09.067) 10. Cancer Pharmacology Totem 22 (10.040-10.044) 11. Clinical Pharmacology, Pharmacokinetics, Pharmacogenomics and Toxicology Totem 14 (11.024-11.029) 12. Drug Discovery and Development Totem 23 (12.014-12.019)
11:00-11:20 am	Coffee-break (Brunch)
11:30 am-12:20 pm	Lectures
Butantan Room (CDI – Audience)	L6 – Preclinical and Phase I Clinical Development of a Novel Multi-Target Drug Candidate for Chronic Neuropathic Pain Zsuzsanna Helyes (University of Pécs, Medical School, Hungary)) Presented by Armando Genazzani (President Italian Society of Pharmacology, University of Torino, Italy) / Soraia K. P. Costa (USP-SP, President SBFTE)
Iris Room (Inova)	L7 – Transforming Molecular Pharmacology into Innovative Drug Discovery Emer Suavinho Ferro (USP-SP) Presented by Gilberto De Nucci (Unicamp, USP-SP)
	Butantan Room (CDI – Audience)
12:30-13:20 pm Closing Lecture	L8 – Molecular Therapeutics Targets for Muscular Dystrophy- the Role of Translational Pharmacology Emilio Clementi (Iuphar Secretary General, University of Milano, Italy) Presented by Soraia K. P. Costa (USP-SP, President SBFTE)
13:20-14:00 pm	Closing Ceremony Cultural presentation Awards Announcement

01. Cellular and Molecular Pharmacology

Totem 09

01.001 SIVA1 as a Key Player in Breast Cancer Development: Evidence from *In Vitro* Approaches. Parducci NS¹, Oliveira BA¹, Garnique AMB, Carvalho, MFL¹, Machado-Neto JA^{1,2} ¹USP, Institute of Biomedical Sciences, Dept. of Pharmacology, Brazil. ²USP, School of Medicine, Dept. of Clinical Medicine, Brazil

01.002 Major Depressive Disorder Patient Serum Induces TLR4 Activation in Human Cells. Soares JV¹, Farias IS¹, Fidalgo FM², Hayashi MAF¹ ¹Unifesp-EPM, Lab of molecular Neuropharmacology, Pharmacology Department, São Paulo, Brazil; ²Unifesp-EPM, Department of Psychiatry, São Paulo Brazil

01.003 Neuroprotective and Antioxidant Effects of the Paracetamol Analog LQFM²91 in a 6-OHDA Mouse Model of Neurotoxicity. Ferreira PYO¹, Santos EEO¹, Uchenna N¹, Campos HM¹, Costa EA¹, Menegatti R², Ghedini PC¹ ¹UFG, Dept Pharmacology, ²UFG, Faculty of Pharmacy

01.004 Nanoplastics Modulate Carboplatin Response in Ovarian Cancer Cell Line: Pharmacological Resistance and Metabolic Paradox. Silva GCA, Lima MC, Portela LMF Aal ME, Rocha VA, Scarano WR, Delella FK¹ Dept Structural and Functional Biology, Unesp, Brazil

01.027 Beyond the Sperm Surface: EPPIN's Distribution and Colocalization with SEMG1 and AKAP4 in Human Spermatozoa. Santos BR¹, Mariani NAP¹, Santos NCM¹, Themer ACF¹, Daroz GA², Martini PV¹, Santos LA², Dias-Melício LA², Teixeira TA³, Hallak J⁴, Santos AGP², Silva EJR¹. ¹IBB-UNESP-Botucatu, ²FMB-UNESP-Botucatu, ³UNIFAP, University Hospital, Division of Urology; ⁴HC-USP, Hospital das Clínicas, Division of Urology

Totem 15

01.006 The Dual Role of ANKHD1 in Gastric Cancer: Insights from Integrative Analyses. Almeida BO¹, Gasparini MVR², Silva JCL¹, Archangelo LF², Machado-Neto JA¹ ¹ICB-USP, Dept Pharmacology, PPG Functional and Molecular Biology, Brazil ²FMRP-USP, Dept Cellular and Molecular Biology and Pathogenic Bioagents, PPG Cell and Molecular Biology, Brazil

01.007 Viability and Cytotoxicity Profile of Beta-Hydroxybutyrate in Neural Progenitor Cells. Menezes IO, Viana DZA, Lima LS, Iwashe EMK USP, Pharmacology

01.008 A Novel Arylpiperazine Derivative (LQFM181) Promotes Antioxidant and Neuroprotective Effects Against 3-Nitropropionic Acid-Induced Neurotoxicity in Swiss Mice. Campos HM¹, Ferreira PYO¹, Pereira R¹, Okoh VI¹, Pruccoli. L³, Uchenna N¹, Leite J¹, Menegatti R², Tarozzi A³, Ghedini PC¹ ¹UFG, Dept Pharmacology, ²UFG, Faculty of Pharmacy, ³University of Bologna, Dept for Life Quality Studies

01.031 Enhanced Neuroprotection by the Glycosylated Chrysin Derivative LQFM280 in an AlCl₃-Induced Neurodegeneration's Model. Uchenna N¹, Campos HM¹, Ferreira PYO¹, Okoh VI¹, Costa EA¹, Menegatti R², Ghedini PC¹ ¹UFG, Dept Pharmacology, ²UFG, Faculty of Pharmacy

02. Neuropharmacology

Totem 01

02.001 **Haloperidol As a Treatment for Wernicke-Korsakoff Syndrome.** Nogueira DL¹, Rocha ME²
¹Centro Universitário de Mineiros, School of Medicine, Brazil, ²Centro Universitário de Mineiros, Brazil

02.002 **Lisinopril Prevents Stress-Induced Fear Generalization and Memory Impairments in Mice.** Valera-Sanchez CC, Solovi SR, Werworn LFM, Resstel LBM. USP, Ribeirão Preto Medical School, Dept Pharmacology, Neuropharmacology Lab, Brazil

02.003 **Effect of 5-HT_{1A} Receptor Participation in Mediation of the Anxiolytic Effect of Resolvin D5: Evaluation of Sex-Based Differences.** Uada TS, Avanço LC, Zanoveli JM ¹Lab. of Neuropsychopharmacology, Brazil, UFPR

02.004 **Copaiba Oil did not Induce Significant Changes in the Locomotor Activity of Adult Zebrafish.** Costa CHL¹, Barros Costa G¹, Andrade KC², Abreu LS², Nicole Maciel NMF², Fonteles MV³, Sales M³, Correia MFS³, Franco VMF⁴, Mesquita DS⁵, Honório Júnior JER⁵ ¹Christus University Center, Psychology, Brazil; ²Christus University Center, Biomedicine, Brazil; ³Nursing student at Christus University Center, Brazil; ⁴UFC, Postgraduate in Pharmacology, Brazil; ⁵Christus University Center, Brazil

Totem 02

02.005 **Evaluation of the Antidepressant Effect of CBD from *Cannabis sativa* by the Forced Swimming Test in a Chronic Model in Mice.** Costa GB¹, Costa CHL¹, Sousa JDDS², Pimentel ISB², Dinelly Neto VC², Izidorio JLG², Paixão LDS³, Franco VMF⁴, Mesquita DS⁵, Honório Júnior JER⁵ ¹Christus University Center, Psychology, Brazil; ²Christus University Center, Biomedicine, Brazil; ³Christus University Center Nursing, Brazil; ⁴UFC, Postgraduate in Pharmacology, Brazil; ⁵Christus University Center, Brazil

02.006 **Neurotoxic effects of N-Nitrosodimethylamine (NDMA) on Oxidative Stress Markers in the Cerebral Cortex and Hippocampus of Prenatally Exposed Rats.** Alves DL¹, Ferreira PYO¹, Santos EEO¹, Aguiar IS¹, Campos HM¹, Ghedini PC¹, Mazzaro R¹, Silva MS², Reis DS², Rodrigues BM², Amaral VCS², Leite JA¹ ¹UFG, Dept Pharmacology, Institute of Biological Sciences, Goiânia, Brazil; ²UEG, Lab Farmacologia e Toxicologia de Produtos Naturais e Sintéticos, Anápolis, GO, Brasil

02.008 **Early-Life Reduction of Parvalbumin in the Medial Prefrontal Cortex (mPFC): Behavioral and Electrophysiological Outcomes in Adolescent and Adult Rats.** Caceres-Silva GH, Silveira JR, Freitas IS, Guimarães FS, Gomes FV FMRP-USP, Dept. of Pharmacology, Brazil

02.061 **Hemodynamic and Histological Alterations Caused by Venom of the Coralsnake (*Micrurus spixii*) in Anesthetized Rats.** Ramos JS^{1,2}, Macedo TCS^{1,2}, Takabayashi LA^{1,2}, Matos-Silva H³, Silva Júnior NJ², Hyslop S¹. ¹FCM-UNICAMP, Depto de Farmacologia, PPG Farmacologia, Brazil; ²PUC-GO, PPG em Ciências Ambientais e Saúde, Brazil; ³NUPMA-UNIRV-Goiânia, Núcleo de Pesquisa da Faculdade de Medicina, Brazil

Totem 10

02.009 **Prolyl Oligopeptidase (POP) Activity in an Animal Model of Congenital Syndrome Associated with Zika Virus (CZS).** Ximenes APX¹, Oyadomari WY¹, Christoff RR², Garcez PP², Hayashi MAF¹ ¹Unifesp-EPM, Lab Neurofarmacologia Molecular, Depto de Farmacologia, São Paulo, Brazil ²ICB-UFRJ, Rio de Janeiro, Brazil

02.010 Evaluation of the Neuroprotective Effect of Monoterpene 1,8-Cineole Complexed with Beta-Cyclodextrin *in vitro* Model of Parkinson's Disease. Araújo-Monteiro L¹, Carvalhinho-Lopes PS¹, Soares-Silva B¹, Donzelli NIZ¹, Pereira EGJ¹, Binatto BVCC¹, Ribeiro TF¹, Quintans-Junior LR², Rando DG³, Ribeiro AM¹ ¹Unifesp-Baixada Santista, PPG in Bioproducts and Bioprocesses, Brazil; ²UFS, PPG in Pharmaceutical Sciences, Brazil; ³Unifesp-Diadema, PPG in Pharmaceutical Sciences; Brazil

02.011 Behavioral and Synaptic Alterations in Offspring Induced by Gestational Valproic Acid Exposure: Sex Differences. Rodrigues BA, Silva JN, Andreotti DZ, Amorim TL, Kawamoto EM. ICB-USP, Lab of Molecular and Functional Neurobiology, PPG Pharmacology, Brazil

02.012 Anticonvulsant and Neuroprotective Potential of Microalgae *Chlorella vulgaris* and *Limnospira platensis* in a Pentylene-tetrazol-Induced Seizure Model. Cancela LCF^{1,2}, Freitas CM¹², Bentes ILG^{1,2}, Araújo TB¹, Pinto DSV¹, Jesus MS¹, Faustino SMM¹, Nascimento JLM², Oliveira FR^{1,2} ¹Unifap, Depto de Ciências Biológicas e da Saúde, Curso de Farmácia, Brazil ²UFPA, Graduate Program in Pharmacology and Biochemistry, Brazil

Totem 11

02.013 *In utero* Exposure to Valproic Acid, a Proposed Model of Autism, Enhances Dopamine System Activity in the Ventral Tegmental Area of Adolescent and Adult Rats. Grigório-de-Sant'Ana M, Freitas IS, Souza AJ, Verza FA, Guimarães FS, Gomes FV FMRP-USP, Dept Pharmacology, Post-Graduation Program in Medicine (Neurology), Brazil

02.014 The Effect of Cannabidiol on Fear Memory Reconsolidation may Involve Neuroactive Steroid Biosynthesis and PPAR γ Activation. Santos MR¹, Cardoso NC¹, Raymundi AM¹, Guimarães FS², Bertoglio LJ³, Pinna G⁴, Stern CAJ¹ ¹UFPR, Dept Pharmacology, ²FMRP-USP, Dept Pharmacology, ³UFSC, Dept Pharmacology, ⁴University of Illinois Chicago The Psychiatric Institute, Chicago, USA

02.015 Evaluation of the Anxiolytic Effect of *Lavandula dentata* Essential Oil. Marco MR, Flores VG, Silva GC, Barcellos ES, Zorzo RR, Veiverberg A, Mattos CB, Betti AH. Feevale University, Institute of Health Sciences, Brazil

02.016 Assessing Microglial Phenotype Alterations in Rats Exposed to Prenatal Dexamethasone and Acute Stress Kamidai NM, Sbardellotto GE, Munhoz CD USP, PGP Functional and Molecular Biology, Brazil

Totem 23

02.017 Ndel1 Activity in Cultured Neuronal Cells and Effects of Treatment with Drugs and Serum from patients with Depression and Depressive Bipolar Disorder. De Farias IS¹, Oyadamari WY¹, Fidalgo T², Hayashi MAF¹ ¹Unifesp-EPM, Dept Pharmacology, São Paulo, Brazil; ²Unifesp-EPM, Dept Psychiatry, São Paulo, Brazil

02.018 Andrographolide Prevents Increases in Hyperlocomotion, GSK3-Beta Levels, 50-kHz Ultrasonic Vocalization Emission and Brain Oxidative Stress in Animal Models of Mania. Kanazawa LKS¹, Radulski DR¹, Pereira GS¹, Nelissen E², Prickaerts J², Schwarting RKW³, Acco A¹, Andreatini R¹ ¹UFPR, PPG Pharmacology, Brazil ²Maastricht University, Dept Psychiatry and Neuropsychiatry, The Netherlands ³Philipps-Universität Marburg, Center for Mind, Brain and Behavior (CMBB), Germany

02.019 Differential Modulation of the ADAM10-NEGR1-FGFR2 Pathway by Cannabidiol and Escitalopram in a Corticosterone-Induced Stress Model: Sex- and Brain Region-Dependent Effects. Vatanabe IP¹, Pereira ACM¹, Magalhães MS², Barros AS², Bilibio JO², Lisboa, SF^{1,2} ¹FCFRP-USP, Dept Biomolecular Sciences, Brazil; ²FMRP-USP, Pharmacology PPG, Brazil

02.021 **Hormone Replacement Therapy During Perimenopause: Analysis of Brain Electrical Activity of Female Wistar Rats Treated With 17 β -Estradiol After Surgical Castration.** Mattos BG^{1,2}, Souza RD^{1,3}, Teixeira LL^{1,3}, Silveira EP^{1,4}, Hamoy AO⁵, Pinto LC¹, Hamoy M^{3,5}, Lopes DCF^{1,3} ¹UFPA, Lab Neuropatologia Experimental, Hospital Universitário João de Barros Barreto, Brasil, ²FSCM, Pediatria, Brasil; ³UFPA, PPG FARMABIO, Brasil. ⁴FSCMPA, Clínica Médica, Brasil. ⁵UFPA, Lab Farmacologia e Toxicologia de Produtos Naturais, Brasil

03. Psychopharmacology

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03.001 **The Neurobehavioral Role of Leptin in a Murine Model of Chronic Stress.** Sampaio LL, Albernaz KA, Munhoz CD ICB-USP, Dept Pharmacology, Neuroendocrinepharmacology and Immunomodulation Lab, PPG Functional and Molecular Biology, Brazil

03.002 **The Role of Adenosinergic Signaling in the Antidepressant-like Effects of Cannabidiol and Potential Molecular Mechanisms Involved.** Silveira JR¹, Sales AJ¹, Ciruela F², Guimarães FS¹ ¹FMRP-USP, Dept. of Pharmacology, Brazil; ²University of Barcelona, Institute of Neurosciences, Dept. of Pharmacology and Experimental Therapeutics, Spain

03.003 **Epigenetic Regulation by HAT p300/CBP is Temporally Controlled by the Endocannabinoid System During Fear Memory Reconsolidation.** Bergmann MF, Raymundi AM, Stern CAJ Dept Pharmacology, UFPR, Curitiba, PR, Brazil

03.004 **Role of the Globus Pallidus Endocannabinoid System in the Expression of Defensive Responses in Mice with a Depressive-Like Phenotype.** Coelho-Silva WC, Coimbra NC FMRP-USP, Ribeirão Preto, São Paulo, Brazil

03.005 **Evaluation of Parvalbumin Interneurons and Perineuronal Nets Across Brain Regions in Relation to Motor and Cognitive Effects Induced by Typical Antipsychotics.** Ponciano, R¹, Issy AC², Gomes FV³, Del-Bel EA^{1,2} ¹USP-RP, Dept Physiology, Brazil; ²USP-RP, Dept Basic and Oral Biology, Brazil; ³USP-RP, Dept Pharmacology, Brazil

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03.006 **Two-Step Pharmacological Treatment with Roflumilast and Cannabidiol Prevents Fear Return After a Reminder Stress.** Sohn JMB¹, Daneluz DM¹, Satori NA², Godinho RO², Stern CAJ¹ ¹UFPR, PGP Pharmacology, Brazil, ²Unifesp-EPM, PGP Pharmacology, Brazil

03.007 **Modeling the Psychocardiac Impact of Social Stress: Behavioral, Cardiovascular, Electrocardiographic, and Endocrine Responses and The Role of Oxytocin Receptors.** Belém-Filho IJAB¹, Suzuki GMF¹, Giati LO², Machado LS³, Santos LB¹, Busnardo C², Corrêa FMA¹ ¹FMRP-USP, Dept Pharmacology; ²FCFAr-Unesp, Dept Pharmaceuticals and Medicines, ³UFPA

04. Inflammation and Immunopharmacology

Totem 03

04.001 **Prenatal Malnutrition Impairs Alveolar Macrophage Function and Increases Infection Susceptibility in Adult Offspring.** Pádua MEB¹, Azevedo GA^{1,2}, Ferreira RR^{2,3}, Giaquinto LRR⁴, Landgraf MA⁴, Landgraf RG¹ ¹Unifesp-Diadema, Dept Pharmaceutical Sciences, Diadema, SP, Brazil; ²UNIFESP, PPG Medicina Translacional, São Paulo, SP, Brazil; ³USP-SP Biotério Central, Brazil; ⁴UNIP-Rangel, Santos, SP, Brazil

04.002 **Second-Line and Biologic Therapies in Pediatric Chronic Nonbacterial Osteomyelitis: A Systematic Review of Multicenter Cohort Data.** Maraia ME^{1,2}, Lemes FS³, Oliveira CJF², Forte GV³,

Miguel CB^{2,3}, Rodrigues WF^{2,3} ¹UFTM, Undergraduate course in Biomedicine, Uberaba, MG, Brazil
²UFTM, Postgraduate Course in Tropical Medicine and Infectious Diseases, Uberaba, MG, Brazil
³Unifimes, Multidisciplinary Lab of Scientific Evidence, Mineiros, GO, Brazil.

04.003 **Evaluation of the Anti-Inflammatory Effect Of hDPSC in an *in vitro* Model of Neuroinflammation Induced By LPS.** Silva MR¹, VF Gonzaga¹, Kerkis I² ¹Butantan Institute, Lab of Genetics, São Paulo, Brazil ²Cellavita Scientific Research Ltda, Valinhos, São Paulo, Brazil

04.004 **Dietary Modulation of Zinc Induces Sex-dependent Responses in Inflammatory Pain by CFA in Mice.** Silva MC¹, Mathias DO², Lima LMTR³, Miranda ALP¹ ¹UFRJ, Lab Estudos em Farmacologia Experimental ²Fiocruz-RJ ³UFRJ, Lab Biotecnologia Farmacêutica e Nutricional

04.019 **Lipid Profile Evaluation in the Lung Response to Silica Particle Exposure in Mice.** Cabral, CL¹, Pezzella-Ferreira GN¹, Pereira-Dutra FS², Espinheira-Silva H², Martins MA¹, Bozza PT², Silva PMR¹ ¹IOC-Fiocruz, Lab of Inflammation, ²IOC-Fiocruz, Lab of Immunopharmacology, Rio de Janeiro, Brazil

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04.005 **Therapeutic Effects of the Rho-Kinase Inhibitor LASSBio-2065 on Silica-Induced Chronic Inflammation in Mice.** Ferreira GG¹, Ferreira TPT¹, Santana ACC¹, Arantes ACS¹, De Souza LM¹, Martins MA¹, Silva PMR, Pinheiro PMS², Lima LM² ¹IOC-Fiocruz, Lab of Inflammation; ²UFRJ, Lab of Evaluation and Synthesis of Bioactive Substances, RJ, Brazil

04.006 **Cholinergic Modulation Attenuates Lung Inflammation *in vitro* and *in vivo* Experimental Model of Asthma-COPD Overlap.** Queiroz VC¹, De Almeida FM¹, Nascimento C¹, Suehiro C², Pinheiro NM¹, Prado MAM¹, Prado VF¹, Tibério IFLC¹, Hamouda AK¹, Prado CM² ¹FM-USP, ²Unifesp

04.007 **Antifungal Action of Rapamycin Might Prolong the Life of Mice with T1DM Infected by *Sporothrix brasiliensis*.** Oliveira MA¹, Albuquerque RC², Migliorini S¹, Tavares YPST¹, Almeida SR², Martins JO¹ ¹FCF-USP, Dept. of Clinical and Toxicological Analyses, Lab. of Immunoendocrinology, Brazil. ²FCF-USP, Dept. of Clinical and Toxicological Analyses, Lab of Mycology, Brazil

04.008 **Enhanced Anti-Inflammatory and Immunomodulatory Action of Geopropolis in Synergism with Low-Dose Dexamethasone in Human Macrophages.** Honorio MS, Lopes EC, Almeida JFS, Sforcin JM IBB-Unesp-Botucatu, Programa de Pós-Graduação em Ciências Biológicas, Brazil

04.009 **Evaluation of the Proliferative Potential of Lavandula dentata Essential Oil on HaCaT Keratinocytes.** Sousa MBN¹, Purgatto, E², Greco KV³, Moreira V¹ ¹Unifesp-EPM, Dept Pharmacology, Brazil, ²FORC-CEPID-USP, School of Pharmaceutical Sciences, Brazil, ³University College London, United Kingdom

04.010 **Nitric Oxide Involvement in the Disability and Active Disease of Multiple Sclerosis: Systematic Review and Meta-Analysis.** Kudsi SQ, Da Silva B, Viero FT, Rodrigues P, Trevisan G, UFSM, Graduate Program in Pharmacology, Brazil

Totem 24

04.011 **Omega-3 Supplementation Attenuates Glucocorticoid Overproduction and Bone Mineralization Loss in a Type 1 Diabetes Mellitus Model.** Amanda Chaves, Lucas Carttman, Brunna Barbosa, Fabiana Pacheco, Livia Bergara, Patrícia Machado Martins e Silva, Marco Aurélio Martins, Vinicius Frias Carvalho IOC-Fiocruz, Lab of Inflammation, Center for Research, Innovation, and Surveillance in COVID-19 and Health Emergencies

04.012 Enhanced Anti-Inflammatory and Antioxidant Effects of H₂S-Releasing NSAID Derivatives on NLRP3-Activated Macrophages. Coavoy-Sánchez SA¹, Teixeira SA¹, Wallace JL², Costa SKP¹, Muscará MN¹ ¹ICB-USP, Dept Pharmacology, São Paulo; ²Antibe Therapeutics Ltd., Toronto, Canada

04.014 DHA-Loaded Nanocapsules Enhance Microcirculation and Reduce Inflammation in Sepsis Experimental Model. Moraes BPT^{1,2}, Silva KP³, Moraes-de-Souza I², Gomes GLS², Saraiva JSG¹, Costa, MF², Cunha, CMC², Almeida, MAP², Estado, V², Bozza PT², Castro-Faria-Neto HC², Ferrarini SR³, Sperandio M⁴, Silva AR², Gonçalves-de-Albuquerque CF^{1,2} ¹Unirio, Immunopharmacology Lab, Rio de Janeiro, Brazil ²IOC-Fiocruz, Immunopharmacology Lab, Rio de Janeiro, Brazil ³UFMT, HealthSciences Post-Graduation Program, Sinop, Brazil; ⁴Ludwig-Maximilians-University Munich, Institute of Cardiovascular Physiology and Pathophysiology, Biomedical Center, Planegg-Martinsried, Germany

04.015 Lack of Effect of Dexamethasone in a Mouse Model of Influenza Infection with Bronchiectasis-Like Features as Monitored by Microcomputed Tomography. Santana ACC¹, Ferreira TPT¹, Gomes HS¹, Santos HBS¹, Arantes ACS¹, Carvalho VF¹, Martins PMRS¹, Ball L², Martins MA¹ ¹IOC-FIOCRUZ, Lab of Inflammation, ²University of Genoa Dept Surgical Sciences and Integrated Diagnostics, Genoa, Italy

04.068 Tyrosine Kinase Inhibitor Reduces Systemic and Cerebral Inflammation in Experimental Sepsis. Cunha CMCD^{1,2,3}, Moraes BPT^{1,3,4}, Abreu VHP^{1,3}, Soares GVM^{1,3}, Moraes-de-Souza IM^{1,2,3}, Almeida MAP¹, Estado V¹, Sayão PGF^{1,3,4}, Souto HA^{1,3}, Bozza PT^{1,2}, Castro-Faria-Neto HC^{1,2}, Silva AR^{1,2}, Gonçalves-de-Albuquerque CF^{1,2,3,4} ¹IOC-Fiocruz, Immunopharmacology Lab, ²IOC-Fiocruz Postgraduate Program in Cellular and Molecular Biology, ³Unirio, Immunopharmacology Lab, Rio de Janeiro, Brazil. ⁴Unirio, Postgraduate Program in Molecular and Cellular Biology

05. Pain and Nociception Pharmacology

Totem 04

05.001 Unravelling the Anterior Cingulate Cortex Nitric Oxide Relevance in the Effects of Electroacupuncture on the Anxiety and Neuropathic Pain Interface in Rats. Ribeiro Siqueira CM¹, Donizetti-Fonseca MA¹, de Souza MBRF¹, da Silva AL^{1,2}, da Silva ML^{1,2}, da Silva JRT^{1,2}, Falconi-Sobrinho LL^{1,2} ¹UNIFAL, Lab. of Neuroscience, Neuromodulation and Study of Pain, Brazil, ²UNIFAL, Postgraduate Program in Biosciences Applied to Health, Brazil

05.002 Antinociceptive Evaluation of the Ethanolic Extract from Bark and Leaves of *Abarema cochliacarpus* in an Animal Pain Model. Guedes GN¹, Macario VSG¹, Oliveira BCCA¹, Rezende B¹, Bazilio YS¹, Maia EP¹, Martins RCC², Montes GC¹ ¹UERJ, Roberto Alcântara Gomes Institute of Biology (IBRAG), Lab of Pharmacology and Psychobiology, Brazil; ²UFRJ, Walter Mors Institute of Research on Natural Products (IPPN), Brazil

05.003 Evaluation of the Antinociceptive and Anti-Inflammatory Profile of New P2X₇ Receptor Antagonist Derivatives. Valério AMF¹, Gaspar LCSG¹, Faria RX², Salles JP¹, Miranda ALP¹ ¹FF-UFRJ, Lab Estudos em Farmacologia Experimental (LEFEx), Brasil. ²IOC- Fiocruz, Lab for Environmental Health Assessment and Promotion, Instituto Oswaldo Cruz, Brasil

05.004 TRPV4 Channel Participates in the Nociception and Spinal Neuroinflammation involved in a Model of Complex Regional Pain Syndrome Type I In Mice. Brito HA¹, Ruviano NA², Rodrigues P¹, Antoniazzi C¹, Viero FT¹, Peres DS¹, Frare JM², Trevisan G^{1,2} ¹UFSM, PPG Pharmacology, Brazil, ²UFSM, PPG Biological Sciences: Toxicological Biochemistry, Brazil

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05.005 Analgesic Effectiveness According to Pain Intensities and Locations in Pediatric Cancer Patients. Marotta FC¹, Leal EL², Oliveira IA¹, Gonçalves TG¹, Santos PCJL¹ ¹Unifesp-EPM, Dept Pharmacology, Brazil, ²Unifesp, Grupo de Apoio ao Adolescente e Criança com Câncer, Brazil

05.006 Chemical Profile and Antinociceptive and Anti-inflammatory Activities of Ethyl Acetate and Butanolic Fractions of the Endophytic Fungus *Schizophyllum commune* Isolated from *Tocoyena bullata*. Santos LL, Melo EDN, dos Santos MB, Guimarães DO, Muzitano MF, Carmo PL, Pinto SC UFRJ-Macaé, Lab of Bioactive Products, Federal University of Rio de Janeiro, Brazil, Multidisciplinary Center

05.007 Learning and Memory Impairment in Preclinical Models of Orofacial Pain: A Systematic Review and Meta-Analysis Morais RIF¹, Viero FT¹, Rodrigues P¹, Kudsi SQ¹, Pereira LG¹, Trevisan G¹ ¹UFSM, Graduate Program in Pharmacology, Brazil

05.008 TRPM3 as a Therapeutic Target for Osteoarthritis Pain: Effects of Local and Systemic Antagonism. Pereira S², Almeida BL², Nunes MC², Andersson D¹, Bevan, SJ¹, Costa R^{1,2} ¹Wolfson SPaRC, King's College London, London, UK; ²UFRJ, School of Pharmacy, Rio de Janeiro, Brazil

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05.009 Pharmacopuncture with Apitoxin and Melittin: A Novel Approach to Chronic Pain Control and Prevention. Boaventura de Oliveira, AM¹, Leonor, MGR¹, Silva, DF¹, Sant'anna, MB¹, Almeida, TC¹, Silva GSA¹, Marques-Porto R², Picolo G¹ ¹Lab of Pain and Signaling, Butantan Institute, São Paulo, Brazil; ²Lab of Development and Innovation, Butantan Institute, São Paulo, Brazil

05.010 Sex Differences in Behavioral Responses Across Rat Models of Migraine. Sex Differences in Behavioral Responses Across Rat Models of Migraine. Baggio DF, Da Luz FMR, Lejeune VBP, Chichorro JG UFPR – Lab of Craniofacial Pain, PPG Pharmacology, Brazil

05.011 Anterior Cingulate Cortex Mediates Cannabigerol-Induced Antinociception in a Mouse Model of Chemotherapy-Induced Peripheral Neuropathy. Ferreira MV¹, Wade QW², Morris NM², Cunha JM¹, Graziane NM² ¹UFPR, Dept Pharmacology, Curitiba, PR, Brazil. ²Dept Anesthesiology and Perioperative Medicine, Pennsylvania State University, Hershey, PA, USA

05.012 Characterization of a Peripheral Neuropathy Model Induced by the Chemotherapeutic Agent Sorafenib in Mice. Characterization of a Peripheral Neuropathy Model Induced by the Chemotherapeutic Agent Sorafenib in Mice Rasga GL, Almeida BL, Pereira S, Costa R, Passos GF UFRJ, Lab Neurofarmacologia e Comportamento, Programa de Pós-Graduação em Ciências Farmacêuticas, Universidade Federal do Rio de Janeiro, Brazil

06. Cardiovascular and Renal Pharmacology

Totem 05

06.001 Treatment with Grape Pomace (*Vitis vinifera*) Improves Cardiac Morphological Parameters in Spontaneously Hypertensive Rats. Magalhães VHL¹, Costa KCM², Pertuzatti PB¹, Freitas RA¹, Giachini FRC^{1,2} ¹UFMT, Institute of Biological Science and Health, Lab. Vascular Biology and Histopathology, Brazil, ²UFG, Institute of Biological Sciences, Brazil

06.002 Serotonin Amides: A Renal Safety Pharmacology Analysis. Brasil JPP, Pereira T, Esteves GAB, Santos MARFD, Morcillo LSL UFRJ

06.003 **The Influence of a Synthetic Broad-Spectrum Metalloprotease Inhibitor on the Coagulotoxic Properties of *Bothrops alternatus* (Viperidae: Crotalinae) Venom.** Azevedo SNS¹, Dias SR¹, Ghirotti HA¹, Souza-Gomes GC¹, Torres-Bonilla KA², Hyslop S², Sant'Anna SS³, Sousa EP⁴, Morais-Zani K⁴, Floriano RS¹ ¹Uneste, Lab. of Toxinology and Cardiovascular Research, Graduate Program in Health Sciences, Brazil, ²Unicamp, Dept Pharmacology, Faculty of Medical Sciences, Brazil, ³IB, Lab. of Herpetology, Brazil, ⁴IBu, Lab. of Pathophysiology, Graduate Program in Toxinology, Brazil

06.004 **Dual-Action Phenylhydrazide Derivatives as Novel MPO Inhibitors and Vasodilators: Therapeutic Potential for Cardiovascular Disease.** Teixeira AP¹, Navarro TR², Aguiar TBS², Pimentel PP², Lima EC², Silva LL¹, Raimundo JM^{1,3} ¹UFRJ-Macaé, FarPBio, Brazil, ²UFRJ-Macaé, LACB, Brazil, ³UFRJ-Macaé, PPG-ProdBio, Brazil

06.005 **Taurine Reduces High Glucose Levels in *Caenorhabditis Elegans*.** Sant'Ana BH¹, Machado JC¹, Viçozzi GP², Vidal BA¹, Laurentino AOM¹, Pereira FSO³, Garcia S², Leal MB¹, Gomez R¹ ¹UFRGS, Programa de Pós-Graduação em Farmacologia e Terapêutica, Brasil ²UFRGS, Lab Toxicologia (LATOX), Programa de Pós-Graduação em Ciências Farmacêutica, Brasil ³Unijuí, Brasil

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06.006 **Phosphodiesterase 5 Inhibition Attenuates Cardiovascular Dysfunction in Sepsis Surviving Rats.** Delfrate G, Neves PG, Albino LB, Moreira DH, Nardi G, Pinheiro LC, Assreuy J, Fernandes D UFSC, PPG Pharmacology, Brazil

06.007 **Cardiovascular Protective Effects of Syrah Grape (*Vitis vinifera*) Pomace Supplementation in Spontaneously Hypertensive Rats.** Costa KCM¹, Magalhães VHL², Soares KS², Freitas RF², Pertuzatti PB², Giachini FR^{1,2}. ¹UFG, Institute of Biological Science, Brazil. ²UFMT, Institute of Biological Science and Health, Brazil

06.008 **Bufalin-Induced Epithelial-Mesenchymal Transition in LLC-PK1 Cells *in vitro*.** Barros GMO, Bagri KM, Mermelstein CS, Quintas LEM ICB-UFRJ Rio de Janeiro, Brazil

06.009 **Treatment with Wedelolactone Reverses AKI Caused by *Bothrops jararacussu* Envenomation.** Santos MARF, Souza PDN, Accioli DSG, Esteves GAB, Pinto HMC, Brito ES, Albernaz L, Pereira T, Oliveira SSC, Amorim CS, Santos AL, Moraes JA, Gonzalez SR, Melo PA, Morcillo LSL UFRJ

07. Endocrine, Reproductive and Urinary Pharmacology

Totem 06

07.001 **6-Cyanodopamine Enhances Norepinephrine- and Dopamine-Mediated Contractions in Rat Isolated Seminal Vesicle.** dos Santos IAR, Pozzo CFSD, Ribeiro LC, Ramos MCC, de Nucci G UNICAMP, Dept Pharmacology, Faculty of Medical Sciences, Campinas, Brazil

07.002 **6-Cyanodopamine Potentiates Electric Field Stimulation-Mediated Contractions in Rat Isolated Seminal Vesicle.** Ribeiro LC, Dal Pozzo CFS, Ramos MCC, dos Santos IAR, de Nucci G Unicamp, Dept Pharmacology, Faculty of Medical Sciences, State University of Campinas, Campinas, Brazil

07.003 **6-Nitrodopamine as a Mediator of Prostate Contractility in Mouse and Man.** Pereira LGS¹, Dias DRM¹, Olivotti AB¹, Oliveira DL², Schenka AA², Souza VB², De Nucci², de Oliveira MG¹ ¹USF, Lab of Pharmacology, PPG Health Sciences, ²Unicamp, Dept Translational Medicine, PPG Pharmacology

07.004 **6-Nitrodopamine as a Novel Mechanism of Nitric Oxide-Mediated Prostate Relaxation.** Dias, JM¹, Curiel AA¹, De Nucci², de Oliveira MG¹ ¹USF, Lab of Pharmacology, PPG Health Sciences, Brazil
²Unicamp, Dept Translational Medicine, PPG Pharmacology, Brazil

07.008 **Lack of 6-Nitrodopamine, a Prostate Relaxant, Leads to Voiding Dysfunction in Mice.** Dias, DR¹, Dias JM¹, De Nucci², de Oliveira MG¹ ¹USF, Lab of Pharmacology, PPG Health Sciences, Brazil
²Unicamp, Dept Translational Medicine, PPG Pharmacology, Brazil

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07.005 **TTP488 (Azeliragon) Enhances Bladder Function in Leptin-Deficient Obese Mice: The Role of RAGE in Diabetic Bladder Dysfunction.** Oliveira AL, Medeiros ML, Antunes E FCM-Unicamp, Dept Pharmacology, PPG of Pharmacology

07.006 **Pharmacological Characterization of Probenecid in Urinary Bladder and Corpus Cavernosum from Human.** Leonardi GR, Moretti MB, Barros JVC, Lima JM, Antunes E, Mónica FZ FCM-Unicamp, Dept Pharmacology

08. Respiratory and Gastrointestinal Pharmacology

Totem 25

08.001 **Methylglyoxal as a Key Mediator in the Exacerbation of Allergic Asthma in Leptin-Deficient Obese Mice.** Medeiros ML, Oliveira AL, Antunes E FCM-Unicamp, Dept Pharmacology

08.002 **Prevention of Gut Microbiota Imbalance: The Role of *Arthrosira platensis* in Hypercaloric Diet-Induced Dysbiosis.** Claudino BFO^{1,2}, Arruda RRA^{1,2}, Diniz AFA^{1,2}, Melo MB¹, Sousa-Filho JEC¹, Colunga AAG⁴, Nobre CSN⁴, Silva BA^{1,2,3} ¹UFPB, Lab of Functional Pharmacology Prof. George Thomas, Institute of Research in Drugs and Medicines, Brazil ²UFPB, Postgraduate Program in Bioactive Natural and Synthetic Products, Health Sciences Center, Brazil ³UFPB, Dept Pharmaceutical Sciences, Health Sciences Center, Brazil, ⁴University of Minho, Fermentations Lab, Centre of Biological Engineering, Portugal

08.003 **Anti-Colitis Effect of Citral *in vivo* and *in vitro* models: Preventive Effect Mediated by Antioxidant and Anti-Inflammatory Action against Intestinal Damage.** Maycon T. Emílio-Silva; Vinícius P. Rodrigues; Antonio J. Ruiz-Malagon; Isabela G. Guidolin; Felipe L. Dario; Mariana M. Fioravanti; Luiz G. S. Branco; Alba Rodriguez-Nogales; Julio Gálvez; Clelia A. Hiruma-Lima Silva MTE^{1,2,3}, Rodrigues VP¹, Ruiz-Malagon AJ³, Guidolin IG¹, Dario FL¹, Fioravanti MM¹, Branco L², Rodriguez-Nogales A³, Galvez J^{3,4}, Lima CAH¹ ¹IBB-Unesp-Botucatu, Dept Structural and Functional Biology, Physiology sector; ²FORP-USP, Dept Basic and Oral Biology; ³University of Granada, Dept Pharmacology, Center for Biomedical Research, Granada, Spain. ⁴Instituto de Salud Carlos III CIBER de Enfermedades Hepáticas y Digestivas, Madrid, Spain

09. Natural Products and Toxinology

Totem 07

09.001 **Action of Marimastat, a Synthetic Broad-Spectrum Metalloprotease Inhibitor, on the *in vitro* coagulotoxicity of *Lachesis muta* (South American Bushmaster) Venom.** Personi JG¹, Guldoni LF¹, Oliveira IN¹, Proença-Hirata VS¹, Silva NJ², Torres-Bonilla KA³, Hyslop S³, Sousa EP⁴, Morais-Zani K⁴, Floriano RS¹ ¹Unoeste, Lab. of Toxinology and Cardiovascular Research, Graduate Program in Health Sciences, Brazil, ²PUC-Goiás, School of Medical and Life Sciences, Brazil, ³UNICAMP, Dept Pharmacology, Faculty of Medical Sciences, Brazil, ⁴IBu, Lab. of Pathophysiology, Graduate Program in Toxinology, Brazil

09.002 The Antioxidant Potential of Extra Virgin Olive Oil on the Prefrontal Cortex of Rats Exposed to a High-fat Diet. Lena MC, Peres, AM, Raphaelli GB, Dantas RM, Ferreira AC, Silveira ACS, de Souza LCP, Santos, ES, Harttfel MHB, Dalmaz C, Bast RKSS UFRGS, Dept Biochemistry, Brazil

09.003 Mechanisms Involved in Hemostatic Disorders Caused by *Bothrops leucurus* and *Bothrops erythromelas* Envenomation. Rodrigues MAF¹, Paulesini ES¹, Felipe AGC¹, Carneiro IB¹, Lima JAF², Lima PHV³, Sant'Anna SS⁴, Morais-Zani K^{1,4} ¹IBu, Lab Fisiopatologia,; ²IBu, Lab Ecologia e Evolução, Instituto Butantan; ³IBu, Lab Farmacologia, Instituto Butantan; ⁴IBu, Lab Herpetologia, Instituto Butantan

09.004 Evaluation of the Toxicity of Venom from the Epidermal Mucus of the Catfish *Pterodoras granulosus* in the Alternative Zebrafish Model. Iwanichen J, Silva EM, Lima C, Ferreira MVAL IBu

09.005 Antiparasitic and Cytotoxic Potential of *Justicia secunda* Extract: Perspectives for the Control of *Schistosoma mansoni*. Baptista B¹, Canossa IC¹, Fragelli BDL^{1,2}, Rodolpho JMA¹, Valentini MB³, Ataíde AMS⁴, Allegretti SM³, Silva CYY⁴, Silva MN⁴, Monteiro MC⁵, Anibal FF¹ ¹UFSCar, Lab of Inflammation and Infectious Diseases, Brazil, ²UFSCar, Center for the Development of Functional Materials, Brazil, ³UNICAMP, Dept Animal Biology, Brazil, ⁴UFPA, Lab of Liquid Chromatography, Brazil, ⁵INCT-PROBIAM-UFPA Pharmaceuticals Amazônia, Brazil

Totem 13

09.006 Antiproliferative Activity of Hinokinin in Luminal Breast Cancer: An *in silico* and *in vitro* Approach. Santos DC¹, Nagata KS¹, Bertolin LR, Mello LP, Ferreira EA, Sciani JM¹, Longato GB¹ ¹USF, Lab of Natural Products, Graduate Program in Health Sciences, Brazil. ²Mackenzie Presbyterian University, School of Engineering, Brazil

09.007 *In vitro* Modulation of Oxidative Stress by a Topical Formulation Containing Red Propolis. Munhoz LLS¹, Pichinelli-Noronha JJ², De Sá, SG³, Severino AM⁴, Favaro-Trindade CS³, Ambrósio CE², Martins DS¹ ¹FZEA-USP, Lab. of Immunohistochemistry and Experimental Physiology, PPG Animal Biosciences, Brazil ²FZEA-USP, Lab. of Cell Culture and Gene Therapy, PPG Animal Biosciences, Brazil ³FZEA-USP, Lab. of Encapsulation and Functional Foods, PPG Food Engineering, Brazil ⁴ESALQ-USP, Multiuser Center for Biochemistry and Instrumental Analysis, Interunit PPG in Integrated Food Systems, Brazil

09.008 Metalloproteases Phenotypic Diversity in *Bothrops jararaca* Venom. Paulesini ES¹, Galizio NC¹, Vidueiros JP², Sant'Anna SS², Lima JAF³, Pimenta DC³ Gonçalves LRC¹, Morais-Zani K¹ ¹IBu, Lab of Pathophysiology; ²IBu, Lab of Herpetology; ³IBu, Lab of Biochemistry and Biophysics

09.009 Omega-3 Fatty Acid Nanoemulsion Attenuates Inflammatory and Myotoxic Effects Induced by *Bothrops asper* Venom in Mice. Almeida CX¹, Lima RO¹, Lemos G¹, Calmasini FB¹, Escalante T², Purgatto E³, Moreira V¹ ¹Unifesp-EPM, São Paulo, PPG Farmacologia, Brasil, ²Universidade da Costa Rica, Faculdade de Microbiologia, Instituto Clodomiro Picado, Costa Rica, ³FCF-USP, PPG Sistemas Integrados em Alimentos, Brasil

Totem 20

09.010 Cytotoxicity of Lysine/Arginine-Substituted M-pontx-dq3a [1–15] Analogues and Effect Against *Trypanosoma cruzi* Y Strain. Magalhães EP¹, Edson EA², Martins BVB¹, Reis MP³, ¹Silva FRC, da Silva Júnior PN¹, Sampaio TL^{1,3}, Oliveira VX⁴, Menezes RRPPB^{1,3}, Martins AMC^{1,2,3} ¹UFC, PPG in Pharmaceutical Sciences, Brazil. ²UFC, PPG in Development and Technological Innovation in Medicines, Brazil. ³UFC, Clinical and Toxicological Analysis Dept, Brazil, ⁴UFABC, Natural and Human Sciences Center, Brazil

09.011 **Gamma-Oryzanol as a Natural Modulator of Hepatic Lipid Metabolism Caused by Obesogenic Diet.** Siqueira JS¹, Palacio TLN¹, Cruzeiro J¹, Garcia JL¹, Aiello G², D'Amato A², Correa CR¹ ¹Unesp-Botucatu, Medical School, Graduate Program in Pathology, Brazil ²University of Milan, Dept Pharmaceutical Sciences, Doctoral Program in Pharmaceutical Sciences, Italy

09.012 **Screening of Snake Venoms for the Identification of Possible Novel Therapeutical Targets and Toxins with Schistosomicidal Potential.** Taveira-Barbosa TC¹, Chaves AFA², Fischer-Carvalho A¹, Oliveira VG¹, Miyasato PA³, Freitas RP³; Nakano E³, Verjovski-Almeida S¹, Serrano SMT², Amaral MS¹ ¹IBu, Lab Ciclo Celular; ²IBu, Lab Toxinologia Aplicada; ³IBu, Lab Parasitologia

09.013 **Evaluation of Cytotoxicity and Mechanisms of Anti-Inflammatory Action of the Hydroethanolic Extract from *Fridericia chica* Leaves in *in vitro* Assays.** Figueiredo FF¹, Arunachalam K¹, Cruz TCD², Muller JAI¹, Serio BFD¹, Martins DTO¹ ¹UFMT, Pharmacology Area, Graduate Program in Health Sciences, Brazil, ²UFMT, Virology Area, Graduate Program in Health Sciences, Brazil

09.014 **Apigenin Administration Protects Against Obesity-related Muscle Atrophy but Fails to Avert Obesity in Middle-aged Rats Treated with a High-calorie Diet.** Stein DJ^{1,2}, de Castro JM^{1,2}, Melo AS^{1,2}, Silveira BL^{1,2}, Martins IAS^{1,2}, Marçal MM^{1,2}, Dal Bosco T^{1,2}, Keingeski MB^{3,4}, de Oliveira ECL^{3,4}, Alvares-da-Silva MR^{3,4}, Ayres LR^{5,6}, Kowalewski LS^{5,6}, Russo MKB^{5,6}, Krause M^{5,6}, Torres ILS^{3,4} ¹HCPA, Lab Pain Pharmacology and Neuromodulation: Preclinical Investigations, ²UFRGS, Postgraduate Program in Medicine: Medical Sciences, Faculty of Medicine, ³UFRGS, Postgraduate Program in Gastroenterology and Hepatology, Porto Alegre, Brazil ⁴HCPA, Experimental Lab Hepatology and Gastroenterology, ⁵UFRGS, Lab Inflammation, Metabolism and Exercise, and Lab Cellular Physiology, Dept Physiology, ⁶UFRGS, Postgraduate Program in Human Movement Sciences, School of Physical Education, Physiotherapy and Dance

09.060 ***Arthrospira platensis* Prevents KCl-Induced Uterine Hypercontractility in Primary Dysmenorrhea through Redox and Prostanoid Modulation.** Soares MFS^{1,2}, Silva JMA^{1,2}, Arruda RRA^{1,2}, Diniz AFA^{1,2}, Barros BC^{1,2}, Melchiades MKN^{1,2}, Melo MB¹, Santos-Filho JEC¹, Sousa TR¹, Silva BA^{1,2,3} ¹UFPB, Lab Functional Pharmacology Prof. George Thomas, Institute of Research in Drugs and Medicines, ²UFPB, Postgraduate Program in Bioactive Natural and Synthetic Products, Health Sciences Center, ³UFPB, Dept Pharmaceutical Sciences, Health Sciences Center

10. Cancer Pharmacology

Totem 08

10.001 **Assessment of Novel HDAC Inhibitors and Venetoclax in a Cellular Model of Oral Cancer.** Guimarães de Souza PO¹, Carlos JAEG¹, Tavares MT^{2,3,4}, de Sá RE^{1,5}, Costa-Lotufo LV¹, Parise-Filho R², Machado Neto JA¹. ¹ICB-USP, Dept. of Pharmacology, São Paulo, Brazil; ²FCF-USP, São Paulo, Brazil; ³Dana-Farber Cancer Institute. Dept. of Cancer Biology, Boston, United States; ⁴Harvard Medical School, Dept. of Biological Chemistry and Molecular Pharmacology, Boston, United States; ⁵UFDFPar, Parnaíba, Brazil.

10.002 **Multi-Target Inhibitor NT157 Exhibits Antineoplastic Activity in Pancreatic Cancer Cells.** Akiyama KM, Parducci NS, Machado-Neto JA ICB-USP, Dept Pharmacology, Brazil

10.003 **NT157 an IRS/IGF1R Inhibitor impacts on Nox4: a Novel Mechanism Involved in Anti-Melanoma Effects.** Rocha DS, Almeida BO, Veríssimo-Filho S, Machado-Neto JA, Lopes LR ICB-USP, Dept Pharmacology, Brazil

10.004 ***In vitro* And *in vivo* Antitumor Properties of Casearin J on Sarcoma 180 Cells.** Santos IKS¹, Sousa RWR¹, Oliveira RAB¹, Ramos CLS¹, Rodrigues DCN¹, Barbosa SLM¹, Dittz D², Zalewski S³,

Camargo JGS³, Carvalho FA³, Santos AG³, Ferreira PMP¹ ¹UFPI, Lab Experimental Cancerology (LabCancer), Dept Biophysics and Physiology, Brazil; ²UFPI, Dept Biochemistry and Pharmacology, Brazil; ³UNESP, Faculty of Pharmaceutical Sciences, Brazil

10.005 **Cannabidiol Nanoformulations Inhibit Cell Proliferation and Cancer-Stem Cell Marker CD133 In *in vitro* Models of Burkitt Lymphoma.** Santos HAF, Souza VB, Nicolosi JS, Vinha P, Perozzo BR, Schenka NGM, Schenka AA Unicamp, School of Medical Sciences, Dept Pharmacology, Brazil

Totem 14

10.006 **Antiproliferative Activity at Lung Cancer Cells and Acute Toxicity of N-Acylhydrazone Derivatives.** Santos YV¹, Souza SM¹, Silva VR², Bezerra DP², Dias RB³, Dos Santos Filho JM⁴, Militão GCG¹ ¹UFPE, Dept. of Physiology and Pharmacology, Biochemistry and Physiology Graduate Program, Brazil; ²Fiocruz, Gonçalo Moniz Institute (IGM), Lab Tissue Engineering and Immunopharmacology, Brazil; ³UEFS, Dept. of Biological Sciences, Brazil; ⁴UFPE, Dept. of Chemical Engineering, Lab Design and Synthesis Applied to Medicinal Chemistry-SintMed®, Brazil

10.007 **Ivermectin Shows *in vitro* and *in vivo* Activity in a Sarcoma Experimental Model.** Sousa RCL, Porto JCS, Santos IL, Freitas MM, Barbosa SLM, Rodrigues DCN, Pinheiro-Neto FR, Ferreira PMP UFPI, Lab Experimental Cancerology (LabCancer), Dept Biophysics and Physiology, Brazil

10.008 **The Role of the Protein Tyrosine Phosphatase Receptor N2 In Inflammatory Breast Cancer: Differential Profiles and Association with Clinicopathological Features.** Alves BES¹, Oliveira PRA¹, Maia IFVC¹, Arruda LM², Carvalho, MAS¹, Silva, RL¹, Cunha MPSS², Leitão RFC³, Almeida PRC⁴, Rogatto SR^{5,6}, Pereira Lima Júnior RC¹, Wong DVT¹ ¹UFC, Dpt of Physiology and Pharmacology, Lab Inflammation and Cancer Pharmacology, Drug Research and Development Center, Brazil; ²Haroldo Juaçaba Hospital, Cancer Institute of Ceara, Brazil; ³UFC, Dpt of Morphology, Brazil; ⁴UFC, Dpt of Pathology and Forensic Medicine, Brazil; ⁵UNESP, Botucatu Medical School Hospital, Brazil; ⁶University Hospital of Southern Denmark, Denmark

10.009 **Protein Disulfide Isomerase Inhibition Decreases Anoikis Evasion in Melanoma Cells Resistant to Chemotherapy** Nascimento A, Pagotti H, Verissimo-Filho S, Agostinho-Neto J, Lopes LR ICB-USP, Pharmacology Dept, São Paulo, Brazil

10.010 **Therapeutic Combination of McLTP1 Protein and Oxaliplatin in Antitumor Activity and Peripheral Sensory Neuropathy in CT26.WT Tumor Bearing Mice.** Abucater BLSM¹, Moura MNS¹, Nascimento RR¹, França JC¹, Pereira AF¹, Bezerra BS¹, Ribeiro MVS¹, Rocha LR¹, Freire DC¹, Oliveira HD², Souza PFN¹, Brito DMS¹, Vale, ML¹ ¹UFC, Lab Inflammation and Cancer Pharmacology, Drug Research and Development Center (NPDM), Dept Physiology and Pharmacology, Faculty of Medicine, Fortaleza, Brazil. ²UFC, Pharmacology and Biochemistry Lab, Drug Research and Development Center (NPDM), Department of Biochemistry and Molecular Biology, Fortaleza, Brazil

Totem 21

10.011 **Investigation of the Prognostic Impact of PIP4K2s and its Pharmacological Citotoxicity in Hepatocellular Carcinoma.** Carvalho MFL, Parducci NS, Lima K, Machado-Neto JA ICB-USP, Dept Pharmacology, São Paulo, Brazil

10.012 **Impact of the TP53 R248Q Mutation on the Interaction Between p53 and APR-246 in Acute Myeloid Leukemia.** Bertoline LMF¹, Lima K^{1,2}, Machado Neto JA¹ ¹ICB-USP, Dept Pharmacology, São Paulo, Brazil. ²FM-USP, Dept Internal Medicine, Hematology Division, São Paulo, SP, Brazil

10.013 Extracellular Vesicles from Obese-Educated Preadipocytes Enhance Migration and Alter Metabolic Profile in MDA-MB-231 Cells. Botelho, LGX^{1,3}, Franco VA^{1,3}, Ramos IR², Barja-Fidalgo, TC³, Moraes JA⁴, Renovato-Martins, M^{1,3} ¹UFF, Lab Inflammation and Metabolism, Brazil, ²University of Miami, Dept Radiation Oncology, USA. ³ UERJ, Bioscience Post Graduation Program, Brazil ⁴UFRJ, Lab Redox Biology, Brazil

10.014 Multifunctional Thermosensitive Nanocarriers for Local Treatment of Non-Invasive Breast Cancer: Tissue Localization, Cytotoxic and Molecular Effects. Passos JS^{1,2}, Machado-Neto JA¹, Panitch A¹, Lopes LB¹ ¹ICB-USP, Dept Pharmacology, Functional and Molecular Biology Program, Brazil; ²Georgia Institute of Technology, Dept Biomedical Engineering, USA

Totem 26

10.015 Proteomic profiling of Bothrops jararaca venom-induced alterations in cancer cells lines Aguiar MRC¹, Silva MR¹, Pimentel IOC¹, Moreira RAW², Kisaki CY¹, Lima IF¹, Iwai LK¹ ¹IBu. Lab Applied Toxinology ²IBu, Lab Cell Cycle, Center of Toxins, Immune-Response and Cell Signaling

10.016 Characterization of Prednisolone Resistance in Acute Lymphoblastic Leukemia Cells. Salgado MTSF¹, Medeiros ACLP¹, Tancredi TB¹, Almeida MS², Fernandes PD¹, Carvalho RS¹ ¹UFRJ Lab. of Pain and Inflammation Pharmacology, Brazil, ²UFRJ Structural Biology and Bioimage Nacional Center, Brazil.

10.017 Are Antidepressants a Reposition Option to Treat Mesenchymal Tumors? Sousa RWR¹, Conceição MLP², Santos IL¹, Rodrigues DCN¹, Nascimento MLLB⁴, Barbosa SLM¹, Freitas MM¹, Pinheiro Neto FR⁶, Sales SLA⁷, Pereira EM⁵, Silva AS⁵, Monte SJH⁵, Pessoa C⁷, Castro e Sousa JM⁴, Ferreira PMP¹ ¹LabCancer-UFPI, Lab Experimental Cancerology, Brazil; ²Lid-USP, Lab Inflammation and Pain, Brazil; ³Lafac-UFPI, Lab Cardiovascular Pharmacology, Brazil. ⁴Lapgenic-UFPI, Lab Toxicological Genetics Research, Brazil. ⁵Lib-UFPI, Lab Immunogenetics and Molecular Biology, Brazil. ⁶UFPI, Lab Pain Pharmacology, Brazil. ⁷UFC, Lab Experimental Oncology, Brazil

10.018 Synergistic Effect of Ionizing Radiation And N-Hydrazinecarbothioamide B-Lapachone (BV3) Derivative Against Lung Cancer. Andrade JKF¹, Goes AJF², Silva MSL², Silva TG², Oliveira APB³, Militao GCG⁴, ¹UFRPE, Dept Animal Morphology and Physiology, Brazil, ²UFPE, Dept. Antibiotics, Bioscience Center, Brazil, ³Institute of Radiotherapy Waldemir Miranda, Brazil, ⁴UFPE, Dept. Physiology and Pharmacology, Bioscience Center, Brazil

10.019 Cephalochromin Potentiates Clinically Used Drugs in Acute Lymphoblastic Leukemia Models. Serra CSM¹, Lima GC¹, Vicari HP¹, Lima K², Nascimento MC², Rego EM², Ferreira MJ³, Costa-Lotufo LV¹, Machado-Neto JA¹ ¹Dept Pharmacology, ICB-USP, Brazil; ²Dept Internal Medicine, Hematology Division, FM-USP, Brazil; ³Dept Botany, IB-USP, Brazil

11. Clinical Pharmacology, Pharmacokinetics, Pharmacogenomics and Toxicology

Totem 22

11.001 Peritoneal Exposure of Anticancer Drugs in Hyperthermic Peritoneal Chemotherapy (HIPEC): Insights from Literature-Based Pharmacokinetic Modeling. Toson N, Santos LG, Araújo BV UFRGS PPG Medical Sciences Porto Alegre Brazil

11.002 Linalool, Linalyl Acetate, and Lavender Essential Oil: Evaluation of Metabolism and Drug Interactions Using Canine Liver Microsomes and Recombinant Enzymes. Soares-Santos RR¹, Soto-Blanco B¹, Ferreira RCM², Zhu Z³, Court MH³ ¹UFMG, Veterinary School, Dept Veterinary Clinic and Surgery, Lab. of Toxicology, Brazil, ²FSCBH, Molecular Biology Lab., Brazil, ³Washington State

University, College of Veterinary Medicine, Dept Veterinary Clinical Sciences, Program in Individualized Medicine, Comparative Pharmacogenomics Lab., USA

11.003 Development of a PBPK Model to Predict Interactions of DMT and Harmine in Ayahuasca and Co-Administration with Synthetic Drugs Ribeiro GSG¹, Paranhos BAPB¹, Dörr F¹, dos Santos RG², Hallak JEC², Villanova B³, Guerra LTLG³, Raminelli AO³, Reis JAS³, de Paula CC³, Zacharias ABV³, Martins FS¹, Marcourakis T¹ ¹USP, São Paulo, Brazil ²National Institute of Translational Science and Technology in Medicine ³FMRP-USP

11.004 Behavioral Responses of Adult Zebrafish (*Danio rerio*) Exposed to Zein-Nanoencapsulated Cinnamon Essential Oil: An Investigation of Toxicity. Da Silva ACF¹, Barros, JM², Pimentel JRL¹, Chamberlain ALV¹, Stadnik MJ³, Müller LG⁴, da Costa C^{2,5}, Siebel AM^{1,6} ¹UFPR, Graduate Program in Pharmacology, Brazil ²UFSC, Graduate Program in Chemical and Food Engineering, Brazil ³UFSC, Graduate Program in Plant Sciences, Brazil ⁴IBET Institute of Experimental and Technological Biology, Portugal ⁵UFSC, Graduate Program in Textile Engineering, Brazil ⁶FURG, Graduate Program in Health Sciences, Brazil

11.005 Pharmacokinetic Profile of the JME-209 in Rats – A Novel Mexiletine Analogue with Potential for COPD Therapy. Santos GCM^{1,2}, Santos HBS², Rocha BM¹, Santos IB³, Vilela LT³, Elidio HSM³, Silva RCPF³, Costa JCS³, Silva PMR¹, Martins MA^{1,2} ¹IOC-Fiocruz, Lab Inflammation, ²Preclinical Pharmacokinetic Platform, ³NBA2 and NBA3 Animal Experimentation Platform, ⁴VPPIIS-Fiocruz

01. Cellular and Molecular Pharmacology

Totem 01

01.009 **Bioactivity of Angiotensin I (AngI) and II (AngII) Peptides in the Aorta of *Crotalus durissus terrificus* and *Oxyrhopus guibei* Snakes.** Nogueira ACS, Breno MC, IBu, Pharmacology Lab., Brazil

01.010 **Biochemical Characterization of the Cofactors Involved in the Phosphatase Activity of Na, K-ATPase and Thermodynamics of its Interaction with Cardiotonic Steroids Aiming at Stimulatory Effects.** Esteves G¹, Azalim, P², Barbosa L³, Villar J³, O'Doherty G⁴, Blanco G⁵, Quintas L¹, Scavone C².¹UFRJ, Lab Farmacologia Bioquímica e Molecular, Brasil ²USP, Lab Neurofarmacologia Molecular, Brasil ³UFSJ, Lab Bioquímica Celular ⁴Northeastern University, Dept Chemistry, Boston, USA ⁵Kansas University Medical Center, Dept Molecular and Integrative Physiology, USA

01.011 **Intracellular Peptides as Novel Modulators of Thermogenesis: Functional Characterization of U2 in Adipocytes.** Jardim GFR¹, Valdivia LFG², Reckziegel P¹ ¹FCF-USP, Depto de Análises Clínicas e Toxicológicas; ²Unesp, Programa de Pós-graduação em Farmacologia

01.012 **Effect of a Synthetic Curcuminoid on H₂O₂-Damaged Raw 264.7 Cells and Involvement of the Keap1/Nrf2/ARE Pathway.** Ananias CF¹, Duarte ALS¹, Cajazeiras GI¹, Silva ME², Magalhães EP², Duque BR², Bezerra VM², Costa MDR², Pinheiro KS¹, Fernandes MO¹, Araújo JR¹, Santos HS¹, Martins AMC¹, Sampaio TL¹, Menezes RRPPB¹ ¹UFC, Faculty of Pharmacy, Dentistry and Nursing, Dept Clinical and Toxicological Analysis, Brazil; ²UFC, Faculty of Pharmacy, Dentistry and Nursing, Post Graduate Program of Pharmaceutical Sciences, Brazil

01.013 **Effects of Zinc Sulfate, Sodium-Pyrithione, and TPEN on the Viability of human endothelial cells.** Pinheiro JVS¹, Muscara MN², Barros PR¹, Rodrigues, SF¹ ¹ICB-USP, Vascular Nanopharmacology Lab, Dept Pharmacology, São Paulo, Brazil; ²ICB-USP Lab Biochemical Pharmacology of Free Radicals, Inflammation and Pain, Dept Pharmacology, São Paulo, Brazil

02. Neuropharmacology

Totem 02

02.022 **Study of the Activity of 8-Benzylidene Digoxin (BD-8), a New Semi-Synthetic Derivative, on LPS-Induced Oxidative Stress in the Murine Brain.** Fonseca MFR¹, Neves EPFI¹, Silva IA¹, Souza VKB¹, Ferreira PYO², Campos HM², Ghedini PC², Costa RM³, VillarJAFP⁴, Ribeiro AAF⁴, Leite JA¹ ¹UFG, Lab Ensino, Pesquisa e Extensão em Neurociências Aplicadas, Goiânia, Brazil. ²UFG, Lab Farmacologia Bioquímica e Molecular, Goiânia, Brazil. ³UFG, Lab Fisiologia e Farmacologia da Reprodução, Goiânia, Brazil. ⁴UFSJ, Lab Síntese Orgânica e Nanoestruturas, Divinópolis, Minas Gerais, Brazil

02.023 **Kynurenine Neonatal Exposure: Behavioral Consequences in Adolescent Mice.** Corrêa AES, Bomfim ANL, Cruz BE, Andrade BS, Guimarães MZP, Neves GA UFRJ, Lab Molecular Pharmacology, Brazil

02.024 **Impact of Cholinergic Blockade on Conditioned and Unconditioned Fear Memory in Middle-aged Adult Male Rats.** Savitski LR, De Souza STF, Souza LC, Stingelin RL, Vital MABF UFPR, Lab Central Nervous System, PPG Pharmacology, Dept Pharmacology, Brazil

02.025 **Effects of Combining Chronic Stress Exposure with Repeated Corticosterone Administration in Female Mice.** Alves MV, Santos LSP, Cruz BE, Silva, ACF, Barros, RP, Neves, GA UFRJ, Lab Molecular Pharmacology, Brazil

Totem 03

02.026 Effects of non-Selective Muscarinic Inhibition on short-Term Recognition and Spatial Memory in Middle-Aged Male Rats. Stingelin, RL, De Souza, STF, Souza, LC, Savitski, LR, Vital, MABF. UFPR, Lab Central Nervous System, PPG Pharmacology, Dept Pharmacology, Brazil

02.027 Anti-Inflammatory Potential of Baricitinib in Cellular Models of Neuroinflammation in Astrocytes and PC12 Cells. Duarte ALS¹, Bandeira SMA², Sales ISL², Costa Júnior DC², Rebouças MO³, Araújo JR¹, Costa GC¹, Ananias CF¹, Fernandes MO¹, Pinheiro KS¹, Lima PJS⁴, Sampaio TL⁵, Sousa FCF⁶ ¹UFC, Faculty of Pharmacy, Dentistry and Nursing, Dept Clinical and Toxicological Analysis, Brazil, ²UFC, Dept Pharmacology, PPG in Pharmacology, ³ICC, Ceará Cancer Institute, ⁴UFC, Graduate Program in Translational Medicine, ⁵UFC, Dept Pharmacy, ⁶UFC, Dept Physiology and Pharmacology, Faculty of Medicine

02.028 Association Between Advanced Oxidation Protein Products (AOPPs) And Anxiety-Like Behavior in a Progressive Multiple Sclerosis Experimental Autoimmune Encephalomyelitis (PMS-EAE) Model In Mice. Dalmolin L¹, Rodrigues P², Frare JM³, Ruviano NA³, Peres DS², Bochi GV², Trevisan G^{2,3} ¹UFSM, Medical Student, Brazil; ²UFSM, Graduate Program in Pharmacology, Brazil; ³UFSM, Graduate Program in Biological Sciences, Brazil

02.029 Sex-Specific Neurobiological Signatures of Adaptive vs. Maladaptive Fear. Novelli MLT^{1,2}, Wegener G¹, Joca S¹, Iglesias LP¹ ¹Aarhus University, Health Graduate School, Denmark; ²USP, School of Pharmaceutical Sciences of Ribeirão Preto, Brazil

Totem 12

02.030 Increased Matrix Metalloproteinase-9 Activity in the Ventral Hippocampus Induced by Adolescent Stress: A Potential Target for the Prevention of Stress-Induced Abnormalities. Andres ESS, Freitas IS, Santos-silva T, Guimaraes FS, Gomes FV FMRP-USP, Dept Pharmacology, São Paulo, Brazil

02.031 Early Intestinal Oxidative Stress Triggered by Intranasal MPTP in the Parkinson's Disease Model. Gomes J, Severo ER¹, Evangelista HC, Rosa SG, Pinton S Unipampa, Uruguaiana, RS, Brazil

02.032 Involvement of the Hypothalamic-Pituitary-Adrenal (HPA) Axis in the Behavioral, Cardiovascular, and Neuroendocrine Effects Evoked by Social Defeat in Rats. Suzuki GMF¹, Belém-Filho IJA¹, Bushardo C², Paz HS², Corrêa FMA¹. ¹FMRP-USP, Dept Pharmacology, Lab Cardiovascular Neuropharmacology, Ribeirão Preto, Brazil. ²FCFAR-Unesp-Araraquara Dept Drugs and Medicines, Araraquara, Brazil

02.048 Neonatal Exposure to Kynurenine: Behavioral Changes Related to Schizophrenia. Bomfim ANL, Cruz BE, Faria ACC, Nunes LED, Andrade BS, Guimarães MZP, Neves GA UFRJ, Lab Molecular Pharmacology, Brazil

Totem 13

02.034 Analysis of the Anticonvulsant and Neuroprotective Potential of Carotenoid-Enriched Extracts from Amazonian Microalgae in an Acute Pentylentetrazol-Induced Seizure Model. Bentes ILG¹, Cancela LCF^{1,2}, Freitas CM^{1,2}, Araújo TB¹, Pinto DSV¹, Jesus MS¹, Faustino SMM¹, Nascimento JLM², Oliveira FR^{1,2} ¹Unifap, Depto de Ciências Biológicas e da Saúde, Curso de Farmácia, Brazil ²UFPA, Graduate Program in Pharmacology and Biochemistry, Brazil

02.035 Impact of Pilocarpine on Angiotensin I-Converting Enzyme (ACE) Activity in Transgenic Rats with Aberrant Neurodevelopment. Avila F, Basso L, Nani J, Janisset N, Cruz F, Oyadomari W,

Hayashi M Unifesp-EPM, Lab Molecular and Cellular Neuropharmacology, Dept Pharmacology, São Paulo, Brazil

02.036 SARS-CoV-2 Spike Protein Causes Behavioral and Neuro-Oxidative Changes Differently in Male and Female Mice. Willrich CH, Cury BJ, Longo B, de Amorim GES, Machado GM, de Oliveira IM, Queiroz LY, Casimiro LM, Santos MR, Cimarosti HI, Prediger RDS, da Silva L UFSC, Dept Pharmacology, Florianópolis, Santa Catarina, Brazil

02.037 Effects of Live and Inactivated *Bifidobacterium lactis* on Cerebral NPSH Levels Induced by MSG in Wistar Rats. Fontoura EA, Arena RVP, Comis-Neto AA, Rodrigues ES, Gomes J, Rodrigues BG, Pinton S, Rosa SG Unipampa – Uruguaiiana, RS, Brazil

Totem 20

02.038 Cannabidiol Attenuates General Neurotoxicity and Paclitaxel-Induced Cognitive Impairment (Chemobrain) in a Sex- and Treatment Regimen-Dependent Manner. Pereira ACMP¹, Queiroz ABWV², Vatanabe IP¹, Barros ASM², Meyer E¹, Lisboa SF¹ ¹FCFRP-USP, Brazil, ²FMRP-USP, Brazil

02.039 Antidyskinetic Effects of Cannabidiol and Cannabigerol in Parkinsonian Rats: Role of 5-HT_{1A} Receptors. Mattos BA¹, Nascimento GC², Crippa JA³ Hallak J³, Raisman-Vozari R⁴, Michel PP⁴, Guimaraes FS⁵, Del Bel EA² ¹FMRP-USP, Dept Physiology, Ribeirao Preto, Brazil; ²FORP-USP, Dept Basic and Oral Biology, Ribeirao Preto, Brazil; ³FMRP-USP, Dept Neuroscience and Behavior, Medical School of Ribeirao Preto; ⁴ICM- CNRS-Inserm, Paris Brain Institute, Sorbonne Université, Hôpital Pitié Salpêtrière, Paris, France; ⁵FMRP-USP, Dept Pharmacology, Brazil

02.040 Microglial Involvement in Adolescent Stress-Induced Behavioral Impairments and Deficits in Parvalbumin Interneurons and their Perineuronal Nets in Mice. Hadera V¹, Bobotis B², Landwehr A², Guimarães FS¹, Tremblay ME², Gomes FV¹ ¹FMRP-USP, Dept Pharmacology, Brazil ²University of Victoria, Medical Sciences Building, Canada

02.041 Evaluation of the Potential Neuroprotective Effects of Klotho Pre-Treatment in Primary Neuronal and Cerebellar Glial Cell Cultures Exposed to Ferrous Sulfate (FeSO₄) and Staurosporine (STS). Santiago TCP, Kawamoto, EM ICB-USP

02.075 Perillyl Alcohol Decreases the Frequency and Severity of Convulsive-Like Behavior in the Adult Zebrafish Model of Acute Seizures. Pereira ACM, Sánchez-Ortíz BL, Melo EL, Hage-Melim LIS, Borges RS, Hu X, Carvalho JCT, José Sánchez-Ortíz CTL USP

03. Psychopharmacology

Totem 14

03.008 Lithium Microdose Enhanced Object and Spatial Memory in Male Rats. De Souza STF, Kanazawa LKS, Saviski LR, Stingelin RL, Ferreira J, Pereira GC, Andreatini R, Vital MABF UFPR, Lab Central Nervous System, PPG Pharmacology, Dept Pharmacology, Brazil

03.009 Effects of Chronic Oral Administration of Cannabidiol on The Gut Microbiota Profile in Mice. Higa S¹, Hassib LC.¹, Ferreira F², Guimarães FS¹ ¹USP, PPG Pharmacology, Brazil ²FIOCRUIZ, Brazil

03.010 Effects of a Single Dose of Ayahuasca or Dextroketa mine on the Menstrual Cycle and Premenstrual Symptoms: A Randomized, Double-Blind Study. Bellucci T¹, Gomes CC¹,

Maekawa RM¹, Hallak JEC^{1,2}, Santos RG^{1,2} ¹FMRP-USP, Dept Neuroscience and Behavior; ²INCT-TM, National Institute for Translational Medicine

03.011 Automated vs. Manual Immobility Analysis: Fluoxetine Effects on a Rat Forced Swim Test Model. Fonseca RD¹, Almeida FB¹, Silva IAG¹, Battú GS², Flores CD³, Nin MS⁴, Barros HMT⁴
¹UFCSPA, PPG Health Sciences, Brazil, ²UFCSPA, Dept. Psychology, Brazil, ³UFCSPA, Dept. Exact and Applied Social Sciences, Brazil, ⁴UFCSPA, Dept. Pharmacosciences, Brazil

04. Inflammation and Immunopharmacology

Totem 04

04.016 Effects of Colchicine on Lipopolysaccharide (LPS)-Induced Neuroinflammation in Astrocytes and PC12 Cells. Araújo JR¹, Ananias CF¹, Bandeira SMA², Costa Júnior DC², Duarte ALS¹, Fernandes MO¹, Pinheiro KS¹, Rebouças MO³, Sales ISL³, Sampaio TL³, Silva MFS³, Sousa FCF³ ¹UFC, Faculty of Pharmacy, Dentistry and Nursing, ²UFC, Dept Pharmacology, PPG in Pharmacology, ³UFC, Center for Research and Development of Medicines, Brazil

04.017 Interference of BDNF in Depressive Disorders: Effects of Cortisol-Modulating Substances. Zara FF¹, Ferrari DN¹, Sauandag GA¹, Melo JME¹, Rossi R¹, Debia SB¹, Souza VCS¹, Santos L², Estrela HFG¹ ¹CUSC and ²UFRP-UAST

04.018 Antioxidant Activity of New Synthetic Chalcones. Marcarini VSM¹, Melo EDN¹, Falcão JS², Costa PRR², Caleffi GS², Muzitano MF¹, da Silva LL¹, Carmo PL¹ ¹UFRJ-Macaé Multidisciplinary Center, Bioactive Products Pharmacology Research Group, Brazil ²UFRJ, Health Sciences Center, Lab. of Bioorganic Chemistry, Brazil

Totem 15

04.020 Effects of the β -Carboline Alkaloids Harmine and Harmaline on Neuroinflammation and Oxidative Stress *in vitro*. Morales-Lima G^{1,2}, Falconi-Souto A¹, Gray NE², Mendes FR¹ ¹UFABC, Center for Natural and Human Sciences, Brazil ²Oregon Health & Science University, Dept Neurology, USA

04.021 Baricitinib Exhibits Antioxidant and Mitochondrial Protective Activity in Astrocytes and PC12 Cells Exposed to Lipopolysaccharide. Peixoto GPA¹, Bandeira SMA², Costa Junior DC³, Rebouças MO⁴, Lopes IS⁵, Silva DMA⁶, Sampaio TL⁷, Magalhães EP⁸, Teixeira IMM⁹, Sousa FCF¹⁰
¹UFC, PPG Pharmacology, Brazil ²UFC, PPG Pharmacology, Brazil ³UFC, PPG Pharmacology, Brazil ⁴UFC, PPG Pharmacology, Brazil ⁵UFC, PPG Pharmacology, Brazil ⁶UFC, PPG Pharmacology, Brazil ⁷UFC, Dept Pharmacy, Brazil ⁸UFC, PPG Pharmaceutical Sciences, Brazil ⁹UFC, Pharmaceutical Sciences, Brazil ¹⁰UFC, PPG Pharmacology, Brazil

04.022 Anti-Inflammatory Property of Crude Geopropolis Extract from *Melipona quadrisfaciata* In Human Macrophages Zapotoski LNK^{1,2}, Tambourgi DV², Pohl PC² ¹PPIB, University of São Paulo ²IBu, Immunochemistry Lab

04.023 Effect of Hypothalamic ETB Receptor Blockade on Serotonin and Dopamine Levels During Systemic Inflammation in Rats. Alencar ST, Costa RA, Santos MO, Schneider VMM, Fernandes SMA, Borges AP, Zamprônio AR UFPR Inflammation and Fever Lab, Dept Pharmacology

04.024 Muscarinic Antagonists Mitigate Structural Lung Damage in Experimental Silicosis. Santos BDF, Ferreira TPT, Arantes ACS, Santana ACC, Martins MA, Silva PMR IOC-Fiocruz, Lab Inflammation, Brazil

Totem 21

04.025 **Hyperglycemia Induces Macrophages Senescence *in vitro***. Dias DS, Pacheco FS, Mota RG, Chaves AS, Barbosa BL, Carvalho VF, Silva PR Martins MA Lab Inflammation, IOC-Fiocruz, Rio de Janeiro, Brazil

04.026 **Nano Emulsion with Omega 3 Fatty Acids Modulates Muscle Skeletal Tissue Degeneration and Regeneration Process in Venom of *Bothrops asper* Induced Lesion**. Roschel GG¹, Sousa MBN¹, Escalante T², Purgatto E³, Moreira V¹ ¹Unifesp, Lab of Pharmacology of Inflammation, PPG Pharmacology, Brazil ²Clodomiro Picado Institute, Faculty of Microbiology, Costa Rica University, Costa Rica ³FORC-USP, Faculty of Pharmaceutical Sciences and Food Research Center, Brazil

04.027 **A Dual FPR1/FPR2 Agonist Suppresses Pulmonary Inflammation and Fibrosis Development in a Murine Model of Silicosis**. Pezzella-Ferreira GN¹, Cabral CL¹, Arantes ACS¹, Martins MA¹, Perretti M², Silva PMR¹ ¹ Lab Inflammation, Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro, Brazil. ²Queen Mary University, William Harvey's Research Institute, UK

04.028 **Maternal Neutrophils Orchestrate Embryo Development and Reshape the Innate Immune System in the Offspring**. Scharf P¹, Borges PP¹, Costa KAM¹, Fock RA¹, Urban S², Farsky SHP¹ ¹USP, PPG in Pharmacy (Physiopathology and Toxicology), São Paulo, Brazil ²UNIFESP, PPG in Functional and Structural Biology, São Paulo, Brazil

04.029 **Environmental enrichment alters FFA1 receptor-mediated responses in a mouse model of chronic pain**. Estrázulas M^{1,2}, Neculqueo GW^{1,2}, Farias ABS², Campos MM^{1,2} ¹PUCRS, Escola de Medicina, PPG Medicina e Ciências da Saúde, Brazil. ²PUCRS, Escola de Ciências da Saúde e da Vida, INTOX, Brazil

04.030 **Aging, Immunity and Metabolism: Divergent Effects of Rapamycin in Senescence-Prone and -Resistant Mice**. Queiroz LAD¹, Barros RS¹, Assis JB², Pantoja KC¹, Bustia SX¹, Sá-Nunes A², Rodrigues SFP³, Turato WM⁴, Silva CCC⁵, Migliorini S¹, Martins JO¹ ¹FCF-USP, Dept. of Clinical and Toxicological Analyses, Lab. of Immunoendocrinology, Brazil; ²ICB-USP, Dept. of Immunology, Lab. of Experimental Immunology, Brazil; ³ICB-USP, Dept. of Pharmacology, Lab. of Vascular Nanopharmacology, Brazil; ⁴IQ-USP, Dept. of Biochemistry, Energy Metabolism Lab., Brazil; ⁵FCF-USP, Dept. of Clinical and Toxicological Analyses, Multi-user Preclinical Imaging Center, Brazil

05. Pain and Nociception Pharmacology

Totem 05

05.013 **Stigmasterol Attenuates Nociceptive Behaviours in a Fibromyalgia Experimental Model**. Lopes JPV¹, Assis ACP¹, Becker G¹, Brum ES², Oliveira SM¹ ¹UFSM, Pain Neurobiology Lab, Graduate Program in Biological Sciences: Toxicological Biochemistry, Brazil. ²UFRGS, Graduate Program in Biological Sciences: Biochemistry, Brazil

05.014 **Sex Differences in Pain and Long-Lasting Analgesia Induced by Apitoxin and Melittin Treatment**. Leonor MGR¹, Boaventura de Oliveira AM¹, Silva DF¹, Sant'anna MB¹, Paulo, MEFV², Picolo G¹ ¹IBu, Lab Pain and Signaling, São Paulo, Brazil; ²IBu, Pharmacology Lab, São Paulo, Brazil

05.015 **Evaluation of the Antinociceptive Effect of Creams Containing *Cannabis sativa* Extracts in an Animal Model of Pain**. Bazilio YS¹, Rezende B¹, Macario VS¹, Oliveira BC¹, Guedes GN¹, Andrade MEB¹, Calumbi KS¹, Maia EP¹, Oliveira L², Maluf D², Antunes F³, Montes GC¹, ¹UERJ, Roberto Alcântara Gomes Institute of Biology (IBRAG), Brazil, ²UFPR, Dept Pharmacy, Brazil, ³UENF, Animal Clinic and Surgery Lab, Brazil

05.016 Decreased Anterior Cingulate Cortex Activity Optimizes the Effects of Electroacupuncture on Anxiety Related to Neuropathic Pain. Souza MBRF¹, Ribeiro-Siqueira CM¹, Donizetti-Fonseca MA¹, da Silva AL^{1,2}, da Silva ML^{1,2}, da Silva JRT^{1,2}, Falconi LLS^{1,2} ¹UNIFAL, Lab. of Neuroscience, Neuromodulation and Study of Pain, Brazil, ²UNIFAL, PPG Biosciences Applied to Health, Brazil

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05.017 Resveratrol Modulates Neurochemical Parameters and Produces Dose-dependent Analgesia in Rats Subjected to a Rat Model of Chronic Orofacial Inflammatory Pain Roland S^{1,2,3,4}, Morais ITS^{1,2,3,4}, Farias VEF¹, Stieven A^{1,2,4}, Melo AS¹, Stein DJ^{1,2,4}, Torres ILS^{1,2,3,4} ¹Lab Farmacologia da Dor e Neuromodulação: Investigações Pré-Clínicas, Centro de Pesquisa Experimental, ²HCPA, Núcleo de Farmacologia da Dor e Neuromodulação, Porto Alegre, RS, Brasil ³UFRGS Programa de Pós-Graduação em Ciências Biológicas: Farmacologia e Terapêutica, ⁴UFRGS, Programa de Pós-Graduação em Medicina: Ciências Médicas, Porto Alegre, RS, Brasil

05.018 Antinociceptive Effect of Repeated Administration of the TRPA1 Antagonist in a Model of Relapsing-Remitting Multiple Sclerosis in Mice. Luiz ACM¹, Peres DS¹, Antoniazzi C¹, Trevisan G^{1,2} ¹UFSM, Graduate Program in Pharmacology, Federal University of Santa Maria, Brazil. ²UFSM, Graduate Program in Biological Sciences, Brazil

05.019 Molecular Docking Study of Cannabinol with Human TRPV1 Channel: Insights to Treatment of Fibromyalgia. Costa AEA^{1,2}, Santos PVS¹, Santos BS¹, Veras JF¹, Ferreira VGF¹ Freire NAEP¹, Silva JLV¹ ¹FMO, ²Unicamp

05.020 Antinociceptive Effects of Different Full Spectrum Oils of Phytocannabinoids in Mice Acute Models of Pain. Junger MG^{1,4}, Matheus ME², Oliveira DR^{3,4}, Miranda ALP^{1,4} ¹LEFEx-FF-UFRJ, Lab Studies in Experimental Pharmacology, Brazil. ²FF-UFRJ, Brazil. ³FF-UFRJ, Lab Bioprospecting and Applied Ethnopharmacology, Brazil. ⁴FF-UFRJ, Postgraduate Program in Pharmaceutical Sciences, Brazil

06. Cardiovascular and Renal Pharmacology

Totem 06

06.010 Bergamot (*Citrus bergamia*) By-Product as a Preventive Strategy on Heart and Kidney Doxorubicin-Induced Oxidative Stress: A Preclinical Model New Perspectives. Berretta F¹, Agostino ME¹, Palacio TLN², Siqueira JS², Corrêa CR³ ¹Unesp, Institute of Biosciences, Undergraduate Program, Brazil, ²Unesp, School of Medicine, Postgraduate Program in Pathology, Brazil ³Unipex-Unesp, Experimental Research Unit () – Laboratório Redox, Brazil

06.011 Semaglutide Treatment Reduced Inflammatory Adipokines on PVAT from Hypertensive Ovariectomized Female Rats with Overweight. Queiroz MCT, Ramos IT, Sousa JH, Eichler RA, Akamine EH, Costa TJ, ICB-USP, Dept Pharmacology, Brazil

06.012 Increase in Oxidative Stress and Organ Injury in Mice Subjected to Hemorrhagic Shock. Simas A¹, Mariot LN², Pinheiro, LC^{1,2}, Sordi R^{1,2} ¹UFSC, Dept Pharmacology, ²UFSC, PPG in Pharmacology

06.013 Resveratrol Administration During Adolescence in Hypertensive Female Rats Does Not Reduce Vascular Matrix Metalloproteinase (MMP)-2 Activity and Hypertension in Adulthood Meneghesso MR, Melo MMB, Bueno EKP, Mazzaron M FMRP-USP, Departament of Pharmacology

06.014 **Estrogen Attenuates Ovariectomy-Induced PVAT Dysfunction in SHR: Role of NOX4, H₂O₂, and Inflammatory Adipokines.** Ramos IT, Queiroz MCT, Sousa JH, Eichler RA, Akamine EH, Costa TJ, ICB-USP, Dept Pharmacology, Brazil

Totem 17

06.015 **Bariatric Surgery Prevents Nitrate Treatment-Induced Decreases in Circulating Matrix Metalloproteinases Concentrations in Overweight Women.** Cássia-Barros A¹, Sanches-Lopes JM², Conde-Tella SO^{1,2}, Coelho EB³, Kemp R⁴, Salgado Júnior W⁴, Moreno Junior H⁵, Tanus-Santos JE^{1,2}
¹FCM-UNICAMP, Dept Translational Medicine, PPG Pharmacology, Brazil; ²FMRP-USP, Dept Pharmacology, PPG Pharmacology, Brazil ³FMRP-USP, Dept Medicine, Brazil ⁴ FMRP-USP, Dept Surgery and Anatomy, Brazil ⁵Unicamp, Dept Internal Medicine, PPG Pharmacology, Brazil

06.016 **Vasorelaxant Effect of Myrtenyl Pelargonate Derived from Biotransformation by *Cladosporium cladosporioides* in Aorta of SHR Rats: *in silico*, *in vitro* and *in vivo* Approach.** Barros LSR^{1,2}, Rego AF^{1,2}, Sena EP^{1,2}, Viana FSC^{2,3}, Barbosa BS^{2,4}, Acha BT^{5,6}, Kutyla M⁹, Trytek M⁹, Sabino JPJ^{7,8}, Oliveira AP^{1,2,7} ¹UFPI, Lab Cardiovascular Pharmacology, NPPM, Brazil ²UFPI, Graduate Program in Pharmacology, Brazil ³UEMA, Brazil ⁴UFPI, Smooth Muscle Lab, NPPM, Brazil ⁵UFPI, Lab Functional and Molecular Studies in Physiopharmacology, Brazil ⁶RENORBIO-UFPI, Graduate Program in Biotechnology, Brazil ⁷UFPI, Dept Biophysics and Physiology, CCS, Brazil ⁸UFPI, Lab Reflex Control of Pulmonary Ventilation, Brazil ⁹Maria Curie-Skłodowska University (UMCS), Poland

06.017 **Salt-Inducible Kinase Regulates (Na⁺+K⁺)-ATPase Activity in Pig Kidney Outer Cortex.** Ribeiro DDS^{1,2}, Hilário-Souza E¹, Sarmiento GC³, Lowe J⁴, Gomes DS^{1,2}, Bastos GA^{1,2}, Dick CF⁴, Lara LS^{1,2}.
¹ICB-UFRJ, ²IBCCF-UFRJ, Centro de Pesquisa em Medicina de Precisão, ³ IBCCF-UFRJ, ⁴UFRJ, Instituto de Bioquímica Médica, Brazil

06.018 **CB₂ Receptors as a Therapeutic Target for Cardiovascular Dysfunction in Pneumonia-Induced Sepsis.** Borges LB, Simas A, Albino LB, Mariot, LN, Da Silva-Santos, JE Sordi R UFSC, PPG in Pharmacology, Dept Pharmacology

Totem 22

06.019 **Impact of Esomeprazole on Nitric Oxide Bioavailability in a Rat Model of Gestational Hypertension.** Da Silva MLS, Da Silva AAF, Toghi CJ, Dragoneti BJ, Da Costa LC, Dias-Junior CA IB-UNESP-Botucatu, Dept Biophysics and Pharmacology

06.020 **Early Hypercaloric Diet: a Trigger for the Development of Contractile Dysfunction in Mice.** Mariot LN, Neves PG, Simas A, Borges LB, Delfrate G, Andrade CA, Dal Secco D, Da Silva-Santos JE, Fernandes D, Victorio JA, Sordi R UFSC, PPG in Pharmacology

06.021 **Nebivolol Modulates Nitric Oxide Independently of eNOS in Endothelial Cells Exposed to Preeclamptic Placental Factors.** Bueno-Pereira TO, Santos KN, Matheus MB, Zampieri GM, Passeti LFP, Nunes PR, Sandrim VC IBB-Unesp-Botucatu, Dept Biophysics and Pharmacology – Brazil

06.022 **Testosterone-driven Cardiac Remodeling in a Gender-Affirming Hormone Therapy Experimental Model (Testo-GAHT) Relies on CD4⁺ T Cell Effector Profile Activated by the Glycolytic Pathway.** Oliveira-Neto JT^{1,4}, Oliveira CV², Machado MR¹, Cebinelli GCM², Rodrigues D¹, Bayer AL⁴, Robbe ZL⁴, Silva CAA³, Silva JS², Salgado HC³, Cunha FQ^{1,2}, Alves-Filho JCF^{1,2}, Santos JD¹ Alcaide P⁴, Tostes RC¹. ¹FMRP-USP, Graduate Programs in Pharmacology, ²FMRP-USP, Graduate Programs in Immunology and ³FMRP-USP, Graduate Programs in Physiology, Brazil. ⁴Tufts University, School of Medicine, Dept Immunology, USA

06.030 **Aging Differentially Affects Male and Female Thoracic Aorta Perivascular Adipose Tissue: A Study Using a Murine Model of Accelerated Aging.** Marques BVD, Akamine EH USP - Dept Pharmacology, São Paulo, Brazil

07. Endocrine, Reproductive and Urinary Pharmacology

Totem 04

07.007 **Basal Release of 6-Cyanodopamine from Rat Isolated Seminal Vesicle and Its Role in Adrenaline-Mediated Contractions.** Ramos MCC, Dal Pozzo CFS, Santos IAR, Ribeiro LC, De Nucci G Unicamp, Dep. of Pharmacology, IC Program, Brazil

07.009 ***Arthrospira platensis* Modulates Inflammatory Cytokines in the Corpus Cavernosum of Obese Rats: A Promising Strategy Against Obesity-Induced Erectile Dysfunction.** Melo MB¹, Barros BC^{1,2}, Diniz AFA^{1,2}, Arruda RRA^{1,2}, Silva JMA^{1,2}, Ribeiro SB⁴, Lima CAS⁴, Sousa Filho JEC¹, Claudino BFO^{1,2}, Soares MFS^{1,2}, Araújo AA⁴, Silva BA^{1,2,3} ¹UFPB, Lab Functional Pharmacology, Institute of Research in Drugs and Medicines, Brazil ²UFPB, Postgraduate Program in Bioactive Natural and Synthetic Products, Health Sciences Center, Brazil ³UFPB, Dept Pharmaceutical Sciences, Health Sciences Center, Brazil ⁴UFRN, Lab Experimental Model of Intestinal Mucositis, PPG Pharmaceutical Sciences, Brazil

08. Respiratory and Gastrointestinal Pharmacology

Totem 04

08.004 **Investigation of the Antioxidant and Gastroprotective Activity of the Hydroethanolic Extract of *Plectranthus barbatus*.** Casimiro LM¹, Ferreira P², dos Santos AC², da Silva LM¹ ¹UFSC, Center of Biological Sciences, Postgraduate Program in Pharmacology, Brazil. ²Univali, Postgraduate Program in Pharmaceutical Sciences, Brazil

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08.005 **Evidence of Biochemical Alterations of Oxidative and Inflammatory Damage in the Esophageal Mucosa of Rats with Autism Spectrum Disorder Induced by Valproic Acid.** Martins BSP¹, Souza Neto AE², Souza YAF², Nunes RKS², Longo B¹, Venzon L 2, Da Silva LM¹ ¹UFSC, Dept Pharmacology, Florianópolis, Santa Catarina, Brazil. ²Univali, Graduate Program in Pharmaceutical Sciences, Itajaí, Santa Catarina, Brazil

08.006 **Toxicological Evaluation and Therapeutic Potential of the Ethanolic Extract of *Combretum leprosum* Mart. Stem Bark in an Ovalbumin-Induced Asthma Model in Rats.** Oliveira MM¹, Souza ORB¹, Silva RLT¹, Sousa HG², Anjos BS², Leal PPMS², Alves MMM², Vieira Júnior GM², Oliveira AP¹, Oliveira RCM¹, Santos RF¹. ¹UFPI, PPG Pharmacology, Brazil, ² UFPI, Lab. of Natural Products, Brazil, ²UFPI, BioLeish - Advanced Research in Leishmania and Alternative Models, Brazil

08.007 **Reactivity Profile of the Colon in Sepsis Survivors: Sex-Related Functional Differences Involving Angiotensin Signaling** Lima-Moura SA, da Silva-Santos JE UFSC, Lab Cardiovascular and Smooth Muscle Biology, Dept Pharmacology

08.008 **Protective Effect of Lauric Acid on Lung Remodeling: Modulation of ROCK1 and α -SMA in Asthmatic Rats** Martins AMO¹, Figueiredo IAD¹, Felício IM¹, Oliveira LN¹, Souza MLB², Alves AF^{1,2}, Vasconcelos LHC^{1,3}, Cavalcante FA^{1,3} ¹PPgPNSB/CCS/UFPB; ²PIBIC/UFPB; ³DCB/CCS/UFPB

08.009 **Uncovering the Gastroprotective Properties of Araucaria Brown Propolis and its Active Compound, Abietic Acid.** Cury BJ¹, Silva HFT², Makowieski LP², Jerônimo DT², Silva LM², Silva TFO², Longo B¹, Santos MR¹, Cruz AB², Bastos JK³, Santos MFC⁴, Silva LM¹ ¹UFSC, Dept Pharmacology, Postgraduate Program in Pharmacology, Brazil. ²UNIVALI, Postgraduate Program in Pharmaceutical

Sciences, Brazil. ³FCF-USP, Brazil ⁴University of Franca, Research Center in Exact and Technological Sciences, Brazil

09. Natural Products and Toxinology

Totem 08

09.015 **Colilen IBS® Attenuates Inflammation and Alleviates Allodynia in DSS-induced Colitis in C57BL/6 Mice.** Santos LBS, Brito MSC, Rech KS, Martins FG1, Werner MFP UFPR

09.016 **Malvidin's Activity in Dextran Sulfate Sodium (DSS)-Induced Inflammatory Bowel Disease.** Ciscato JW¹, Baggio C², MacNaughton W², Santos RC¹ ¹USF, Lab. de Produtos Naturais, Bragança Paulista, SP, Brasil. ²University of Calgary, Cumming School of Medicine, Dept Physiology and Pharmacology, Snyder Institute for Chronic Diseases, Canada

09.017 **Evaluation of Hematological and Biochemical Effects of Apitoxin and Melittin** Vila JRM¹, Oliveira AMB¹, Leonor MGR¹, Misaca RC¹, Silva GSA¹, Dallazen JL¹, Santoro ML², Picolo G¹ ¹Ibu, Lab Pain and Signaling, São Paulo, Brazil; ²Ibu, Central Animal Facility, São Paulo, Brazil

09.018 **Determination of the Effective Dose for Neutralizing the Coagulant Activity of *Bothrops matogrossensis* Snake Venom by Antivenom.** Carneiro IB¹, Galizio NC¹, Sousa EP¹, Rodrigues MAF¹, Felipe AGC¹, Sant'anna SS², Morais-Zani K^{1,2} ¹Ibu, Lab Fisiopatologia; ²Ibu, Lab Herpetologia

09.019 **Effect of Bergamot Leaf Extract on Renal Antioxidant System and Proteinuria of Obese Rats.** Caccia CM¹, Cruzeiro J², Berretta F¹, Almeida JC¹, Santos MPS², Siqueira JS², Corrêa CR³ ¹IBB-Unesp-Botucatu, Undergraduate student in Nutrition, Brazil. ²Unesp-Botucatu, Dept Pathology, Botucatu Medical School, PostGraduation Program in Pathology, Brazil. ³Unesp-Botucatu, Dept Pathology, Botucatu Medical School, Brazil

09.051 **Development and Characterization of a Food Gel Based on *Mauritia flexuosa* Mesocarp.** Teixeira MC¹, Amorim VR¹, Oliveira JHM¹, Santos WB¹, Almeida AAC¹, Porto JCS¹, Pereira-Freire JA², Silva JN³, Ferreira PMP¹ ¹UFPI, Lab Experimental Cancerology (LabCancer), Dept Biophysics and Physiology; ²UFPI, Dept Nutrition; ³UFPI, Dept Chemistry

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09.020 **Ability of Suramin to Antagonize Some Activities of American Rattlesnake Venoms.** Rocha-Junior JRS¹, Nogueira-Souza PD¹, Pinheiro AN¹, Gomes da Costa PI¹, Strauch MA², Melo, PA¹ ¹ICB-CCS-UFRJ, Lab Farmacologia das Toxinas, Rio de Janeiro, RJ, Brazil; ²ICBS-UFRRJ, Depto de Ciências Fisiológicas, Seropédica RJ, Brazil

09.021 **In Vitro Investigation of the Anti-*T. cruzi* Potential of Natural Products Isolated from *Artemisia umbelliformis*.** Carvalho YAM¹, Karoutzou O², Cezar IS^{1,3}, Neves MVG^{1,3}, Silva JW¹, Mikros E², Skaltsounis LA², Soares MBP^{1,3}, Meira CS^{1,3} ¹FIOCRUIZ-BA, PPG Biotechnology in Healthy and Investigative Medicine, Brazil, ²National and Kapodistrian University of Athens, Greece, ³ SENAI CIMATEC-BA, Brazil

09.022 **Potential of Myristicin in Reversing Multidrug Resistance in Chemotherapy Treatments.** Bertholdo LJ, Carvalho FG, Seneme EF, Sciani JM, Longato, GB. USF, Lab Natural Products, Graduate Program in Health Sciences, Brazil

09.023 **Galactomannan- and Silver Nanoparticle-Based Dressing Accelerates Wound Healing *in vitro* and *in vivo* Experimental Models.** Silva FA¹, Silva ELES¹, Lucena LCP¹, Flor JF¹, Almeida JH¹, Silva EC¹, Nunes DM², Albuquerque PBS², Lima LRA², Rodrigues NER², Correia ACC², Ferro JNS¹

09.024 **Influence of the Geographical Variability on the Neurotoxic Potential and Reactivity with Commercial Antivenom of Venoms from Four Subspecies of *Crotalus durissus* (Viperidae-Crotalinae) Distributed in Brazil** Demico PJ¹, Azevedo SNS¹, Gonçalves de Lima IG¹, Sant'Anna SS², Morais-Zani K³, Floriano RS¹ ¹Unoeste, Lab. of Toxinology and Cardiovascular Research, Graduate Program in Animal Science, Brazil, ²IBu, Lab. of Herpetology, Brazil, ³IBu, Lab. of Pathophysiology, Brazil

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09.025 **Agro-Industrial Waste Upcycling: Polysaccharides from Passion Fruit Peels as Protective Shields Against the Toxicity of 5-Fluorouracil.** Schiebel CS¹, Oliveira NMT¹, Braga LLVM¹, Silva KS¹, Abboud KY², Cordeiro LMC², Maria-Ferreira, D¹ ¹FPP, Instituto de Pesquisa Pelé Pequeno Príncipe, PPG Biotecnologia Aplicada à Saúde da Criança e do Adolescente, Brazil; ²UFPR, PPG Ciências-Bioquímica, Brazil

09.026 **Impact of Marine Animal Extracts on Neuronal Viability and Inflammation in Alzheimer's Disease Models.** Alves MM¹, Vigerelli H², Kitahara MV³, Kerkis I⁴, Sciani JM⁵ ¹IBu, PPG Sciences – Toxinology, Lab. of Biochemistry, Brazil, ²IBu, Center of Excellence in Target Discovery, Brazil, ³USP, Center for Marine Biology, Brazil, ⁴IBu, Lab. of Genetics, Brazil, ⁵USF, Lab. of Natural Products, Brazil

09.027 **Apigenin Promotes Ulcer Healing in a Chronic Acetic Acid-Induced Murine Model.** Bezerra MCF¹, Cechinel Filho V², Colodel EM³, Figueiredo FF¹, Rodrigues DW², Silva WDM³, Cavasani JPS³, Martins DTO¹ ¹UFMT, Pharmacology Area, Graduate Program in Health Sciences, Brazil, ²Univali, Center for Chemical and Pharmacological Investigations, Graduate Program in Pharmaceutical Sciences, Brazil ³UFMT, Veterinary Pathology Lab, Graduate Program in Veterinary Sciences, Brazil

09.028 **Proteomic and Functional Analysis of *Acanthophis antarcticus* Venom: An Integrative Approach for Biochemical Characterization.** Falla MVA¹, Sousa EP³, Morais-Zani K³, Valladao R¹, Santos NG¹, Galizio NC³, Rodrigues MS², Almeida HIF¹, Rocha ARL¹, Moyses MN¹, Lebrun I¹, Spancer P², Pimenta DC¹, Coelho GR¹ ¹ IBu, Laboratório Bioquímica e Biofísica, ²IPEN, ³ IBu, Lab Fisiopatologia

09.029 **Evaluation of a Polysaccharide from *Acrocomia corumbaensis* (Bocaiuva) in DSS-Induced Colitis in Mice.** Brito MSC¹, Taporoski AK², Rech KS¹, Santos LBS¹, Martins FG¹, Cordeiro LMC², Werner MFP¹ ¹UFPR, Pharmacology, ²UFPR, Biochemistry and Molecular Biology

09.030 **Neuroprotective Potential of *Randia formosa* Fruit Extract in a Murine Model of Aluminum-Induced Neurotoxicity.** Okoh VI¹, Campos HM¹, Ferreira PYO¹, Uchenna N¹, Silva GNM², Leite JA¹, Costa EA¹, dos Santos FCA¹, Gil ES², Ghedini PC¹ ¹UFG, Institute of Biological Sciences, Goiânia, GO, Brazil; ²UFG, Faculty of Pharmacy, Goiânia, GO, Brazil

10. Cancer Pharmacology

Totem 09

10.020 **Investigation of Cytotoxic Potential of Melittin and Apitoxin in Bladder Cancer Cell T24.** Brayn GA¹, Poiato GEH¹, Oliveira AMB¹, Porto RM², Silva GN³, Almeida TC¹, Picolo G¹. ¹IB, Lab Pain and Signaling, São Paulo, Brazil; ²IB, Lab Development and Innovation, São Paulo, Brazil; ³UFOP, Toxicogenetic, Epidemiological and Clinical Study and Research Group, Ouro Preto, Brazil

10.021 **Evaluation of Hepcidin and Heparanase Expression in the Treatment of DU-145 and PC-3 Cell Lines with Heparin.** Kawamura JK, Santos PCJL, Vicente CM, Porto CS. Unifesp-EPM, Dept Pharmacology, Brazil

10.022 **PEGylation Reduces Nanoparticle Cytotoxicity in Non-Cancer Cells as a Function of PEG Content.** Maia RA¹, Miguel RA^{1,2}, Costa-Lotufo LV^{1,2}, Fenical W³, Clair JLL⁴, Lopes LB¹ ¹ICB-USP, Dept Pharmacology, PPG Functional and Molecular Biology, Brazil. ²University of Cape Town, Faculty of Health Sciences, Dept Human Biology, South Africa. ³University of California at San Diego, Scripps Institution of Oceanography, Center for Marine Biotechnology and Biomedicine, USA ⁴ University of California at San Diego, Dept Chemistry and Biochemistry, USA

10.023 **BR4Cl Inhibits Migration and Adhesion of SW-480 Colorectal Cancer Cells *in vitro*.** Alves BS¹, Oliveira MP¹, Pereira FIA¹, de Sá RE¹, Pérez CN², Marinho-Filho JDB¹, Araújo AJ¹.¹UFDPAR, Lab Cultura de Células do Delta, Brazil, ²UFG, Instituto de Química, Brazil

10.024 **Characterization of the Cytotoxicity of Seriniquinone in Glioma Cell Lines.** Leal AGB¹, Hirata AS¹, Fenical W², Costa-Lotufo LV¹ ¹ICB-USP, Dept Pharmacology Brazil; ²Scripps Institution of Oceanography University of California in San Diego USA

Totem 25

10.025 **Combining Hypericin-Mediated Photodynamic Therapy and Low-Dose Cisplatin Boosts Cell Death and Necrosis of Cervical Cancer Cells.** Carobeli LR¹, Santos JJ², Martins LF², Caetano W², Consolaro MEL¹ ¹UEM, PGP Biosciences and Physiopathology, ²UEM, PGP Chemistry

10.026 **Melanoma and IAPs: Unraveling Therapeutic Opportunities through Integrated Bioinformatics and Experimental Approaches.** Silva CSMR, Machado-Neto JA, Costa-Lotufo LV ICB-USP

10.027 **Antitumoral Effect of Lemon Gum (Citrus X Latifolia) In A Murine Colon Carcinoma Model (CT26.WT) *in vivo* and *in vitro*.** Oliveira MMF¹, Alves GOM¹, Araújo DD¹, Araújo GS¹, Machado FS¹, Cajado AG², Lima-Júnior RCP², Lopes ALF³, Medeiros JVR³, Ribeiro FOS⁴, Silva DA⁴, Souza LP⁵, Silva FBF⁵, Araújo AJ¹, Marinho JDB¹ ¹UFDPAR, Lab Cultura de Células do Delta, Brasil, ²UFC, Lab Farmacologia da Inflamação e do Câncer, Brasil, ³UFDPAR, Lab Inflamação e Gastroenterologia Translacional, Brasil, ⁴UFDPAR, Núcleo de Pesquisa em Biodiversidade e Biotecnologia, Brasil, ⁵UFDPAR, Brasil

10.028 **IL-33 Expression and M1 Macrophage Polarization in Triple-Negative Breast Cancer: A Translational Study.** Maia IFVC¹, Vidal DBD¹, Alves BES¹, Freitas GL¹, Maia CB¹, Barrozo AEO¹, Silva JMR¹, Almeida PRC², Cunha MPSS³, Júnior-Lima RCP¹, Wong DVT¹ ¹UFC, Dpt of Physiology and Pharmacology, Lab Inflammation and Cancer Pharmacology, Drug Research and Development Center; ²UFC, Dpt of Pathology and Forensic Medicine; ³ICC, Haroldo Juaçaba Hospital

10.029 **Aurelianolides Downregulate Macroautophagy and HSP90 Activity, with Mitochondria Disruption.** Silva GWS¹, Marques AM², Fontão APGA¹, Figueiredo MR², Alves, SV³, Santos, LMG³, Sampaio ALF¹ ¹Farmanguinhos-FIOCRUZ, Lab Farmacologia Molecular; ²Farmanguinhos-FIOCRUZ, Lab Tecnologia para Biodiversidade em Saúde/TecBio; ³INCQS-FIOCRUZ Lab Alimentos

11. Clinical Pharmacology, Pharmacokinetics, Pharmacogenomics and Toxicology

Totem 10

11.006 **Fc Gamma Receptor Polymorphisms as Potential Biomarkers of Natalizumab Therapeutic Failure in Multiple Sclerosis.** Pavani GK¹, Cardoso RC¹, Matos MD¹, Duarte LA^{2,3}, Guimenes BD¹, Gutman EG^{2,4}, Ferreira HFA², Pereira VCSR⁴, Domingues VB⁵, Farinhas JGD⁵, Fernandez KA⁵,

Schmidt FR⁵, Montes GC¹, Alves-Leon SV^{2,4#}, Fontes-Dantas FL^{1 1}UERJ, NeuroPharmacoGenetics Lab, Dept. of Pharmacology and Psychobiology, Brazil; ²UNIRIO, Biomedical Institute, Translational Neuroscience Lab (LabNet), Brazil; ³UFRJ, PPG Clinical Medicine, Brazil; ⁴UFRJ, Dept. of Neurology, Hospital Universitário Clementino Fraga Filho, Brazil; ⁵UERJ, Pedro Ernesto University Hospital, Neuroimmunology Outpatient Clinic, Brazil

11.007 Exposure to Apresa Herbicide leads to Oxidative Stress in brain of Male and Female Wistar Rats. Aguiar IS¹, Ferreira PYO¹, Santos EEO¹, Souza VKB¹, Campos HM¹, Ghedini PC¹, Reis DS², Rodrigues BM², Santiago ALG², Almeida LM², Amaral VCS², Mazzaro R¹, Damin V³, Leite JA^{1 1}ICB-UFG, Goiânia, Brazil; ²UEG, Anápolis, Brazil; ³UFG, School of Agronomy, Goiânia, Brazil

11.008 Validation of a Liquid Chromatography Coupled with Mass Spectrometry (LC-MS/MS) Method to Quantify Artepillin C in Microdialysate Samples from Prostate Rats: Application to Preclinical Pharmacokinetic Study. Menin RH, Toson N, Olivo LB, Dias BB, Lemos JLS, Rodrigues VJ, Barnett L, Bastos JK, Araujo BV Menin RH¹, Toson N¹, Olivo LB¹, Dias BB¹, Lemos JLS¹, Rodrigues VJ¹, Barnett L¹, Bastos JK², Araujo BV^{1 1}UFRGS, ²USP

11.009 Agmatine Exerts Anti-Ferroptotic Effects in HEK-293 Cultured Cells. Cells Caversan S, Santer M, Ilha SH, Sperlich CL, Jacques MT, Farina M UFSC, Neuropathology Experimentation Lab, Dept Biochemistry

11.010 Comorbidities in People Living with HIV/Aids (PLWHA) on Antiretroviral Therapy (ARV) in a Brazilian Hospital. Lima-Franco I², Umanã-Rivas M¹, Velázquez D¹, Soares, LM¹, Noronha EF¹, Meiners MMA^{1,3}, Metzger IF^{1,2 1}UnB, Graduate Program in Tropical Medicine, Center for Tropical Medicine, Faculty of Medicine, Brasília, Brazil, ²UnB, Dept Pharmacy, Faculty of Health Sciences, Brasília, Brazil ³UnB, Faculty of Health Sciences and Technologies, Brasília, Brazil

11.011 Impact of Non-Steroidal Anti-Inflammatory Drugs (Nsaids) During Gestation and Lactation on Hypothalamic Sexual Differentiation in Male Rats. Magalhães L¹, Castro RLM¹, Santos STC¹, Stein J¹, Nagaoka LT¹, Aquino JVF¹, Rosalem GF¹, Jorge BC¹, Arena AC^{1,2 1}IBB-Unesp,, Brazil ²CIATOX-Unesp, Center for Toxicological Information and Assistance, Brazil

12. Drug Discovery and Development

Totem 11

12.001 Synergistic Anti-Schistosomal Efficacy of Curcumin-Anchored Silver Tungstate (AW-HRL-C). Guimarães VH¹, Forde-Thomas J⁵, Anselmo MC¹, Duarte FO¹, Bianchi J¹, Fragelli BDL², Assis M³, Líbero LO², da Silva EL², Hoffmann KF⁵, Anibal FF^{1 1}UFSCar, Lab. of Inflammation and Infectious Disease, Dept. of Morphology and Pathology, Brazil, ²UFSCar, Center for Development of Functional Materials, Dept. of Chemistry, Brazil, ³Universitat Jaume I, Dept. of Physical and Analytical Chemistry, Spain, ⁴Unicamp, Inst. of Biology, Dept. of Animal Biology, Brazil, ⁵Aberystwyth University, IBERS, United Kingdom

12.002 Autophagy and Stress-Responsive Long Non-coding RNAs as New Putative Antischistosomal Targets in *Schistosoma mansoni*. Fischer-Carvalho A, Poli PJ, Tahira AC, Verjovski-Almeida S, Amaral MS IBu, Cell Cycle Lab, São Paulo, Brazil

12.003 Arylboronic Acids as Dual Inhibitors of Butyrylcholinesterase and β -Secretase 1 for Alzheimer's Disease Treatment. Bussinger ER¹, Hiller NJ², Faria RX³, Martins DL², Raimundo JM^{1,4}, Romeiro NC⁴, Silva LL^{1 1}UFRJ-Macaé, FarPBio, Brazil, ²UFF, LabCSI, Brazil, ³Fiocruz/RJ, LAPSA, Brazil, ⁴UFRJ-Macaé, PPG-ProdBio, Brazil

12.004 Neuroprotective Effect of Polymeric Resveratrol Nanoparticles in Parkinson's Disease. Fernandes MO¹, Ananias CF¹, Pinheiro KS¹, Duarte ALS¹, Araújo JR¹, Reis AVF¹, Teixeira IMM², Pinheiro NML², Duque BR², Magalhães EP², Costa MDR², Eloy JO², Martins AMC^{1,2}, Menezes RRPPB^{1,2}, Sampaio TL^{1,2} ¹UFC, Dept Clinical and Toxicological Analysis, Brazil; ²UFC, Post Graduate Program of Pharmaceutical Sciences, Brazil

12.005 Privileged Indole and N-Acylhydrazone Scaffolds in Multitarget Drug Discovery for Alzheimer's Therapy. Leite BF¹, Romeiro NC², Farias AB², Silva LL¹, Raimundo JM^{1,2} ¹UFRJ-Macaé, FarPBio, Brazil, ²UFRJ-Macaé, PPG-ProdBio, Brazil

12.006 Pharmacological Potential of Small Molecules from Marine Bacteria and Extremophiles: A Search for Antitrypanosomal Candidates. Albuquerque V¹, Lago JHG², Santos C³, Pimenta DC⁴, Tempone AG¹ ¹IB, Lab. of Pathophysiology, PPG Toxinology, Brazil, ²UFABC, Center of Natural Sciences and Humanities, Brazil, ³Universidad de La Frontera, Dept Chemical Science and Natural Resources, Chile, ⁴IB, Lab. of Ecology & Evolution, PPG Toxinology, Brazil

12.007 Development of a Poly(lactic-co-glycolic acid) Nanoparticle for the Delivery of Pradimicin-IRD for Colorectal Cancer Treatment. Gomes MAM, dos Anjos R, Costa-Lotufo LV, Lopes LB ICB-USP, Institute of Biomedical Sciences, Dept Pharmacology

14. Pharmacology: Other

Totem 19

14.001 Influence of Treatment Duration and Administration Routes on Therapeutic Approaches for Melasma: A Systematic Review and Meta-analysis. Dalagrana H¹, Ribeiro MM², Galiciolli MEA², Oliveira CL², Garlet QI^{1,3} ¹UFPR, Curso de Farmácia, Brasil, ²IPPPP, Brasil, ³UFPR, PPG Farmacologia, Brasil

14.002 Economic Evaluation of Pharmaceutical Care in Warfarin-Anticoagulated Patients. Oliveira IA, Vasconcelos PENS, Marcatto L, Santos PCJL Unifesp-EPM, Depto de Farmacologia, Brasil

14.003 Evaluation of the Incidence of Electrolyte Disturbances in Patients Undergoing Allogeneic Hematopoietic Cell Transplantation Treated With Calcineurin Inhibitors. Camargo MCF¹, Costa-Junior LC^{2,3}, Leão AML⁴, Fernandez TS⁴, Santos PCJL^{1,2} ¹Unifesp-EPM, São Paulo, PPG Farmacologia, Brasil, ²FMUSP, PPG Ciências Médicas ³INCa, Bone Marrow Transplant Center, Instituto Nacional de Câncer, Rio de Janeiro, RJ, Brasil, ⁴INCa, Cytogenetic Lab, Cell and Gene Therapy Program,, Rio de Janeiro, RJ, Brasil

14.004 Alternative Models to Murine Lethality Tests: Snake Venoms and Antivenoms. Luders SS¹, Silva GSA¹, Schiess MC¹, Hosch NG¹, Dallazen JL¹, Tasima LJ², Azevedo AMT², Zambelli VO¹ ¹IBu, Lab Pain and Signaling, PPGTox, Brazil, ²IBu, Lab Development and Innovation, PPGTox, Brazil

14.005 Fluorescence Microplate Reading for Evaluation of Calcium Influx in Mice Cortical Synaptosomes and Study of PEP2. Alvarenga CC, Pereira TL, Moraes TR, Castro CJ FSCBH, Programa de Pós-graduação Stricto sensu em Medicina e Biomedicina, Belo Horizonte, MG

Totem 26

14.006 Latex Agglutination Assay Conjugated with Dispersin: A New Diagnostic Approach for Enteroreggregative *E. coli*. Florencio C, Moraes CTP, Silva MA, Pollato JM, Piazza RMF, Elias WP IB, Bacteriology Lab, Graduate Program in Toxinology, Brazil

14.007 Characterization of Depression- and Anxiety-Like Behaviours in a Mouse Model of Relapsing-Remitting Multiple Sclerosis. Peres DS¹, Viero FT¹, Rodrigues P¹, Ruviaro NA², Machado

AC¹, Frare JM², Lima IR³, Martins G³, Silveira PCL³, Ferreira MA⁴, Silva AM⁴, Ferreira J⁴, Santos GT^{1,2},
¹UFMS, Graduate Program in Pharmacology; ²UFMS, Graduate Program in Biological Sciences:
Toxicological Biochemistry; ³Unesc, Graduate Program in Health Science; ⁴UFSC Graduate
Program in Pharmacology

14.008 Zinc-alpha2-Glycoprotein as a Predictive Biomarker and Therapeutic Target in Late-Onset Preeclampsia: Results from Proteomic Analyses. Bertozzi-Matheus M¹, Kaihara JSN¹, Chaguri JL², Santos LD³, Barbosa PO⁴, Cardoso VC⁵, Cavalli RC⁴, Sandrim VC¹. ¹IBB-Unesp-Botucatu, Dept Biophysics and Pharmacology, ²IBB-Unesp-Botucatu, Center of Toxicological Assistance, ³IBB-Unesp-Botucatu, ⁴FMRP-USP, Dept Gynecology and Obstetrics ⁵FMRP-USP, Dept Pediatrics, Brazil

14.009 Cinnamaldehyde Modulates 5-FU-Induced Mucositis in a Murine Model: Involvement of Cytokines and Inflammatory and Proliferative Markers Sousa IJO¹, Barbosa BS¹, Silva RLT¹, Pinheiro Neto FR¹, Nogueira KM², Gonçalves RLG¹, Reis ML¹, Oliveira FA¹, Oliveira RCM¹. ¹UFPI, PPG Farmacologia, Brasil; ²UFC, Brasil

14.010 A Systematic Review of Predictive Clinical and Imaging Factors in Pediatric Chronic Recurrent Multifocal Osteomyelitis Lemes FS¹, Maraia ME², Forte GV¹, Rodrigues WF^{1,3}, Miguel CB^{1,3}
¹Unifimes, Multidisciplinary Lab Scientific Evidence. University Center of Mineiros, Mineiros, GO, Brazil. ²UFTM Undergraduate Course in Biomedicine, Uberaba, MG, Brazil. ³UFTM, Postgraduate Course in Tropical Medicine and Infectious Diseases, Uberaba, MG, Brazil

01. Cellular and Molecular Pharmacology

Totem 01

01.014 Involvement of the Transient Receptor Potential Ankyrin 1 (TRPA1) in Oxidative and Inflammatory Damage Induced By AOPPs In Microglial Cells. Giovelli M¹, Brum GF², Lovis EP², Montagner TRS³, Machado AK³, Bochi GV^{1,2} ¹UFMS, Dept. of Physiology and Pharmacology, Brazil, ²UFMS, PPG Pharmacology, Brazil, ³Franciscan University, PPG Nanosciences, Brazil

01.015 Evidence of CD39 Upregulation in Mesenteric Microvasculature Following SARS-CoV-2 Infection in a Murine Model. Monteiro MMLV¹, Dutra LS², Carvalho-Barbosa NC², Savio LEB², Coutinho-Silva R², Silva CLM¹ ¹ICB-UFRJ, Biochemical and Molecular Pharmacology Lab., Institute of Biomedical Sciences, Brazil; ²UFRJ, Immunophysiology Lab., Institute of Biophysics Carlos Chagas Filho, Brazil

01.016 BAG2 Favors Metformin-Induced Reduction in Metabolic Viability in H4 Neuroglioma Cells: Implications for TAU Pathology in Alzheimer's Disease. Duarte GZ, Souza ACS, Carrettiero DC, Almeida MC UFABC, Centro de Ciências Naturais e Humanas, PPG Biossistemas, Brazil

01.017 Effect of Dietary Supplementation with Chia Oil on Adipose Tissue Remodeling Induced by Caloric Restriction in Obesity. Pontes-Castro G, Oliveira-Malafaia T, Muniz-Cassuce G, Barja-Fidalgo C, Vargas-Silva S UERJ, Lab Farmacologia Celular e Molecular, Rio de Janeiro/RJ, Brasil

01.018 The Role of Ric-8b Protein in Energy Homeostasis of Brown Adipose Tissue. Pires CVB, Alves JM, Leiria LOS FMRP-USP, Ribeirão Preto, São Paulo, Brazil

Totem 26

01.019 Heme induces oxidative stress and inflammation in gastric epithelial cells via NADPH oxidase activation. Souza MJ¹, Barcellos-de-Souza P¹, Berger M², Oliveira ACP³, Nicolau LAD³, Figueiredo-Júnior AT⁶, Guimarães JA², Santos CAN⁴, Nasciutti LE⁴, Arruda MA⁵, Moraes JA⁶, Barja-Fidalgo TC¹ ¹UERJ, Lab Cellular and Molecular Pharmacology, Brazil. ²UFRGS, Experimental Research Center, Hospital de Clínicas de Porto Alegre, Brazil. ³UFDFPar, Translational Inflammation and Gastroenterology Lab, Brazil. ⁴UFRJ, Institute of Biomedical Sciences, Brazil. ⁵CNPEM, National Lab Biosciences, Brazil. ⁶UFRJ, REDOX Biology Lab, Brazil

01.020 P2Y6 Receptor Downregulation in Endothelial Cells as a Putative Mechanism for Reduced Intestinal Inflammation in Schistosomiasis. Oliveira NF, Savio LEB, Silva RC, Silva UFRJ, Lab. Farmacologia Bioquímica e Molecular, Instituto de Ciências Biomédicas, Brazil

01.021 Caveolin-1 Knockdown Disrupts P2Y2/P2X7 Receptors Crosstalk in Schistosomiasis-Primed Mesenteric Endothelial Cells. Oliveira NF, Mainieri NS, Silva CLM UFRJ, Lab. Farmacologia Bioquímica e Molecular, Instituto de Ciências Biomédicas, Brazil

01.022 Multidrug Resistance Proteins Regulate Intracellular Cyclic Nucleotide Levels in Prostate Tissue and Cell Lines. Passos GR¹, O'Brien S², D'Ancona CA³, Fregonesi, A³, Antunes E¹, Calebiro D², Monica FZ¹ ¹Unicamp, Dept Pharmacology, Faculty of Medical Sciences,, Brazil ²University of Birmingham, Institute of Metabolism and Systems Research, College of Medical and Dental Science, United Kingdom ³Unicamp, Dept Surgery (Urology), Faculty of Medical Sciences, Brazil

01.023 **Caveolin-1 Modulates Monocyte Adhesion Mediated by P2X7 Receptor Activation in Endothelial Cells During Schistosomiasis.** Mainieri NS, Oliveira NF, Silva CLM ICB-UFRJ Biochemical and Molecular Pharmacology Lab., Brazil

01.024 **Plasmatic C3a is Reduced While Cytokines and Matrix Metalloproteinase (MMP)-2 Are Increased in Early-onset Pre-eclampsia** Mello MMB¹, Ramos LVR¹, Palei AC², Santos JETD¹, Cavalli RC³, Sandrim V⁴, Mazzaron M¹ ¹FMRP-USP, Dept Pharmacolgy, Brazil, ²University of Mississippi Medical Center, School of Medicine, Dept Surgery, USA, ³FMRP-USP, Dept Gynecology and Obstetrics, Brazil, ⁴IBB-Unesp, Dept Biophysics and Pharmacology, Brazil.

01.025 **Identification of Intracellular Peptides Present in Adipose Tissues and their Roles in Thermogenic Function.** Reckziegel P^{1,2}, Jardim GFR¹, Valdivia LFG², Souza E¹, Moreira RJ¹, Freire PP¹ ¹FCF-USP, Dept Clinical and Toxicological Analyses, Brazil ²Unifesp, Graduate Program in Pharmacology, Brazil

02. Neuropharmacology

Totem 02

02.042 **Unveiling Dexamethasone's impact on adolescent male and female rat neurodevelopment** Sbardellotto GE, Kamidai N, Munhoz CD ICB-USP, Dept Pharmacology

02.043 **Neurobiological Alterations Induced by Fipronil in *Nauphoeta cinerea* Cockroaches.** Silva AB¹, Souza ABG¹, Araujo SOM¹, Rosa ME¹, Oliveira IN², Talone SC², Hyslop S², Vinadé L¹, Dal Belo CA^{1,2,3} ¹Unipampa-São Gabriel, ²Unicamp, ³Unifesp-Osasco

02.044 **Study of the Effects of the Exposure to Uremic Serum on Healthy Astrocytes.** Custodio GT, de Sá LL, Andreotti DZ, Lima TAS, Kawamoto EM. ICB-USP, Dept Pharmacology

02.045 **Adolescent Exposure to a Synthetic Cannabinoid Affects the late Rewarding Response to the Same Drug?** Moutinho IC, Corrêa EAS, Lemos LC, Freire LC, Souza INO, Neves GA UFRJ, Lab Molecular Pharmacology, Brazil

Totem 10

02.033 **Cannabidiol Attenuates Neurotoxic Effects of Anhydroecgonine Methyl Ester (AEME), a Crack Pyrolysis Product, in Hippocampal Astrocytes: Role of CB1 and TRPV1 Receptors** Patrocínio TB, Silva SC, Leitão SGR Unifal, PPG Biotecnologias aplicadas à Saúde, Brasil

02.046 **Preliminary Assessment of Mice Impulsive Behavior in the Delay Discounting Task.** Scafi H¹, Kondo T², Gogola J², Dalagrana H², Rosa E³, Pradella G³, Garlet QI^{1,2,3}. ¹UFPR, Graduate Program in Pharmacology, Brazil. ²UFPR, Course of Pharmacy, Brazil. ³UFPR, Biomedical Science Course, Brazil.

02.047 **Differential Effects of Probiotic and Paraprobiotic *Lactobacillus casei* on Intestinal ROS in a Rat Model of Alzheimer's Disease.** Severo ER, Gomes J, Meus SS, Riella D, Comis AA, Rosa, SG, Pinton S Unipampa

02.048 **Neonatal Exposure to Kynurenine: Behavioral Changes Related to Schizophrenia.** Bomfim ANL, Cruz BE, Faria ACC, Nunes LED, Andrade BS, Guimarães MZP, Neves GA UFRJ, Lab Molecular Pharmacology, Brazil

02.049 **Modulating Effects of Acai Extract on Neuroinflammation after Ischemic Stroke: Evaluation of Astrocytic Reactivity.** Teixeira LL^{1,2}; Ferreira LO²; Rodrigues JCM; Pinto LC²; Rogez HLG^{1,3}; Lopes DCF^{1,2} ¹ICB-UFGA, Graduate Program in Pharmacology and Biochemistry, ²UFGA, Lab Experimental

Totem 11

02.050 The Role of Cingulate Cortex Orexin-1 Receptors in the Expression and Organization of Innate Fear Carvalho MLC¹, Oliveira GVM¹, Hernandez PM², Coimbra NC², Almada RC¹ ¹FCLAS-UNESP-Assis, Dept Biological Sciences, Lab Neurobiology and Neurobiotechnology, ²FMRP-USP, Ribeirão Preto, Dept Pharmacology, Lab Neuroanatomy and Neuropsychobiology, Postgraduate Program in Medicine (Neurology), Brazil

02.051 *In Vitro* Effects of Exposure to Anhydroecgonine Methyl Ester (Byproduct of Crack/Cocaine) on the Viability, Morphology, and Activity of Hippocampal Astrocytes. Silva SC, Santos SO, Patrocínio TB, Leitão SGR, UNIFAL, Programa de PósGraduação em Biociências Aplicadas à Saúde, Brasil

02.052 Early Exposure to a Synthetic Cannabinoid Effects on Anxiety and Depressive-Related Behaviors. Freire LC, Moutinho IE, Côrrea AES, Lemos LC, Souza IO, Neves GA UFRJ, Lab Molecular Pharmacology, Brazil

02.053 Effects of the Prebiotic Inulin on Behavioral Changes in a Model of Autism Spectrum Disorder (ASD) In Adolescent Rats. Amorin BM¹, Bertotti G², Haddad NM², Longo B¹, Silva LM¹ ¹LAFATI-UFSC, Lab Gastrointestinal Tract Pharmacology and Interactions, Brazil ²Univali, Medical School, Brazil

Totem 18

02.054 *Baccharis dracunculifolia* Extract Induces Anxiolytic-Like Behavior and Enhances Brain Antioxidant Activity in Male Rats. Souza RML¹, Batista MAC², Silva AO¹, Moura SAL³, Cardoso LM², Oliveira LB¹, Ceron CS² ¹UNIFAL, Dept Food and Medicine, PPG Pharmaceutical Sciences, Brazil, ²UFOP, Research Center in Biological Sciences, PPG Cipharm, Brazil, ³UFOP, Dept Biological Sciences, PPG Cipharm, Brazil

02.055 Effects of Maternal Melatonin Deficiency on Klotho Protein Expression in the Cerebellum of Male and Female Rat Offspring. Silva, JRM¹, Aliaga ALB², Lima PH², Tida-Oliveira CH², Andreotti, DZ¹, Sandoval, MRL², Cipolla-Neto J³, Amaral FG⁴, Abdalla FMF², Afeche SC², Kawamoto, EM¹ ¹ICB-USP, Lab Molecular and Functional Neurobiology; ²IB, Lab Pharmacology; ³ICB-USP, Pineal Gland Neurobiology Lab; ⁴Unifesp, Dept Physiology

02.056 Activation of GPER Promotes Functional Recovery and Modulates Neuronal Degeneration Following Transient Focal Cerebral Ischemia in Male Rats. Jucá PM1 Munhoz CD LaNEFI-ICB-USP, Neuroendocrinepharmacology and immunomodulation Lab, Dpt of Pharmacology

02.057 Antioxidant and Anticholinesterase Activities of a Semisynthetic Derivative of (–)-Myrtenol as a Potential Therapeutic in Alzheimer’s Disease. Souza RP¹, Freitas MM², Fernandes GS², Oliveira MYCM¹, Pimentel VD³, Nobre TA⁴, Nascimento MLLB⁴, Furtado PVOS⁵, Feitosa CM⁵, Castro e Sousa JM⁴, Viana AFSC⁶, Kutyla M⁷, Trytek M⁷, Ferreira PMP², Oliveira AP¹ ¹UFPI, Lab Cardiovascular Pharmacology (Lafac), Brazil; ²UFPI, Lab Experimental Cancerology (LabCancer), Brazil; ³UFPI, Lab Inflammation Pharmacology (LAFIN), Brazil; ⁴UFPI, Lab Toxicological Genetics (Lapgenic), Brazil; ⁵UFPI, Lab Natural Products and Experimental Neurochemistry (Lpnex), Brazil; ⁶UEMA, Campus Santa Inês, Brazil; ⁷Maria Curie-Skłodowska University, Lublin, Poland

03. Psychopharmacology

Totem 03

03.012 **Effects of Subchronic Treatment and Withdrawal of Cannabigerol on Anxiety-like Behaviors Related to Acute Stress in Female Wistar Rats.** Hirabayashi MH, Storgatto FJ, Zanolini JM UFPR, Dept Pharmacology, Brazil

03.013 **Neuronal Activity of the Periaqueductal Gray in Response to Reward-Associated Cues: An Investigative Analysis.** Gouvea IN, Costa BY, Camarini R ICB-USP, Dept Pharmacology, Lab Neurochemistry and Behavioral Pharmacology, Brazil

03.014 **Investigation of the Involvement of Cannabinoid Receptors CB1 and CB2 in the Mediation of Resolvin D5 Effects on Anxiety-Like Responses: A Comparative Study Between Sexes.** Avanço LC, Ueda TS, Zanolini JM UFPR, Dept Pharmacology

03.016 **Effects of TNFR1 Deletion on the Behavior of Female Mice.** Neves MCV, Lima TAS, Kawamoto, EM ICB-USP, Lab Molecular and Functional Neurobiology Dept Pharmacology

03.017 **Effects of Muscimol Administration in the Dorsal Hippocampus of Wistar Rats on the Strength and Specificity of Contextual Memory During its Recall.** Castro JC, Quintiliano dos Santos AC, Vido Lopes L, Glória VTL, Boscariol Lourenço WT, Fornari RV UFABC, Centro de Matemática, Computação e Cognição

03.018 **Z-Score Standardization for the Characterization of Resilient and Non-Resilient Female Mice in a Chronic Restraint Stress Model.** Pereira GJS^{2,3}, Schneider GK³, Lovis EP^{3,4}, Zanchet EM^{1,3,4}, Bochi GV^{1,3,4}. ¹UFSM, Dept Physiology and Pharmacology, Brazil, ²UFSM, Faculty of Pharmacy, Brazil, ³UFSM, Lab. of The Study of Depression – LEDep, Brazil, ⁴UFSM, PPG Pharmacology, Brazil

03.031 **Efeito da Bufotenina em Modelo de Depressão em Ratos.** Oliveira PS, Queiroz KS, Paulo MEFV, Gonçalves TCP, Nencioni ALA Fundação Butantan

04. Inflammation and Immunopharmacology

Totem 04

04.031 **Evaluation of Different Estradiol Treatment Strategies in Lung Inflammation of Brain Death Donors After Lung Transplantation.** Wellichan G¹, Sousa PS, Vidal-dos-Santos M, Correia CJ¹, Breithaupt-Faloppa AC, Moreira LFP, Ricardo-da-Silva FY InCor-FM-USP Lab Cirurgia Cardiovascular e Fisiopatologia da Circulação, São Paulo, Brasil

04.032 **Methyl Gallate as a Modulator of Pro-Inflammatory Macrophage.** Rodrigues CS^{1,2}; Correa LB¹, Seito LN¹, Rosas EC¹ ¹FIOCRUZ, Lab Applied Pharmacology, Farmanguinhos, Rio de Janeiro, RJ, Brazil. ²IFRJ, Biological Sciences, Rio de Janeiro, RJ, Brazil

04.033 **Effectiveness of Nonsteroidal Anti-Inflammatory Drugs as First-Line Therapy in Pediatric Chronic Nonbacterial Osteomyelitis: A Systematic Review of Multicenter Cohorts.** Forte GV¹, Maraia ME^{2,3}, Lemes FS¹, Miguel CB^{1,3}, Rodrigues WF^{1,3} ¹Unifimes, Multidisciplinary Lab Scientific Evidence, Mineiros, GO, Brazil. ²UFTM, Biomedicine, Uberaba, MG, Brazil. ³UFTM, Postgraduate Course in Tropical Medicine and Infectious Diseases, Uberaba, MG, Brazil

04.034 **Effect of Gedunin on the Polarization of Differentiated Human Macrophages.** Nascimento JAD^{1,3}, Guedes LS², Rosas EC³, Penido C^{3,4} Costa TEMM^{3,4} ¹UFRJ, Faculdade de Farmácia, Brazil, ²UERJ, Faculdade de Farmácia, Brazil, ³Farmanguinhos-Fiocruz, Lab Farmacologia Aplicada, Brazil, ⁴CDTS-Fiocruz, Brazil

Totem 12

04.035 **A Novel Sulfonylhydrazone Derivative of LASSBio-1632 as Potential Therapeutic for Inflammatory Pulmonary Diseases.** Ota RSN¹, Oliveira MS², Souza LM¹, Gomes HS¹, Ferreira TPT¹, Carvalho VF¹, Lima LM², Martins MA¹, Silva PMR¹, ¹Fiocruz-IOC, Lab of Inflammation, Rio de Janeiro, Brazil, ²UFRJ, LASSBio, Rio de Janeiro, Brazil

04.036 **Melatonin Attenuates Vascular Hyporeactivity Induced by TLR4 and P2X7 Receptor Coactivation.** Brandão BJ, Silva CLM UFRJ, Lab. of Biochemical and Molecular Pharmacology, Brazil

04.037 **Sexual Dimorphism in a Model of Systemic Inflammation of Viral Origin.** Schneider VMM, Costa RA, Borges AP, Alencar ST, Santos MO, Zamprônio AR UFPR, Inflammation and Fever Lab, Dept Pharmacology

04.038 **Bornyl Acetate Enhances Phagocytosis and M2-Associated Gene Expression in Macrophages.** Silva GRS, Barros ABB, Ferreira EGA, Fidelix MSP, Santana JS, SilvaJP, Reis MDS Barreto E UFAL, Lab Biologia Celular, UFAL, Brasil

04.039 **Neutrophils Polarization as a New Approach for Cancer Treatment.** Amorim NS¹, Amorim CS¹, Valença LS¹, Almeida PP², Moraes J¹ ¹UFRJ, Lab Biologia Redox, RJ; ²UERJ, Depto de Biologia Celular

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04.040 **Estrogen Modulation of Febrile Response via GPER Receptors in Systemic Inflammation in rats.** Costa RA, Santos MO, Alencar ST, Schneider VMM, Borges AP, Zamprônio AR. UFPR, Inflammation and Fever Lab, Departament of Pharmacology

04.041 **Zika Virus (ZIKV) and Dengue Virus (DENV) NS1 Proteins Impair Blood-Epididymis Barrier Integrity in Mice.** Camargo IA¹, Martini PV¹, Andrade AD¹, Kushima H¹, Amarilla AA², Modhiran N^{3,4}, Watterson D^{3,4}, Costa SM⁵, Alves AMB⁵, Silva, EJR¹ ¹UNESP, Institute of Biosciences of Botucatu, PPG Biomolecular and Pharmacological Sciences, Brazil; ²Commonwealth Scientific and Industrial Research Organization, Australian Centre for Disease Preparedness, Australia. ³University of Queensland, School of Chemistry and Molecular Biosciences, Australia; ⁴University of Queensland, Australian Infectious Diseases Research Centre, Australia; ⁵Fiocruz, PPG Cellular and Molecular Biology, Brazil

04.042 **Modulatory Effects of Cholecalciferol on Inflammatory Cytokines and Autophagic Flux Proteins in RAW 264.7 Cells Stimulated with Lipopolysaccharide.** Freire ALP, Bella LM, Migliorini S, Martins JO FCF-USP, Dept. of Clinical and Toxicological Analyses, Lab. of Immunoendocrinology, Brazil

04.043 **Effects of *Tityus paraguayensis* and *Tityus confluens* venoms on the *in vitro* macrophages cell response.** Fernandes MML¹, Bonfá IS¹, Lencina JS¹, Lossavaro PKMB¹, Lencina DS¹, Toffoli-Kadri MC¹, Lucena MN², Silva-Filho SE¹ ¹UFMS, Pharmaceutical Sciences, Food and Nutrition College, Brazil, ²UFMS, Biosciences Institute, Brazil

04.044 **Evaluation of the Antimicrobial Effect of Anacardic Acid on Multidrug-Resistant *Staphylococcus epidermidis* and its Immunomodulatory Action on Macrophages.** Lopes EC, Honorio MS, Almeida JFS, Sforcin JM. UNESP, Institute of Biosciences, Brazil

04.045 **Targeting PAR-1 to Attenuate Lung Fibrosis Induced by Silica Particles.** Souza LM¹, Ferreira TP¹, Ferreira GG¹, Pezzella-Ferreira GN¹, Villar LS¹, Cotias AC¹, Arantes AC¹, Lagente V², Martins

MA¹, Silva PMR¹ ¹Lab Inflammation, Oswaldo Cruz Institute/FIOCRUZ, Rio de Janeiro, Brazil; ²University of Rennes I, Faculty of Pharmacy, Rennes, France

04.046 Effects of Intestinal Ischemia-Reperfusion (I/R) in Mice with Cholinergic Deficiency. Queiroz VC¹, Silva CN¹, Lyoko CS², Silva AF¹, Freitas PLZ¹, Almeida FM¹, Tibério IFLC¹, Soriano FG¹, Barbeiro DF¹, Breithaupt-Faloppa AC¹, Prado CM² ¹FMUSP, School of Medicine, Brazil, ²Unifesp, Dept Bioscience, Brazil

05. Pain and Nociception Pharmacology

Totem 05

05.021 Search for Therapeutic Targets against Multiple Sclerosis Through Global Gene Expression Analysis: Use of the Crotoxin:SBA-15 Complex and Genetically Susceptible Mice in the Experimental Autoimmune Encephalomyelitis Model. Moraes JG¹, Corcino LFC¹, Sant'Anna MB¹, Lopes FR¹, Boaventura de Oliveira AM¹, Borrego A², De Franco, M³, Sant'Anna OAE⁴, Picolo G¹ ¹IB, Lab Pain and Signaling; ²IB, Development and Innovation Lab; ³IB, Diagnostic Section, Pasteur Institute; ⁴IB, Immunochemistry Lab

05.022 Targeting Neuronal Nitric Oxide Synthase to Reduce Migraine-Like Nociception Demyelination, and Anxiety-Like Behavior in a Mouse Model of Multiple Sclerosis. Fim YV¹, Da Silva B¹, Viero FT², Antoniazzi CTD¹, Kudsi SQ¹, Peres DS¹, Morais RIF¹, Pereira LG¹, Trevisan G¹ ¹UFSM, Graduate Program in Pharmacology, Federal University of Santa Maria, Santa Maria, RS, Brazil. ²IQ-USP, Neuroscience Lab, Dept Biochemistry, São Paulo, SP, Brazil

05.023 Flavonoid Diosmetin Reduces Paclitaxel-Induced Nociceptive Symptoms in Acute and Chronic Pain Models in Rodents. Dias R¹, Atuati SF^{1,2}, Becker G^{1,2}, Oliveira SM^{1,2} ¹UFSM, Pain Neurobiology Lab, ²UFSM, Graduate Program in Biological Sciences: Toxicological Biochemistry, Brazil

05.024 Antinociceptive Effect of Cannabigerol in Experimental Diabetic Neuropathic Pain Depends on CB1 and CB2 Receptors. Rampim LR¹, Ferreira MV¹, Miranda JM¹, Rauchbach L¹, Wolaniuk L¹, Demeu TA¹, Crippa JA², Zanolli JM¹, Cunha JM¹ ¹UFPR, Dept Pharmacology, Curitiba, PR, Brazil. ²FMRP-USP, Ribeirão Preto, SP, Brazil

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05.025 Evaluation of the Antinociceptive Potential of Neryl Acetate in Neuropathic Pain Model- Investigation of Its Molecular Targets. Gomes LS¹, Martins RYC¹, Dos Reis ACF¹, Castro JLS¹, Barbosa BS¹, Santos ALS¹, Ferreira MKA², Menezes JES², Santos HS², Almeida FRC¹. ¹UFPI, Graduate Program in Pharmacology, Teresina-PI, Brazil; ²UECE, Graduate Program in Natural Sciences, Fortaleza-CE, Brazil

05.026 Diosmetin Reduces Nociception Caused by Antineoplastic Anastrozole in a Preclinical Study. Favarin A, Atuati SF, Becker G, Oliveira SM UFSM, Graduate Program in Biological Sciences: Toxicological Biochemistry, Centre of Natural and Exact Sciences, Brazil

05.027 Aldehyde Dehydrogenase-2 Plays a Key Role in the Development of Alcoholic Neuropathy. Silva JCB, Souza DF, Alcantara QA, Hosch NG, Martins BB, Olzon V IB, Pain and Signaling Lab (LDS), Butantan Institute, Brazil

05.028 Influence of Calcitonin Gene-Related Peptide on Cutaneous Healing and Nociception: Insights from CGRP-Knockout and Wild-Type Mice. Jamba MF¹, Tavares YPST¹, Teixeira SA¹, Brain

SD², Muscará MN¹, Costa SKP¹ ¹ICB-USP Depto de Farmacologia; ²Kings College, School of Cardiovascular Medicine & Sciences, Waterloo Campus, London

Totem 20

05.029 **Investigation of the Antinociceptive Potential of Geranyl Acetate in an Animal Model of Non-Inflammatory Diffuse Pain.** Pereira SAP¹, Martins RYC¹, Castro JLS¹, Ferraz SLNS¹, Martins DFA¹, Acha BT¹, Sousa DP², Oliveira, FA¹, MLS¹, Almeida, FRC¹ ¹UFPI, Lab Pain Pharmacology, Medicinal Plants Research Center ²UFPB, Dept Pharmaceutical Sciences, Health Sciences Center

05.030 **Standardization of a Bilateral 6-OHDA Mouse Model for Nociceptive Assessment in Parkinson's Disease and Evaluation of Pregabalin as a Therapeutic Strategy.** Pereira LG¹ Brito HA¹ Moraes RIF¹ Luiz. ACM¹ Peres DS¹ Kudsi SQ¹ Viero FT² Bressan GN¹ Antoniazzi CTD¹ Fachinetto R¹ Trevisan G¹ ¹UFSM, PPG Pharmacology, Brazil. ²IQ-USP, Brazil

05.031 **Joint Pain Modulation by *Hibiscus sabdariffa*: Functional Evaluation in a Monoiodoacetate-Induced Osteoarthritis Model.** Cruz ACSN, Mesquita MJTAM, Reis ATF, Correia LCP, Carvalho TGPM, Frota GM, Lima RF, Sousa LR, Garcia JBS, Carvalho RC, Andrade MS, Cartagenes MSS UFMA, Experimental Lab for Pain Studies, Brazil

05.032 **Targeting Drp1, a Key Regulator of Mitochondrial Fission, to Mitigate Paclitaxel-Induced Neurotoxicity.** Schiess MC¹, Troitino VC¹, Souza MM² deOcesano-Pereira C² Bufalo MC² ChudzinskiTavassi AM² Zambelli VO^{1,2} ¹IB, Lab Pain and Intracellular Signalization, Brazil, ²IB, Centre of Excellence in New Target Discovery, Brazil

06. Cardiovascular and Renal Pharmacology

Totem 14

06.023 **Metformin-Mediated Cardiac and Antitumor Effects in Doxorubicin-Treated Mice with Triple-Negative Breast Cancer.** Cavalcante KDM^{1,2}, Cavalcante FAS¹, Ferreira LPF^{1,2}, Lago JPAD³, Nagai MA⁴, Mangone FR⁴, Pavanelli AC⁴, Silva MTB⁵, Leal FLT⁶, Sousa JMC³, Silva VC⁷, Ferreira PMP ², Amaral DT8, Dittz D^{1,2} ¹UFPI, Antineoplastic Pharmacology Lab, Brazil; ²UFPI, PPG Pharmacology, Brazil; ³UFPI, PPG Pharmaceutical Sciences, Brazil; ⁴FMUSP-ICESP, Molecular Genetics Lab, Brazil; ⁵University of Porto, Institute of Biomedical Sciences Abel Salazar, Portugal; ⁶UFPI, Dept Biophysics and Physiology, Brazil; ⁷Fiocruz, Campus Piauí, Brazil; ⁸UFABC, Center for Natural and Human Sciences, Brazil

06.024 **Hypertension Impairs Tissue Accumulation of Nitric Oxide Metabolites after Oral Nitrite Administration.** Lima-Silva AK¹, Vitorino TR¹, Rebelo MA¹, Conde-Tella SO², Tanus-Santos JE² ¹FCM-Unicamp, PPG Pharmacology, Brazil. ²FMRP-USP, Lab. Cardiovascular Pharmacology, Brazil

06.025 **Supplementation with *Arthrospira platensis* Prevents Arterial Blood Pressure Increase in Asthmatic Rats.** Oliveira GNM¹, Vieira MM¹, Oliveira TA², Cavalcanti CO³, Cruz JC^{2,3}, Vasconcelos LHC^{1,4} ¹UFPB, Programa de Pós-graduação em Produtos Naturais e Sintéticos Bioativos, Brazil; ²UFPB, Programa Multicêntrico de Pós-graduação em Ciências Fisiológicas, Brazil; ³UFPB, Depto de Biotecnologia, Brazil; ⁴UFPB, Depto de Ciências Biomédicas, Brazil

06.026 **Effect of High Salt and Ouabain on Renal Proximal Tubular Cells *In Vitro*.** Borges GT, Quintas LEM ICB-UFRJ

Totem 21

06.027 **Renal Impact of Subacute Exposure to Endocrine Disruptors in Female Rats: Evidence of Preserved Function.** Esteves GAB^{1,2}, Buhler GV, Accioli DSG^{1,2}, Alves LM¹, Morcillo LSL^{1,2} ¹ICB-UFRJ, ²IBCCF-UFRJ, Centro de Pesquisa em Medicina de Precisão

06.028 Chronic Lead and Fluoride Exposure During Early Life Reduces Circulating and Cardiac Nitric Oxide Metabolites in Rats. Conde-Tella SO^{1,2}, Figueiredo JT³, Ribeiro NM³, Rebelo MA¹, Gerlach RF³, Tanus-Santos JE^{1,2} ¹Unicamp, Faculty of Medical Sciences, Dept Translational Medicine, PPG Pharmacology, Brazil ²FMRP-USP, Dept Pharmacology, PPG Pharmacology, Brazil ³FORP-USP, Dept Basic and Oral Biology, Brazil

06.029 Resistance versus Conduit Artery Endothelial Cells: Divergent Roles in T Cell Activation. Santos CVD³, Fontes MT¹, Costa TJ³, Priviero F¹, Carneiro FS³, Webb RC¹, Wenceslau CF¹, Bomfim GF² ¹University of South Carolina, Cardiovascular Translational Research Center, Dept Cell Biology and Anatomy, Columbia. ²UFMT, Institute of Health, Brazil ³. University of Sao Paulo - Brazil

06.031 Sodium Nitrite Lowers sFlt-1 Levels, Increases NO Production and Endothelial Cell Migration in an *in vitro* Model of Preeclampsia in Hypoxic Conditions. Zampieri GM, Kaihara JNS, Bueno-Pereira TO, Pasetti LF, Sandrim VC IBB-Unesp-Botucatu, Dept Biophysics and Pharmacology, PPG in Biomolecular and Pharmacological Sciences, Brazil

07. Endocrine, Reproductive and Urinary Pharmacology

Totem 15

07.010 Probenecid Potentiates the Relaxant Effect of β -Adrenergic Agonists in the Prostate of Rats. Moretti MB, Leonardi GR, Barros JVC, Lima JM, Monica FZ Unicamp, PPG Pharmacology, Brazil

07.011 Intermittent Glucocorticoid Exposure Programs Dexamethasone Resistance and Alters Adult Metabolic Outcomes. Peixe CMS¹, Giusti-Paiva A², Rafacho A² ¹UFSC, PPG em Farmacologia, Brazil; ²UFSC, Dept. of Physiological Sciences, Brazil

07.012 Acute Activation of the MGO-AGE-RAGE Axis Promotes Bladder Hypercontractility. Horioka CP, Oliveira AL, Medeiros ML, Antunes E FCM-Unicamp, Dept Translational Medicine – Pharmacology area

07.013 Progression of Lower Urinary Tract Symptoms in the Face of Obesity: Interaction between H₂S and Cyclic Guanosine Monophosphate. Souza ALC¹, Gonzaga L³, Alves MCO¹, Teixeira SA¹, Antunes E², Muscara MN¹, Costa SKP¹ ¹ICB-USP, Dept. of Pharmacology, São Paulo, Brazil. ²FCM-Unicamp, Dept. of Translational Medicine, São Paulo, Brazil. ³IB

08. Respiratory and Gastrointestinal Pharmacology

Totem 22

08.010 Sex-Specific Differences in Gastric Ulcer Healing in Normotensive and Spontaneously Hypertensive Rats: Preliminary Findings. Rizzon-Santos M, Willrich CH, Fontoura LB, Cury BJ, Longo B, Moura, SAL, De Oliveira IM, Casimiro LM, De Amorim GES, Prediger RD, Da Silva-Santos JE, Da Silva LM. UFSC, Dept Pharmacology, Florianópolis

08.011 Lauric Acid Modulates Nitric Oxide and Cyclooxygenase Pathways to Prevent Tracheal Hyperresponsiveness in Asthmatic Wistar Rats. Oliveira LN¹, Figueiredo IAD¹, Martins AMO¹, Felício IM¹, Vasconcelos LHC^{1,2}, Cavalcante FA^{1,2} ¹PPgPNSB/CCS/UFPB; ²DCB/CCS/UFPB

08.012 *In silico*, *in vitro* and *in vivo* Approach for Evaluation of the Ethyl Acetate Fraction of *Platonia insignis* Mart. in a Model of Ovalbumin-Induced Asthma. Souza ORB¹, Oliveira MM¹, Barbosa BS¹, Nascimento ER², Moreira RA², Freitas SDL², Oliveira AP¹, Oliveira RCM¹, Santos, RF¹ ¹UFPI, PPG Pharmacology, Brazil. ²UFPI, Lab. Natural Products, Dept Chemistry, Brazil

08.013 **Modulation of Pulmonary Inflammation and the Redox Pathway *in vitro* and *in vivo* by Fumaric Esters.** Mineiro PCO¹, Cardoso AOP², Figueiredo-Júnior ATF¹, Mota EC¹, Amorim CS¹, Valença HM¹, Gomes ACCA³, Serra SSS³, Silva PL³, Takiya CM³, Lima LM², Moraes JA², Valença SS², Deus MLD² ¹ICB-UFRJ, ²UFRJ, Postgraduate Program in Immunology and Inflammation, Federal University of Rio de Janeiro, ³IBCCF-UFRJ

08.014 **Lauric Acid Reduces Oxidative Stress to Prevents Tracheal Hyperresponsiveness in Asthmatic Wistar Rats.** Felício IM¹, Figueiredo IAD¹, Martins AMO¹, Oliveira GNM¹, Oliveira LN¹, Alves BFL², Golzio AMFO^{1,3}, Vasconcelos LHC^{1,4}, Cavalcante FA^{1,4} ¹PPgPNSB/CCS/UFPB, ²PIBIC/UFPB, ³DTA/CTDR/UFPB, ⁴DCB/CCS/UFPB

09. Natural Products and Toxinology

Totem 06

09.031 **Effect of Galactomannan and Silver Nanoparticles-Based Wound Dressing on the Cutaneous Wound Healing Assessed by Raman Spectroscopy.** Silva EC¹, Oliveira JNSR², Silva FA¹, Lucena LCP¹, Nunes DM³, Correia ACC³, Albuquerque PBS³, Lima LRA³, Rodrigues NER³, Souza ST², Ferro JNS¹ ¹UFAL, Lab Biologia Celular, ²UFAL, Grupo de Óptica e Nanoscopia, ³UPE, Lab Bioprospecção e Etnofarmacotoxicologia Aplicada e de Biologia Celular

09.032 **Anti-Obesogenic and Anti-Hyperglycemic Effect of Citral: Preventive Effect Against the Development of Metabolic Endotoxemia in Obese C57BL/6J Mice Challenged with Lipopolysaccharide.** Fioravanti MM, Emílio-Silva MT, Assunção R, Dario FL, Trinas JG, Guidolin IG, Hiruma-Lima CA Unesp- Botucatu

09.033 **Coagulotoxicity *in vitro* of Two South American Snake (Viperidae: Crotalinae) Venoms and Inhibition by Marimastat and Commercial Antivenoms.** Gonçalves de Lima IG¹, Proença-Hirata VS¹, Souza-Gomes GC¹, Demico PJ¹, Giuffrida R¹, Silva NJ², Torres-Bonilla KA³, Hyslop S³, Sant'Anna SS⁴, Morais-Zani K^{4,5}, Vaiyapuri S⁶, Floriano RS¹ ¹Unoeste, Lab. of Toxinology and Cardiovascular Research, Graduate Program in Health Sciences, Brazil, ²PUC-Goiás, School of Medical and Life Sciences, Brazil, ³Unicamp, Dept Pharmacology, Faculty of Medical Sciences, Brazil, ⁴IB, Lab. of Herpetology, Brazil, ⁵IB, Lab. of Pathophysiology, Brazil, ⁶University of Reading School of Pharmacy, Reading, United Kingdom

09.034 **Involvement of K⁺ Channels in the Tocolytic Action of (S)-8-formyl-3',5-Dihydroxy-7-Methoxy-6-Methylflavanone and 3'-Formyl-3,4',6'-Trihydroxy-2'-Methoxy-5'-Methylchalcone, a Flavonoid Mixture from *Piper montealegreanum* Yuncker (Piperaceae): *in vitro* and *in silico* Study.** Souza MLB¹, Oliveira LN², Martins AMO², Felício IM², Figueiredo IAD², Silva JLV³, Santos BVO⁴, Cavalcante FA^{2,5} ¹PIBIC/CNPq/UFPB; ²PPgPNSB/CCS/UFPB; ³FMO; ⁴UACEN/UFCG; ⁵DCB/CCS/UFPB

09.035 **Antioxidant and Anticholinesterase Effect of Guarana Seed Extract (*Paullinia cupana* Kunth).** Piristrello BS, Souto AF, Mendes FR UFABC

09.036 **The Anti-Inflammatory Activity of Extra Virgin Olive Oil in the Hypothalamus And Hippocampus of Rats Exposed to a High-Fat Diet.** Harttfel-Bajerski MH, Peres AM, Dantas RM, Ferreira AC, Silveira ACS, Machado DN, Souza LCP, Raphaelli GB, Lena MC, Netto CA, Dalmaiz C, Bast RKSS UFRGS Porto Alegre, Lab Pesquisa da Programação Neurobiológica do Comportamento Alimentar (PROCAL), PPG Ciências Biológicas: Bioquímica, Brazil

Totem 16

09.037 **Methyl Cinnamate Down-Regulates Epithelial Mesenchymal Transition Through Modulating NF- κ B/p65 Pathway in Alveolar Epithelial Cell.** Ferreira EGA, Barros ABB, Fidelix MSP, Silva GRS, Silva JP, Barreto E UFAL, Lab Biologia Celular

09.038 **Neuroprotective Effect of *Cannabis sativa* Oil-Rich in Delta-9-tetrahydrocannabinol (Δ 9-THC) *in vitro* Epilepsy Model Is Modulated by Ionotropic Glutamatergic Receptors.** Tavares BLC¹, Aquino DN², Andrade GLR³, Marreiro VS², Ramalho IGS², Nunes VRV², Cipriano MSFL^{1,2}, Diniz MFFM², Donato MF⁴ ¹UFPE, PPG Ciências Farmacêuticas, Brazil, ² UFPB, PPG em Produtos Naturais e Sintéticos Bioativos, Brazil, ³UFPB, Brazil, ⁴UNILA, PPG em Biociências, Brazil

09.039 **Methyl Cinnamate Attenuates TGF- β 1-Induced Fibrotic Responses in Fibroblast via Inhibition of the Smad3 Pathway.** Barros ABB, Ferreira EGA, Fidelix MSP, Silva GRS, Silva JP, Santana JR, Barreto E UFAL, Lab Cell Biology

09.040 ***Spondias tuberosa* Arruda Extract Attenuates Liver Damage in Streptozotocin-Induced Diabetic Animals by Modulating Oxidative Enzymes.** Silva CPC¹, Sena V¹, Pires LAB¹, Freitas L¹, Araujo RFA¹, Abreu BJA¹, Araujo IGA², Medeiros IA², Santos, DD³, Gil CD³ Medeiros KCP¹ ¹UFRN, Dept Morphology, Brazil, ²UFPB, Dept Health Sciences, Brazil. ³Unifesp-EPM, Dept Morphology and Genetics, SP, Brazil

09.041 ***Boswellia serrata* Dry Extract with Intestinal Anti-Inflammatory Properties Also Accelerates Gastric Ulcer Healing in Rats.** Oliveira IM¹, Serafim FGS², Breguez L², Venzon L², Santos AC², Longo B¹, Nunes RK¹, Gutiérrez MV³, Roman-Junior WA⁴, Silva LM¹ ¹UFSC, Center of Biological Sciences, PostGraduation Program of Pharmacology, Brazil ²Univali, Post-Graduation Program of Pharmaceutical Sciences, Brazil ³University of Sonora, Dept Chemistry, Biology and Agricultural Sciences, Mexico ⁴Unochapecó, Post-graduation Program of Health Sciences, Brazil

Totem 23

09.042 **Antivenom potential of *Jatropha mutabilis* (Pohl) Bail. (Euphorbiaceae) against *Bothrops leucurus* venom.** Veras BO, Souza FS, Ximenes RM UFPE, Lab. of Ethnopharmacology and Phytochemistry, Brazil

09.043 **Luteolin Ameliorates Valproic-Acid Autism-Like Behaviors in Rats through the Attenuation of Neuroinflammation, Oxidative Stress, and Neural Damage.** Longo B¹, Nunes RKS¹, De Siqueira MCB², Ferreira PC², Cazarin CA², De Queiroz e Silva TF², Dos Santos AC², Venzon L², Da Silva LM², Pagliochi AC², Sievers J², De Souza MM², Da Silva LM¹. ¹UFSC, Dept Pharmacology, ²Univali, Graduate Program in Pharmaceutical Sciences

09.044 **Regenerative Effects of Citral in Human Epithelial Cells and Colon Organoids.** Fagundes FL¹, Aal MCE², Luo X³, Okhovat A³, Castillo F³, Delella FK², Villablanca EJ³, Hiruma-Lima CA¹ ¹Unesp-Botucatu, Dept Structural and Functional Biology, sector of Physiology, Lab Biological Assays with Natural Products, Brazil ²Unesp-Botucatu, Dept Structural and Functional Biology, sector of Morphology, Interactions and Cancer Biology Lab, Brazil ³Karolinska Institutet and University Hospital, Dept Medicine, Division of Immunology and Respiratory Medicine, Stockholm, Sweden

09.045 **Assessment of *Bothrops jararacussu* Venom-Induced Cardiotoxicity in an Isolated Heart (Langendorff) Model.** Almeida RACS¹, Santos JS¹, Amorim LS¹, Bueno GS¹, Melo RL¹, Monteiro-Machado M², Melo PA², Olivares EL¹, Strauch MA¹ ¹UFRRJ. Lab Fisiologia e Farmacologia Cardiovascular. Depto de Ciências Fisiológicas, Instituto de Ciências Biológicas e da Saúde. ²UFRJ.

09.046 Investigation of the Protective Effect of Polysaccharides Extracted from Tamarillo (*Solanum betaceum*) in an Ulcerative Colitis Model. Braga LLVM¹, Schiebel CS¹, Sauruk KS¹, Maia MHS¹, Fernandes ACVU¹, Nascimento GE², Issa TA³, Gois MB³, Cordeiro LMC², Maria-Ferreira D¹ ¹FPP-IPPPP, Programa de Pós-Graduação em Biotecnologia Aplicada à Saúde da Criança e do Adolescente; ²UFPR, Programa de Pós-Graduação em Ciências-Bioquímica, Faculdade de Ciências da Saúde, ³UFR

09.047 Cockroaches (*Nauphoeta cinerea*) as an Experimental Model for Screening Coralsnake Venoms. Oliveira IN¹, Oliveira RS¹, Dias DRC², Guimarães MP³, Vinadé LHC⁴, Dal Belo CA^{1,3}, Silva NJ Jr⁵, Hyslop S¹ ¹FCM-UNICAMP, Depto de Farmacologia, PPG Farmacologia, Brazil; ²UFES, Depto de Estatística; ³EPPEN-Unifesp-Osasco, Depto Multidisciplinar, ⁴Unipampa-São Gabriel, ⁵PUC-GO, PPG em Ciências Ambientais e Saúde

10. Cancer Pharmacology

Totem 17

10.030 Impact of LGALS3 Gene Silencing on the Sensitivity to Chemotherapy in Acute Lymphoblastic Leukemia Cells. Haus LLFA^{1,2}, Salgado MTSF¹, Almeida MS³, Carvalho RS¹ ¹ICB-UFRJ, Lab. of Pain and Inflammation Pharmacology, Brazil; ²UFRJ, PPG Biological Systems Engineering, Brazil, ³CENABIO-UFRJ, National Center for Structural Biology and Bioimaging, Brazil

10.031 Antiproliferative Activity of a Natural Clerodane Diterpene on Breast Cancer Cells. Barbosa SLM¹, Almeida ML¹, Ramos RWS¹, Freitas MM¹, Feitoza LLS¹, Zalewski S², Camargo JGS², Carvalho FA², Santos AG², Castro e Sousa JM³, Dittz D³, Ferreira PMP¹ ¹UFPI, Dept Biophysics and Physiology, Lab Experimental Cancerology (LabCancer), PP Pharmaceutical Sciences Brazil, ²UNESP, Faculty of Pharmaceutical Sciences, Lab Pharmacognosy, Brazil, ³UFPI, Dept Biochemistry and Pharmacology, Brazil

10.032 Cytotoxic and Cell Death Mechanisms Induced by Cannabidiol in Colorectal Cancer: Role of Cannabinoid and Vanilloid Receptors. Jesus MFIO, Pereira THR, Zamarioli LDS, Trindade CB Unifesp-EPM, Depto de Farmacologia

10.033 Oxidative Stress Induction by Thiosemicarbazone DT-10 as a Therapeutic Strategy in Cholangiocarcinoma Cell Lines. Vasconcelos BV¹, Moraes ADTO², Da Costa VCM¹, Leite ACL³, Rêgo MJBM¹ ¹UFPE, Programa de Pós-Graduação em Inovação Terapêutica ²UFPE, Programa de Pós-graduação RENORBIO ³UFPE, Programa de Pós-Graduação em Ciências Farmacêuticas

10.034 Assessment of Diaporthein B Cytotoxicity in Gastric Cancer Cells. Domingos HV¹, Peña-Hidalgo M², Ferreira MJP², Miguel RA³, Lima K¹, Machado-Neto JA¹, Costa-Lotufo, LV¹ ¹ICB-USP Department of Pharmacology, Brazil, ²IB-USP Dept Botany, Brazil, ³UFDPAR, Delta Cell culture Lab, Brazil

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10.035 Nanoemulsion Containing Limonene: A Promising Strategy for Topical Transdermal Approach to Chemoprevention Breast Cancer. Nunes JR, Teixeira MAS, Lopes LB ICB-USP

10.036 Exploring Kinin Receptor Modulation as a Therapeutic Approach in Head and Neck Squamous Cell Carcinoma. Neculqueo GW^{1,2}, Farias ABS², Estrázulas M^{1,2}, Campos MM^{1,2} ¹PUCRS, Escola de Medicina, PPG Medicina e Ciências da Saúde, Brazil. ²PUCRS, Escola de Ciências da Saúde e da Vida, INTOX, Brazil

10.037 Integrative Network Analysis Reveals Stage-Specific Molecular Modules in Glioblastoma Progression and Potential Intervention Points for Thiazolidinedione Derivatives. Silva Junior CLC¹, Pitta MGR², Rego MJB², Rosa MM² ¹UFPE, PPG Therapeutic Innovation, Brazil. ²NUPIT-SG-UFPE, Research Center for Therapeutic Innovation - Suely Galdino, Brazil

10.038 ACEA, A Selective Cannabinoid Receptor 1 Agonist, enhances the antitumoral activity of Oxaliplatin and inhibits the Associated Peripheral Sensory Neuropathy in a Murine Colorectal Adenocarcinoma Model. Nascimento RR^{1,3}, Carvalho VM¹, Pereira AF¹, França JC¹, Moura MNS¹, Alves BWF¹, Muniz PTG¹, Rodrigues EDD¹, Freire DC¹, Ribeiro MVS¹, Rocha LR¹, Abucater BLSM¹, Bezerra BS¹, Nicolau LAD², Vale ML¹ ¹UFC, Dpt of Physiology and Pharmacology, Brazil; ²UFDP, Biotechnology and Biodiversity Center Research, Brazil; ³Medical University of South Carolina, Charleston, Dpt of Regenerative Medicine and Cell Biology, USA

10.039 Friedelin Induces Caspase-3-Dependent Apoptosis Without Causing Systemic Toxicity in Mice Bearing Ehrlich Ascitic Carcinoma. Silva ELES¹, Silva FA¹, Souza TPM¹, Silva JP¹, Santana JR¹, Flor JF¹, Figueiredo CG¹, Santos EC², Bassi JE², Leal DC³, Silva JC³, Reis MDS¹, Lins MP¹, Barreto EO¹, Ferro JNS¹ ¹UFAL, Cellular Biology Lab and ² Lab Virology and Immunology Research; ³UNCISAL, Brazil

11. Clinical Pharmacology, Pharmacokinetics, Pharmacogenomics and Toxicology

Totem 07

11.012 *Crotalus durissus terrificus* Venom: Complexity of PLA2 Isoforms. Felipe AGC¹, Galizio NC¹, Carneiro IB¹, Sousa EP¹, Rodrigues MAF¹, Vidueiros J², Lima JAF³, Coelho GR⁴, Pimenta D³, Sant'anna S², Morais-Zani K^{1,2} ¹IB, Lab Fisiopatologia, ²IB, Lab Herpetologia, ³IB, Lab Ecologia e Evolução, ⁴IB, Lab Bioquímica, Instituto Butantan

11.013 Nanoencapsulation of Cinnamon Essential Oil in Zein: The Toxicity Evaluation in the Non-Target Organism Zebrafish (*Danio rerio*). Chamberlain ALV¹, Barros JMH², Pimentel JRL¹, da Silva ACF¹, Stadinik MJ³, Müller LG⁴, da Costa C^{2,5}, Siebel AM^{1,6} ¹UFPR, Dept Pharmacology, Brazil, ²UFSC, Dept Chemical and Food Engineering, Brazil, ³UFSC, Dept Plant Sciences, Brazil, ⁴Institute of Experimental and Technological Biology (iBET), Portugal, ⁵UFSC, Dept Textile Engineering, Brazil, ⁶UFRGS, Dept Health Sciences, Brazil

11.014 Effects of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) Exposure during Pregnancy and Lactation: Maternal Toxicological Assessment and Early Development of Female Wistar Rat Offspring. Castro RLM¹, Dos Santos STC¹, Magalhães L¹, Stein J^{1,2}, Nagaoka LT^{1,2}, Aquino JVF¹, Rosalem GF¹, Vitale OG¹, Jorge BC¹, Arena AC^{1,3} ¹IBB-Unesp-Botucatu, ²UNESP, PPG Biomolecular and Pharmacological Sciences, ³CIATOX-UNESP-Botucatu, Center for Toxicological Information and Assistance

11.015 Precision Medicine in Pediatric Transplants: Developing a Population Pharmacokinetic Model for Busulfan. Rodrigues VJ¹, Olivo LB¹, Wermann S¹, Dias BB¹, Pinhatti AV^{2,3}, Zuckerman J^{2,3}, Daudt LE⁴, Dalla-Costa T¹, Araújo BV^{1,2} ¹UFRGS, Faculty of Pharmacy, Pharmacokinetics and PK/PD Modeling Lab, Brazil, ²UFRGS, PPG Medical Sciences, Porto Alegre, Brazil, ³HCPA-UFRG, Pharmacy Service, Porto Alegre, Brazil, ⁴HCPA-UFRGS Hematology Service, Hospital de Clínicas de Porto Alegre, Porto Alegre, Brazil

11.016 Analysis of the Humoral Response Against SARS-CoV-2 of Immunocompromised Healthcare Professionals Participating in the SEVACOV-PRO Study. Pereira, BM¹, Silva VEG¹, Silva DLM², Duarte, DB^{1,3} ¹UnB, Lab Pharmacological Assays, Dept Pharmacy, School of Health

Sciences, University of Brasília, Brasília, Brazil, ²HUB, University of Brasília Hospital, Brasília, Brazil, ³UnB, Graduate Program in Tropical Medicine, School of Medicine, University of Brasília, Brasília, Brazil

11.017 Dopaminergic Cell Model Comparison to Evaluate Harmine's Neuroprotective Potential Against Cocaine Toxicity. Terra LAG, Ribeiro JLF, Pereira ML, Dörr F, Marcourakis T University of Sao Paulo

Totem 25

11.018 Exposure to Nitrosodimethylamine (NDMA): Toxicological and Reproductive Effects in Male Wistar Rats. Nagaoka LT¹, Stein J¹, Vitale OG¹, Marraschi GK¹, Aquino JVF¹, Castro RLM¹, Rosalem, GF¹, Santos STC¹, Magalhães L¹, Alves, JT¹, Jorge BC¹, Arena AC^{1,2} ¹Unesp, Dept Structural and Functional Biology, Embryology Sector, Lab Toxicology of Natural and Synthetic Products, PPG Biomolecular and Pharmacological Sciences, Brazil; ²CIATOX-Unesp, Toxicological Information and Assistance Center, Brazil

11.019 Context-Dependent Modulation of Neuroinflammation and CD4+ T Cell Activation by Heated Tobacco and Cigarette Smoke. Scharf P¹, Sandri S¹, Borges P¹, Xavier LF¹, Silva T², Faria L², Olival GS³, Peron JPS², Farsky SHP¹ ¹USP, PPG in Pharmacy (Physiopathology and Toxicology), São Paulo, Brazil; ²USP, PPG in Immunology, São Paulo, Brazil; ³Hospital Santa Casa de São Paulo, Dept. of Neurology, São Paulo, Brazil

11.020 From Plasma to Effect: Integrating Interstitial Pharmacokinetics and MRSA Kill Dynamics with PBPK/PD Modeling. Olivo LB¹, Lemos JLS¹, Rodrigues VJ¹, Dias BB¹, Kretschmer DB², Cruz WA², Staudt KJ², Annaert P³, Araújo BV¹ ¹UFRGS, PPG Pharmaceutical Sciences, Brazil, ²URI-Santo Angelo, Brazil, ³KULeuven, Pharmaceutical and Pharmacological Sciences, Belgium

11.021 Parental and Postnatal NDMA Exposure Alters Reproductive and Toxicological Profiles in F1 Rats. Stein J^{1,2}, Nagaoka LT^{1,2}, Castro RLM¹, Rosalem, GF¹, Aquino JVF¹, Alves, JT¹, Vitale OG¹, Marraschi GK¹, Santos STC¹, Magalhães L¹, Jorge BC¹, Arena AC^{1,3} ¹UNESP, Dept Structural and Functional Biology, Embryology Sector, Lab Toxicology of Natural and Synthetic Products, Brazil ²UNESP, PPG Biomolecular and Pharmacological Sciences, Brazil ³UNESP, Toxicological Information and Assistance Center (CIATOX), Brazil

11.022 Using Microdialysis Free Concentrations to Model Tissue-Level Targets for Therapeutic Success in Glycemic Control of Type 2 Diabetes. Dias BB, Olivo LB, Braga A, Rodrigues VJ, Araujo BV UFRGS, Faculty of Pharmacy, PK/PD Modeling Lab, Pharmaceutical Sciences Graduate Program, Brazil

11.023 Physiologically Based Pharmacokinetic (PBPK) Modeling of Empagliflozin in Pediatric Populations with Healthy and Obese Weights. Milhm, GP¹, Moreira, FL², Abrahim-Vieira, BA¹ UFRJ, Lab Molecular Modeling & QSAR (ModMolQSAR), PPG Pharmaceutical Sciences, Brazil. ²UFRJ, Pharmacometrics Lab (LabFarma), Brazil

12. Drug Discovery and Development

Totem 08

12.008 Analysis of Cell Membrane Integrity of *Trypanosoma cruzi* Epimastigote Forms under Treatment With (2E,4E)-1-(2-hydroxy-3,4,6-trimethoxyphenyl)-5-phenylpenta-2,4-dien-1-one. Pinheiro KS¹, Duarte ALS¹, Fernandes MO¹, Ananias CF¹, Araújo JR¹, Ribeiro LR², Magalhães EP², Silva FRC², Gomes NDB², Maia MMG², Santos HS³, Martins AMC², Menezes RRPPB². ¹UFC, Faculty of Pharmacy, Dentistry and Nursing, Dept Clinical and Toxicological Analysis, Brazil; ²UFC, Faculty

of Pharmacy, Dentistry and Nursing, Dept Clinical Analysis and Toxicological, Post Graduate Program of Pharmaceutical Sciences, Brazil;³UVA, Center for Exact Sciences and Technology, Brazil

12.009 Synthesis and Antiproliferative Activity of Triazol-Containing Derivatives Based on Minor Groove Binders and LSD1 Inhibitors. Peripolli JB¹, Theisen MC², Borba IAS², Castro AM¹, Maia BC², Librelotto DRN², Fumagalli F² ¹UFSM, Graduação em Farmácia, Brasil; ²UFSM, PPG Ciências Farmacêuticas, Brasil

12.010 Recombinant Expression of *Mycobacterium tuberculosis* Caseinolytic Protease (Clp) Components for Structural Analysis and Therapeutic Targeting. Ribeiro PHC, Silva CS, Rossini NO, Dias MVB USP, Lab. of applied structural biology, Brazil

12.011 Exploration of Cytotoxic Microorganisms from *Palythoa caribaeorum* collected at Brazilian Oceanic Islands. Moura, MHS¹, Silva, CSMR², Sahm, BDB³, Costa-Lotufo, LV² ¹FCF-USP, Brazil, ²ICB-USP, Brazil, ³FCFRP-USP, Brazil

12.012 Investigation of Bioavailability, Taste Masking Effect and Antimalarial Activity of Chloroquine-Ion Exchange Resin Complex. Passos MXV¹, Mascarenhas ED¹, Sousa EGR², Guimarães TF², Rosas EC¹, Pádua TA¹ ¹Farmanguinhos-Fiocruz, Lab Applied Pharmacology, Rio de Janeiro, Brazil. ²Farmanguinhos-Fiocruz, Educação, Pesquisa e Inovação, Rio de Janeiro, Brazil

12.013 Pharmacology in the Shadows: Extracts of Bacteria Recovered from Brazilian Caves show Anticancer Potential. Ferraro ET^{1,3}, Lopes NP², Kato N², Costa-Lotufo LV¹, Sahm BDB^{1,2} ¹ICB-USP, Brasil; ²FCFRP-USP, Brasil; ³USP, Escola de Artes, Ciências e Humanidades, Brasil

13. Pharmacology Education and Technology

Totem 09

13.001 Assessment of Healthcare Professionals in Public Hospital Knowledge on the Use of Pressurized Metered-Dose and Dry Powder Inhalers. Moreira EM¹, Silva MCB¹, Oliveira HR², Nunes GM², Mendes GC², Araújo LCG², Borges AC², Nascimento AR¹, Fontes MLS¹, Silva GS¹, Gonçalves YS¹, Jacobina KF¹, Murcy, ACI, Castro PFS¹ ¹UEG, UnU Goiânia - ESEFFEGO, Brasil, ²UEG, UnU Itumbiara, Brasil

13.002 Do Primary Health Care Professionals Know How to Use Inhaler Devices? A Study in Central-West Brazil. Oliveira HR¹, Borges TS¹, Vasconcelos CO¹, Fonseca FV¹, Silva MCB², Nascimento AR², Fontes MLS², Juliano FR¹, Castro PFS² UEG, UnU Itumbiara, Brasil, ²UEG, UnU Goiânia - ESEFFEGO, Brasil

13.003 Simulated Jury as an Active Learning Strategy for Teaching Medication Deprescribing to Medical Students. Rosa JG, Barros HMT UFCSPA, Porto Alegre, Brazil

13.004 Pharmacology UNO and the Hypertension Card Deck: Gamified Strategies to Teach Lipid-Lowering and Antihypertensive Agents. Santa Clara-Amorim RC, Higashijima, AN, Lívero FADR UFPR, Lab. of Cardiometabolic Pharmacology, PPG Pharmacology, Brazil

01. Cellular and Molecular Pharmacology

Totem 07

01.026 Resveratrol in the Pre-Gestational, Gestational and Breastfeeding Phases of Spontaneously Hypertensive Rats (SHR) Reduces Hypertension and Matrix Metalloproteinase (MMP)-2 Activity In The Aortas of Adult Offspring. Oliveira RR¹; Mello MMB¹; Gomes BQ¹; Dourado TMH^{1,2}; Assis VO¹; Tirapelli CR^{1,2}; Castro MM¹ ¹Dept Pharmacology, Ribeirão Preto Medical School; ²Cardiovascular Pharmacology Lab, Ribeirão Preto School of Pharmaceutical Science University of São Paulo (USP)

01.028 Prospecting for Bioactive Substances in Microorganisms Recovered from Caves of the Parque Estadual Turístico do Alto Ribeira (São Paulo, Brazil). Rivero BES¹, Sahm BDB^{1,2}, Lopes NP², Costa-Lotufo, LV¹. ¹ICB-USP, ²FCFRP-USP

01.029 Doxycycline Attenuates Cytokine Responses in a Mouse Model of Lipopolysaccharide-Induced Epididymitis: A Potential Role of Matrix Metalloproteinase Inhibition. Martini PV¹, Andrade AD¹, Ferreira CT¹, Camargo IA¹, Bizzotto JQ¹, Portela LMF², Kushima H¹, Justulin-Junior LA², Fernandes CJC¹; Silva, EJR¹ ¹Unesp, PPG Biomolecular & Pharmacological Sciences, Brazil; ²Unesp, PPG General & Applied Biology, Brazil

01.030 Modulation of NF- κ B by TLR4 Activation in Doxorubicin-Stimulated MCF-7 and MDA-MB-231 Breast Cancer Cells. Quispe C¹ Buitron Mercado MF², Riera Perez AC³, Concha Escobedo PA⁴, Vallejos Paredes MF⁵, Villanueva-Salas JA⁶, Capio Carpio JM⁷ ^{1,2,4,5,6}Escuela Profesional Farmacia y Bioquímica, Facultad de Ciencias Farmacéuticas, Bioquímicas y Biotecnológicas de la Universidad Católica de Santa María, Perú. ^{3,7}Escuela Profesional Ingeniería Biotecnológica, Facultad de Ciencias Farmacéuticas, Bioquímicas y Biotecnológicas de la Universidad Católica de Santa María, Perú

Totem 15

01.005 TRPV1 Antagonist SB366791: An *in Silico* and *in vitro* Evaluation of Physicochemical and Neurobiological Properties. Barros ASM¹, Lisboa SF^{1,2} ¹FMRP-USP, Pharmacology PPG, Brazil; ²FCFRP-USP, Dept Biomolecular Sciences, Brazil

01.032 Identification of MRP/ABCC Transporters Involved in cAMP Efflux from Skeletal Muscle Fibers. Satori NA and Godinho RO Unifesp-EPM, Dept. of Pharmacology, Div. of Cellular Pharmacology; PPG Pharmacology, São Paulo, Brazil

01.033 Mouse SEMG1 Reduces PKA Phosphorylation Without Changing cAMP Levels in Spermatozoa: A Non-canonical Pathway with Pharmacological Implications. Santos NCM¹, Mariani NAP¹, Santos BR¹, Fernandes CJC¹, Novero AG², Oliveri LG³, Steeman TJ², Stival C², Luque GM³, Krapf D², Buffone MG³, Silva EJR¹ ¹UNESP, PPG Biomolecular & Pharmacological Sciences, Brazil, ²National University of Rosario, Institute of Molecular and Cellular Biology of Rosario, Rosario, Argentina, ³Institute of Biology and Experimental Medicine, Buenos Aires, Argentina

01.034 Microglial Alpha 7 Nicotinic Acetylcholine Receptor's: Characterization of Immediate Response. Bello-Santo, VG; Oiveira, LN; Castro, NG UFRJ, Instituto de Ciências Biomédicas, Lab Farmacologia Molecular, PPGFQM, Brazil.

02. Neuropharmacology

Totem 01

02.007 **Repeated CBD Treatment Increased Hippocampal Pro-Caspase-1 in Socially Isolated Female Mice.** Pedro AP¹, Bilibio JO², Barros ASM², Lisboa SF^{2,3} ¹FFCLRP-USP, Brazil; ²FMRP-USP, Pharmacology PPG, Brazil; ³FCFRP-USP, Brazil

02.058 **Redox Imbalance and Rescue: Very Long Chain Fatty Acids Drive Neuroinflammation in X-ALD, Reversed by Monomethyl Fumarate.** Pôças P¹, Gomes A¹, Mendes F², Moraes JA¹ ¹UFRJ, Lab. of Redox Biology, Brazil, ²UFRJ, Lab. of Cell Signaling and Mechanobiology, Brazil

02.059 **Nicotine-Induced Conditioned Place Preference: Different Responses Between Female and Male Mice.** Lemos LC, Corrêa AE, Costa LF, Nunes LED, Souza INO, Neves GA UFRJ, Lab Molecular Pharmacology, Brazil

02.060 **Acute Administration of Fluoxetine Reverses Stress-Induced Deficits in Contextual Fear Extinction in Rats.** Franzoni ACM, Moraes-Neto TB, Belem-Filho IJA Furtado ASJ, Bertacchini GL, Resstel LBM FMRP-USP, Dept Pharmacology

Totem 08

02.062 **Preclinical Evaluation of the Therapeutic Potential of Doxycycline in Male Rats of the MAM Schizophrenia Model.** Dassow PZ, Ferrarezi ESM, Mendes V, Toscano LF, Vargas L, Salum C UFABC, Post-Graduation Program in Neuroscience and Cognition, Brazil

02.063 **Dorsal Hippocampal Orexin 1 Receptors Modulate the Expression of Contextual Fear Conditioning in Rats.** Rodrigues JS, Santos L B, Rossato GO, Bertacchini GL, Morars-Neto TB, Resstel LBM FMRP-USP, Dept Pharmacology, Ribeirão Preto, São Paulo Brazil

02.064 **Sex vs Stress: Their Role in a Mice Model of Depression.** Silva ACF, Barros RP, Cardoso AR, Souza RV, Neves, GA UFRJ, Lab Molecular Pharmacology, Brazil

02.065 **Neuroprotective Effects of Environmental Enrichment in a PTZ-Induced Seizure Model.** Sulzbach AC¹, de Lima-Rosa G², Grotto LG³, Padilha RB², de Lima AMDL⁴, Guzzo EFM², Barbosa BB⁵, Coitinho AS^{1,2,3}. ¹UFRGS, Postgraduate Program in Biological Sciences, Pharmacology, and Therapeutics, Brazil. ²UFRGS, Postgraduate Program in Biological Sciences, Physiology, ³UFRGS, Brazil. ⁴UFRGS, Postgraduate Program in Biological Sciences: Biochemistry. ⁵UFRGS, Postgraduate Program in Neurosciences

Totem 09

02.066 **Involvement of Glutamatergic and Nitrergic System in Memory Processes Deficits and Aversion Induced by Stress.** Solovi SR, Valera Sanchez CC, Bertacchini GL, Resstel LBM FMRP-USP, Dept Pharmacology, Ribeirão Preto, São Paulo, Brazil

02.067 **Kynurenine Neonatal Exposure: Deficits in Mice Development.** Cruz BE¹, Corrêa AES², Bomfim ANL¹, Andrade BS¹, Guimarães MZP², Neves GA¹ ¹UFRJ, Lab Molecular Pharmacology, Postgraduate Program in Pharmacology and Medicinal Chemistry, Brazil ²UFRJ, Lab Molecular Pharmacology, Brazil

02.068 **Voltage-Gated Sodium Channel Modulation by Mexiletine Analogues.** Rosado-Santos PL, Bello-Santos VG, Castro NG ICB-UFRJ, Lab Farmacologia Molecular, PPGFQM, Brazil

02.069 **Klotho Silencing Decreases AMPA Receptor Expression in Mixed Cerebellar Culture.** dos Santos GRO¹, Kawamoto EM¹ ICB-USP, Functional and Molecular Biology Post-Graduation Program, Brazil

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02.070 **Chronic Social Stress in C57BL Female Mice Impairs Cognition, but Not Memory or Social Behavior.** Covre LHH^{1,2}, Munhoz CD¹ ¹USP-ICB, Lab of Neuroendocrine Pharmacology and Immunomodulation, Brazil, ²USP, PPG Functional and Molecular Biology, Brazil

02.071 **Molecular Mechanisms Underlying Photobiomodulation-Induced Neuroplasticity: Implications for Psychiatric Therapeutics.** Schons T^{1,3}, Bortoluzzi L^{1,2}, Colombo R², Viscardi LH², Padilha AT², Agostini JF², da Cunha SB², Rosa AR^{1,3,4} ¹IBCS-UFRGS, Postgraduate Program in Biological Sciences: Pharmacology and Therapeutics, ²UCS, Caxias do Sul, Brazil. ³HCPA, Lab Molecular Psychiatry, 4UFRGS, Postgraduate Program in Psychiatry and Behavioral Sciences, Faculty of Medicine, Porto Alegre, Brazil

02.072 **Esterification of Geraniol to Geranyl Acetate Enhances its Therapeutic Potential in Adult Zebrafish.** Bastos LM¹, Gallas-Lopes M¹, Rosa GO¹, Patelli-Alves A¹, Portela SM¹, Costa PM³, Herrmann AP¹, Piato A^{1,2} ¹UFRGS, Porto Alegre, PPG Farmacologia e Terapêutica, Brazil; ²UFRGS, Porto Alegre, PPG Neurociências, Brazil; ³UFCSPA, Porto Alegre, Brazil

02.073 **Effect of TNFR1 Deletion on Different Types of Memory in Mice with LPS-Induced Neuroinflammation.** Lima TAS, de Sá LL, Andreotti DZ, Kawamoto EM. ICB-USP, Dept Physiology and Pharmacology

02.074 **Ouabain Modulates BDNF Expression in Lung and Brain Regions in a Murine Model of Mixed Asthma.** Ribeiro MR¹, Santos AS¹, Leite JA², Alves VF¹, Melhado IVS¹, Lima TAS¹, Suaiden AS¹, Lima LS¹, Viana DZA¹, Oliveira MA¹, Kawamoto EM¹, Tavares-de-lima W¹, Scavone C¹ ¹ICB-USP, Dept. of Pharmacology, São Paulo Brazil. ²ICB-UFG, Dept. of Pharmacology, Goiás, Brazil

03. Psychopharmacology

Totem 02

03.015 **Standardization of a Depression Model for Assessing the Antidepressant Potential of Bufotenine in Rats.** Oliveira PS, Queiroz KS, Paulo MEFV, Gonçalves TCP, Nencioni ALA IB, Lab. of Pharmacology, Brazil

03.019 **Evaluation of Anxiety- And Depression-like Behaviors in Tumor-Free Mice Treated with Paclitaxel: Potential Neuroprotective Effects of Cannabidiol** Queiroz ABWV¹, Pereira ACM², Lisboa SFS² ¹FMRP-USP, Neuropsychopharmacology Lab, Brazil; ²FCFRP-USP, PPG Pharmaceutical Science

03.020 **Effects of *Lactiplantibacillus plantarum* 286 and *L. plantarum* 81 on Ethanol-Induced Reinstatement in Conditioned Place Preference Associated with Sleep Deprivation in Mice.** Figuera YM, Silva KSO, Resende GR, Tamura EK UESC, Chronobiology Research Group, Ilhéus, BA

03.021 **Effects of Aripiprazole on Social and Locomotor Behavior in Adult Zebrafish Acutely Exposed to Ketamine.** Patelli-Alves A, Becker SZ, Gallas-Lopes M, Bruck SM, Müller DV, Bastos LM, Stahlhofer-Buss T, Piato A, Herrmann AP. UFRGS, Pharmacology Dept

03.022 **Effect of Lisdexamfetamine on the Reactivity Behavior of Mice in the Looming Test across Sex and Lineages.** Rosa EB, Kondo TA, Pradella GR, Gogola JV, Dalagrana H, Garlet QI UFPR, Biological Sciences Sector, Dept Pharmacology, Brazil

03.023 Psychogenic Profile of Early Life Trauma Induced by Single Prolonged Stress in an Experimental Model of Post-Traumatic Stress Disorder. Silveira EP^{1,2}, Teixeira LL^{1,3}, Andrade AS¹, Pinto LC¹, Hamoy M⁴, Lopes DCF¹ ¹UFPA, Lab Neuropatologia Experimental, Hospital Universitário João de Barros Barreto, Brasil, ²FSCMPA, Programa de Residência Médica em Clínica Médica, Brasil, ³UFPA, Programa de Pós-graduação em Farmacologia e Bioquímica, Brasil, ⁴ UFPA, Lab Farmacologia e Toxicologia de Produtos Naturais, Instituto de Ciências Biológicas, Brasil

03.024 Acute Effects of Cannabigerol on Anxiety-Like Behavior Associated with Acute Restraint Stress in Male and Female Rats. Storgatto FJ, Hirabayashi MH, Zanoveli JM UFPR, Dept Pharmacology, Curitiba, Paraná, Brazil

Totem 17

03.025 Modulating the Reconsolidation of Generalized Fear Memories: A Preclinical Model of Combined Pharmacological Intervention Soares LA^{1,2}, Gazarini L³, Guimaraes FS⁴, Bertoglio LJ^{1,2} ¹UFSC, Lab Neuropsychopharmacology, Dep. of Pharmacology; ²UFSC. ³UFMS, 4.FMRP-USP, Dep. of Pharmacology

03.026 Lipopolysaccharide Preconditioning Disrupts the Behavioral and Inflammatory Response to Restraint Stress in Male Mice. Lovis EP¹, Pereira GC¹, Viero FT², Arboit F³, Becker G⁴, Zanchet EM¹, Trevisan G¹, Oliveira SM⁴, Pillat MM¹, Bochi GV¹. ¹UFMS, PPG Pharmacology, Brazil. ²USP, Institute of Chemistry, Brazil. ³UFMS, PPG Veterinary Medicine, Brazil, ⁴UFMS, PPG Biological Sciences: Toxicological Biochemistry, Brazil

03.027 The CB1 Receptor Negatively Modulates the Recent Reconsolidation of Long-Term Spatial Memory. Queiroz R, da Silva WC Unicentro Neuropsychopharmacology Lab

03.028 Pubertal Treatment Of N-Acetyl-L-Cysteine or Brilliant Blue G Prevents Behavioral Deficits and Inflammatory Cytokines in Male Rats of Schizophrenia MAM Model. Toscano LF¹, Vargas L¹, Ribeiro DE², Ulrich H², Salum C¹ ¹UFABC, Post-Graduation Program in Neuroscience and Cognition, Brazil. ²IQ-USP, Dept Biochemistry, Institute of Chemistry

03.029 Ayahuasca Reduces Stress-Induced Deficits In The Process Of Contextual Conditioned Fear Extinction in Male Rats. Furtado ASJ¹, dos Santos, RG², Resstel, LBM¹ ¹FMRP-USP, Pharmacology Dept, Lab. of Neuropharmacology, PPG Pharmacology, Brazil ²HC-FMRP-USP, Neuroscience and Behaviour Dept, Lab. of Psychopharmacology

03.030 Primary Cortical Neurons from Maternal Immune Activation Animal Model Presents Disruptions on Mitochondrial Function, Dendritic Arborization and Synaptic Events: Relevance to Schizophrenia Pathophysiology. Santos ASD^{1,2}, Araujo VC^{1,2}, Ribeiro MR^{1,2}, Pérez MMM², Hernández DM², Madrigal JLM², Scavone C¹, Leza JC² ¹ICB-USP, São Paulo, Brasil ² University Complutense de Madrid, Medical School, Madrid, Spain

04. Inflammation and Immunopharmacology

Totem 03

04.047 Differential Phenotype Modulation and Molecular Mechanisms of Lipoxin on Tumor Associated Macrophages. Villanova Amaral MF, Cunha-da-Costa H, Duncan-Moretti J, De-Brito NM, Simões RL, Barja-Fidalgo TC. UERJ, Lab Farmacologia Celular e Molecular, Rio de Janeiro

04.048 Eicosapentaenoic Acid- Supplementation Decreases Adrenal Glucocorticoid Steroidogenesis in a Murine Model of Type 1 Diabetes. Barbosa BLSS, Chaves AS, Carttman L,

Pacheco FS, Bergara L, Martins PMRS, Martins MA, Carvalho VF IOC-Fiocruz, Lab Inflammation, Center for Research, Innovation, and Surveillance in COVID-19 and Health Emergencies

04.049 **Red LED Therapy Led to Tissue Remodulation and Decrease of Immune Cells in Skin of Murine Imiquimod-induced Psoriasis Experimental Model.** Rodrigues-Silva N, Correia-Silva RD, Santos DD, Corrêa MP, Gil CD Unifesp-EPM, Brazil

04.050 **Effects of Nor-JNJ-7777120, a Novel Potent Histamine H4 Receptor Antagonist, on a DSS-Induced Colitis in Mice.** Silva PS, Fernandes JPS, Landgraf RG Unifesp-Diadema, Dept Pharmaceutical Sciences, Brazil

04.051 **Hydrogen Sulfide Pathways in a Male Murine Model of Experimental Cystitis.** Sombra VT^{1,2}, Santos LG¹, Costa de Souza AL¹, Teixeira AS¹, Muscara MN¹, Costa SKP¹.¹ICB-USP, Depto de Farmacologia, São Paulo, SP, ²Associação Crescer Sempre, São Paulo, SP, Brazil

Totem 10

04.052 **Reduction of Pro-Inflammatory Cytokines by Resveratrol and AGK-2 in vivo and in vitro Stimulated with *Klebsiella pneumoniae*.** Costa M^{1,3}, Thiele, E¹, Almeida MAP^{1,2}, Castro LVG¹, Bozza PT¹, Castro-Faria-Neto HC¹, Gonçalves-de-Albuquerque CF^{1,2,3}, Silva AR^{1,3} ¹IOC-Fiocruz, Immunopharmacology Lab and Postgraduate Program in Cellular and Molecular Biology, Rio de Janeiro, Brazil ²UFF, Post-Graduation Program in Neuroscience, ³UFRJ, Immunopharmacology Lab and Postgraduate Program in Molecular and Cellular Biology, Rio de Janeiro, Brazil

04.053 **Nanostructured DHA Attenuates Inflammatory Effects in BV-2 Microglial Cells Stimulated with *E. coli*.** Gomes-Reis GLS¹, Moraes BPT², Saraiva JSG², Ferrarini SR³, Santos FS⁴, Ferreira AM¹, Bozza PT¹, Castro-Faria-Neto HC¹, Silva AR¹, Gonçalves-de-Albuquerque CF^{1,2} ¹IOC-FIOCRUZ, Immunopharmacology Lab and Postgraduate Program in Cellular and Molecular Biology, Rio de Janeiro, Brazil, ²UFRJ, Immunopharmacology Lab and Postgraduate Program in Molecular and Cellular Biology, Rio de Janeiro, Brazil, ³UFMT, Institute of Health Sciences, Mato Grosso, Brazil, ⁴UFF, Post-Graduation Program in Neuroscience, Niterói, Brazil

04.054 **PPAR-γ Profiling in Macrophage-Mediated Inflammatory Dysregulation Induced By *Klebsiella pneumoniae* Infection.** Guedes LD, Carvalho MV, Castro LGV, Costa MF, Souza BB, Dutra FSP, Bozza PT, Farias Neto HCC, Albuquerque CG, Silva AR IOC-Fiocruz

04.055 **Systemic Endotoxemia Differently Modulates the Expression of NLRP3 Inflammasome Complex in Mice Epididymis: A Pilot Study.** Bizzotto JQ, Martini PV, Torres RM, Andrade AD, Silva EJR IBB-Unesp-Botucatu, PPG Biomolecular and Pharmacological Sciences, Brazil

04.056 **Anti-Inflammatory and Antiedematogenic Activity of the Isolated Trypsin Inhibitor from *Inga laurina* seeds (ILT_i).** Lencina DS¹, Bonfá IS¹, Lossavaro PKMB¹, Venancio GS¹, Fernandes MML¹, Lencina JS¹, Silva SE¹, Jacobowski AC², Macedo MLR², Kadri MCT¹ ¹UFMS, Lab. of pharmacology and inflammation; ²UFMS, Lab Protein Purification and its Biological Functions

Totem 18

04.057 **Hydroethanolic Extract from *Schinus terebinthifolius* Raddi as Therapeutic Alternative for Joint Inflammation.** Nascimento, Sarah N.1;2; Correa, Luana B.1; Berenger, Ana L.R3.; Figueiredo, Maria R3; Rosas, Elaine C.1;2. 1Farmanguinhos-Fiocruz, Lab Applied Pharmacology, Rio de Janeiro, Brazil. 2. Rio de Janeiro State University IBRAG, Postgraduate Program in Biosciences. 3. Lab Natural Products (TecBio), Institute of Pharmaceutical Technology - Farmanguinhos, Oswaldo Cruz Foundation, Rio de Janeiro, RJ, Brazil

04.058 **Influenza A Virus Infection Exacerbates the Inflammatory Response and Bronchiectasis Induced by Cigarette Smoke Exposure in Mice Assessed by Microcomputed Tomography.** Nobrega MEGC^{1,2}, Cotias AC¹, Gomes HS¹, Lopes RR¹, Gaze LA¹, Arantes ACS¹, Ferreira TPT¹, Santos BDF¹, Carvalho VF¹, Silva PMR¹, Ball L³, and Martins, MA¹ ¹IOC-Fiocruz, Lab Inflammation, Rio de Janeiro, Brazil; ²UFRJ, PPG Farmacologia e Química Medicinal, Brazil; ³University of Genoa, Dept Surgical Sciences and Integrated Diagnostics, Genoa, Italy

04.059 **Metformin Effect on *Klebsiella Pneumoniae* Infection in vitro and in vivo: Focus on Brain Cells.** Ferreira AM^{1,2}, Ferreira-Costa M^{1,2}, Castro LVG^{1,2}, Gomes-Reis GLS^{1,2}, Santos FS^{1,2,3}, Souza BB^{1,2}, Bozza PT¹, Castro-Faria-Neto HC¹, Gonçalves-De-Albuquerque CF^{1,2,3}, Silva AR^{1,2,3} ¹IOC-Fiocruz Immunopharmacology Lab and Postgraduate Program in Cellular and Molecular Biology, Rio de Janeiro, Brazil; ²UFRJ, Immunopharmacology Lab and Postgraduate Program in Molecular and Cellular Biology, Brazil; ³UFF, Postgraduate program in Neuroscience, Brazil

04.060 **Evaluation of Phenotypic changes Induced by Lipoxin in Macrophages Derived from Breast Cancer.** Duncan-Moretti J, Cunha-da-Costa H, Villanova Amaral MF, Simões RL, Barja-Fidalgo TC. UERJ, Lab Farmacologia Celular e Molecular, Rio de Janeiro

04.061 **Microglial Cannabinoid Receptor 2 Activation and Its Therapeutic Potential in Sepsis** Castro LVG¹, Guedes, LD¹, Souza BB^{1,2}, Pereira-Dutra F¹, Bozza PT¹, Castro-Faria-Neto HC¹, Gonçalves-de-Albuquerque CF^{1,2}, Silva AR¹ ¹IOC-Fiocruz, Immunopharmacology Lab and Postgraduate Program in Cellular and Molecular Biology, Rio de Janeiro, ²Unirio, Immunopharmacology Lab and Postgraduate Program in Molecular and Cellular Biology, Rio de Janeiro, Brazil

04.062 **Exploring the Therapeutic Potential of Luteolin and Quercetin Co-treatment in E. coli-induced Inflammation in BV-2 Cells.** Brito MADSM¹, Santos FSD², Cunha CMC³, Gomes-Reis GLS³, Ferreira AMS³, Costa MF³, Dutra FSP³, Bozza PT³, Farias-Neto HCC³, Silva AR³, Albuquerque CFG¹ Unirio¹, ²UFF, ³Fiocruz

04.063 **Therapeutic Potential of Citral in the Treatment of Periodontal Disease Induced by Systemic Microbial Challenge.** Solon IG¹, Passaglia P⁴, Trajano⁴IP, Silva WS¹, Salvador SLS², Bulle D³P, Nascimento GC¹, Branco LGS¹ ¹FORP-USP, Dept Basic and Oral Biology, ²FCFRP-USP Dept Clinical Analyses, ³FORP-USP, Dept Oral and Maxillofacial Surgery and Periodontology, ⁴FMRP-USP, Dept Physiology

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04.013 **N2-like Neutrophil-Derived Extracellular Vesicles Enhance Malignancy of MDA-MB-231 Breast Tumor Cells.** Amorim CS¹, Amorim NS¹, Frony AC², Miranda BT³, De-Freitas-Junior JCM², RenovatoMartins M⁴, Barja-Fidalgo TC², Moraes JA¹ ¹UFRJ, Lab Biologia Redox; ²UERJ, Depto de Biologia Celular; ³IBCCF- UFRJ,, RJ; ⁴UFF, Depto de Biologia Celular e Molecular

04.064 **LPS- and LTA-Induced Epididymitis Modifies the Transcriptional Profile of Protein Disulfide Isomerase (PDI) Genes in Mice.** Andrade AD^{1,2}, Silva EJR¹, Avellar MCW² ¹Unesp, Dept Biophysics & Pharmacology, Brazil. ²Unifesp-EPM Dept Pharmacology, Brazil

04.065 **Oral JME-209, a Mexiletine Analogue, Prevents Glucocorticoid-Resistant Pulmonary Neutrophilic Inflammation and Atelectasis in a Mouse Model of Acute Lung Injury.** Gomes HS¹, Coutinho DS¹, Cotias AC¹, Gaze LA¹, Costa JCS², Carvalho VF¹, Silva PMR¹, Martins MA¹ ¹IOC-FIOCRUZ, Lab Inflammation, Brazil. ²Fiocruz, Production and Innovation in Health, Brazil

04.066 *Arthrospira platensis* Modulates Inflammatory Cytokines in the Intestine and Liver: A Preventive Approach Against Obesity Induced by a Hypercaloric Diet. Diniz AFA^{1,2}, Arruda RRA^{1,2}, Silva JMA^{1,2}, Ribeiro SB⁴, Lima CAS⁴, Sousa Filho JECS³, Melo MB³, Soares MFS^{1,2}, Claudino BFO^{1,2}, Barros BC^{1,2}, Araújo AA⁴, Silva BA^{1,2,3} ¹UFPB, Prof. George Thomas Functional Pharmacology Lab, Institute of Research in Drugs and Medicines, Brazil ²UFPB, PPG Bioactive Natural and Synthetic Products, Brazil ³UFPB, Department of Pharmaceutical Sciences, Health Sciences Center, Brazil ⁴UFRN, Lab Experimental Model of Intestinal Mucositis, PPG Pharmaceutical Sciences, Brazil

04.067 Micro-Computed Tomography (MicroCT) as a Non-Invasive Tool for Longitudinal Assessment of Lung Alterations in an Experimental Model of Elastase-Induced Pulmonary Emphysema. Ferreira TPT, Arantes ACS, Silva KN, Cotias AC, Martins MA, Silva PMR IOC-Fiocruz, Lab Inflammation, Rio de Janeiro, Brazil

05. Pain and Nociception Pharmacology

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05.033 Investigation of Alpha-Terpinene in Chemotherapy-Induced Nociception: Insights from *in vivo* Model and *in silico* ADMET Analysis. Gouvea MC^{1,2,3}, Romeiro NC³, Carmo PL^{1,2}, Barth T¹, Raimundo JM², Bonavita AGC^{1,2} ¹UFRJ, Lab Produtos Bioativos, Centro Multidisciplinar UFRJ-Macaé. ²UFRJ, Grupo de Pesquisa em Farmacologia de Produtos Bioativos, Centro Multidisciplinar UFRJ-Macaé. ³UFRJ, Laboratório Integrado de Computação Científica, Centro Multidisciplinar UFRJ-Macaé

05.034 Oleic Acid Alleviates Mechanical Hypersensitivity in an Experimental Inflammatory Pain Model. Dambros LV¹, Serafini PT^{1,2}, Brum ES^{1,2}, Favarin A^{1,2}, Becker G^{1,2}, Brusco I^{1,2}, Oliveira SM^{1,2} ¹USFM Santa Maria, Neurotoxicity and Psychopharmacology Lab – Pain Research Group, Brazil; ²USFM Santa Maria PPG Biological Sciences: Toxicological Biochemistry, Brazil

05.035 Anterior Cingulate Cortex NMDA Receptors Regulate the Effects of Electroacupuncture on Allodynia in Rats with Peripheral Neuropathy. DonizettiFonseca MA¹, de Souza MBRF¹, Ribeiro-Siqueira CM¹, da Silva AL^{1,2}, da Silva ML^{1,2}, da Silva JRT^{1,2}, Falconi-Sobrinho LL^{1,2} ¹Unifal, Lab. of Neuroscience, Neuromodulation and Study of Pain, Brazil, ²Unifal, Postgraduate Program in Biosciences Applied to Health, Brazil

05.036 Neryl Acetate, derived from the Esterification of Nerol, shows a Possible Neuroprotective Effect in Glioblastoma(U-87) Tumor Cells Exposed to Paclitaxel Induced Chemotherapy. Martins RYC, Gomes LS, Cavalcante MLS, Dittz Junior D, Cavalcante AACM, Ferreira PMP, Almeida FRC UFPI, PPG Farmacologia, Teresina, Brasil

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05.037 Long-Lasting Thermal Sensitization Following SARS-CoV-2 Spike Protein Injection in Mice. Goldoni FC¹, Fernandes ON¹, Ribeiro MER¹, Almeida BL², Pereira S², Costa R², Santin JRS¹, Quintão NLM¹ ¹Univali, Postgraduate Program in Pharmaceutical Sciences, Santa Catarina, Brazil. ²UFRJ, Postgraduate Program in Pharmaceutical Sciences, Rio de Janeiro, Brazil

05.038 Physiopharmacological Evaluation of Anthelmintics against Inflammatory, Neuropathic and Oncological Pain. Rodrigues DCN¹, Sousa RWR¹, Santos IL¹, Conceição MLP², Freitas MM¹, Acha BT³, Porto JCS¹, Feitoza LLS¹, Almeida ML¹, Santos IKS¹, Silva JN¹, Barbosa SLM¹, Dittz, D⁴, Almeida FRC³, Ferreira PMP¹ ¹UFPI, Lab Experimental Cancerology (LabCancer), Dept Biophysics and Physiology, Brazil; ²USP, Lab Inflammation and Pain (Lid), Ribeirão Preto Medical School, Brazil; ³UFPI, Lab Pain Pharmacology, Brazil; ⁴UFPI, Antineoplastic pharmacology Lab (Lafan), Brasil

05.039 **FLT3L Dependent Neuro-Immune Interaction in Peripheral Neuropathic Pain.** Ajas D^{1,2}, Aníbal E¹, Lucena-Silva GV¹, Pigatto G¹, Rivat C², Cunha TM¹ ¹FMRP-USP, Dept Pharmacology, Ribeirão Preto, Brazil. ²University of Montpellier, Institut for Neurosciences of Montpellier (INM) INSERM, Montpellier, France

05.040 **Mechanistic Insights into the Anti-Inflammatory Action of IMT504.** Miguel B¹, Anibal Silva, CE², Brumovsky PR¹, Cunha TM² ¹Universidad Austral Instituto de Investigaciones en Medicina Traslacional (CONICET-Universidad Austral), Argentina. ²FMRP-USP, Dept Pharmacology, Brazil

05.041 **Advanced Oxidation Protein Products (AOPPs) Activate TRPA1 in a Neuropathic Multiple Sclerosis Model.** Rodrigues P¹, Frare JM², Ruviaro NA², Peres DS¹, Moraes RIF¹, Antoniazzi C¹, Machado AC¹, Araújo DSM⁴, Marini M⁴, Landini L⁴, De Logu F⁴, Geppetti P⁴, Nassini R⁴, Bochi GV¹, Trevisan G. ¹UFSM, Graduate Program in Pharmacology, Brazil. ²UFSM Graduate Program in Biological Sciences, Brazil. ³UFSM, Graduate Program in Pharmaceutical Sciences, Brazil. ⁴University of Florence, Dept Health Sciences, Italy

05.042 **TRPV1 Channel Sensitization Mediates Orofacial Thermal Allodynia in Mice Mucositis Model.** Benso B¹, Bustos D², Madrid D³ ¹Pontificia Universidad Catolica de Chile, School of Dentistry, Faculty of Medicine, Santiago, Chile ²Universidad Católica del Maule, Laboratorio de Bioinformática y Química Computacional, Depto de Medicina Traslacional, Facultad de Medicina, Talca, Chile ³Universidad de Santiago de Chile, Facultad de Química y Biología, Santiago, Chile

06. Cardiovascular and Renal Pharmacology

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06.032 **Evaluation of Cardiac Autonomic Tone in Rats with Heart Failure with Preserved Ejection Fraction.** Oliveira CGA¹, Alflen L², Betat A^{1,2}, Lataro RM¹ ¹UFSC, Dept Physiological Sciences, Brazil, ²UFSC, Dept Pharmacology, Brazil

06.033 **Resveratrol Reduces Vascular Hypercontractility in Rats with Obesity and Metabolic Syndrome through Reducing Oxidative Stress, Visfatin and MMP-2 Activity.** Manoel LB¹, Blascke de Melo MM¹, Dourado TMH¹, Assis VO¹, Bueno EKP¹, Fazan Jr R², Tirapelli CR¹, Castro MM¹ ¹FMRP-USP, Dept Pharmacology, ²FMRP-USP

06.034 **Testosterone Modulates Autonomic and Cardiovascular Responses to Stress in a Rat Model of Gender-Affirming Hormone Therapy.** Martins SCA, Belem IJA, Suzuki GMF, Correa FMA, Tostes RC, Santos JD FMRP-USP, Dept Pharmacology, Brazil

06.035 **Effects of the Hydrogen Sulfide (H₂S)-Releasing Non-Steroidal Anti-Inflammatory Drug (NSAID) ATB-346 on the Proliferation and Migration of Cultured Vascular Smooth Muscle Cells.** Silva FB, da Costa Marques LA, Teixeira SA, Costa SKP, Muscará MN ICB-USP, Dept Pharmacology

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06.036 **O-GlcNAcylation Increases the Activity of Matrix Metalloproteinase (MMP)-2 in Aortas Incubated with Glucosamine and in Rat Aortas with Metabolic Syndrome.** Bueno EKP¹, Manoel LB¹, Assis VO¹, Ferreira GM², Neves VGO¹, Blascke de Mello MM¹, Tostes RC¹, Castro MM¹ ¹FMRP-USP, Dept Pharmacology; ²FCF-USP, Dept Clinical and Toxicological Analysis

06.037 **Effects of Metformin on Anti-Angiogenic Factors and Mitochondrial Function in an *in vitro* Model of Early and Late-Onset Preeclampsia.** Passeti LFP, Pereira DA, Bertozzi-Matheus M, Kaihara JSN, Bueno-Pereira TO, Santos KN, Zampieri GM, Nunes PR, Sandrim VC IBB-UNESP-Botucatu, Dept Biophysics and Pharmacology, Brazil

06.038 **A Model of Salt-Sensitive Hypertension with Early Renal Dysfunction: Investigating New Therapeutic Targets.** Pereira T¹, Araújo GVP², Brasil JPP¹, Esteves GAB¹, Santos AVZ², Filho HM¹, Morcillo LSL¹ ¹UFRJ, ²Unirio

06.039 **Sodium Nitrite Attenuates NLRP3 Associated Genes in a Hypoxia *in vitro* Model of Early-Onset Preeclampsia.** Santos KN, Nunes PR, Pereira DA, Zampieri GM, Bueno-Pereira TO, Passeti LFP, Matheus MB, Sandrim VC IBB-Unesp- Botucatu, Dept Biophysics and Pharmacology, Brazil

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06.040 **Renin-Angiotensin System and Abdominal Aortic Aneurysm: An Imbalance Between Circulating and Local Environments.** Dugaich VF¹, Mestriner F¹, Dantas PB¹, Kovacs HZ¹, Jordani MC¹, Ribeiro MS¹, Becari C^{1,2} ¹FMRP-USP, Vascular Disease Lab, Dept Surgery and Anatomy, Brazil. ²FOB-USP, Dept Biological Science, Bauru, Brazil

06.041 **Effects of Carvedilol in the Cognition of Rats that Survived Sepsis.** Betat A¹, Bialeski MEG², Candido AJS², Caixeta LFAF², Oliveira CGA², Fernandes D³, Lатарo RM² ¹UFSC, PPG Pharmacology, Brazil; ²UFSC, Dept Physiological Sciences, Brazil; ³UFSC, Dept Pharmacology, Brazil

06.042 **Effects of a Single Dose of Methylmercury on Nitrosative Mechanisms Involved in the Regulation of Vascular Tone.** Caetano ESP, Tella SOC, Rebelo MA, Barros AC, Cunha MS, Santos JET FMRP-USP, Dept Pharmacology, Ribeirao Preto Medical School, University of Sao Paulo, Ribeirao Preto, Brazil

06.043 **Antioxidant Effect of Ascorbic Acid on Heart Damage Associated with Doxorubicin in Murine Breast Carcinoma.** Ferreira LPF^{1,2}, Cavalcante KDM^{1,2}, Cavalcante FAS¹, Silva ACA¹, Santos BLB³, Nascimento MCA¹, Leal BS², Silva MTBS⁴, Amaral DT⁵, Ferreira PMP², Dittz D^{1,2,3}. ¹UFPI, Antineoplastic Pharmacology Lab, Brazil; ²UFPI, PPG Pharmacology, Brazil; ³UFPI, PPG Pharmaceutical Sciences, Brazil; ⁴University of Porto, Institute of Biomedical Sciences Abel Salazar, Portugal; ⁵UFABC, Center for Natural and Human Sciences, Brazil

06.044 **Dopaminergic Receptor Expression in the Aorta of *Chelonoidis carbonarius*: An Immunohistochemical Study.** Santos EXD¹, Souza VB¹, Britto-Júnior JB¹, Campos R¹, Nucci G^{1,2}, Schenka AA¹ ¹Unicamp, Dept Pharmacology, Faculty of Medical Sciences, Brazil ²ICB-USP, Dept Pharmacology, Brazil

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06.045 **Therapeutic Potential of Glibenclamide and Metformin as Endothelial Protective Agents in a Preeclampsia *in vitro* Model.** Nunes PR, Passeti LFP, Pereira DA, Bueno-Pereira TO, Sandrim VC IBB-Unesp-Botucatu, Dept Biophysics and Pharmacology

06.046 **Pharmacological Potential of Cafestol, a Bioactive Substance in Coffee, in Preventing Ischemia-Reperfusion-Induced Acute Kidney Injury.** Gomes DS^{1,2}, Romanelli MA^{1,2}, Gomes SPS^{1,2}, Brand ALM³, da Silva RMV³, Oliveira SSC⁴, Santos ALS⁴, Rezende CM³, Lara LS^{1,2} ¹UFRJ, ICB, Brazil. ²CPMP-IBCCF-UFRJ, ³IQ-CCMN-UFRJ, ⁴IMPG-UFRJ

06.047 **SC1, a Novel AT1 Receptor Antagonist, Inhibits Angiotensin II-Mediated Vascular Responses and Lowers Blood Pressure in Rats with Chronic Kidney Disease.** Aires RS¹, Ferreira JG¹, Barbosa FM¹, Ferreira LFL², Anjos JV², Araujo AV¹ ¹Centro Acadêmico de Vitória, UFPE, Brasil; ²Lab Síntese Orgânica Medicinal, Centro de Ciências Exatas e da Natureza, UFPE, Brasil

06.048 Protective Effects of β -Caryophyllene, a CB2 Agonist, on Renal and Vascular Alterations in Sepsis. Dal-Secco D, Mariot LN, Simas A, Sordi R UFSC, Center of Biological Sciences, PPG Pharmacology, Brazil

06.049 Differently Expressed Genes Regulated by Metformin in an *in Vitro* Model of Preeclampsia. Pereira DA, Passeti LFP, Nunes PR, Sandrim VC Unesp - Botucatu, Institute of Biosciences, Dept Biophysics and Pharmacology - Brazil

06.050 Dependent on Concentration Procainamide is Either a Negative or a Positive Inotropic Agent in Guinea Pig Heart. Sigler W^{1,2}, Oliveira AC¹ ¹ICB-USP, Dept Pharmacology, Institute of Biomedical Sciences, University of São Paulo; São Paulo, Brazil; ²Faculty Oswaldo Cruz, São Paulo, Brazil

06.051 Effects of Hyperbaric Oxygen Therapy on the Remodeling of Intramural Coronary Arteries in Diabetic Rats. Silva FS¹, Moraes MV², Abreu BJ³, Ishikawa U³, Oliveira Albuquerque ECS³, Medeiros KCP³, Farias NBS³, Araújo Júnior RF³, Carmo BA¹, Augusto dos Santos JR¹, Vidal TIR¹, Kennedy Feitosa E¹, Moacir FO⁴ ¹UFERSA, Dept Health Sciences,; ²UFRN, Dept Surgery; ³UFRN, Dept Morphology,; ⁴UFERSA, Dept Animal Sciences

07. Endocrine, Reproductive and Urinary Pharmacology

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07.014 Antioxidant Effects of Morosil® [*Citrus sinensis* (L.) Osbeck var. Moro] on the Reproductive System of Rats Treated with High Fat Diet. Rosa, GA; Oliveira, DA; Oliveira, LV; Lima, GF; Brazão, SC; Mendes, ABA; Bragança, LAR; Ribas, JAS; Santos, CM; Brito, FCF; Marostica, E. LAFE-UFF, Dep. Physiology and Pharmacology

07.015 Evaluation of the Participation of Pannexin Channels -1 in Relaxation of the Corpus Cavernosum of Rats: Implications for the Treatment of Erectile Dysfunction - An *ex-vivo* study. Barros JVC, Moretti MB, Leonardi GR, Lima JM, Passos GR, Antunes E, Mônica FZ UNICAMP, Faculty of Medical Sciences, Dept Translation Medicine, PPG Pharmacology, Brazil

07.016 Toll-Like Receptor 4 (TLR4) as Mediator of High-Fat Diet-Induced Structural Remodeling and Inflammation in Mouse Epididymis: Mechanistic Link to Male Fertility Dysfunction? Silva CP¹, Cruz Machado SS¹, Santiago MSA², Perobelli JE², Avellar MCW¹, ¹Unifesp-EPM, Dept. Pharmacology, Brazil; ²Unifesp-Baixada Santista, Dept. Marine Sciences, Brazil

07.017 Pharmacological Characterization of the Newest Phosphodiesterase (PDE) Type 4 Inhibitor, Roflumilast in Isolated Detrusor from Pig Bladder: An *in vitro* Assay. Lima JM, Barros JVC, Passos GR, Moretti MB, Leonardi GR, Antunes E. Mônica FZ, Unicamp, Dept Pharmacology, Faculty of Medical Sciences, Brazil

07.018 Effects of the Non-Phthalic Plasticizer DEHT on the Morphophysiology of the Male Genital System. Lima LE, Silva Junior EP, Nogueira, LS, Lambertucci RH, Perobelli JE Unifesp, Experimental Toxicology, Brazil

09. Natural Products and Toxinology

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09.048 Acute Toxicity Evaluation and Gastroprotective Activity of the Flavone Chrysin in Female Rodents. Pereira MC¹, Pereira QC², Santos RC³ ¹USF, School of Medicine, Brazil; ²USF, Graduate Program in Health Sciences, Brazil; ³USF, Graduate Program in Health Sciences, Brazil

09.049 **Brown Propolis Produced by *Apis mellifera* Bees from Crateús, CE Prevents Tracheal Hyperreactivity in Wistar Rats with Allergic Asthma.** Alves BFL¹, Martins AMO³, Felício IM³, Oliveira LN³, Figueiredo IAD³, Souza MLB¹, Silva SO², Cordeiro KM², Silva TMS⁴, Cavalcante FA^{3,5} ¹UFPB, PIBIC, Brazil; ²UFPB, PIVIC, Brazil; ³ UFPB, CCS, PPgPNSB, Brazil; ⁴UFRPE, DCM, Brazil; ⁵UFPB, DCB, CCS, Brazil

09.050 ***Arthrospira platensis* Prevents Intestinal Reactivity Induced by Obesity by Modulation of Oxidative Stress and Nitric Oxide Pathways: A Promising Nutraceutical Strategy** Sousa Filho JEC¹, Arruda RRA^{1,2}, Melo MB¹, Diniz AFA^{1,2}, Silva JMA^{1,2}, Claudino BFO^{1,2}, Barros BC^{1,2}, Soares MFS^{1,2}, Silva BA^{1,2,3} ¹UFPB, Lab Functional Pharmacology Prof. George Thomas, Institute of Research in Drugs and Medicines, Brazil ²UFPB, PPG Bioactive Natural and Synthetic Products, Health Sciences Center, Brazil ³UFPB, Dept Pharmaceutical Sciences, Health Sciences Center, Brazil

09.052 **Cytotoxic and Anti-inflammatory Potential of the Extract of *Oenocarpus bacaba* Mart.** Da Silva, LB^{1,2}; Figueiredo, RO^{3,4}; Gomes, PWP⁴; Figueiredo, PLB⁴; Lopes, DCF²; Pinto, LC² ¹UNIFAMAZ, ²UFPA, Lab. Experimental Neuropathology, Brazil, ³UFPA, PPG Chemistry, Brazil, ⁴UEPA, Lab. Natural Products Chemistry, Brazil

09.053 **Exploring the Toxicity, Antioxidant, and Anticholinesterase Potential of *Ilex paraguariensis* Extract.** Penha MEF¹, Morales-Lima G^{1,2}, Gray, NE², Lago, JHG¹, Teodorov, E³, Mendes FR¹ Affiliation: ¹Centro de Ciências Naturais e Humanas, UFABC, Santo André, SP, Brazil ²Oregon Health and Science University, Dept Neurology, United States ³Centro de Matemática, Computação e Cognição, UFABC, Santo André, SP, Brazil

09.068 **Effect of the Lipophilic and Hydrophilic Fractions of Pequi Oil on the Extracellular Matrix During Cutaneous Repair.** Matsnaka DL, Silva MEU, Mimura KKO e Oliveira SM UFMT

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09.054 **Characterization and Evaluation of Biological Activities of the Polysaccharide Obtained from *Anadenanthera colubrina* (Vell.) Brenan Seeds.** Araújo AAB¹, Santos AF¹, Almeida ERM¹, Matos TS¹, Santos HLS², Silva, FCO³, 4Freitas, JD, Castanha ER¹, Albuquerque PBS², Correia ACC¹ ¹UPE, PPG Saúde e Desenvolvimento Socioambiental, Brasil, ²UPE, PPG Biologia Celular e Molecular Aplicada, Brasil, ³UFRJ, Lab Bioquímica e Biologia Celular de Glicoconjugados, Brasil, ⁴IFAL, Laboratório Multiusuário de Tecnologias Ambientais, Brasil

09.055 **Hepato and Nephroprotective Effects of *Petiveria alliacea* L. in a Preclinical Model of Metabolism Associated Fatty Liver Disease.** Higashijima AN, Santa Clara-Amorim RC, Kluck AJ, Silva GR, Lívero FADR UFPR, Lab. of Cardiometabolic Pharmacology, PPG Pharmacology, Brazil

09.056 **Hemodynamic Alterations Caused by Venom of the Semiaquatic Coralsnake (*Micrurus surinamensis*) in Anesthetized Rats.** Talone SC^{1,2}, Carvalho WA^{1,2}, Matos-Silva H3, Silva Jr NJ ², Hyslop S¹ ¹Unicamp, Faculdade de Ciências Médicas, Depto de Farmacologia, PPG Farmacologia, Brazil; ²PUC-GO, PPG em Ciências Ambientais e Saúde, Brazil. NUPMA-UNIRV-Campus Goiânia, Núcleo de Pesquisa da Faculdade de Medicina, Brazil

09.057 ***Arthrospira platensis* Prevents Endometrial Damage and the Increase of Pro-Inflammatory Cytokines in the Uterus with Primary Dysmenorrhea.** Silva JMA^{1,2}, Soares MFS^{1,2}, Arruda RRA^{1,2}, Diniz AFA^{1,2}, Ribeiro SB³, Lima CAS³, Melo MB¹, Sousa-Filho JEC¹, Sousa, TR¹, Barros BC^{1,2}, Alves, AF⁴, Araújo, AA³ Silva BA ^{1,2,5} ¹UFPB, Lab Functional Pharmacology Prof. George Thomas, Institute of Research in Drugs and Medicines, Brazil ²UFPB, Postgraduate Program in Bioactive Natural and Synthetic Products, Health Sciences Center, Brazil ³UFRN, Lab Experimental Model of Intestinal

Mucositis, Postgraduate Program in Pharmaceutical Sciences, Brazil ⁴UFPB, Dept Biomedical Sciences, Health Sciences Center, Brazil ⁵UFPB, Dept Pharmaceutical Sciences, Health Sciences Center, Brazil

09.058 **Modulation of Acute Inflammation by trans-Cinnamic Acid via Neutrophil Recruitment and Downregulation of ICAM-1.** Fidelix MSP, Santos SLD, Santana JR, Barros ABB, Ferreira EGA, Silva GRS, Silva JP, Barreto E UFAL Lab Biologia Celular

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09.059 **Citrus sinensis (L.) Osbeck var. Moro Attenuates Deleterious Effects on Liver of Hypercholesterolemic Rats.** Bragança LAR¹, Brazão SC¹, Lima GF¹, Mendes ABA¹, Pereira NCA¹, Proença AB^{2,3}, Alexandre-Santos B^{2,3}, Frantz EDC^{2,3}, Motta NAV¹, Brito FCF¹ ¹FF, Lab Experimental Pharmacology, Dept. of Physiology and Pharmacology, Brazil. ²UFF, Lab Exercise Sciences, Dept. of Physiology and Pharmacology, Brazil. ³UFF, Research Center in Morphology and Metabolism, Dept. of Morphology

09.061 **Immunomodulatory Effects of a Peptide Derived from *Phoneutria nigriventer* Venom, on PBMCs co-cultured with Glioblastoma: Preliminary Data and Ongoing Translational Studies.** Silva MVR¹, Santos NBD¹, Sciani J², Vitorino-Araujo JL³, Verinaud L³, Carneiro CRD¹ ¹Unicamp, Faculty of Pharmaceutical Sciences, Campinas, SP, Brazil ²USF-Bragança Paulista, Brazil ³FCMSCSP, Division of Neurosurgery, Dept Surgery, Brazil. ⁴Unicamp, Dept Structural and Functional Biology, Institute of Biology, University of Campinas, Campinas, SP, Brazil

09.062 **Dual Antinociceptive and Cardioprotective Actions of *Vasconcellea cundinamaricensis* Cysteine Proteases in a Chemotherapy-Induced Toxicity Model.** Lima KFN^{1,2}, Sousa FM¹, Mendes NO¹, Silva ACA², Gomes LS², Lopes MTP⁴, Ferreira PMP⁶, Rodrigues GA³, Silva WC³, Almeida FRC², Silva MTB⁵, Silva IS⁶, Dittz D^{1,2} ¹UFPI, Lab de Farmacologia Antineoplásica, Brasil; ²UFPI, PPG em Farmacologia, Brasil; ³UFPI, Depto de Química, Brasil; ⁴UFMG, Depto de Farmacologia, Brasil; ⁵Universidade do Porto, Depto de Imunofisiologia e Farmacologia, Portugal; ⁶UFPE, Depto de Fisiologia e Farmacologia, Brasil

09.063 **Investigation of the Antiobesogenic Action of Myrcene in Obese C57BL/6J Mice and in 3T3-L1 Cells.** Assunção R¹, Pinto TS², Andrade AFC², Emílio-Silva MT¹, Fioravanti MM¹, Dario FL¹, Guidolin IG¹, Aquino MLA¹, Fogaça MS¹, Zambuzzi WF², Hiruma-Lima CA¹ ¹Unesp, Dept Structural and Functional Biology, Brazil ²Unesp, Dept Chemical and Biochemical, Brazil

Totem 26

09.065 **Effects of *Rosmarinus officinalis* Extract on Human Neutrophil Extracellular Traps Formation and Neutrophil Apoptosis ex vivo.** Seito LN1, Tappin MR2, Pádua TA1, Costa TEMM1, Rosas EC1, ¹Fiocruz-Farmanguinhos, Dept Pharmacology, Lab Applied Pharmacology, Brazil; ²Fiocruz-Farmanguinhos, Dep Analytical Methods, Analytical Methods Service, Brazil.

09.066 **In Silico Screening admetSAR Profiles from Brazilian Red Propolis.** Silva JLV¹, Cruz RO¹, Borini GB², Berretta AA², Cavacante FA³, Oliveira TKB¹, Oliveira PLV¹, Correia ACC⁴ ¹FMO, Lab. Práticas Funcionais, Brasil, ²Apis Flora Industrial e Comercial Ltda, Depto. P&D&I, Brasil, ³UFPB, Lab. Farmacologia Funcional, Brasil, ⁴UPE, PPG Saúde e Desenvolvimento Socioambiental, Brasil

09.067 **Effects of Prodigiosin on Zebrafish in vitro Outcomes.** Teixeira SA, Rezende-Teixeira P, Jimenez PC², Machado-Neto JA, Machado-Santelli GM, Costa-Lotufo LV, ¹ICB-USP, ²Unifesp, Dept Marine Science, Brazil

10. Cancer Pharmacology

Totem 22

10.040 **In Vitro Cytotoxic Action of Anthelmintics on Glioblastoma Cells.** Santos IL¹, Feitoza LLS¹, Sousa RWR¹, Rodrigues DCN¹, Porto JCS¹, Machado FS², Marinho Filho JDB², Araújo AJ², Sales SLA³, Pessoa C³, Ferreira PMP¹ ¹UFPI, Lab Experimental Cancerology (LabCancer), Dept Biophysics and Physiology, Teresina, Brazil; ²UFDPAr, Lab Cell Culture from Delta (LCCDelta), Parnaíba, Brazil; ³NPDM-UFC, Dept Physiology and Pharmacology, Center for Drug Research and Development, Fortaleza, Brazil

10.041 **Synthetic Hydroxylated Chalcone A3 Impairs Migration and Adhesion in Oral Cancer Cells.** Machado FS¹, Penha NS¹, de Sá RE¹, Alves BS¹, Pereira FIA¹, Santos JC^{1,2}, Perez CN³, Marinho-Filho JDB¹, Araujo AJ¹. ¹UFDPAr, Lab Cultura de Células do Delta, Brazil, ²UNIFAP, Hospital Universitário, Brazil, ³UFG, Instituto de Química, Brazil

10.042 **Mechanistic Insights into the Cytotoxic Action of a Withanolide from *Athenaea velutina*: DNA Damage and Autophagy in Colorectal Cancer Cells.** Oliveira MP¹, Sá RE¹, Machado FS¹, Almeida BO¹, Garnique ADMB², Costa-Lotufo LV², Machado-Neto JA², Rocha KAD³, Pessoa ODL³, Araujo AJ¹; Marinho-Filho JDB¹ ¹ UFDPAr, Lab Cultura de Células do Delta, LCCDelta, Brasil, ²USP, Depto de Farmacologia, Instituto de Ciências Biomédicas, Brasil, ³UFC, Depto de Depto de Química Orgânica e Inorgânica, Brasil

10.043 **Analysis of the Antiangiogenic and Cytotoxic Effects of Dihydromyricetin in Preclinical Models.** Gomes JGF¹, Barbosa BS¹, Acha BT², Leite NFB³, de Sousa AA⁴, do Lago JPAD⁵, Castro e Sousa JMC⁶, Carvalho GD⁷, Freitas GBL⁸ ¹UFPI, PPG Farmacologia, Brasil; ²UFPI, PPG Biotecnologia, Brasil; ³UFPI, PPG Química, Brasil; ⁴UFPI, PPG Ciências e Saúde, Brasil; ⁵UFPI, Biomedicina, Brasil; ⁶UFPI, PPG Ciências Farmacêuticas, Brasil; ⁷Universidade Federal do Maranhão, Brasil; ⁸UEM, PPG Farmacologia, Brasil

10.044 **In Vitro Antiproliferative Activity of Oleoresin, Volatile and Resinous Fractions of *Copaifera reticulata* Ducke from the Tapajós National Forest – Belterra/Pará.** Caetano-Frota JK^{1,2}, Oliveira JC¹, Pontes RS¹, Sartoratto A³, Marsaioli AJ⁴, Oliveira ASS³, Freitas AS¹, Silva MC⁵, Oliveira ECP⁶, Foglio MA¹, Ruiz ALTG¹, Carvalho JE¹ ¹UNICAMP, Lab Phytochemistry, Pharmacology, and Experimental Toxicology, Brazil, ²UFOPA, Institute of Water Science and Technology, Brazil, ³Unicamp, Multidisciplinary Center for Chemical, Biological, and Agricultural Research, Brazil, ⁴Unicamp, Lab Biocatalysis and Organic Synthesis, Brazil, ⁵ IFPA, Postgraduate Program in Environment and Development, Brazil, ⁶ UFOPA, Lab Biotechnology of Medicinal Plants, Brazil

11. Clinical Pharmacology, Pharmacokinetics, Pharmacogenomics and Toxicology

Totem 14

11.024 **Meropenem Dosing Regimens in Critically Ill Pediatric Patients Undergoing Continuous Renal Replacement Therapy.** Vieira JW¹, Matsuno VK¹, Delgado AF², Benzi JRL¹ ¹Dept Pharmacy, School of Pharmaceutical Sciences, University of São Paulo, São Paulo, SP, Brazil. ²Pediatric Critical Care Unit, Dept Pediatrics, Faculty of Medicine, University of São Paulo, São Paulo, Brazil

11.025 **Pharmacokinetic Evaluation and Marker Residue Determination of Topical Fipronil in Cattle.** Ferreira SM¹, Gotardo AT¹, Raspantini LER¹, Barreto F² ¹CEPTOX-FMVZ-USP Research Centre for Veterinary Toxicology, Dept Pathology, Pirassununga, Brazil, ²LFDA-RS, Federal Agricultural Defense Lab, Ministry of Agriculture and Livestock, Porto Alegre, Brazil

11.026 **Effects of Low-Dose DEHP Exposure on Brain Function and Behavior: Gut Microbiota as a Potential Modulator of Its Toxicity.** Silva LN¹, Carvalhinho-Lopes PS², Santiago MSA¹, Monteiro LA²,

Ribeiro AM², Perobelli JE¹ ¹UNIFESP, Lab Experimental Toxicology, Brazil, ²UNIFESP, Lab Neuroscience and Bioprospecting of Natural Products, Brazil

11.027 Locomotion Parameters as Indicators of Toxicity: Effects of Nanoencapsulated Cinnamon Essential Oil in Adult Zebrafish (*Danio rerio*). Pimentel JRL¹, Barros JM², da Silva ACF¹, Chamberlain ALV¹, Stadinik MJ³, Müller LG⁴, da Costa C^{2,5}, Siebel AM^{1,6} ¹UFPR, PPG Pharmacology, Brazil ²UFSC, PPG Chemical and Food Engineering, Brazil ³UFSC, PPG Plant Sciences, Brazil ⁴Institute of Experimental and Technological Biology, Portugal ⁵UFSC, PPG Textile Engineering, Brazil ⁶FURG, PPG Health Science, Brazil

11.028 A LC-MS/MS Method for the Simultaneous Determination of Nicotine and Cotinine in Mice's Plasma and Brain Samples after Exposure to Vaping Compared to Cigarette Smoking. Rocha, BM¹, Santos, GCM^{1,2}, Santos, HBS², Nobrega, MEG¹, Gomes, HS¹, Cotias, AC¹, Silva, PMR¹, Martins, MA^{1,2} ¹IOC-Fiocruz, Lab Inflammation, ²Fiocruz, Preclinical Pharmacokinetic Platform, Rio de Janeiro – Brazil

11.029 Adverse Reaction to COVID-19 Vaccines in Healthcare Professionals in Brazil: An Analysis of the Associated Factors at the SEVACOV-PRO Study. Rufino LG^{1,3}, Silva DLM^{1,2}, Duarte DB^{1,3} ¹UnB, Lab Pharmacological Assays, Dept Pharmacy, School of Health Sciences, Brasília, Brazil ²HUB-UnB, University of Brasília Hospital, Brasília, Brazil ³UnB, Graduate Program in Tropical Medicine, School of Medicine, Brasília, Brazil

12. Drug Discovery and Development

Totem 23

12.014 Mechanistic Modeling of Biopharmaceutics Data to Enhance the Bioavailability of a Weakly Basic Drug: Ketoconazole–Succinic Acid Cocrystal Mitigates pH-dependent Absorption. Goes AKS^{1,2}, Soliman A^{1,3}, Olivera PR², de Moraes NV¹ ¹University of Florida, College of Pharmacy, Dept. of Pharmaceutics, Center for Pharmacometrics and Systems Pharmacology, USA ²Unicentro, Dept Pharmacy, Guarapuava, PR, Brazil. ³Helwan University, School of Pharmacy, Dept Pharmacy Practice, Egypt

12.015 Beyond Solubility: How Nanoencapsulation Boosts Seriniquinone Pharmacological Activity. Miguel RA¹, Hirata AS¹, Garnique AMB¹, de Jesus DFF², de Melo GB¹, Barroso VM², Ishida K², Machado-Neto JA¹, Machado-Santelli GM³, Lepique AP⁴, Martins TS⁵, Lopes LB¹, Costa-Lotufo LV¹ ¹USP, PPG Functional and Molecular Biology, Brazil, ²USP, PPG Microbiology, Brazil, ³USP, PPG Systems Biology, Brazil, ⁴USP, PPG Immunology, Brazil, ⁵Unifesp, PPG Chemistry, Brazil

12.016 Overcoming Drug Resistance in Osteosarcoma: Investigating Palladium-oxime Complexes as Potential Antitumor Agents. Pereira THR¹, Guarache GC¹, Moura TR², Godoy Netto AV², Bincoletto C¹ ¹Unifesp-EPM, PPG Pharmacology, Brazil. ²Unesp, Institute of Chemistry, Brazil

12.017 Drug Discovery: New PAD-4 Inhibitors HITs for the Treatment of Inflammatory Diseases. Augusto PSA, Costa VF, Cunha TM, Cunha, FQ FMRP-USP

12.018 AI-Driven Analysis Reveals Macrophage Reprogramming by TPPU for Anti-inflammatory Therapeutic Repurposing. Rodrigues WF¹, Miguel CB², Leite MS¹, Maraia ME³, Lazo-Chica JE⁴, Soares SC², Góes-Neto A¹, Santos MA¹ ¹UFMG, Postgraduate Program in Bioinformatics, Belo Horizonte, MG, Brazil ²UFTM, Postgraduate Program in Tropical Medicine and Infectious Diseases, Uberaba, MG, Brazil ³UFTM, Undergraduate Program in Biomedicine, Uberaba, MG, Brazil ⁴UFTM, Lab Cell Biology, Dept Structural Biology, Institute of Biological and Natural Sciences, Uberaba, MG, Brazil

12.019 The role of ERO1 α as a drug target for the inhibition of cellular proliferation. Aljofan M, Khojayeva K, Moldeshva A. Nazarbayev University, Dept Biomedical Sciences, School of Medicine, Kazakhstan

1º Simpósio de Inovação e Produtos Naturais Rumo a Novos Medicamentos (1st Symposium on Innovation and Natural Products Towards New Medicines)

Lectures

Research and Development of New Drugs from the Amazon Biome (Pesquisa e Desenvolvimento de Novos Fármacos do Bioma Amazônico). José Carlos Tavares Carvalho - Universidade Federal do Amapá - UNIFAP

Research and development of new drugs from the Amazon biome constitutes a strategic area for pharmaceutical innovation and the strengthening of the national bioeconomy. The Amazon, home to approximately 20% of known plant species, offers unique potential for the discovery of bioactive metabolites with therapeutic applications in infectious, inflammatory, chronic, and neglected diseases. This field of research integrates knowledge from ethnopharmacology, nanotechnology, metabolomics, and translational pharmacology, aiming to transform natural compounds into effective and safe pharmaceutical products. The sustainable use of natural resources, combined with the valorization of traditional knowledge and the use of advanced biological screening and molecular modeling technologies, has driven the development of innovative phytopharmaceuticals and nutraceuticals. Advances in this area contribute significantly to the country's scientific and technological sovereignty, promoting biodiversity conservation and generating positive impacts on public health, social inclusion, and sustainable regional development. (A pesquisa e desenvolvimento de novos fármacos a partir do bioma amazônico constitui uma área estratégica para a inovação farmacêutica e o fortalecimento da bioeconomia nacional. A Amazônia, que abriga cerca de 20% das espécies vegetais conhecidas, oferece um potencial único para a descoberta de metabólitos bioativos com aplicações terapêuticas em doenças infecciosas, inflamatórias, crônicas e negligenciadas. Este campo de investigação integra conhecimentos da etnofarmacologia, nanotecnologia, metabolômica e farmacologia translacional, visando transformar compostos naturais em produtos farmacêuticos eficazes e seguros. A utilização sustentável dos recursos naturais, aliada à valorização do conhecimento tradicional e ao emprego de tecnologias avançadas de triagem biológica e modelagem molecular, tem impulsionado o desenvolvimento de fitofármacos e nutracêuticos inovadores. Os avanços nesta área contribuem significativamente para a soberania científica e tecnológica do país, promovendo a conservação da biodiversidade e gerando impactos positivos na saúde pública, inclusão social e desenvolvimento regional sustentável.)

Symposia

SS1 – New Approaches in Bioprospecting (Novas Abordagens em Bioprospecção)

Zebrafish Model: Importance in the Discovery of Bioactive Molecules (Modelo Zebrafish: Importância na Descoberta de Moléculas Bioativas). Monica Valdyrce dos Anjos Lopes Ferreira (IBu)

Zebrafish possess advantageous characteristics that make them a valuable experimental model. With the growing demand for natural products in the development of functional foods, nutraceuticals, natural cosmetics, and bioactive molecules, zebrafish have emerged and been validated as an unprecedented tool for rapidly and economically investigating the bioactivities and safety of natural products with potential benefits for human health. Financial Support: FAPESP, Butantan Foundation (O zebrafish possui características vantajosas que o torna um valioso modelo experimental. Com a crescente demanda por produtos naturais *no desenvolvimento* de alimentos funcionais, nutracêuticos, cosméticos naturais, moléculas bioativas, o zebrafish surgiu e foi validado como uma ferramenta sem precedentes para investigar rápida e economicamente as

bioatividades e a segurança dos produtos naturais com potenciais benefícios à saúde humana. Suporte Financeiro: FAPESP, Fundação Butantan)

Drug Development from Microbial Biodiversity (Desenvolvimento de Fármacos a Partir da Biodiversidade Microbiana). Spartaco Astolfi Filho, Divisão de Biotecnologia, Centro de Apoio Multidisciplinar, UFAM

A história tem mostrado que a microbiota é uma fonte riquíssima de metabólitos secundários e enzimas com as mais diversas aplicações industriais, inclusive na área farmacêutica. Os microrganismos produzem uma ampla variedade de substâncias com propriedades antimicrobianas, antivirais, anti-helmínticas, anticancerígenas, anti-inflamatórias e outras propriedades terapêuticas. Nessa palestra serão apresentadas diferentes abordagens utilizadas na prospecção de novos fármacos partir da biodiversidade microbiana e como exemplo será mostrada a rota de desenvolvimento da tecnologia de produção de collagenase pela Cristália Produtos Químicos Farmacêuticos Ltda. *(History has shown that the microbiota is a rich source of secondary metabolites and enzymes with a wide range of industrial applications, including pharmaceuticals. Microorganisms produce a wide variety of substances with antimicrobial, antiviral, anthelmintic, anticancer, anti-inflammatory, and other therapeutic properties. This lecture will present different approaches used in the exploration of new drugs from microbial biodiversity, and, as an example, will demonstrate the development path of collagenase production technology by Cristália Produtos Químicos Farmacêuticos Ltda.)*

SS2 – Pesquisa Pré-Clínica para o Desenvolvimento de Fármacos (Preclinical Research for Drug Development)

The Challenges of Proof of Concept on the Bench to Scale-Up and Production (Os Desafios da Prova de Conceito em Bancada ao Escalonamento e Produção). Elizabeth Angelica Leme Martins (IBu)

Process development for any product goes through the phases of idea conception, small-scale product generation, and purification with sufficient quality for proof of concept. This phase begins with market, cost, and economic feasibility studies. Concurrently, the development of a robust and reproducible production model begins, which will then be scaled up to the quantities required for testing, analytical quality tests, reproduction of proof of concept tests, and preclinical testing. Production conducted under Good Lab Practices will proceed to pilot scale in a "Good Manufacturing Practices" environment, required for clinical trial proposals. Specialists must propose test quantities and analysis methods in phases I, II, and III. In this way, pilot production can guide industrial production when the product proves interesting in clinical trials. It is also important to design a robust quality system around equipment and process validation in compliance with all applicable regulatory standards. We will discuss these aspects of process development and product differences. Financial Support: Butantan Foundation *(O desenvolvimento de processo para obtenção de qualquer produto passa pelas fases de concepção da ideia, geração de produto em pequena escala, purificação com qualidade suficiente para provas de conceito. Nesta fase começam os estudos de mercado, custos e viabilidade econômica. Paralelamente, inicia-se o desenvolvimento de um modelo robusto e reprodutivo de produção, o qual será então escalonado para quantidades necessárias para testes, provas analíticas de qualidade, reprodução das provas de conceito e testes pré-clínicos. A produção realizada em Boas Práticas de Laboratório seguirá para escala piloto em ambiente "Boas Práticas de Fabricação", requeridas para proposta de testes clínicos. Os especialistas devem propor quantidade de testes e formas de análises em fases I, II e III. Dessa forma, a produção piloto pode desenhar a produção industrial quando o produto se mostra interessante nos testes clínicos. Importante o desenho robusto também do sistema de qualidade em torno de equipamentos, validação de processos no cumprimento de todas as normas regulatórias impostas. Conversaremos sobre esses aspectos dos desenvolvimentos de processos e diferenças entre produto. Suporte Financeiro: Fundação Butantan)*

Avanços no Estabelecimento de uma Rede Pré-clínica no Instituto Butantan. Durvanei Augusto Maria (IBu)

Preclinical studies are crucial to ensuring the safety, efficacy, and quality of new drugs, vaccines, and advanced therapies before they are used in humans. They allow for the assessment of potential adverse effects, safe doses, and mechanisms of action, helping to prevent risks and optimize results for subsequent phases of human clinical research. Furthermore, they provide essential data for obtaining regulatory approvals and ensuring that the developed products are reliable and safe for future clinical phases. (A importância dos estudos pré-clínicos é fundamental para garantir a segurança, eficácia e qualidade de novos fármacos, vacinas e terapias avançadas antes de serem utilizados em humanos. Permitem avaliar possíveis efeitos adversos, doses seguras e mecanismos de ação, ajudando a prevenir riscos e a otimizar os resultados para as fases subsequentes da pesquisa clínica em humanos. Além disso, fornecem dados essenciais para obter aprovações regulatórias e garantir que os produtos desenvolvidos sejam confiáveis e seguros para futuras fases clínicas. Suporte Financeiro: FAPESP, Fundação Butantan

The Challenges of Developing a Project from the Bench to Clinical Trial (Os desafios de desenvolver um projeto da bancada ao estudo clínico). Lucindo José Quintans Júnior (UFS)

The lecture "The Challenges of Developing a Project from the Bench to Clinical Trial" will address the stages and obstacles in translational research involving medicinal plants from the folk medicine of Northeast Brazil. It will highlight the importance of in vitro and in vivo preclinical studies to assess safety, efficacy, and mechanisms of action. Translation to clinical trials requires standardization of extracts, proof of toxicological safety, and rigorous scientific validation. Ethical and logistical issues will be discussed, highlighting the need for institutional partnerships and adequate infrastructure. The process seeks to integrate traditional knowledge and science, aiming for the qualified inclusion of phytotherapeutics in the Unified Health System (SUS) or for veterinary use. *(A palestra "Os desafios de desenvolver um projeto da bancada ao estudo clínico" abordará as etapas e obstáculos na pesquisa translacional com plantas medicinais da medicina popular do Nordeste brasileiro. Destacará a importância dos estudos pré-clínicos in vitro e in vivo para avaliar segurança, eficácia e mecanismos de ação. A translação para o estudo clínico exige padronização de extratos, comprovação de segurança toxicológica e validação científica rigorosa. Serão discutidas questões éticas e logísticas, ressaltando a necessidade de parcerias institucionais e infraestrutura adequada. O processo busca integrar saberes tradicionais e ciência, visando a inserção qualificada de fitoterápicos no SUS ou no uso veterinário.)*

SS3 – Farmacologia Geral, Celular, Molecular e Bioquímica (General, Cellular, Molecular and Biochemical Pharmacology)

From Animal Toxins to Plant Compounds: Antitumor Action and its Translational Potential (Das Toxinas Animais aos Compostos Vegetais: Ação Antitumoral e seu Potencial Translacional). Paulo Michel Pinheiro Ferreira UFPI

Microorganisms, algae, plants, and animals are nearly inexhaustible sources of inspiration for drug development. Thus, even compounds considered toxic have great potential for treating diseases, including chronic and multifactorial ones. In this context, molecules such as terpenes and bufadienolides, although originating from different chemical classes and biosynthetic pathways, may have a common pharmacological action: killing tumor cells. Fractions and compounds from *Casearia sylvestris*, *Mimosa caesalpiniiifolia*, and frogs of the genus *Rhinella* have demonstrated cytotoxic and antiproliferative action, with cell cycle arrest and apoptotic death of tumor cell lines in vitro, and antitumor activity in vivo in various animal models. Such preclinical studies highlight the importance of understanding lead molecules in the fight against cancer. (Microorganismos, algas, plantas e animais, são fontes quase inesgotáveis de inspiração para o desenvolvimento de medicamentos. Assim, mesmo compostos considerados tóxicos têm grande potencial para tratar enfermidades, inclusive doenças de base crônica e multifatorial. Nesse contexto, moléculas como os terpenos e os bufadienolídeos, embora originadas de diferentes classes químicas e vias biossintéticas, podem ter uma ação farmacológica em comum: matar células tumorais. Frações e compostos de *Casearia sylvestris*, *Mimosa caesalpiniiifolia* e de sapos do gênero *Rhinella* têm

revelado ação citotóxica, antiproliferativa, com parada de ciclo celular e morte por apoptose de linhagens tumorais *in vitro* e ação antitumoral *in vivo* em diferentes modelos animais. Tais estudos pré-clínicos destacam a importância de se conhecer moléculas-líder na luta contra o câncer)

Oil from the Visceral Fat of the Pantanal Alligator (*Caiman yacare*) as a Cardiorenal Protector: an Alternative Source to Polyunsaturated Fatty Acids from Cold-Water Marine Fish? Arquimedes Gasparotto Junior UFGD

Caiman yacare, commonly known as the Pantanal alligator (*Jacaré-do-Pantanal* in Portuguese), is the most prevalent alligator species in the Brazilian states of Mato Grosso and Mato Grosso do Sul. Research has shown that Pantanal alligator fat oil is rich in various fatty acids, particularly omega-6. Fat oil derived from various *Caiman* species has been traditionally used in Brazil to prevent and treat cardiovascular complications, including stroke and thrombosis. Despite its widespread traditional use, there is currently no scientific data confirming the efficacy of these preparations. This study aims to evaluate the potential cardiovascular protective effects of long-term treatment with *C. yacare* fat oil (YO) in hypertensive rats with multiple cardiovascular risk factors. Male spontaneously hypertensive rats (SHR) received an atherogenic diet for 8 weeks to induce cardiovascular and metabolic dysfunction. Treatments with YO (19, 56, and 168 mg/kg), rosuvastatin (5 mg/kg), and fish oil (56 mg/kg) started after 4 weeks and continued for 4 weeks. Renal function, blood pressure, and cardiac activity were monitored. Serum lipids, inflammatory and oxidative stress markers, as well as liver and kidney function parameters, were assessed. Vascular reactivity of mesenteric vascular beds was evaluated, and tissue samples from the kidneys, heart, and arteries were collected for histopathological analyses. Treatment with YO at 168 mg/kg significantly reduced serum lipids, urea, creatinine, and oxidized LDL levels while preventing arterial endothelial dysfunction and intima-media thickening. The data obtained in our study showed that the presence of unsaturated fatty acids in *C. yacare* fat oil is associated with a partial reduction in cardiovascular risk in hypertensive dyslipidemic rats. Renoprotective effects were observed through a decrease in serum urea and creatinine levels, alongside an improvement in endothelial function. While the oil's ability to reduce cardiovascular risk factors, including its effects on VLDL-C, LDL-C, and oxLDL, is promising, its impact on tissue redox state and systemic inflammation remains unclear. Further preclinical *in vivo* studies are necessary to fully elucidate the benefits and risks associated with the use of YO, particularly in the context of cardiovascular diseases. This research was funded by the Fundação de Apoio ao Desenvolvimento do Ensino, Ciência e Tecnologia do Estado de Mato Grosso do Sul (FUNDECT, Brazil; no. 83/013.186/2023), the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, Brazil; no. 305742/2022-1), and the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Brazil; process number 88887.910272/2023-00 and Finance Code 001).

Use of Wine Industry Waste in the Treatment of High Blood Pressure. Fernanda Regina Casagrande Giachini Vitorino (UFMT)

Wine by-products, particularly those rich in polyphenols, have garnered attention for their potential in supporting cardiovascular health. Their antioxidant properties play a crucial role in mitigating oxidative stress, a key contributor to hypertension and related cardiovascular disorders. As interest grows in alternative and adjunctive therapies, natural compounds with bioactive potential are increasingly being explored as candidates for novel antihypertensive treatments. This emerging area of research underscores the importance of comprehensive scientific evaluation to establish the therapeutic efficacy and safety profiles of these natural interventions. This study aimed to identify and quantify the phenolic compounds present in Syrah (*Vitis vinifera*) grape pomace, and to evaluate the effects of its dietary supplementation on blood pressure, vascular reactivity, and cardiac remodeling. Grape pomace was subjected to identification and quantification using HPLC-DAD-ESI-MSn. Normotensive male rats (Wistar) and spontaneously hypertensive rats (SHR) received grape pomace-enriched (300 mg/kg/day, 14 days) or standard chow. Indirect arterial pressure was assessed indirectly by tail plethysmography. Endothelium-dependent and -

independent relaxation, as well as vascular contractile response were evaluated in mesenteric resistance arteries. Histological procedures were done in the heart to evaluate the number of cardiomyocytes, intercellular spaces, cardiomyocyte diameter, collagen deposition. Pomace samples exhibited higher total phenolic compound concentrations and five derivatives of hydroxycinnamic acids and twelve flavonols were identified. Quercetin-3-glucoside and ethyl caffeate were the most abundant phenolic compounds. Grape pomace-enriched chow promoted hypotensive effect [diastolic blood pressure (mmHg) 144 ± 1.2 vs. 165 ± 0.8 non-treated SHR]. Supplementation improved the endothelium-dependent ($E_{\max}$ $93.7 \pm 4.1\%$ vs. $73.3 \pm 3.1\%$ non-treated SHR), but not independent, relaxation, as well as reduced phenylephrine induced contraction ($E_{\max}$ 23.8 ± 1.8 vs. 30.2 ± 1.8 non-treated SHR) in resistance arteries from SHR. There were no changes in the number of cardiomyocytes or in the number of intercellular spaces between the groups evaluated. However, SHR showed augmented cardiomyocyte diameter (μm), compared to the normotensive group (11.0 ± 0.02 vs 9.8 ± 0.02 respectively), and grape pomace supplementation reverted it in the hypertensive treated rats (9.4 ± 0.18). Greater collagen deposition was observed in the SHR group compared to the normotensive control group ($33.6 \pm 1.8\%$ vs. $23.9 \pm 2.3\%$ respectively), and grape pomace-treatment evoked the reduction of deposited collagen ($30.2 \pm 0.5\%$). SHR rats showed greater deposition of type I collagen compared to the Wistar group, and grape pomace treatment reduced type I collagen in the hypertensive rats. The high amounts of flavonols and hydroxycinnamic acids found in the grape pomace contributed both to the hypotensive and cardioprotective effects observed in hypertensive rats submitted to chow-enrichment.

SS4 – Farmacologia Clínica e os Desafios Translacionais (Clinical Pharmacology and Translational Challenges)

Perspectives on the Application of Bioactives from Amazonian Biodiversity in the Development of New Medicines (Perspectivas da Aplicação de Bioativos oriundos da Biodiversidade Amazônica no Desenvolvimento de Novos Medicamentos). Emerson Silva Lima Faculdade de Ciências Farmacêuticas-UFAM

The Amazon's biodiversity is rich in flora and fauna and can be explored for the development of active molecules or extracts rich in bioactives for human health. Scientific research in the Amazon has been ongoing for over 30 years, with institutions dedicated to studying this biodiversity. Amazonian bioactives can be applied to a wide range of human health needs, including nutritional aspects, cosmetic applications, and medicinal applications such as herbal medicines or phytopharmaceuticals. In the pharmaceutical field, many of these bioactives have been developed for the prevention or treatment of chronic non-communicable diseases such as diabetes, obesity, and chronic inflammation. The lecture will present some practical results of the speaker's research on active ingredients studied for the prevention or treatment of conditions such as diabetes, obesity, non-alcoholic fatty liver disease, fatty liver disease, and chronic inflammatory processes. ((A biodiversidade amazônica é rica enquanto a sua flora e sua fauna e pode ser explorada quanto ao desenvolvimento de moléculas ativas ou extratos ricos em bioativos aplicados a saúde humana. A pesquisa científica na Amazônia tem mais de 30 anos com instituições dedicadas ao estudo dessa biodiversidade. Os bioativos amazônicos podem ser aplicados as mais diversas necessidades da saúde humana, como aspectos nutricionais, aplicações em cosméticos e também em medicamentos como fitoterápicos ou fitofármacos. Na área de medicamentos muitos desses bioativos tem sido desenvolvidos para prevenção ou tratamento de doenças crônicas não-transmissíveis como diabetes, obesidade e inflamações crônicas. A palestra apresentará alguns resultados práticos da pesquisa do palestrante com ativos estudados na prevenção ou tratamento de agravos como diabetes, obesidade, doença gordurosa não-alcoolica, esteatose hepática e processos inflamatórios crônicos.)

The Challenges of Translational Research in Brazil: From Idea to Product (Os Desafios da Pesquisa Translacional no Brasil: Da Ideia a Produto). Adriano Antunes de Souza Araújo. UFS

Translational research involves a process known as knowledge translation, in which the focus of scientific investigation acquires a bidirectional flow of information between basic and clinical research, resulting in the real applicability of knowledge and new technologies with clinical application in new therapeutic concepts, providing direct health benefits. Over the past twenty years, our research group has focused on developing new products using primarily pharmaceutical nanotechnology, focusing on skin lesions and inflammatory and autoimmune diseases. Thus, the development of biomaterials that accelerate healing dynamics in burn patients; the development of topical products containing drugs impregnated into tissues for chronic venous insufficiency; and the development of screening tests to aid in the diagnosis of peripheral neuropathies using a computational solution, including a smartphone app that assesses membrane reactions through colorimetric quantification in a practical, accurate, and rapid manner. The main results show that the topical application of membranes or textile materials containing drugs incorporated into liposomes and nanocapsules modulates important events in the healing process, particularly by stimulating the development and maturation of the granulation reaction and substantially increasing collagen deposition. Furthermore, these nanosystems control drug penetration into the skin, increasing efficacy and serving as important colorimetric markers for rapid neuropathy testing. Therefore, through this presentation, we aim to demonstrate that continued investment in this area is essential to translate Lab innovations into tangible clinical benefits. (A pesquisa translacional envolve um processo conhecido por translação do conhecimento, no qual o foco da investigação científica adquire um fluxo de informação bidirecional entre as pesquisas básica e clínica, resultando na aplicabilidade real do conhecimento e novas tecnologias com aplicação clínica em novos conceitos terapêuticos proporcionando benefícios diretos para saúde. Nos últimos vinte anos nosso grupo de pesquisa tem se voltado para o desenvolvimento de novos produtos utilizando principalmente a nanotecnologia farmacêutica com foco nas lesões sobre a pele e doenças inflamatórias e autoimunes. Desta forma o desenvolvimento de biomateriais que atuam acelerando a dinâmica cicatricial para pacientes queimados; o desenvolvimento de produtos tópicos contendo fármacos impregnados em tecidos aplicados a insuficiência venosa crônica, assim como o desenvolvimento de testes de triagem para auxiliar no diagnóstico de neuropatias periféricas a partir de uma solução computacional, incluindo um aplicativo para smartphones, o qual avalia a reação de membranas por meio da quantificação colorimétrica, de modo prático, preciso e rápido, tem sido a tônica do nosso grupo de pesquisa. Os principais resultados mostram que a aplicação tópica de membranas ou materiais têxteis contendo fármacos incorporados em lipossomas e nanocápsulas modulam eventos importantes do processo cicatricial, representados especialmente por estímulo ao desenvolvimento e maturação da reação de granulação e incremento substancial da deposição colagênica. Além disso, esses nanossistemas controlam a penetração de fármaco na pele aumentando a eficácia e sendo importantes marcadores colorimétricos para o teste rápido de neuropatias. Desta forma, através desta apresentação pretendemos demonstrar que investimentos contínuos nessa área são essenciais para traduzir inovações laboratoriais em benefícios clínicos tangíveis.)

Development of Recombinant Onco BCG Against Bladder cancer (Desenvolvimento da Onco BCG Recombinante Contra Câncer de Bexiga). Luciana Cezar de Cerqueira Leite (IBU)

Our laboratories have developed an improved bladder cancer treatment based on a recombinant BCG strain. A new strain without the antibiotic resistance gene was constructed through auxotrophic complementation for human use, and a seed bank was produced under Good Manufacturing Practices (GMP). A bioreactor production process was developed in compliance with GMP standards. Clinical batches of the product are being produced under GMP, and clinical trials are outlined. This trajectory will be discussed. Financial Support: FAPESP, CNPq, BNDES, Butantan Foundation. Nossos laboratórios desenvolveram um tratamento aprimorado contra câncer de bexiga baseado numa cepa de BCG recombinante. Foi construída uma nova cepa sem gene de resistência a antibióticos pela complementação auxotrófica para uso em humanos e produzido um banco semente em Boas Práticas de Fabricação (BPF). Foi desenvolvido um

processo de produção em biorreatores, em conformidade com a produção em BPF. Estão sendo produzidos os Lotes clínicos de produto em BPF e os ensaios clínicos estão delineados. Esta trajetória será discutida. Suporte Financeiro: FAPESP, CNPq, BNDES, Fundação Butantan.

Courses

Cr1 – Principles of Systematic Review (Princípios de Revisão Sistemática)

Overall Concepts of Systematic Review (Conceitos gerais de revisão sistemática). Adriane Ribeiro Rosa (UFRGS)

A systematic review is a robust and structured methodological approach for identifying, evaluating, and synthesizing all available relevant evidence on a specific research question. Unlike traditional narrative reviews, this type of review follows rigorous, predefined, and transparent steps, aiming to minimize bias and provide a reliable scientific basis for clinical, political, and academic decision-making. This introductory lecture will cover the fundamentals of systematic reviews, including their origins, purposes, and their growing role in evidence-based medicine and other areas of science. The principles governing this type of study will be presented, such as the need for a well-defined research question, the importance of a prior protocol, and the standardization of study inclusion and exclusion criteria. The distinction between qualitative and quantitative systematic reviews will also be discussed, as well as the integration of meta-analyses into the synthesis process.

Research Question, Eligibility and Data Extraction (Questão de pesquisa, elegibilidade dos estudos e extração de dados). Patrícia Martins Bock (UFRGS)

Properly defining the research question is the essential starting point for any successful systematic review. In this class, we will explore formulating structured questions using models such as PICO (Population, Intervention, Comparison, and Outcome), which help guide the search and selection of evidence. Once the question has been defined, the next step is to establish clear eligibility criteria, which determine which studies will be included or excluded from the review. These criteria are crucial to ensuring the consistency, reproducibility, and relevance of the results obtained. Strategies for objectively defining inclusion and exclusion criteria will be discussed, considering aspects such as study type, target population, evaluated interventions, outcomes, and language. The class will also cover in detail the data extraction process, which should be conducted in a standardized manner and generally by independent reviewers to ensure accuracy and minimize errors. Examples of extraction forms, best practices for data collection, and ways to deal with missing or poorly reported data will be presented.

Bias Risk, Concepts and Interpretation of Meta-Analysis (Risco de Viés, conceitos e interpretação de metanálise). Patrícia Martins Bock (UFRGS)

Assessing the risk of bias in studies included in a systematic review is a critical step in correctly interpreting its results. This class will address the main types of bias that can compromise the validity of findings, such as selection bias, performance bias, detection bias, attrition bias, and reporting bias. Widely recognized tools, such as the Cochrane Risk of Bias Tool and ROBINS-I, will be presented, highlighting their practical application and limitations. Based on the bias assessment, the discussion will focus on confidence in the available evidence and its usefulness in clinical or scientific practice. The second part of the class will focus on the fundamentals of meta-analysis, a statistical technique that allows combining the results of independent studies to obtain a more accurate estimate of the effect of an intervention or combination. Key concepts such as heterogeneity, fixed vs. random models, effect size, confidence interval, and interpretation of forest plots will be explained. The class will also discuss how to critically interpret the results of a meta-analysis, recognizing its limitations and potential publication bias.

Cr2 – Applications of Physiology-Based Pharmacokinetics in the Drug Development (Aplicações de Farmacocinética Embasada em Fisiologia na área de desenvolvimento de novos fármacos)

Key-Parameters in PBPK Model Building: Volume of Distribution and Clearance. Parâmetros chave em PBPK: volume de distribuição e clearance). Natalia de Moraes Center for Pharmacometrics and Systems Pharmacology, College of Pharmacy, University of Florida, Orlando, Florida, USA

Physiologically-Based Pharmacokinetic (PBPK) models are essential tools in drug development and regulatory science, providing a mechanistic framework to predict the absorption, distribution, metabolism, and excretion of compounds in various biological systems. This lecture will explore two critical parameters in PBPK model building: Volume of Distribution (Vd) and Clearance (CL). Volume of Distribution is a fundamental concept that describes the extent to which a drug disperses into body tissues relative to plasma. Understanding Vd is crucial for determining the loading dose and interpreting plasma concentration data. Clearance, on the other hand, represents the body's efficiency in eliminating a drug, encompassing renal, hepatic, and other pathways. Accurate estimation of CL is vital for predicting drug half-life and optimizing dosing regimens. Through this lecture, we will explore the theoretical underpinnings of Vd and CL, their roles in PBPK modeling, and practical approaches for parameter estimation. Through case studies, the course will highlight strategies for in vitro in vivo extrapolation, scaling across populations, and parameter identification. Attendees will gain insights into the challenges and advancements in PBPK modeling, fostering a deeper understanding of its significance in clinical pharmacology and drug development.

Real-World Impact of PBPK: Applications Across Drug Development (Impacto da PBPK no mundo real: aplicações no desenvolvimento de fármacos). Valva Vozmediano (Model Informed Development, Clinical Trial and Consulting Services, USA)

Physiologically-based pharmacokinetic (PBPK) modeling is now an essential part of model-informed drug development (MIDD), enabling the translation of mechanistic knowledge into quantitative predictions that inform decision-making across all phases of drug development. This lecture will highlight real-world applications of PBPK that demonstrate its value in supporting efficient and informed development strategies. The presentation will begin with a case study showcasing how PBPK was used to integrate preclinical data to predict human pharmacokinetics and anticipate drug-drug interactions (DDIs) for a planned Phase II clinical trial. By leveraging in vitro and in vivo inputs, and accounting for enzyme and transporter-mediated processes, PBPK modeling enabled proactive identification of potential interaction liabilities and informed clinical protocol design—reducing development risk and timelines. The second example will focus on the application of PBPK modeling to predict target-site disposition in the central nervous system (CNS). In this context, PBPK supported the estimation of brain exposure using a physiological brain models. This approach was particularly valuable in the development of neurotherapeutics, where direct CNS measurements are often unavailable or infeasible. The use of PBPK modeling, allowed the prediction of levels at the site of action and allowed to inform and optimize the formulation strategy as well as to understand the contribution of absorption pathways in brain exposure. Finally, we will discuss the application of PBPK in evaluating DDIs in pediatric populations. Pediatric drug development is often constrained by ethical and logistical limitations, making PBPK a powerful tool to extrapolate interaction risks by incorporating developmental physiology and enzyme maturation. This case illustrates how PBPK supported safe and rational dose selection in children and provided regulatory confidence without requiring extensive clinical DDI studies. These examples underscore the practical impact of PBPK modeling when used strategically-bridging knowledge gaps, informing clinical development plans, and supporting regulatory interactions.

Cr3 – Neuropsychopharmacology of Reinforcement and Reward Behaviors (Neuropsicofarmacologia de Comportamentos de Reforço e Recompensa)

Neuropsychopharmacology of Reinforcement and Reward Behaviors. Cristina A J Stern (UFPR, Curitiba, PR), Luana Freese (Northwestern Univ, Chicago, Ill), Carine Lampert (Northwestern Univ, Chicago, Ill), Helena M T Barros (UFCSPA, Porto Alegre, RS)

The neuropsychobiology of aversive and rewarding behaviors connected to mental health issues reflects a dynamic and multidisciplinary field that incorporates neuroscience, pharmacology, psychiatry, and behavioral science. This evolving area of research considers not only the biological

view of behavior but also the individual variability in reward and aversive responsiveness tied to sex, age, developmental stage, and environmental exposure. This course will explore the state of the art related to "nature and nurture," reviewing genetic and environmental factors on addictions and drug dependence. Even though the core reward system remains centered on the mesolimbic dopamine pathway, many other brain areas and neuromodulators are involved. These include findings from pharmacological studies using novel biased agonists, allosteric modulators, and interactive modulation of reward and aversive circuits. This course will discuss a) molecular insights into the genetics of risk alleles for addiction and aversive sensitivity, b) the epigenetics linked to long-term adaptations to drug exposure and stress, c) animal learning models and how the phases of memory maintenance might be explored as important opportunities for pharmacological interventions, d) clinical and translational implications related to learning and memory aspects, regarding comorbidities and application of the role of the neurobiology of reward and reinforcement, in the pharmacotherapies for addiction, polysubstance use, and comorbidity with other psychiatric disorders, such as post-traumatic stress disorder. Finally, ethical concerns around pharmacologically changing motivation or pleasure and the need for better animal models that reflect complex human behaviors will empower and engage the audience's participation. In the context of "The New Era of One Health," this course will contribute to a broader understanding of the interconnectedness between individuals and their environments, highlighting how mental health is connected to the individual experiences, regarding the physical environments, and the social human interactions.

Cr4 – Experimental Models in Cancer and their Interface with Environmental Pollutants. Débora Barbosa Vendramini Costa^{1,2}, Juliana Elaine Perobelli³. ¹Henry Ford Pancreatic Cancer Center, Henry Ford Health, Detroit MI, USA, ²Michigan State University, Lansing MI, USA, ³Unifesp Baixada Santista, Santos SP, Brazil

Cancer is one of the leading causes of death worldwide. It is predicted that by 2045, there will be 12.6 million new cases of cancer and 7 million deaths. Among the causes of cancer are genetic predisposition, chronic inflammatory diseases, obesity, and lifestyle habits, such smoking, western diet and alcohol consumption. A notable factor is the exposure to environmental pollutants, and despite many of them having well studied causative effects, novel emerging pollutants require further investigation into the tumor initiating and promoting effects. Cancer is a systemic disease; therefore, it should be studied as such. Currently, there are several refined models to study the different types of cancer: 1.) *In vitro* techniques modeling the three-dimensional structure of the tumor, their metabolic needs and the influence of other components of the tumor environment, such as stromal cells and immune cells; 2.) *In vivo* models phenocopying the genetic predisposition to different types of cancer, or modeling the disease orthotopically, so that the biology of the affected organ is properly taken into consideration; 3.) *Ex vivo* models using primary cells and their secreted factors, which provide great information on the biology of the tumor microenvironment. Finally, leveraging the several new multiomics techniques, the study of tumor biopsies and resections bridge bench to bedside. In this course we will briefly discuss some relevant aspects of experimental models of cancer, considering not only the biology of cancer cells, but also the influence of their surrounding environment. Topics to be discussed include: the biology of the tumor microenvironment; overview of refined *in vitro* models for cancer research; and different strategies to study cancer using *in vivo* models. Given the close relationship between the exposure to environmental pollutants and the incidence of cancer, we will discuss how to apply these different models for the study of environmental pollutants as initiators, promoters and modulators of cancer and their micro/macroenvironments.

L2 – Skeletal Muscle and Transsulfuration Pathway. Giuseppe Cirino (University of Naples Federico II, Italy)

Hydrogen sulfide (H_2S) is a gaseous signaling molecule endogenously produced in mammalian tissues through a multi-step enzymatic pathway, called the transsulfuration pathway, involving cystathionine β -synthase (CBS), cystathionine γ -lyase (CSE), and 3-mercaptopyruvate sulfurtransferase (3MST). The endogenous production of H_2S contributes to various physiological functions, including redox balance, vascular homeostasis, neuromodulation, angiogenesis, and cytoprotection. In recent years, H_2S has emerged as a critical modulator of skeletal muscle (SKM) physiology. It participates in the regulation of muscle cell bioenergetics, mitochondrial function, and myogenesis. H_2S enhances mitochondrial biogenesis and efficiency, supports antioxidant defence mechanisms, and modulates contractile function. Moreover, it exhibits anti-inflammatory and cytoprotective properties that are particularly relevant in conditions such as muscle atrophy and age-related sarcopenia. Alterations in endogenous H_2S production and/or signaling have been associated with impaired muscle regeneration, oxidative stress, and metabolic dysfunction. For instance, it has been demonstrated that the H_2S pathway is impaired in Duchenne muscular dystrophy (DMD) and sarcopenia, while H_2S signaling is increased in human malignant hyperthermia. Understanding the role of H_2S in SKM physiopathology opens new perspectives for therapeutic strategies targeting muscle-wasting diseases and metabolic disorders.

L3 – Leveraging Physiology-based Models in Perinatal Pharmacology and in Drug-Induced Liver Injury. Pieter Anaert Drug Delivery and Disposition, Department of Pharmaceutical and Pharmacological Sciences, KU Leuven, Leuven), and BioNotus CommV, Niel (Belgium)

The first part of my lecture will highlight use cases in perinatal pharmacology. A first example will cover the development of a pregnancy PBPK model for antenatal sildenafil, illustrating the importance of protein binding differences in sildenafil distribution to the fetus. A second example in the field of perinatal pharmacology will be the development and evaluation of a platform for PBPK-based prediction of secretion of medicines to human breast milk. In addition, I will highlight the complementary utility of an *in vitro* model of the blood-milk barrier and of Göttingen Minipigs as animal model for studying lactation pharmacokinetics. The second part of my lecture will provide an overview of recent insights obtained by integrating *in vitro* models of DiCho, primarily sandwich-cultured human hepatocytes, with quantitative systems pharmacology (QSP) modelling. Our group has demonstrated the utility of bile acid profiling in said *in vitro* models to elucidate the cholestatic signature of marketed drugs (e.g. bosentan). We have shown that disturbance of bile acid homeostasis in hepatocytes represents a key mechanism contributing to DiCho in the clinic. By combining QSP models for bile acids along with an advanced PBPK model for bosentan, the clinical DiCho signature of bosentan can be recapitulated. Recent advances in longer term culturing of primary human hepatocytes are improving existing *in silico* models for mechanistic understanding and prediction of DiCho. The predictive performance of these modelling tools will be demonstrated by simulations of clinically relevant effects of cholestatic compounds at therapeutically relevant concentrations. I will conclude my lecture by defining future opportunities for studying interindividual variability in perinatal pharmacology and in DiCho susceptibility. **Financial support:** The ConcePTION project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No. 821520. Several parts of this research were supported by FWO scholarships and projects including sabbatical project K803624N.

L4 – Nicotinamide Phosphoribosyl-Transferase (NAMPT) and Nicotinic Acid Phosphoribosyl Transferase (NAPRT) are Novel Cytokines Involved in Inflammation. Armando A. Genazzani, President Italian Society of Pharmacology, Department of Pharmaceutical Sciences, Università di Torino, Italy

Nicotinamide phosphoribosyl transferase (NAMPT) and nicotinic acid phosphoribosyl transferase (NAPRT) are key intracellular enzymes that participate in the synthesis of NAD⁺. While the first utilizes nicotinamide, the second instead uses nicotinic acid, to yield NMN and NaMN, respectively. The up-regulation of NAMPT in many tumours, which presumably is required to provide an energetic boost to rapidly proliferating cells, has led to intense medchem campaigns and a few inhibitors have entered clinical trials. No significant progress has, instead, been made to develop drug-like molecules that inhibit the activity of NAPRT. Both proteins are also actively secreted by a number of cell types, and in the extracellular space act as cytokines independently of their enzymatic activity. While TLR4 is a possible receptor, it would appear that NAMPT and NAPRT act also via other, yet unidentified, targets. High NAMPT and NAPRT serum levels are elevated in cancer and in acute and chronic inflammatory states, and each of these cytokines is regulated separately of the other. The lab has developed specific neutralizing murine antibodies that reduce circulating levels but that do not affect the enzymatic bioenergetic activity of the proteins. With these, we have shown that the anti-NAMPT antibody ameliorates colitis in a number of experimental models. Alongside, the same antibody also significantly reduces the growth and metastatic potential of triple negative breast cancer. It does so by counteracting T cell immune evasion and by reducing neoangiogenesis, reducing the presence of pericyte in the tumoural mass. On the other hand, NAPRT appears to be up-regulated in acute inflammation and the anti-NAPRT antibody is able to significantly reduce the damage of sepsis in a murine model.

L5 – Microbiota-Derived Polyphenol Metabolites Modulate the T Helper 17 to Regulatory T Cell (Th17/Treg) Imbalance, Promoting Resilience to Stress-Induced Anxiety- and Depressive-Like Behavior. Giulio Maria Pasinetti M.D., Ph.D., Department of Neurology, Icahn School of Medicine at Mount Sinai, New York

Chronic stress disrupts immune homeostasis, while metabolites derived from gut microbiota attenuate inflammation, thus promoting resilience to stress-induced immune and behavioral abnormalities. There are both peripheral and brain region-specific maladaptations of the immune response to chronic stress that produce interrelated mechanistic considerations necessary for designing novel therapeutic strategies to prevent stress-induced psychological impairment. This study shows that a combination of probiotics and polyphenol-rich prebiotics, a symbiotic, mitigates the chronic stress-induced inflammatory responses in the ileum and the prefrontal cortex, enhancing resilience to the resultant depressive and anxiety-like behaviors in male mice. Pharmacokinetic studies revealed that this effect may be attributed to specific symbiotic-produced metabolites, including 4-hydroxyphenylpropionic acid, 4hydroxyphenylacetic acid, and caffeic acid. Using a chronic unpredictable stress model, behavioral abnormalities were associated with significant immune cell activation and recruitment in the ileum. Concurrently, inflammasome pathways were implicated in the prefrontal cortex and hippocampus. Chronic stress also upregulated the ratio of activated proinflammatory T helper 17 (Th17) to regulatory T cells (Treg) in the liver and ileum. It was predicted through Ingenuity Pathway Analysis (IPA) that the aryl hydrocarbon receptor (AHR) could be driving the symbiotic effect on the ileum's inflammatory response to stress. Symbiotic treatment effectively attenuated the stress-induced immune and behavioral aberrations in the ileum and the brain. This study characterizes a novel symbiotic treatment for chronic stress-induced behavioral impairments while defining a putative mechanism of gut-microbiota host interaction for modulating the peripheral and brain immune systems. Supported by NIH AT010835 to GMP

L6 – Preclinical and Phase I Clinical Development of a Novel Multi-Target Drug Candidate for Chronic Neuropathic Pain. Zsuzsanna Helyes^{1,2,3,7}, Ádám István Horváth^{1,2}, Nikolett Szentes^{1,2}, Valéria Tékus^{1,2}, Tamás Kálai⁴, Szilárd Pál⁵, Péter Mátyus⁶ ¹Department of Pharmacology and Pharmacotherapy, Medical School, University of Pécs, H-7624 Pécs, Hungary ²HUN-REN-University of Pécs, Chronic Pain Research Group, Pécs, Hungary ³ALGONIST Biotechnologies GmbH, 1030 Vienna, Austria ⁴Institute of Organic and Medicinal Chemistry, Faculty of Pharmacy,

University of Pécs, H-7624 Pécs, Hungary ⁵Institute of Pharmaceutical Technology and Biopharmacy, Faculty of Pharmacy, University of Pécs, H-7624 Pécs, Hungary ⁶University of Veterinary Medicine, Budapest, Hungary ⁷PharmInVivo Ltd., H-7629 Pécs, Hungary

SZV-1287 (3-(4,5-diphenyl-1,3-oxazol-2-yl)propanal oxime) is a novel and unique multi-target drug, which irreversibly inhibits semicarbazide-sensitive amine oxidase (SSAO) metabolizing primary amines to irritants like methylglyoxal and formaldehyde activating the transient receptor potential ankyrin 1 (TRPA1) and vanilloid 1 (TRPV1) receptors. SZV-1287 also directly antagonizes these non-selective cation channels located predominantly on nociceptive primary sensory neurons, immune cells and several central nervous system regions. Furthermore, its main metabolite, oxaprozin, is a clinically used cyclooxygenase inhibitor, which might have synergistic effect with the mother compound in several pain conditions. We provided proof-of-concept for the ability of single SZV-1287 administration (10-50 mg/kg i.p.) to significantly reduce acute and chronic pain behaviors including the sciatic nerve injury-induced neuropathic hyperalgesia in mice via inhibiting TRPA1 and TRPV1 activation. Repeated 20 mg/kg SZV-1287 injections attenuate chronic arthritis (edema, myeloperoxidase activity, histopathological changes) and related hyperalgesia, L4-L6 spinal dorsal horn neuroinflammation (microgliosis) even in the late phase of the model when neuropathic mechanisms are important. To avoid SZV-1287 conversion to oxaprozin in the stomach, enterosolvent capsule was formulated and tested during preclinical development. SZV-1287 is quickly absorbed, its plasma concentration is stable for 2 h and enters the brain. It does not induce any considerable toxicity either in rodents or in dogs. Phase IA clinical trial (single ascending dosing: 75, 150, 300, 450 mg) has successfully been completed without considerable side effects. If the candidate proves to be safe in humans after repeated dosing for 10 days, it can progress to phase II studies in neuropathic pain patients. Funding: Hungarian Research Network (Chronic Pain Research Group), Pécs, National Brain Research Program 3.0, TKP2021-EGA-16, RRF-2.3.1-21-2022-00015

L7 – Transforming Molecular Pharmacology into Innovative Drug Discovery. Emer Suavinho Ferro (USP-SP)

The discovery of bradykinin by Professor Mauricio Oscar Rocha e Silva and his colleagues in the early 1950s was more than just a scientific achievement - it was a turning point that reshaped our understanding of how the body regulates blood pressure and inflammation. This breakthrough not only put Brazilian biomedical research on the global map but also paved the way for major medical advances, including the development of captopril, a revolutionary antihypertensive drug still used by millions today. Our Lab has focused on exploring the enzymes that break down bradykinin and related peptides. Using site-directed mutagenesis and guided by strong scientific hypotheses, we uncovered several previously unknown natural peptides with potential clinical applications – we have coined these “intracellular peptides”. One of the most promising intracellular peptides is Pep19, a small decapeptide currently undergoing early-stage clinical trials. So far, Pep19 has shown exciting potential to improve metabolic health and reduce visceral fat, two key factors in chronic diseases like diabetes and obesity. In this conference, we will revisit the story of bradykinin’s identification and show how it inspired a new path of research that led us to Pep19 - a candidate that could represent a pharmacological innovation. Financial support: Fapesp, CNPq

L8 – Molecular Therapeutics Targets for Muscular Dystrophy- The Role of Translational Pharmacology. Emilio Clementi Dept Biomedical Sciences, University of Milano, Milano, Italy, Secretary General, IUPHAR

This lecture will start by representing the physiopathology of degenerative muscle disease, in particular of the Duchenne Muscular Dystrophy touching upon molecular mechanisms and their interaction. Diagnosis and connected challenges will be then briefly introduced. The current therapeutic scenario will then be presented including conventional and innovative drugs and more recent molecular approaches with adenoviral vectors, gene therapy and exon skipping strategies. Then a particular angle will be developed, on the role of mitochondrial function and bioenergetics

and how targeting these organelles may be exploited for therapeutic purposes in muscle degeneration. Mechanisms that can be exploited will be presented and therapeutics and their efficacy described. These novel therapeutic strategies include both pharmacological and genetic approaches.

S1 – Novel Approaches to Cardio- and Neuroprotection

Development of miRNA Therapeutics for Cardioprotection and other Indications. Péter Ferdinandy MD, PhD, MBA, Center for Pharmacology and Drug Development, Semmelweis University, Budapest, Hungary; Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary; Pharmahungary Group, Szeged, Hungary

Despite intensive research in the last almost 4 decades, drug treatment of myocardial infarction and the consequent post-infarction heart failure is still an unmet clinical need, possibly due to its complex molecular mechanism. Therefore, targeting a molecular network rather than a single molecular drug target may be the adequate strategy for cardioprotective therapy. Unbiased, comprehensive analysis of gene expression pattern in normal and protected ischemic myocardium may lead to exploration of the transcriptomic and proteomic molecular network to identify novel molecular targets for cardioprotection. The non-coding oligonucleotides microRNAs (miRNA) targeting multiple mRNAs form a transcriptomic dynamic molecular network. Targeted perturbation of the molecular network by miRNA oligonucleotide compounds is a promising approach for treatment of diseases of complex molecular mechanisms. By a systematic unbiased analysis of the transcriptomic network of healthy, infarcted, as well as protected by ischemic conditioning heart samples, we have discovered endogenous cardioprotective miRNAs (e.g. miR125b-3p, miR-450a-3p) and validated them in vitro and in vivo and patented the nucleotide sequences of around these miRNAs. We termed these miRNAs protectomiRs. We are developing a miRNA discovery platform consists of bioinformatic software packages (see miRNAtarget.com) to build the transcriptomic and proteomic molecular network and identify potential oligonucleotide drug candidates to treat myocardial infarction and other diseases with unmet clinical need. We improve the performance of these algorithms by machine learning to reach an effective drug discovery enabling platform. In conclusion, miRNA therapy may represent a breakthrough in the treatment of diseases with complex molecular mechanisms such as myocardial infarction. Here we developed a miRNA discovery enabling platform and currently developing miRNA compound pipeline towards clinical phases for several indications.

Pharmacological Modulation of Endothelial Precursor Cell-Mediated Brain Capillary Repair Mechanisms. István Krizbai^{1,4}, Duc Lam Tri^{1,2}, István Tóth^{1,3}, Imola Wilhelm^{1,4}, Claudine Kieda⁵, and Attila E. Farkas¹ ¹HUN-REN Biological Research Centre, Institute of Biophysics, Szeged, Hungary, ²University of Szeged, Doctoral School of Biology, Szeged, Hungary, ³Szeged Scientists Academy, Foundation for the Future of Biomedical Sciences in Szeged, Szeged, Hungary, ⁴Vasile Goldis Western University of Arad, Institute of Life Sciences, Arad, Romania, ⁵Military Institute of Medicine-National Research Institute, Lab of Molecular Oncology and Innovative Therapies, Warsaw, Poland

One of the most severe consequences of ageing is cognitive decline, which is associated with the dysfunction of brain microvasculature. Thus, repairing brain vasculature could result in healthier brain function. To better understand the potential beneficial effect of endothelial progenitor cells (EPCs) in vascular repair, we studied the adhesion and integration of EPCs using the early embryonic mouse aorta-gonad-mesonephros – MAgEC 10.5 endothelial cell line. The EPC interaction with brain microvasculature was monitored ex vivo and in vivo using epifluorescence, laser confocal and two-photon microscopy in healthy young and old animals. The effects of senolysis, EPC activation and ischaemia (two-vessel occlusion model) were analysed in BALB/c and FVB/Ant:TgCAG-yfp_sb #27 mice. MAgEC 10.5 cells rapidly adhered to brain microvasculature and some differentiated into mature endothelial cells (ECs). MAgEC 10.5-derived endothelial cells integrated into microvessels, established tight junctions and co-formed vessel lumens with pre-existing ECs within five days. Adhesion and integration were much less pronounced in aged mice, but were increased by depleting senescent cells using abt-263 or a combination of dasatinib plus quercetin. Furthermore, we demonstrated that MAgEC 10.5 interaction with brain vessels could be upregulated by ischaemic conditions and the inflammatory mediator TNF α . Thus, even though

progenitor cell therapy using MAgEC 10.5 was rendered less effective by aging, it could be improved by modulating EPC-vasculature interactions through senolytics or by activating EPCs before treatment. Combining progenitor cell therapy with senolytic therapy and the prior activation of EPCs are promising for improving EPC adhesion to and integration into the cerebral vasculature and could help rejuvenate the ageing brain.

Harnessing Single-Cell and Spatial Omics to Unveil Novel Therapeutic Targets. Alexandre Denadai Souza Lab of Mucosal Biology, Department of Chronic Diseases and Metabolism, KU Leuven, Leuven, Belgium.

Biological therapies have brought the prospect of remission to many with inflammatory bowel disease (IBD). However, about half of these patients continue to experience persistent and debilitating gastrointestinal symptoms, including severe abdominal pain, despite clinical remission. Within the gut epithelium, rare chemosensory cells such as enteroendocrine cells mediate signal transduction between the lumen and enteric nervous system. Although their role in abdominal pain has been suggested, underlying mechanisms remain unclear. We hypothesized that transcriptional changes in colonic epithelial cells may drive persistent symptoms in IBD patients in remission. To test this, we applied single-cell and spatial omics to profile the colorectal epithelium of healthy individuals and patients with Crohn's disease in remission, with or without symptoms. Among genes defining enteroendocrine cells, we identified PCSK1N as a marker of mature subsets, with low expression in progenitors and other epithelial cells. This was validated by sequencing- and imaging-based spatial transcriptomics. PCSK1N expression inversely correlated with abdominal pain severity and was reduced in symptomatic patients. This gene encodes ProSAAS, precursor of BigLEN, the endogenous agonist of GPR171, an analgesic mediator in preclinical models of somatic pain. Multiplex immunofluorescence revealed PCSK1N⁺ enteroendocrine cells in proximity to GPR171⁺ nerve fibers in the mucosa. In mice, GPR171 antagonism induced visceral hypersensitivity, while agonist treatment restored normosensitivity after colitis. Our study reveals a novel analgesic pathway linking enteroendocrine cells to visceral nociceptors, disrupted in symptomatic patients, and highlights a potential therapeutic target for abdominal pain in IBD. **Financial support:** This study was supported by the Helmsley Charitable Trust (USA), FWO (Belgium), and KU Leuven start-up and C1 grants.

S2 – Research and Innovation in Natural Product: From Biodiversity to Therapeutic Application

Pharmacological potential of food compounds in the management of immuno-mediated inflammatory diseases. Marina Sánchez-Hidalgo^{1,2}, ¹Instituto de Biomedicina de Sevilla, IBiS/Hospital Universitario Virgen del Rocío/CSIC/Universidad de Sevilla, Seville, Spain, ²Faculty of Pharmacy, University of Seville, Seville, Spain.

Certain nutritional components influence the cellular metabolism and interfere in the pathological inflammatory process, so that they may act as a coadjutant in the treatment of many chronic immuno-mediated inflammatory. In recent years, the therapeutic potential of functional foods or those foods that, regardless of their nutritional properties, have a beneficial effect on the body has been revealed; that is, its daily intake, within a balanced diet, contributes to maintaining or improving the state of health and well-being. In this regard, the Mediterranean diet rich in plant-based foods such as whole grains, legumes, fruit, vegetables, and extra virgin olive oil (EVOO), might have the potential to contribute to well-being and has protective effects against cardiovascular, neurodegenerative disease, and cancer. One of the Mediterranean diet characteristic habits is the daily consumption of 25–50g/day of EVOO. Specifically, EVOO consumption in the diet leads to protective effects in the Mediterranean population. In fact, it is well-known that EVOO contains significant amounts of monounsaturated fatty acid (MUFA) (oleic acid) and other minor but highly bioactive components including triterpenic alcohols, sterols, hydrocarbons, vitamins, β -carotene, phytosterols and numerous functional phenolic compounds (e.g., hydroxytyrosol, oleocanthal and oleacein). In this context, we have demonstrated the functionalism of EVOO and its phenolic compounds in autoimmune inflammatory pathologies revealing their anti-inflammatory and

immunomodulatory potential in experimental models of collagen-induced arthritis (CIA) and pristane-induced systemic lupus erythematosus (SLE). Additionally, we have also described the beneficial effects of its phenolic compounds in lipopolysaccharide (LPS)-stimulated murine peritoneal macrophages and human synovial fibroblasts SW982. The observations reported are promising and suggest that EVOO and its minor components play a remarkable role in these healthy benefits, influencing the course of the experimental diseases, ameliorating the symptoms and severity at and downregulating the inflammation at molecular level. Nevertheless, future studies should therefore focus more on understanding the efficacy of EVOO and its polyphenols in humans and bringing them to the forefront of treatment of chronic immuno-mediated inflammatory diseases. **Financial support:** PID2021-125488OB-I00; PROYEXCEL_00547; AGL2017-89342-P; AGL2011-26949; P10-AGR-6609

S3 – Understanding Psychedelics and Non-Hallucinogenic Psyche-delic Analogs

Beyond the Trip: The Molecular Basis of Lasting Behavioral Effects of 2-Br-LSD and Its Comparison to Classical Psychedelics. Vern Lewis¹, Daniel Kot¹, Gianfilippo Coppola² and Argel Aguilar-Valles¹

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Major depressive disorder (MDD) affects 280 million people worldwide, with 80 million experiencing treatment-resistant depression (TRD). Axon hypomyelination and neuronal hypotrophy in the prefrontal cortex (PFC) are hallmark features, underscoring the need for treatments targeting these deficits. Psychedelics, like psilocybin, produce rapid antidepressant effects via 5-HT_{2A} receptor activation, but their hallucinogenic properties limit widespread use. This study explores whether 2-Bromo-LSD (2-Br-LSD), a non-hallucinogenic analog, promotes neuroplasticity and myelination. Adult C57BL/6/J mice received saline or 2-Br-LSD (0.3, 1.0, 3.0 mg/kg) and were tested in the open field and forced swim 24 hrs later. Chronic stress-exposed mice received saline, 2-Br-LSD (1–3 mg/kg), or psilocybin (1 mg/kg) and underwent behavioral assays. Post-treatment, brains were processed for Golgi staining and bulk RNA sequencing, with cell-type deconvolution via CIBERSORTx. 2-Br-LSD promoted active coping in the forced swim test, effects blocked by volinanserin (α5-HT_{2A} antagonist). It reversed stress-induced behaviors akin to psilocybin. Transcriptomic analysis identified differentially expressed genes related to synaptic plasticity and myelination, particularly in PFC oligodendrocytes. These findings suggest 2-Br-LSD mimics classic psychedelics' antidepressant effects via 5-HT_{2A}, supporting its potential as a novel MDD therapy. **Funding:** This work was funded by a Mitacs Accelerate grant to AAV, with additional contributions by BetterLife Pharma.

The Antidepressant Potential of Ayahuasca: From Preclinical Studies to Clinical Trials. Rafael Guimarães dos Santos (USP-RP)

Major depressive disorder (MDD) is a psychopathology with a high rate of patients refractory to current medications. Furthermore, these medications can take weeks to begin producing their effects. Ayahuasca and other serotonergic hallucinogens such as LSD and psilocybin have been investigated in patients with MDD, with promising results. The main active ingredient in ayahuasca is dimethyltryptamine (DMT), a psychedelic tryptamine that acts as an agonist of 5-HT_{2A} serotonergic receptors, similar to LSD and psilocybin. Furthermore, ayahuasca is rich in beta-carbolines such as harmine, which act as selective inhibitors of the enzyme monoamine oxidase (MAO). Preclinical studies demonstrate that both DMT and harmine have antidepressant effects, and an open-label, placebo-controlled clinical trial of a single dose of ayahuasca or DMT in volunteers with treatment-resistant MDD demonstrated a significant reduction in depressive symptoms, and these effects appear to begin within the first few hours after consumption and last between one and three weeks. Therefore, the objective of this lecture is to present the main results of these studies with ayahuasca in patients with MDD, as well as unpublished data in cancer patients and those with post-traumatic stress disorder.

Translational Approach for a Pharmacological Modulation of Neurological and Psychiatric Symptoms due to Disorders of the Striatal Pathway. Louise-Laure Mariani, Associate Professor, Paris Sorbonne University, Pitié-Salpêtrière Hospital, Departments of Neurology and Department of Medical Pharmacology APHP, Paris Brain Institute, INSERM, CNRS, Paris, France

Movement disorders can be classified into hyperkinetic and hypokinetic. Hyperkinetic movement disorders are characterized by the presence of abnormal involuntary movements, comprising most notably of dystonia, chorea, myoclonus, dyskinesia and tremor. They can be extremely disabling and impair the quality of life. The striatum plays a critical role in the control of movements through balanced and coordinated activity of two populations of striatal projection neurons (SPNs): direct striatonigral neurons (dSPNs) and indirect striatopallidal neurons (iSPNs) which facilitate and inhibit movement execution, respectively. AC5 is the main enzyme for cyclic AMP (cAMP) production in striatal neurons, and can be either activated or inhibited by various G protein-coupled receptors (GPCRs) upon binding of neurotransmitters in these two neuronal populations. Dysregulation of the cAMP pathway and associated striatal dysfunction have been implicated in numerous movement disorders, including many genetic causes such as Huntington Disease, and drug-induced causes such as levodopa-induced dyskinesia in Parkinson's disease. We will discuss herein the dysregulations of the cAMP pathway using translational pharmacological approaches in disorders such as Parkinson Disease but also of mixed hyperkinetic movement disorders linked to pathogenic variants of the *Adcy5* gene encoding AC5, a disorder with a primary disturbance of AC5 the striatal enzyme producing cAMP, giving a unique opportunity to unravel the common mechanisms underlying these different types of movement disorders or underlying adverse effects of dopaminergic medications such as levodopa or dopamine agonists used in Parkinson Disease. Fundings: Sponsor of clinical trials were « Assistance Publique - Hôpitaux de Paris » and Inserm. Works were funded by the French Foundation for rare diseases (Fondation maladies rares), Fondation de France, the patient association Association France Parkinson, the Paris Brain Institute, the patient association ADCY5.org, Amadys, the Dystonia Medical Research Foundation DMRF grant, Merz Pharma, Aguetant, Enjoy Sharing and Agence Nationale de la Recherche ANR.

Unraveling the anti-inflammatory mechanisms of the two synthetic cannabinoids 4'-F-CBD and HU-910 on brain glial cells. Patrick Pierre Michel^{1*}, Mauricio dos Santos Pereira^{1,2}, Bruna Maitan Santos^{1,2}, Rocio Gimenez^{1,3}, Francisco Silveira Guimaraes⁴, Rita Raisman-Vozari¹, and Elaine Del Bel² ¹Sorbonne Université, Paris Brain Institute-ICM, Inserm, CNRS, APHP, Hôpital de la Pitié Salpêtrière, Paris, France, ²Department of Basic and Oral Biology, FORP, Campus USP, University of São Paulo, Ribeirão Preto, Brazil, ³IREN Center, National Technological University, Buenos Aires, Argentina, ⁴Pharmacology Department, FMRP, Campus USP, University of São Paulo, Ribeirão Preto, Brazil

Among cannabinoids found in *Cannabis sativa*, cannabidiol (CBD) has attracted the most attention because it displays interesting pharmacological properties while having no psychotomimetic effects contrary to delta-9-TetraHydroCannabinol. Several studies, including ours, have shown that CBD effectively restrains brain microglial activation in cell culture and animal models that reproduce brain neuroinflammatory processes (Martin-Moreno et al, Mol Pharmacol, 2011; dos Santos Pereira et al, Glia, 2020; Landucci et al, Neurobiol Dis, 2022; Rodrigues da Silva et al, BioRxiv, 2024). Here, we examined the anti-inflammatory potential of 4'-F-CBD, a hemi-synthetic derivative of CBD, and HU-910, another synthetic cannabinoid, using post-natal cultures of microglia and astrocytes. We found that 4'-F-CBD and HU-910 efficiently reduced the release of TNF- α , IL-6, and IL-1 β in microglia and astrocyte cultures exposed to the bacterial Toll-Like Receptor (TLR)4 ligand LPS. Upon LPS challenge, 4'-F-CBD and HU-910 effectively prevented the induction of phenotypic activation markers specific to microglia and astrocytes, that is, Iba-1 and GFAP, respectively. In microglial cultures, these two compounds also efficiently restrained LPS-stimulated release of glutamate, a non-cytokine inflammation marker for these cells. The immunosuppressive effects of

the two cannabinoid compounds were concentration-dependent (1-10 μ M). Cannabinoid and cannabinoid-like receptors did not play a significant role in these effects. Interestingly, both 4'-F-CBD and HU-910 were also capable of mitigating glial inflammatory responses mediated by Pam3CSK4, a lipopeptide that acts as a ligand for TLR2, as well as by BzATP, a prototypic agonist of P2X7 purinergic receptors, suggesting that these two cannabinoid compounds may exert anti-inflammatory effects across a wide range of inflammatory conditions. Using LPS-stimulated microglia and astrocytes, we established that the immunosuppressive action of 4'-F-CBD and HU-910 resulted from their capability to inhibit ROS produced by NADPH oxidase and, subsequently, NF- κ B-dependent signaling events. Overall, our results suggest that 4'-F-CBD and HU-910 may have therapeutic utility in pathological conditions characterized by prominent neuroinflammatory processes. **Funding information:** Capes/Cofecub Research Program between Brazil and France: 88887.710933/2022-00 and Me928/19; FAPESP BEPE grant 03482-0; FAPESP post-doctoral fellowship: 2017/14207-7.

Dyskinesia and protection in preclinical models of Parkinson's disease. Elaine Del-Bel^{1,3*}, Mariza Bortolanza¹, Glaucelino Crivelaro do Nascimento¹, Patrick Pierre Michel², Mauricio dos Santos Pereira^{1,2,3}, Rita Raisman-Vozari^{2,1} Department of Basic and Oral Biology, FORP, Campus USP, University of São Paulo, Ribeirão Preto, Brazil; ²Sorbonne Université, Paris Brain Institute-ICM, Inserm, CNRS, APHP, Hôpital de la Pitié Salpêtrière, Paris, France Elaine Del Be; ³Physiology Department, FMRP, Campus USP, University of São Paulo, Ribeirão Preto, Brazil

There is substantial preclinical evidence from multiple rodent and invertebrate models demonstrating that doxycycline exerts neuroprotective and anti-dyskinetic effects relevant to Parkinson's disease (PD) pathophysiology. In various mouse and rat models, doxycycline has been shown to inhibit α -synuclein aggregation, reduce neuroinflammation, protect dopaminergic neurons, and attenuate L-DOPA-induced dyskinesia without impairing the antiparkinsonian efficacy of L-DOPA. Mechanistically, these effects are associated with suppression of microglial and astrocytic activation, downregulation of pro-inflammatory cytokines (e.g., TNF- α , IL-1 β), inhibition of COX-2 and prostaglandin E2, reduction in metalloproteinase activity, and decreased production of reactive oxygen species in the basal ganglia and upregulation of axonal and synaptic proteins critical for neuronal integrity. ^[1-7] Beyond dyskinesia, doxycycline has demonstrated neuroprotective properties in several preclinical PD models. It inhibits α -synuclein aggregation and associated neurotoxicity, reduces glial activation, and restores synaptic and axonal protein expression, which are critical in early neurodegenerative processes of PD. ^[1,3,5] In transgenic mouse models expressing human α -synuclein mutations, doxycycline improved motor and cognitive deficits, reduced neuroinflammation, and prevented synaptic loss. ^{[1][5]} In vitro studies further support its role in inhibiting α -synuclein aggregation and mitigating mitochondrial oxidative stress. ^[5] Doxycycline has also demonstrated efficacy in transgenic mouse models expressing human α -synuclein mutations, where it improved both motor and cognitive deficits, reduced α -synuclein oligomerization, and restored synaptic function. ^[4] In vitro and in vivo studies further support its ability to prevent ferroptosis-mediated dopaminergic neurodegeneration and to inhibit pathological α -synuclein aggregation and neuroinflammatory responses. ^{[4][5]} However, there are no studies in the medical literature documenting the effects of doxycycline in non-human primate models of PD. All available in vivo evidence is derived from rodent models (mice and rats) invertebrate systems (e.g., *C. elegans*) and non-human primates, with one published data from primate/human studies. ^[1-7] Thus, while the preclinical data are robust in lower species, translational studies in primates are lacking. **Bibliography** 1-Bortolanza M, Nascimento GC, Raisman-Vozari R, Del-Bel E. [Doxycycline and Its Derivative, COL-3, Decrease Dyskinesia Induced by L-Dopa in Hemiparkinsonian Rats](#). British J. Pharmacology. 2021; 178(13): 2595-2616 doi:10.1111/bph.15439. 2-Lazzarini M, Martin S, Mitkovski M, et al. [Doxycycline Restrains Glia and Confers Neuroprotection in a 6-OHDA Parkinson Model](#). Glia. 2013;61(7):1084-100. doi:10.1002/glia.22496. 3-Amaral L, Dos Santos NAG, Sisti FM, Del Bel E, Dos Santos AC. [Doxycycline Inhibits Dopaminergic Neurodegeneration Through Upregulation of Axonal and Synaptic Proteins](#). Naunyn-Schmiedeberg's Archives Pharmacology.

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S5 – Sex, Hormones and Age as Determinants for Disease Severity and its Implications on Therapeutics

Using Functional MRI to Assess Sex and Age Differences in Pain Processing at the Whole-Brain Level. Joyce T. da Silva (Department of Neural and Pain Sciences. University of Maryland, Baltimore USA)

Old age and female sex are risk factors for the development of osteoarthritis (OA) and chronic pain, yet the underlying mechanisms are poorly understood and available treatments are largely ineffective. Increasing evidence suggests that the high OA prevalence in the elderly does not reflect a simple consequence of the biological changes associated with age but rather the complex interplay between age, sex, and pain processing at multiple levels of the neuroaxis. Alterations in descending pain modulatory networks may contribute to the pathophysiological basis for chronic OA pain, and potential therapies could target these networks. This session will present new data on functional connectivity of the periaqueductal gray (PAG), a main core of the descending pain inhibitory pathways, with the whole brain and sex- and age-related changes in a healthy state and during chronic pain progression in rats (early and late phases of OA pain). Data reflecting how sex and age differences in PAG connectivity may affect pain-like behaviors in a preclinical OA model will be presented as well as their clinical applications. Moreover, it will be demonstrated that consistently with human studies age significantly impacts OA-like pain making the elderly, particularly females, more vulnerable to chronic pain. Funding: National Institute of Neurological Disorders and Stroke (NINDS); National Institutes of Health (NIH) - Grant R33NS113269

Sex Differences and Influence of Female-Sex Hormones on Systemic Inflammation. Aleksander Roberto Zampronio, Regina Azevedo Costa, Thais Alencar de Souza UFPR - Federal University of Paraná.

Males are at higher risk for developing more severe systemic inflammation (SI) than females. The female sex-hormones, and particularly estrogen, contributes to this differences and particularly changes at older age when this hormone production reduces. However, the underlying mechanisms involved in these differences and changes are poorly understood. This section will show new pre-clinical data on these differences between males and females regarding neuroinflammation observed during SI induced by different stimulus (bacterial and viral models). Data will focus on central nervous system-associated responses observed during SI, e.g. fever, hyperalgesia and hypolocomotion. Moreover, it will explore the effects of the reduction of female sex hormones on these responses as a model of menopause and the implications of estrogen reposition. Changes in some systems, particularly involving serotonin and endothelin-1, which are profoundly affected by sex differences will be discussed. Financial support: CNPq and CAPES

S6 – Impact of Sex Hormones on Vascular Biology: New Perspectives and Insights

Beyond Genders: Unraveling the Impact of Testosterone in the Vasculature. Santos, J.D., Tostes, R.C. Department of Pharmacology, Ribeirao Preto Medical School, University of São Paulo, Brazil. Changes in testosterone levels are linked to increased cardiovascular risk, highlighting the critical role of androgens in cardiovascular homeostasis. Sub- and supraphysiological levels of testosterone are associated with impaired vascular function and remodeling, leading to poor

vascular health. Pharmacologically, testosterone is used in masculinizing gender-affirming hormone therapy (M-GAHT) to produce male sexual secondary characteristics in transmasculine individuals. Although considered safe, M-GAHT triggers undesired cardiovascular effects, including dyslipidemia, impaired vasodilation, and increased blood pressure, events related to increased testosterone levels. Due to the modulatory effects of immunological components on vascular function, the contribution to the potential role of immune-related mechanisms in the vascular effects of M-GAHT has gained increased attention. Clinical studies show that GAHT-M increases the release of pro-inflammatory cytokines from immune cells, and leukocyte-endothelial interactions in transmasculine individuals, events associated with impaired vasodilatory responses. Experimental studies also reveal those immunological pathways – innate- and adaptive-related immune cells e.g. – as key to testosterone-induced vascular dysfunction. Although still preliminary, studies indicate a close relationship between sex factors and the immunological system that can lead to cardiovascular (dys)function, indicating that the immune system is a potential therapeutic target for interventions in the context of GAHT-M. The comprehension of the mechanisms involved in the cardiovascular effects of GAHT-M will contribute to a more precise evaluation of the potential benefits and risks of GAHT, improving healthcare for transgender individuals. Financial support from FAPESP, 2023/02962-6 and 2023/12013-1.

S7 – Basic Pulmonary Pharmacology and Clinical Practice for Respiratory Diseases

CT Imaging for Respiratory Diseases: Bridging Experimental Models and Clinical Practice. Lorenzo Ball (University of Genoa, Italy)

Preclinical research is a cornerstone in the development of novel therapeutic approaches for respiratory diseases, including both pharmacological treatments and respiratory support tools such as mechanical ventilation strategies. Micro-computed tomography (microCT) has emerged as a valuable tool for obtaining *in vivo* images, analogous to what is commonly used in clinical research through conventional computed tomography (CT). Although the technique has limitations in soft tissue discrimination, in lung research microCT offers significant advantages due to the high contrast between air and lung structures, enabling accurate mapping of disease extent and ventilation distribution. In this lecture, we will explore how this innovative, non-destructive, *in vivo* imaging tool can enhance preclinical research by bridging the translational gap that often exists between bench and bedside. We will discuss its role in refining animal models, improving reproducibility, and identifying preclinical endpoints aligned with clinically relevant imaging-derived biomarkers. The potential for back-translating quantitative radiographic assessments — widely adopted in clinical research — into the preclinical domain will also be addressed. A section will be dedicated to the integration of microCT with histology, lung mechanics, and omics data, to deepen our understanding of disease pathophysiology. As imaging continues to evolve towards functional and molecular domains, microCT may serve as a proof-of-concept platform for experimental designs with direct relevance to clinical applications.

Nicotinic Receptors in Respiratory Diseases: Insight from Experimental Models. *Carla Máximo Prado – Full professor – Departamento de Biociências – Instituto de Saúde e Sociedade - Universidade Federal de São Paulo (UNIFESP) – Campus Baixada Santista*

Nicotinic acetylcholine receptors (nAChRs) have emerged as key modulators of respiratory function and pulmonary inflammation. This presentation will explore findings from experimental models that shed light on the role of specific nAChR subtypes—particularly the $\alpha 7$ subtype—in regulating inflammatory pathways in the lungs. *In vitro* studies will also be presented to illustrate the therapeutic potential of targeting these receptors in respiratory diseases. By integrating mechanistic insights with preclinical evidence, this talk will highlight how modulating cholinergic signaling via nAChRs may contribute to the development of new pharmacological interventions for respiratory disorders, in line with current efforts to innovate treatment approaches in a rapidly evolving health landscape.

Development and Validation of a Methodology for Preclinical Evaluation of Candidate Compounds for the Treatment of Chronic Pulmonary Diseases Using microCT. Patrícia M. R e Silva – Senior Researcher – Lab Inflamação – Instituto Oswaldo Cruz – Fundação Oswaldo Cruz (FIOCRUZ) – Rio de Janeiro-Brazil.

The development of effective therapies for chronic pulmonary diseases requires robust and reproducible preclinical models. This study presents the development and validation of a non-invasive imaging methodology using micro-computed tomography (microCT) for the longitudinal evaluation of candidate therapeutic compounds in animal models. The approach enables detailed assessment of lung structure and function over time, allowing the monitoring of disease progression and therapeutic responses. Importantly, this microCT-based system is being applied to the identification and evaluation of novel therapies for inflammatory lung diseases. We will present experimental data generated through this approach, highlighting its potential to support translational research and accelerate the discovery of effective treatments.

S8 – Role and Modulation of Cardiac and Neural Ion Channels

Decreased Repolarization Reserve as a Possible Mechanism behind Drug Induced Proarrhythmia.

Andras Varro. Department of Pharmacology and Pharmacotherapy, Albert Szent-Györgyi Medical School, University of Szeged, Szeged, Hungary HUNREN SZTE Research Group for Cardiovascular Pharmacology, Hungarian Research Network, Szeged, Hungary

Several cardiac and even more importantly not cardiac drugs were reported to induce serious, sometimes lethal arrhythmias or sudden cardiac death. These events are usually attributed to torsade de pointes chaotic ventricular tachycardia or ventricular fibrillation. The incidence of such arrhythmias are rare but since they may danger life it can stop new drug developments or even can result withdrawal of drugs from the market. Therefore, drug induced arrhythmias represent a major issue in safety pharmacology. Drug induced torsade pointes arrhythmia or ventricular fibrillation usually attributed to cardiac repolarization lengthening - often but not always - manifested as QTc interval prolongation in the surface ECG. However, since – as it was mentioned previously - the incidence of such fatal arrhythmias can be very rare (less than 1:10 000) and/or can be manifested without prior noticeable QTc change, the preclinical and clinical predictive screening is very difficult. In this lecture the possible cellular mechanism of such drug induced arrhythmias is discussed based on the “repolarization reserve” theory. With better understanding the ion channel mechanism of these arrhythmias it would be possible to develop more effective and better predictive preclinical and clinical screening methods to avoid drug induced proarrhythmias. This work was supported by the National Research Development and Innovation Office (NKFIH K 135464, K 142738, K 147212, FK 138223, the Albert Szent-Györgyi Medical School institutional grant (SZTE AOK-KKA 2022) and by Hungarian Research Network (HUN-REN TKI project to A.V., N.J, and I.B).

Lipid Raft Disruption Around Nociceptive TRP Channels Provides Novel Analgesic Opportunity Éva Szőke, Andrea Kinga Nehr-Majoros, Maja Payrits, Noémi Bencze, Zsuzsanna Helyes University of Pécs, Medical School, Department of Pharmacology and Pharmacotherapy, Pécs, National Lab for Drug Research and Development, Budapest, Centre for Neuroscience, University of Pécs, HUN-REN PTE Chronic Pain Research Group, Pécs

Chronic pain conditions are unmet medical needs, the available drugs, opioids, non-steroidal anti-inflammatory/analgesic drugs and adjuvant analgesics do not provide satisfactory pain relief. There is a great need to find novel alternative therapeutic approaches. Since Transient Receptor Potential (TRP) ion channels especially the Vanilloid 1 and Ankyrin 1 (TRPV1 and TRPA1) were identified as potential targets for chronic pain, great efforts were made to develop novel analgesic drugs acting on these receptors. Unfortunately, due to the hyperthermic side effects and/or impaired noxious heat sensation of TRPV1 antagonist drug candidates, these compounds were never commercialized. A potential novel approach may be the disruption of the hydrophobic connections between these proteins and the cell membrane microdomains rich in cholesterol, sphingomyelins

and glycosphingolipids, the lipid rafts. Cholesterol depletion by cyclodextrins (CDs), hydrolyzation of sphingomyelin by sphingomyelinase (SMase) or inhibition of or inhibition of glycosphingolipid synthesis by Myriocin (Myr) are suitable for disintegration of lipid rafts. We proved that these compounds were able to inhibit TRPV1, TRPA1 and TRP Melastatin 8 but not TRP Melastatin 3 and voltage-gated calcium ion channel activation on receptor-expressing cells and sensory neuronal culture. They had analgesic and anti-inflammatory effect in *in vivo* mouse models of pain involving TRPV1, TRPA1 and TRPM8 ion channels. We provided *in silico* evidence for mechanism of action of CDs. Targeting the lipid rafts by sphingolipid or cholesterol depletion might open future perspectives for the therapy of chronic inflammatory, neuropathic or cancer pain, acting at the periphery. Support: TKP2021-EGA-16 (Development and Innovation Fund of Hungary); TKP2021-EGA-13 (Development and Innovation Fund of Hungary); RRF-2.3.1-21-2022-00015 (Pharmalab); ELKH-PTE, Hungarian Research Network (HUN-REN PTE Chronic Pain Research Group), Pécs, Hungary; NKFIH K 138936, ÚNKP-22-3-I-PTE-1616

Regulatory Role of TRPA1 Receptors in Neurodegenerative Disorders Associated with Dementia.

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Introduction: The Transient Receptor Potential Ankyrin 1 (TRPA1), receptor is involved in the mediation of pain and inflammation. However, little is known about its expression patterns and functions in the brain. WT mice injected with A β 1-42 exhibited significant cholinergic cell and fiber loss and memory deficit as well, which was attenuated in TRPA1^{-/-} animals. Elderly KO mice also showed milder memory loss. We have shown by RNAscope in situ hybridization technique that the EWcp area is the site of abundant Trpa1 mRNA expression in the mouse CNS, localizing to peptidergic, UCN1-containing neurons. We hypothesized that TRPA1 plays a role in AD-associated neurodegeneration of EW peptidergic neurons. **Methods:** In the present study, we used triple transgenic (3xTg) animals overexpressing 3 genes (amyloid precursor protein (APP), presenilin-1 (PSEN1) and tau protein) each of them predisposing to AD. The Trpa1 mRNA expression and the UCN1 peptide content were assessed in five age groups of 3xTg and C57BL6 mice (2, 6, 9, 12 and 18 months) using RNAscope in situ hybridization (ISH) technique combined with immunofluorescence labelling in the EWcp. The site-specific deletion of TRPA1 in the urocortinergic EWcp neurons in a mouse model of AD was also investigated. We have constructed an adeno-associated virus vector (AAV) that carries an UCN1 promoter-driven Cre recombinase (Ucn-Cre-AAV). The AAV vector was injected into the EW nucleus of the stopflox-td-Tomato mice. The Trpa1 mRNA expression was determined by RNA scope in situ hybridization technique and the urocortin protein was labelled by immunohistochemistry. **Results:** Higher Trpa1 expression was observed in the 2 and 6 months-old control groups compared to the 3xTg counterparts, however no genotype-dependent differences were detectable in the elder groups due to the progressive age-related reduction of Trpa1 copy number in the C57BL6 strain. On the contrary, the Trpa1 expression of transgenic mice persisted at basally lower levels that was not affected by aging. In the brain of the stopflox-td-Tomato reporter mice, we have proven that Ucn-Cre-AAV was introduced into urocortinergic cells, where the promoter activated the Cre recombinase and td Tomato, turning them red. By also performing the RNA scope technique on the sections, the complete co-localization is clearly visible, showing that the virus is specific for TRPA1 expressing urocortinergic cells. **Conclusions:** The altered age-dependent dynamics of Trpa1 expression in urocortinergic neurons of AD mice suggest that peptidergic neurons may also be affected by AD. Our data indicate that TRPA1 may play a crucial role in neurotoxicity and age-related dementia. On the basis of histological findings in the stopflox-td-Tomato reporter mice, we can say, that our Ucn-Cre-AAV construct is available to create the EW specific conditional TRPA1 KO mice. These mice are suitable to investigate the role of the TRPA1 receptor expressed in EWcp in mouse models of

neurodegenerative diseases. Research supports: Supported by the Hungarian Brain Research Program (NAP3) 2022-2025, TKP2021-EGA-16, HUNREN

S9 – Therapeutic Potential of Natural Products: From Ancient uses to Modern Practices

Natural products for Neurodegenerative Diseases: Potential Candidates, Current Studies And Perspectives. Fúlvio Rieli Mendes – Center for Natural and Human Sciences, Federal University of ABC

The use of medicinal plants and other natural products is an ancient practice. Ethnopharmacology is an excellent tool for the search for bioactive compounds, including substances with potential for the prevention and treatment of neurodegenerative diseases. The current therapeutic options available for Alzheimer's disease and Parkinson's disease are scarce and are not capable of promoting a cure. Therefore, the search for substances that act through mechanisms different from those of the drugs currently available has great potential. Natural products stand out as a source of chemical compounds with diverse structures and activities, and can serve as inspiration for the creation of new drugs. Furthermore, modern techniques allow the preparation of innovative formulations, increasing the bioavailability and effectiveness of active ingredients. In this presentation, some examples of well-known substances and other potential candidates for the treatment of neurodegenerative diseases will be discussed.

Effects of Natural Polysaccharides in Cancer Models. Alexandra Acco. Department of Pharmacology - Federal University of Paraná (UFPR)

New anticancer agents with fewer adverse effects are necessary for several kinds of cancer. Our team has been investigated the antitumor effect of polysaccharides extracted from sweet green pepper (*Capsicum annuum* – CAP) in breast cancer and melanoma models, both *in vitro* and *in vivo*. CAP is composed mainly of uronic acids (67%), with minor amounts of rhamnose (1.6%), arabinose (6.4%), xylose (0.3%), galactose (6.7%), and glucose (4.4%) and consisted of a highly methoxylated homogalacturonan, together with type I arabinogalactan anchored to rhamnogalacturonan. CAP treatment reduced cell colonies in three human tumor lineages (MCF-7, MDA-MB-231 and MDA-MB-436), but did not reduce the viability of the non-tumor mammary HB4a cells. CAP reduced VEGF gene expression in MCF-7 and MDA-MB-436 cells, but not in MDA-MB-231 cells. Also *in vitro*, the CAP+methotrexate (MTX) association decreased the viability of the MDA-MB-231 and MDA-MB-436 cells more significantly when compared to both compounds isolated. *In vivo*, oral CAP treatment (100 mg/kg) was able to reduce the mammary Ehrlich tumor volume by 40% in female Swiss mice, while CAP+MTX treatment reduced in 95% the tumor development. CAP elevated the tumor levels of IL-6 and TNF- α , and decrease the *Vegf* gene expression and the vascular area in Ehrlich tumors. Similarly, higher concentration of CAP reduced the melanoma B16F10 cell viability after 72 h of exposure, as well as the CAP oral treatment (100 mg/kg) reduced in 72% the melanoma growth in C57BL/6 female mice. Also in melanoma tissue, CAP induced a reduction of *Vegf* and *Hif1 α* , and an increase of *Ripk1* and *Bax* gene expression, which was accompanied of increased tumor necrosis area, increased TNF- α level and reduced number of PCNA-positive tumor cells. Additionally, CAP was nontoxic to both mice lineages (Swiss and C57BL/6). Therefore, CAP has antineoplastic effect against mammary and melanoma tumor cells. Its anticancer mechanism seems to depend on the regulation of inflammation and angiogenesis. CAP is promising as an assistant therapy for mammary tumors and melanoma. Financial support: CNPq and CAPES.

S10 – Placenta, Preeclampsia, and Neuroprotection: Emerging Therapies and Future Horizons

AQP3: More than a Water Channel – A potential Therapeutic Target of Aspirin in Preeclampsia. Mariano Reynoso^{1,2}, Matías Sierra^{1,3}, Nazarena Fernández^{1,3}, Alicia E. Damiano^{1,3} ¹Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Ciencias Biológicas, Cátedra de Biología Celular y Molecular, Ciudad Autónoma de Buenos Aires, Argentina. ²Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Fisicomatemática, Cátedra de Matemática, Ciudad Autónoma de Buenos Aires, Argentina. ³CONICET-Universidad de Buenos

Aires, Instituto de Fisiología y Biofísica Bernardo Houssay (IFIBIO Houssay), Facultad de Medicina, Laboratorio de Biología de la Reproducción, Ciudad Autónoma de Buenos Aires, Argentina.

Aquaporin-3 (AQP3), a membrane channel permeable to water, urea, glycerol, and H₂O₂, plays critical roles in placental development beyond its classical function in fluid transport. As a peroxiporin, AQP3 contributes to redox homeostasis by facilitating H₂O₂ efflux and is involved in key inflammatory signaling pathways. It is also essential for extravillous trophoblast (EVT) migration and endovascular differentiation, two fundamental processes in early placentation. AQP3 is the most abundant aquaporin in first-trimester chorionic villi. In pregnancies complicated by preeclampsia (PE), placental AQP3 expression is markedly reduced at both gene and protein levels. We have recently demonstrated that elevated levels of tumor necrosis factor- α (TNF- α), a hallmark of the pro-inflammatory environment in PE, downregulate AQP3 expression, thereby contributing to placental dysfunction. Low-dose aspirin (LDA, 150 mg/day), administered between 16 and 36 weeks of gestation, remains one of the few pharmacological strategies with proven efficacy in reducing the incidence of early-onset PE. Although historically associated with cyclooxygenase (COX) inhibition, growing evidence points toward COX-independent mechanisms. Based on our previous findings, we hypothesized that LDA attenuates the TNF- α -mediated repression of AQP3, thereby enhancing EVT function, reducing oxidative stress, and improving placental development. In our study, LDA treatment in preeclamptic pregnancies was associated with milder clinical presentations, a higher incidence of deliveries beyond 37 weeks, and improved neonatal birth weight. At the molecular level, LDA significantly increased placental AQP3 expression (and reduced the percentage of syncytial nuclear aggregates, an established marker of trophoblast stress. In contrast, LDA had no significant effects in normotensive pregnancies. Notably, in obese women, LDA failed to improve neonatal outcomes, indicating a reduced pharmacological response in this population. These findings suggest a potential COX-independent mechanism of aspirin involving modulation of the TNF- α /AQP3 axis. AQP3 may represent a promising molecular target for pharmacological intervention in preeclampsia and could act as a mediator of aspirin's placental protective effects during gestation. Financial support: UBACYT-20020220300097BA

Placental and Amnion Derivatives: from Preclinical Research to Therapeutic Innovation. San Martin S (1,2), Quevedo K (1,3), Lira A (1,3), Buendía D (4), Garrido M (1,5), Montedónico S (1,5), Córdova C (1), Egaña T (6). 1) Centro Interdisciplinario de Investigación Biomédica e Ingeniería para la Salud-MEDING, Escuela de Medicina, Universidad de Valparaíso, Chile. 2) Group of Research and Innovation in Vascular Health, GRIVAS Health, Chillán, Chile. 3) Doctorado en Ciencias e Ingeniería para la Salud, Universidad de Valparaíso, Chile. 4) Escuela de Ingeniería Civil Biomédica, Universidad de Valparaíso, Chile. 5) Unidad de Cirugía Pediátrica y Servicio de Maternidad, Hospital Carlos Van Buren de Valparaíso, Chile. 6) Institute for Biological and Medical Engineering, Faculty of Engineering, Pontifical Catholic University of Chile, Santiago, Chile.

Amniotic membrane (AM) is a tissue that to date has proven to be an excellent option for regenerative medicine, given a series of proregenerative properties, its phenotypic plasticity and immunomodulatory capacity; being effectively used in preclinical and clinical trials. Some examples of such applications, developed in our group, correspond to its use as a therapeutic tool in a model of hepatic fibrosis and as a treatment alternative in clinical trials for ophthalmologic and dermatologic pathologies. Advances in the treatment of wounds for reconstructive purposes have also been promising, given their anti-inflammatory, epithelializing and antifibrotic properties. However, the adequate supply of oxygen to the wound area remains one of the major challenges in the development of dermal substitutes, in this sense our group has developed strategies for the creation of a temporary symbiotic dressing based on MA and microalgae, whose single implant would allow greater benefits from the point of view of treatment efficacy, via the conjugation of photosynthetic and regenerative properties of MA. This allowed demonstrating biocompatibility with this microorganism, allowing its viability, proliferative and photosynthetic capacity, generating the first photosynthetic symbiotic allograft of human amniotic membrane. The implementation of

this type of therapy brings with it important challenges, such as guaranteeing the safety of the grafted tissue through the application of strict donation criteria and quality control. In Chile, the National Tissue Bank (BNT), an institution under MINSAL, centralized the control and process of the public health system, making all tissue used in patients must be sterilized by gamma radiation, with the logistical complications and those of the radiation itself representing a challenge to expand the use of this type of therapy. Several low-cost, local, non-radioactive sterilization methods can be developed for application to tissues. Ozonation is currently used to sterilize environments and inert materials for clinical use. We have developed the implementation and validation of an ozone sterilization prototype for use in tissues, allowing with this method to eliminate microorganisms while maintaining the structural properties of the AM. In recent years, our Lab has developed a series of strategies for the study and use of AM in different conditions, which has allowed us to characterize its properties and to continue searching for future therapeutic alternatives to solve different pathological conditions in the population. **Financial Support:** Fondef VIU 20P0096; LICHEN proyecto UVA20993 Universidad de Valparaíso, Fondecyt Reg 1200280 y ANID-Subdirección de Capital Humano Avanzado/Doctorado Nacional/2023-21231783. **La patente** “Andamio biológico humano fotosintético para el tratamiento y regeneración de heridas” se encuentra actualmente protegida por la siguiente patente: Patente No. 70.240/Patent Granted: 70.240.

RT2 – 55 Years of the Postgraduate Course in Pharmacology at the Escola Paulista de Medicina - Unifesp: Training Researchers Who are Up to Date with Science and Innovation in Brazil (55 anos da Pós-Graduação em Farmacologia da Escola Paulista de Medicina – Unifesp: Formando Pesquisadores Atualizados com Ciência e Inovação no Brasil)

Current results, Technologies and Innovation (Resultados Atuais, Tecnologias e Inovação). Miriam Akemi Furuie Depto Farmacologia, EPM/UNIFESP

Innovation is driven not only by technology, but also by market demands, social factors, and environmental factors. Effective management of technological innovation involves aligning with business strategy, fostering a culture of innovation, and investing in research and development. Collaborative and open innovation approaches are also crucial to driving advances in scientific knowledge. The "valley of death" in drug discovery refers to the significant gap between promising preclinical discoveries and successful clinical trials, leading to a high failure rate. It is a challenging phase in drug development, where many potential therapies struggle to become effective treatments, often due to factors such as difficulties in translating preclinical models to humans, scientific limitations, and methodological approaches. Biomarkers can contribute to building bridges to overcome the "valley of death" by connecting basic research and clinical translation with pragmatic and innovative solutions. Various cutting-edge technologies have enabled the development of innovative solutions for pathologies without therapeutic possibilities using classical pharmacology based on the use of chemical or natural compounds, confirming the importance and necessity of interdisciplinary approaches for the development of innovative treatments for complex pathologies such as mental disorders and other neurodevelopmental pathologies. Although there is still no cure for these pathological conditions, especially psychiatric ones, advances in knowledge of the molecular mechanisms involved have pointed to promising therapeutic possibilities that could be applied in the near future. Recent results obtained by the group in recent years, which have enabled the filing of several patent applications, will be presented to illustrate the main impacts and opportunities created by research initiated and developed by Brazilians and in Brazil. Financial support: FAPESP, CNPq, INCT, CAPES, and FINEP.

Perspectives and Aspects of the Program (Perspectivas e Aspectos do Programa). Miriam Galvonas Jasiulionis Department of Pharmacology, Escola Paulista de Medicina, UNIFESP

The Postgraduate Program in Pharmacology at the Escola Paulista de Medicina, UNIFESP, is one of Brazil's most prestigious and long-standing programs, having begun its activities in 1970. Since its foundation, its mission has been to train highly qualified professionals to teach and conduct research in Pharmacology and Physiology. With strong integration between basic and clinical Departments and interinstitutional collaborations, the program maintains excellence in training master's and doctoral students, many of whom have contributed to the creation and consolidation of other Postgraduate programs across Brazil. It currently has 25 faculty members and approximately 100 researchers in training and postdoctoral studies. It has a high degree of internationalization, with co-tutorship agreements, exchange programs, and active participation in the CAPES-PrInt program. The faculty stands out for its high scientific productivity and impact, including a substantial number of publications in collaboration with international researchers. The majority of the program's supervisors are recipients of CNPq productivity grants. The recently reorganized research lines cover current and interdisciplinary topics, such as molecular pharmacology, neuropsychopharmacology, biomarkers, and aging. The Program also promotes outreach and scientific dissemination activities, such as the Pharmacology Vacation Course, as well as actions in public schools and partnerships with primary education institutions. Rated with a score of 6 by CAPES, the program reaffirms its commitment to quality science, ethical training, social inclusion, and national and international impact. Support: FAPESP, CAPES

RT3 – Beyond the Academy (Além da Academia)

If my career doesn't fit within the University, what then? (Moving from academia to pharma without losing scientific identity). Lorena C. Corrêa Fehlberg (Scientific Manager Pfizer)

What if all the effort dedicated to research is not meant to end in a classroom or a Lab? What if the knowledge we build could create an impact on people's lives far beyond publications and conferences? These questions, though uncomfortable, can mark the beginning of a fascinating transformation. The truth is that an academic career is not the only legitimate way to set science in motion. Often, academia does not teach us how to leave academia. The corporate world, for instance, demands exactly the skills we cultivate at the University: critical thinking, the ability to solve complex problems, resilience, communication, and innovation. What changes is the stage and the way we translate our background into this new setting. When science changes its badge, making this transition does not mean losing our scientific identity. On the contrary, it means continuing to seek opportunities to expand science's impact in the world through knowledge. It means accepting that our path can - and should - be reinvented. So, what if your career doesn't fit inside the University? Maybe that's exactly where your true journey begins: when you realize you don't have to fit into a mold but can create your own. I invite you to join me as we explore these (un)certainities that once set me free... and, in many ways, keep setting me free every day.

Anatomy of an Entrepreneur Scientist (Anatomia de um Cientista Empreendedor). Fabrício Alano Pamplona Vital Green Holding / Mind the Graph

The transition from academic researcher to entrepreneur represents a paradigm shift that requires the development of new competencies beyond traditional scientific training. This presentation explores the anatomy of the scientist-entrepreneur journey, examining the essential skills, mindset changes, and strategic decisions required to successfully bridge the gap between academia and business. Drawing from personal experience transitioning from pharmaceutical research to founding and scaling Mind the Graph - a platform that democratizes scientific communication through visual tools - we will dissect the critical components of entrepreneurial success in science-based ventures. The discussion will cover the importance of understanding market needs, building interdisciplinary teams, securing funding, and maintaining scientific rigor while pursuing commercial objectives. We will also address common challenges faced by academic professionals entering the entrepreneurship ecosystem, including risk assessment, intellectual property management, and the development of business acumen. The presentation aims to provide young pharmacologists and researchers with practical insights and actionable frameworks for evaluating and pursuing entrepreneurial opportunities beyond traditional academic careers. By understanding the anatomical structure of successful scientist-entrepreneurs, attendees will be better equipped to make informed decisions about their own career trajectories and contribute to innovation at the intersection of science and business.

Is the Entrepreneurial Spirit an Innate or Adaptive Force? (O espírito empreendedor é uma força inata ou adaptativa?) Andreas Kaasi (Eva Scientific)

Having an 'entrepreneurial spirit' is often seen in a positive light, by laymen and scholars alike. But just like immunity, being either innate or adaptive, what can we say of the entrepreneurial spirit - is it inborn or can it be learned? Before we explore this further, if the entrepreneurial spirit is something you can study and learn, or if it is a gift, much like the original meaning of the word charismatic, a word which we often associate with those endowed with the entrepreneurial spirit, charisma being a "God-given gift", we should ponder some on the complex nature of this trait. In fact, using a Google search methodology aiming to find the commonest definitions of the trait, searching for "entrepreneurial spirit is a" and taking the statistics of the subsequent word or words, 'mindset' tops the list, followed by 'way', 'process', 'approach', 'desire', 'attitude', and only then, 'force'. 'Mindset' was, precisely, the word originally picked out for the purpose of the title here, yet 'force' was given precedence, not only to invoke the philosophy of Yoda but also to highlight, or rather, to answer the previous question, how 'entrepreneurial spirit' is a force that can, indeed, be trained much like muscular or rhetorical force. As previously described ([Kaasi, 2017](#)), an entrepreneur can be characterized as "confident" and "resourceful", characteristics that can, indeed, be learned and

that are positive, yet at the same time he is characterized as “opportunistic” and “selfish”, characteristics that are mostly inborn and that are viewed as negative, at least in general terms. In other words, an entrepreneur exhibits positive and negative traits at the same time, and his characteristic essence has both an innate and an adaptive component! Upon studying US-based star entrepreneurs such as Steve Jobs and John D. Rockefeller, and Brazilian-based entrepreneurs such as Roberto Justus and Andreas Kaasi, we recognize patterns: they have innate traits, but many of their traits are adaptive in nature and were learned. It is a classical chicken and egg problem, which comes first? It is concluded that while star entrepreneurship may have an innate component, core entrepreneur characteristics such as confidence and resourcefulness can be learned and trained. Anyone can exhibit entrepreneurial spirit, whereas it might just be that charisma, in the true sense of the word, is required to stand out. Kaasi, A. (2017). “Startup in Brazil”—Current Trends in Drug Delivery—UFG. SlideShare. <https://www.slideshare.net/AndreaskKaasi/current-trends-in-drug-delivery-andreas-kaasi-Goiania-startup-in-Brazil>

01. Cellular and Molecular Pharmacology

01.001 SIVA1 as a Key Player in Breast Cancer Development: Evidence from *in vitro* Approaches. Parducci NS¹, Oliveira BA¹, Garnique AMB, Carvalho, MFL¹, Machado-Neto JA^{1,2} ¹USP, Institute of Biomedical Sciences, Dept. of Pharmacology, Brazil. ²USP, School of Medicine, Dept. of Clinical Medicine, Brazil

Introduction: Breast cancer remains a major clinical challenge, with disease progression still occurring despite therapeutic advances. Its development involves dysregulation of key cellular processes, including proliferation, cell death, and differentiation—events controlled by complex gene and protein networks. Among the molecules implicated in these processes, SIVA1 has been associated with apoptosis, migration, cytoskeletal dynamics, and epithelial-mesenchymal transition. However, its precise role in breast cancer biology remains unclear, representing a critical gap in our understanding that warrants further investigation. **Objectives:** To explore the cellular mechanisms associated with SIVA1 activity and its molecular network, with the aim of characterizing its potential anti-oncogenic role. **Methods** The cell lines BT549 (high SIVA1 expression) and Hs578T (low SIVA1 expression) were selected based on a screening of breast cancer cell lines. Knockdown models were generated via lentiviral transduction and treated with doxorubicin, paclitaxel, and topotecan. The models were subjected to assays for cell viability (MTT), colony formation, apoptosis, cell cycle, migration, and spheroid formation. Protein content and gene expression were assessed by Western blot and qPCR. Statistical analyses included Student's t-test, ANOVA, Kruskal-Wallis test, and Bonferroni post hoc test, with significance set at $p < 0.05$. **Results:** The knockdown models exhibited lower IC_{50} values for paclitaxel treatment ($0.004 \mu M$ for both), showing higher basal apoptosis rates ($p_{Hs578T} = 0.001$; $p_{BT549} = 0.016$) and increased Sub-G1 arrest ($p_{Hs578T} = 0.041$; $p_{BT549} = 0.017$) compared to non-silenced cells. Spheroids derived from the knockdown were smaller in size ($p_{Hs578T} = 0.002$; $p_{BT549} = 0.005$) and more responsive to taxane treatment, exhibiting decreased ATP levels ($p_{Hs578T} = 0.017$; $p_{BT549} = 0.021$). Their migratory capacity was significantly enhanced by SIVA1 inhibition ($p_{BT549} < 0.0001$). Protein analysis confirmed increased sensitivity of the models to treatment, reflected by elevated levels of cleaved PARP1 and p-H2AX. Additionally, SIVA1 downregulation induced accumulation of phosphorylated p53 (active form), along with decreased levels of phosphorylated Stathmin1 (inactive form) and ubiquitin labeling. **Conclusion:** SIVA1 knockdown enhances breast cancer cell sensitivity to paclitaxel by increasing apoptosis and cell cycle arrest. It also reduces spheroid growth and elevates DNA damage markers, while promoting migration. These findings reinforce the protective role of the gene. **Financial support:** CNPq, CAPES (001), and FAPESP (#2023/11752-5).

01.002 **Major Depressive Disorder Patient Serum Induces TLR4 Activation in Human Cells.** Soares JV¹, Farias IS¹, Fidalgo FM², Hayashi MAF¹ ¹Unifesp-EPM, Laboratory of molecular Neuropharmacology, Pharmacology Department, São Paulo, Brazil; ²Unifesp-EPM, Department of Psychiatry, São Paulo Brazil

Introduction: Major depressive disorder (MDD) affects about 20% of adults and is a leading cause of disability and suicide. Immune dysregulation and chronic inflammation, particularly involving Toll-like receptor 4 (TLR4), have been implicated in its pathophysiology. Increased gut permeability may allow bacterial translocation, activating TLR4 and triggering oxidative/nitrosative damage. In mice, stress upregulates TLR-4 pathway in the frontal cortex, activating NF- κ B and pro-inflammatory enzymes. Similarly, clinical studies have reported upregulation of the TLR4 signaling pathway in newly diagnosed and untreated patients with MDD. Antidepressant treatment has anti-inflammatory effects, reducing the expression of TLR-4 and NF- κ B, although some studies report higher TLR4 levels even in remission. **Objective:** To investigate whether serum from MDD patients could activate TLR-4 proinflammatory pathway *in vitro*. **Methods:** HEK-Blue™ hTLR4 cells were divided into two experimental groups: one treated with pooled serum from healthy control (HC) individuals, and another treated with pooled serum from patients diagnosed MDD who were undergoing pharmacological treatment with antidepressants, such as selective serotonin reuptake inhibitors, and/or atypical antipsychotics. Exclusion criteria for all participants included comorbid substance abuse or drug dependence (with the exception of tobacco use), as well as any intellectual disability that could impair the ability to understand and provide informed consent. HC volunteers were screened to ensure they had no history of psychiatric illness and were not using any psychoactive medications, including antidepressants or antipsychotics. Cells were treated with 1% pooled human serum and incubated for 48 hours. Culture supernatants were then collected and analyzed using the QUANTI-Blue™ assay, a colorimetric method for detecting secreted embryonic alkaline phosphatase (SEAP). SEAP expression is induced by NF- κ B activation downstream of TLR4 stimulation; thus, increased absorbance readings reflect enhanced activation of the TLR4 signaling pathway. **Results:** TLR4 activation was lower in cells exposed to MDD patient serum compared to HC. Given that the patient group was undergoing antidepressant treatment, these findings suggest a positive correlation between antidepressant use and reduced TLR4 activation. However, since patients were under different pharmacological regimens, it is not possible to attribute the observed effect to a specific class or drug. **Conclusion:** Antidepressant treatment has recognized anti-inflammatory effects and may contribute to normalizing TLR4 levels in patients with MDD. Our results are consistent with this clinically relevant mechanism and validate the use of this assay for future studies across different pathologies and treatment conditions. However, treatment heterogeneity limits conclusions about drug-specific effects, highlighting the need for studies with controlled pharmacological groups. **Ethics:** This study was approved by the Research Ethics Committee of UNIFESP and CEP [CAAE: 57957122.1.0000.5505], and written informed consent was obtained from all recruited participants. **Financial support:** FAPESP, CNPq, CAPES, and FINEP

01.003 Neuroprotective and Antioxidant Effects of the Paracetamol Analog LQFM²91 in a 6-OHDA Mouse Model of Neurotoxicity. Ferreira PYO¹, Santos EEO¹, Uchenna N¹, Campos HM¹, Costa EA¹, Menegatti R², Ghedini PC¹

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Introduction: Oxidative stress and neuroinflammation play key roles in the pathogenesis and progression of neurodegenerative diseases (NDs). Within a research program aimed at developing novel treatments for NDs, LQFM291 was synthesized by molecular hybridization of paracetamol and butylated hydroxytoluene. Previous studies demonstrated antinociceptive, anti-inflammatory, and antioxidant properties of LQFM291 in inflammatory animal models. Based on these findings, this study aimed to evaluate the antioxidant and neuroprotective effects of LQFM291 in a 6-hydroxydopamine (6-OHDA)-induced neurotoxicity model in male Swiss mice. **Methods:** Male Swiss mice (12 weeks old) were randomly allocated into six groups (n=10): Control, 6-OHDA (15 µg/µL), L-DOPA (10 mg/kg), and LQFM291 at doses 0.03 mM (LQFM1), 0.1 mM (LQFM2), and 0.3 mM (LQFM3). Animals were anesthetized with ketamine (80 mg/kg) and xylazine (15 mg/kg), followed by stereotaxic injection of 30 µg 6-OHDA dissolved in 2 µL 0.9% NaCl containing 0.2 µg/µL ascorbic acid into the right striatum (coordinates: 0.9 mm anterior, 1.8 mm lateral, and 3.0 mm ventral). Seven days post-surgery, oral treatments were administered for 30 consecutive days. Motor performance was assessed using the rotarod (RR) and chimney (CM) tests. After 30 days the animals were euthanized for striatal analyses, including lipid peroxidation (LPO) expressed in nmol of MDA/mg of protein, and the activities of superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), all expressed in U/mg of protein. All procedures were approved by the Ethics Committee on Animal Use of UFG (nº 074/2022). Statistical analysis was performed with significance set at $p < 0.05$. **Results:** Behavioral tests showed that LQFM291 at 0.1 mM and 0.3 mM significantly improved locomotor performance in the CM test compared to the 6-OHDA group ($p < 0.05$). All LQFM291 doses increased latency to fall in the RR test (LQFM1: 11.65 ± 0.90 s; LQFM2: 11.75 ± 1.05 s; LQFM3: 13.26 ± 1.25 s) compared to 6-OHDA (7.73 ± 0.56 s) ($p < 0.05$). Biochemically, LPO levels were increased by 97% in the 6-OHDA group compared to the Control, while treatment with LQFM291 significantly reduced these levels by 53%, 68%, and 82% for LQFM1, LQFM2, and LQFM3, respectively ($p < 0.05$). CAT activity was reduced by 63% in the 6-OHDA group compared to the Control, while treatment with LQFM291 restored enzyme activity by 34%, 42%, and 58% for LQFM1, LQFM2, and LQFM3, respectively ($p < 0.05$). Similarly, SOD activity was decreased by 49% in the 6-OHDA group compared to the Control, while treatment with LQFM291 partially restored this activity by 17%, 26%, and 35% for LQFM1, LQFM2, and LQFM3, respectively ($p < 0.05$). GPx activity was reduced by 59% in the 6-OHDA group compared to the Control, while treatment with LQFM291 led to a partial recovery of 21%, 37%, and 46% for LQFM1, LQFM2, and LQFM3, respectively ($p < 0.05$). **Conclusion:** These preliminary results demonstrate that LQFM291 exerts significant antioxidant and neuroprotective effects against 6-OHDA-induced neurotoxicity in male Swiss mice. LQFM291 shows potential as a novel therapeutic agent for neuroprotection and warrants further investigation. **Financial support:** CAPES

01.004 **Nanoplastics Modulate Carboplatin Response in Ovarian Cancer Cell Line: Pharmacological Resistance and Metabolic Paradox.** Silva GCA, Lima MC, Portela LMF Aal

Introduction: Nanoplastics (NPs) resulting from plastic degradation pose environmental and health risks due to their ability to cross biological membranes and accumulate in tissues. Epithelial ovarian cancer (EOC) can exhibit resistance to chemotherapeutic agents like carboplatin (CB), which presents a significant challenge. **Methods:** This study explored the dual role of polystyrene NPs (100 nm) in modulating chemoresistance in the epithelial ovarian cancer cell line (SKOV-3), assessing both NPs alone (40 µg/mL) and in combination with carboplatin (CB, 100 µM) over 24 hours, based on preliminary assays indicating viability modulation within an NP exposure range of 10-80 µg/mL. We performed evaluations of cell viability, cell migration, Live/Dead staining, and gene expression related to tumor progression. Statistical analyses employed one-way ANOVA followed by Tukey's test ($p < 0.05$). **Results:** NPs group showed a significant increase in metabolic viability (25%) and cell migration, along with upregulation of pro-survival markers (mTOR, 2.08x; BCL2, 2.3x) and a pro-inflammatory shift characterized by an increase in IL-6 and a decrease in IL-10, despite downregulation of the PI3K/AKT/MAPK pathways. Notably, while MTT assays suggested enhanced viability, Live/Dead staining revealed underlying structural damage (85.5% viability vs control). In the NPs+CB co-exposure group, striking metabolic hyper-viability (182.3%) contrasted sharply with reduced structural integrity (79.8% Live/Dead viability), demonstrating NPs' capability to sustain metabolic activity while compromising membrane integrity. This group maintained BCL2 (1.7x) and mTOR (1.3x) expression with EMT induction (increased vimentin by 4.65x), yet displayed 80.7% migration inhibition likely due to CB-induced cytoskeletal damage. Both treatment groups exhibited apoptosis resistance, characterized by decreased CASP3/8 gene expression, despite BAX induction, confirming the chemoprotective effect of NPs. Higher NP concentrations (60 and 80 µg/mL) decreased metabolic viability, indicating direct cytotoxicity. **Conclusion:** These findings reveal that at environmental doses, NPs promote ovarian cancer survival through mTOR/BCL2 activation and IL-6-mediated inflammation while causing sublethal damage, and critically impair CB efficacy through dual metabolic-structural disruptions. The findings underscore the impact of NP pollution on the progression of EOC and treatment efficacy. **Keywords:** nanoplastics, cancer, chemoresistance, tumor microenvironment. **Financial support:** FAPESP (24/04120-5), CAPES (Finance Code 001)

01.005 TRPV1 Antagonist SB366791: An *in Silico* and *in vitro* Evaluation of Physicochemical and Neurobiological Properties. Barros ASM¹, Lisboa SF^{1,2}. ¹FMRP/USP, Pharmacology PPG, Brazil; ²FCFRP/USP, Department of Biomolecular Sciences, Brazil.

Introduction: TRPV1 is a predominantly calcium-permeable ion channel that responds to various stimuli, such as noxious heat and capsaicin. In the Central Nervous System, this receptor is expressed in several cells and can be activated by endogenous ligands such as anandamide. In postsynaptic neurons, TRPV1 activation generates an increase in Ca²⁺ influx, which can lead to increased glutamate release and, consequently, excitotoxicity. In microglia, TRPV1 activation is associated with a greater release of pro-inflammatory cytokines. Thus, TRPV1 inhibition may be a promising strategy to mitigate neuroinflammation. Characterizing the physicochemical, pharmacokinetic, toxicological, and biological properties of TRPV1 antagonists is critical. In this work, the antagonist SB366791 was evaluated. **Methodology:** *In silico* analyses were conducted using ChemsSketch (chemical structure), Molecule Property Calculator (physicochemical properties), Preadmet (ADMET prediction), Protox II (toxicity), and Pass Online (biological activity). *In vitro* studies were performed with a human microglia line (T0251) and a human neuroblastoma line (SH-SY5Y, SH) differentiated into a mature neuronal phenotype by adding retinoic acid (10 µM) and BDNF (50 ng/mL) to the culture medium for 8 days. Cells were treated with vehicle (cell medium) or SB366791 (0.01, 0.1, 1, 10, 100 and 1000 µM) for 24 hours. **Results:** *In silico* analysis: SB366791 has a molecular mass of 287.73 g/mol, logP of 4.07, polar surface area of 38.33, hydrogen bond acceptors of 3 and hydrogen bond donors of 1, thus fulfilling the requirements of Lipinski's rule. Moreover, the molecule has a high skin permeability (-2.63, since high permeability: <0.1, and low permeability: > 0.1) and a high human intestinal absorption (96.3%), and is able to cross the blood-brain barrier in a moderate way (1.6, since the molecule passes through moderately when the value is in the 2-0.1 range). SB366791 also exhibits potential toxicity to marine organisms and is carcinogenic to mice. Moreover, one of the main targets of interaction may be Monoamine Oxidase A, and the molecule may act as an inhibitor of ubiquinol cytochrome-c reductase. *In vitro* studies: All concentrations of SB366791 reduced the cell viability of microglia cells, analyzed by the colorimetric method of 3-(4,5-Dimethylthiazol-2-yl) 2,5-Diphenyl Tetrazolium Bromide (MTT). Moreover, a significant release of lactate dehydrogenase (LDH assay) was observed after treatment with 100 and 1000 µM of the drug in these cells. In differentiated neurons (SH cells), viability decreased at 100 and 1000 µM, while LDH release increased significantly at 10, 100 and 1000 µM of SB366791. **Conclusion:** The TRPV1 antagonist SB366791 displays favorable physicochemical and pharmacokinetic properties. However, its potential mitochondrial interference (e.g., ubiquinol-cytochrome-c reductase inhibition) and cytotoxicity in microglia and neurons underscore the need for further safety and dosage studies. **Financial support:** FAPESP and CAPES.

01.006 **The Dual Role of ANKHD1 in Gastric Cancer: Insights from Integrative Analyses.** Almeida BO¹, Gasparini MVR², Silva JCL¹, Archangelo LF², Machado-Neto JA¹ ¹ICB-USP, Dept Pharmacology, PPG Functional and Molecular Biology, Brazil ²FMRP-USP, Dept Cellular and Molecular Biology and Pathogenic Bioagents, PPG Cell and Molecular Biology, Brazil

Background: The ANKHD1 gene encodes a scaffold protein involved in protein and nucleic acid interactions, implicated in the regulation of cancer-related signaling pathways. However, its role in gastric cancer (GC) remains poorly understood. **Aims:** To investigate the biological role of ANKHD1 and its associated signaling pathways in gastric cancer. **Methods:** Data from The Cancer Genome Atlas (TCGA) GC cohort were analyzed, stratifying patients into high and low ANKHD1 expression groups based on quartiles. Kaplan-Meier survival analysis and chi-square tests were used to explore associations with clinical and molecular features. Differentially expressed genes (DEGs) were identified and subjected to Gene Ontology (GO)-based enrichment analysis. The ANKHD1 interactome was determined via immunoprecipitation followed by mass spectrometry, with protein enrichment assessed using STRING. CRISPR/Cas9 was used to generate ANKHD1 knockout models in AGS and HGC-27 gastric cancer cell lines. Clonogenic, Sulforhodamine B (SRB), and Transwell migration assays assessed proliferation and migration capacity. To evaluate chemotherapy response, control and ANKHD1-knockout cells were treated with increasing concentrations of 5-fluorouracil (5-FU), paclitaxel, and doxorubicin for 72 hours, and viability was evaluated by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide) assay. **Results:** ANKHD1 expression was significantly higher in GC tissues compared to normal gastric samples ($p = 0.037$). High ANKHD1 levels correlated with larger tumor size ($p = 0.003$), advanced TNM stage ($p = 0.046$), and molecular subtype ($p = 0.001$). Among GC cell lines, HGC-27 (a metastatic cell line) exhibited the highest ANKHD1 protein levels ($p = 0.047$). High ANKHD1 expression was also associated with worse progression-free survival in the intestinal and microsatellite instability (MSI) subtypes. ANKHD1 knockout in AGS cells reduced colony formation and enhanced migratory capacity ($p < 0.05$). ANKHD1 depletion conferred resistance to doxorubicin and reduced paclitaxel efficacy. Enrichment analyses of DEGs and interactome data suggested that ANKHD1 is involved in regulating cell adhesion, transcription, and translation. Immunolocalization revealed ANKHD1 accumulation at cell-cell junctions and distinct nuclear puncta. **Conclusions:** ANKHD1 plays a multifaceted role in gastric cancer, influencing tumor growth, migration, and chemotherapy response in a context-dependent manner. Its association with poor progression-free survival in specific molecular subtypes, such as intestinal and microsatellite instability (MSI) tumors, underscores the importance of dissecting its function within defined biological contexts. A deeper understanding of ANKHD1's molecular interactions and regulatory mechanisms is essential for evaluating its potential as a selective therapeutic target in gastric cancer. **Financial support:** CAPES (001); FAPESP (2022/11038-8 and 2023/12246-6).

01.007 Viability and Cytotoxicity Profile of Beta-Hydroxybutyrate in Neural Progenitor Cells.

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Introduction: Beta-hydroxybutyrate (BHB), the main endogenous ketone body, has been studied for its neuroprotective and epigenetic modulating properties. However, its direct effects on proliferation, viability, and cytotoxicity in neural progenitor cells (NPCs) remain unclear. To evaluate the effects of BHB under different treatment schedules on the proliferation, viability, and cytotoxicity of murine NPCs cultured in proliferative medium. **Methods:** NPCs isolated from neonatal mouse (Animal Use Ethics Committee approved 3952300622) hippocampus were cultured to form clusters of neural progenitors (neurospheres). BHB concentrations ranging from 0.25 to 10 mM were tested under three treatment schemes: single treatment at sixth day *in vitro* DIV6; intermittent treatment every 2 days (DIV1, 3, 5, and 7); and intermittent treatment every 3 days (DIV1, 4, and 7). Proliferation was assessed by neurosphere number and diameter; viability and cytotoxicity were measured by MTT and LDH release assays, respectively, at DIV7 and DIV8. Data were expressed as mean $\pm$ standard error of the mean (SEM) and analyzed by ANOVA with Dunnett's test ($p < 0.05$). Note: DIV stands for "days *in vitro*," indicating the number of days cells have been in culture. **Results:** In the single treatment, no BHB concentration significantly altered neurosphere proliferation (number and diameter), viability, or cytotoxicity after 24 and 48 hours. In the intermittent every 2 days treatment, only the 10 mM concentration significantly reduced neurosphere diameter ($p = 0.0034$), with no change in number, viability, or cytotoxicity. The intermittent every 3 days treatment showed no effect on proliferation or viability/cytotoxicity parameters at any tested concentration. **Conclusion:** The data indicate that BHB up to 10 mM does not exert cytotoxic or viability effects on NPCs, even under multiple and prolonged treatment schemes. Only the highest concentration (10 mM) under the intermittent every 2 days treatment showed an impact on reducing neurosphere diameter, suggesting a mild antiproliferative effect without associated cytotoxicity. These results support the safety of BHB for NPCs within the studied concentration range and emphasize the need for careful dose evaluation in therapeutic strategies. **Financial support:** Fapesp, CNPQ, Capes.

01.008 A Novel Arylpiperazine Derivative (LQFM181) Promotes Antioxidant and Neuroprotective Effects Against 3-Nitropropionic Acid-Induced Neurotoxicity in Swiss Mice. Campos HM¹, Ferreira PYO¹, Pereira R¹, Okoh VI¹, Pruccoli. L³, Uchenna N¹, Leite J¹, Menegatti R², Tarozzi A³, Ghedini PC¹
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Introduction: In the pursuit of novel antioxidant therapies for the prevention and treatment of neurodegenerative diseases, a new arylpiperazine derivative, LQFM181, was synthesized through a molecular hybridization strategy using piribedil and butylated hydroxytoluene (BHT) as lead compounds. Piribedil is known for improving cognitive function, while BHT has recognized antioxidant properties. This strategy aimed to combine both neuroprotective and antioxidant actions into a single, more effective compound—LQFM181—designed to enhance therapeutic potential through the integration of multiple beneficial effects. **Aim:** To evaluate the antioxidant and neuroprotective effects of LQFM181 in *in vitro* and *in vivo* models of 3-nitropropionic acid (3-NP) induced neurotoxicity. **Methods:** For the *in vitro* assays, the human SH-SY5Y neuroblastoma cells, were acquired by the Experimental Zootechnical Institute of Lombardy and Emilia Romagna (Italy). The cells were cultured in Dulbecco's Modified Eagle Medium supplemented with phenol red, fetal bovine serum, L-glutamine, penicillin, and streptomycin, and maintained at 37 °C in a 5% CO₂ incubator. Cell viability was assessed using the MTT assay to evaluate the protective effects of LQFM181 in the presence or absence of 3-NP. For the *in vivo* experiments, male Swiss mice were randomly divided into five groups (n = 10): Control, 3-NP (15 mg/kg), and LQFM181 at 10 mg/kg (LQFM10), 25 mg/kg (LQFM25), and 50 mg/kg (LQFM50). Mice received daily oral doses of LQFM181 or vehicle (Control and 3-NP groups) for 31 days. Behavioral assessments included the chimney, open field, and rotarod tests. After euthanasia, the striatum was collected for biochemical analyses, including lipid peroxidation (LPO), protein carbonyl (CP) levels, superoxide dismutase (SOD), catalase (CAT), and succinate dehydrogenase (SDG) activities. All procedures were approved by the Animal Research Ethics Committee of UFG (Protocol No. 053/2016). Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test, with p < 0.05 considered statistically significant. **Results:** *In vitro* results demonstrated that LQFM181 reduced the neurotoxic effects induced by 3-NP (p < 0.05). *In vivo*, behavioral tests revealed that the 3-NP group exhibited reduced motor coordination, exploratory behavior, and resistance when compared to the control group (p < 0.05). Treatment with LQFM181 at all doses improved the locomotor activity when compared to the 3-NP group (p < 0.05). Biochemical analyses showed that SDG, SOD, and CAT activities were reduced in the 3-NP group when compared to control (p < 0.05), and the treatment with LQFM181 at all doses restored SDG, SOD and CAT activities (p < 0.05). LPO and CP levels were elevated in the 3-NP group (p < 0.05), and LQFM181 at all doses prevented this increase (p < 0.05). **Conclusion:** LQFM181 exerts neuroprotective effects in SH-SY5Y cells against 3-NP-induced toxicity. Additionally, *in vivo* results demonstrated significant locomotor protection, supporting the neuroprotective efficacy of LQFM181 against 3-NP-induced motor deficits. These behavioral outcomes were corroborated by biochemical data, indicating that LQFM181 mitigates oxidative stress. Therefore, LQFM181 represents a promising therapeutic candidate for further research in the treatment of neurodegenerative conditions. **Financial support:** CAPES and CNPq.

01.009 Bioactivity of Angiotensin I (AngI) and II (AngII) Peptides in the Aorta of *Crotalus durissus terrificus* and *Oxyrhopus guibei* Snakes. Nogueira ACS, Breno MC, IBu, Pharmacology Lab., Brazil

The AngII receptor and others renin-angiotensin system (RAS) components have been characterized in some species of vertebrate. RAS is one of the endogenous systems that maintain cardiovascular homeostasis in the vertebrate. Previous studies carried out in the Brazilian snakes *Bothrops jararaca* and *Crotalus durissus terrificus* (*Cdt*) - Viperidae family, and *Oxyrhopus guibei* (*Og*) - Colubridae family have characterized the presence of an AngII receptor and an active angiotensin-converting enzyme, *in vivo* and *in vitro*, in the cardiovascular system of these reptiles. This study aims to evaluate the action of the AngII analogue in the aorta of the snakes *Cdt* and *Og*, as well as the participation of the Ca^{2+} -ATPase pump from sarcoplasmic reticulum (SERCA) in the AngII dynamic response in the *Cdt* aorta. The action of the *Goosefish* AngI was also obtained in two *Cdt* aorta segments - at rostral or caudal positions. Adult snakes *Cdt* (350-800g) and *Og* (100-200g), both gender, were kept under controlled environmental conditions (12h light/dark, 21-27°C) at Instituto Butantan. Snakes were anesthetized (sodium pentobarbital, 30 mg/kg) and euthanized (CEUA 9073080518 and 9738030616). Aortic rings (~1cm) were removed free of connective tissues and suspended between two L-shaped steel hooks into a 5 ml organ chamber, containing nutritive solution at 37°C, aerated with carbogen. The aortic muscle activity was recorded using the Biopac MP100WS data acquisition system. Isometric tension induced by cumulative concentration-response curves to AngII or AngI were obtained in aorta of *Cdt* or *Og* to evaluate the AngII analogue and *Goosefish* AngI responses. AngII-cumulative curve was also obtained in the absence and in the presence of cyclopiazonic acid (CPA- 3×10^{-5} M, 30 min.) to evaluate the Ca^{2+} -ATPase pump, as well as AngII activity at caudal aorta segment. The pharmacological parameters pD_2 (the negative logarithm of the molar concentration of AngII required to produce 50% of the maximum effect - potency) and $E_{\max}$ (maximum effect) were calculated from the concentration-response curves. The non-mammalian AngII analogue ([Asn¹, Val⁵] AngII - $pD_2 4.33 \pm 0.20$) was less potent than the human AngII ([Asp¹, Ile⁵] AngII - $pD_2 6.56 \pm 0.13$) in the *Cdt* aorta (n=7). Similar result was obtained in *Og* aorta (n=3) being the non-mammalian AngII ($pD_2 3.91 \pm 0.05$) less potent than the human AngII ($pD_2 6.30 \pm 0.19$). The *Goosefish* AngI ($pD_2 4.62 \pm 0.38$) was also less potent than the human AngII ($pD_2 6.97 \pm 0.26$) n=3, in both, the *Cdt* rostral and caudal aorta segments (*Goosefish* AngI $pD_2 4.54$ / human AngII $pD_2 7.19$ n=1). The cyclopiazonic acid (3×10^{-5} M, 30 min.) did not modify the human AngII vasoconstriction effect in the *Cdt* aorta (in the presence $pD_2 7.01 \pm 0.11$; in the absence $pD_2 6.97 \pm 0.07$, n=4). These results indicate that the *Goosefish* AngI (inactive peptide) seems to be converted to AngII (active form) in the aorta, producing a vasocontraction effect as potent as the non-mammalian AngII. [Asn¹Val⁵] AngII was less potent than human AngII in both snake species. The CPA results suggest no participation of a SERCA in the AngII response. The data together with our previous results in other Brazilian snakes allow understanding the pharmacology and the physiology of the RAS in the cardiovascular system of these reptiles. Supported: Fundação Butantan

01.010 **Biochemical Characterization of the Cofactors Involved in the Phosphatase Activity of Na, K-ATPase and Thermodynamics of its Interaction with Cardiotonic Steroids Aiming at Stimulatory Effects.** Esteves G¹, Azalim, P², Barbosa L³, Villar J³, O'Doherty G⁴, Blanco G⁵, Quintas L¹, Scavone C². ¹UFRJ, Laboratório de Farmacologia Bioquímica e Molecular, Brasil ²USP, Laboratório de Neurofarmacologia Molecular, Brasil ³UFSJ, Laboratório de Bioquímica Celular ⁴Northeastern University, Dept Chemistry, Boston, USA ⁵Kansas University Medical Center, Dept Molecular and Integrative Physiology, USA

Introduction: Although cardiotonic steroids (CTSs) are traditionally known as inhibitors of Na,K-ATPase (NKA) activity, some studies have reported their stimulatory capacity, suggesting potential new therapeutic approaches for Alternating Hemiplegia of Childhood (AHC), a disease associated with mutations that affect the alpha3 isoform of NKA. NKA, however, undergoes several conformational states during its catalytic cycle, including an E1 state for ATP hydrolysis and Na⁺ binding, and an E2 state for phosphatase activity and K⁺ binding — the latter being poorly addressed in the literature. Since various cofactors influence its function, characterizing NKA requires new approaches beyond traditional Michaelis-Menten models. Moreover, to identify ligands, the kinetics and thermodynamics of interaction may be more critical to understanding activity than equilibrium constants alone. Therefore, our goal is to characterize phosphatase activity as a step toward drug discovery for AHC. **Methods:** We measured the activity of NKA from porcine kidney (alpha1/beta1 isoform, obtained from a commercial slaughterhouse) using increasing concentrations of the chromogenic substrate p-NPP in 96-well plates, under varying concentrations of cofactors via a curve progression assay. We subsequently identified the interaction kinetics of 40 CTSs (novel and commercial) and assessed the effect of temperature (20–40°C) on rate constants associated with distinct NKA conformations. **Results:** Our data suggest that only Mg²⁺ and K⁺ displayed a bell-shaped profile in initial velocity (v_0), with $CE_{50} = 1.26$ mM (Hill coefficient, $nH = 1.69$) and $IC_{50} = 50$ mM ($nH = -2.32$), respectively. K⁺ exhibited distinct CE_{50} and IC_{50} values at each p-NPP concentration, sharing only the stimulatory $nH = 1.76$ and inhibitory $nH = -1.35$, suggesting that only K⁺ exerts an allosteric effect capable of altering substrate affinity. All other ligands — Na⁺, Pi, and ATP — merely reduced the V_{max} with IC_{50} values of 42 mM ($nH = -2$), 1.4 mM ($nH = -1.3$), and 0.97 mM ($nH = 0.9$), respectively. The v_0 vs. p-NPP relationship presented a bell-shaped curve in the presence of low K⁺ or high Na⁺ concentrations. Plotting $\log(p\text{-NPP affinity})$ vs. $\log(K^+)$ revealed a biphasic behavior with IC_{50} values at 0.22 mM and 446 mM, both with $nH = 1$. Regarding the inhibitory nature of 15 CTSs, three showed enthalpy-dominant inhibition, while the others were entropy-dominant. These three molecules also exhibited the fastest kinetics. None of the 15 compounds tested showed stimulatory or bell-shaped activity. **Conclusion:** These data reveal how the active site responds to cofactors and the structure of the tested ligands, guiding us in evaluating future compounds and moving toward the next experimental phase using porcine brain preparations, which contain the alpha3 isoform of NKA. **Financial support:** PIBIC-CNPq, CNPq, FAPESP, FAPERJ, CAPES, FINEP

01.011 **Intracellular Peptides as Novel Modulators of Thermogenesis: Functional Characterization of U2 in Adipocytes.** Jardim GFR¹, Valdivia LFG², Reckziegel P¹ ¹FCF-USP, Depto de Análises Clínicas e Toxicológicas; ²Unesp, Programa de Pós-graduação em Farmacologia

Introduction: For at least 20 years, several researchers have been working to understand the specific functions of intracellular peptides, a distinct group of protein fragments generated during protein metabolism. In the process of identifying the intracellular peptides profile in brown and white adipose tissues during thermogenesis induced by cold and the peroxisome proliferator-activated receptor gamma (PPAR γ) agonist pioglitazone (Valdivia, L. F. G., et al., 2023. Cold acclimation and pioglitazone combined increase thermogenic capacity of brown and white adipose tissues, but this does not translate into higher energy expenditure in mice. American Journal of Physiology-Endocrinology and Metabolism, 324(4), E358–E373), one peptidase fragment from uncoupling protein 1 (UCP1), the main protein related to thermogenesis in adipose tissues, was identified. Notably, this peptide was upregulated in all experimental groups involving brown adipose tissue (BAT) activation and browning promotion. The elucidation of the effects of this peptide, named U2, in adipocytes could reveal a novel peptide with thermogenic effects, potentially useful in the treatment of obesity and associated diseases. **Methods:** Primary cultures of brown and white adipocytes were obtained from adipose tissues of three-week-old male C57BL6 mice (Comissão de Ética no Uso de Animais da Faculdade de Ciências Farmacêuticas da Universidade de São Paulo [CEUA-FCF/USP] #028.2023). After six days of differentiation, cells were treated with a fragment from uncoupling protein 1 (UCP1), conjugated with a cationic TAT sequence (U1T), at concentrations of 1 μ M and 10 μ M. Cells were co-exposed to water or norepinephrine (NE, 5 μ M), the key physiological activator of brown and beige adipocytes. After 24 hours of exposure, cells were harvested for gene expression analysis focusing on thermogenic markers – UCP1, carnitine palmitoyltransferase 1A (CPT1 α), and peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1 α) – as well as the adipogenic marker fatty acidbinding protein 4 (FABP4). **Results:** The U2 peptide at 10 μ M significantly upregulated UCP1 (57,8%) and CPT1 α (5%), suggesting a potential to induce the "browning" of white adipose cells, a promising strategy to enhance energy expenditure. In the presence of NE, the effect was potentiated, showing upregulation of UCP1 (16%), PGC1 α (92%), and CPT1 α (76,4%), indicating a synergistic action with adrenergic stimuli. In brown adipocytes, U2 at the same concentration increased FABP4 expression only under adrenergic stimulation, suggesting a more tissue-specific effect (59%). Regarding brown adipocytes, an upregulation of UCP1 expression was observed without NE (49%). Interestingly, U2 at a lower concentration (1 μ M) elicited a different gene expression profile, indicating dose-dependent and possibly biphasic activity. This concentration- dependent effect is crucial for pharmacological development, as varying doses may trigger distinct cellular responses. Overall, U2 (10 μ M) emerged as a pharmacologically promising peptide capable of modulating adipose tissue metabolism. **Conclusion:** The differential effects observed for peptides U2 and F1 in brown and white adipocytes highlight their potential as selective modulators of adipose tissue metabolism. U2, particularly at 10 μ M, demonstrated strong thermogenic induction in white adipocytes and synergistic activation when combined with norepinephrine, suggesting its role in promoting white fat browning and increasing metabolic activity. F1, on the other hand, exhibited a more complex and context-dependent modulation of gene expression, influencing both thermogenic and adipogenic markers in a stimulus-specific manner. Together, these findings reinforce the concept that intracellular peptides, depending on their origin and cellular context, can elicit distinct and potentially therapeutic metabolic responses. This study supports the exploration of U2 and F1 as lead molecules for the development of peptide-based interventions targeting obesity and related metabolic disorders, paving the way for novel strategies focused on enhancing energy expenditure rather than solely suppressing caloric intake. This work was supported financially by the University of São Paulo and FAPESP. Process numbers: 2023/02579-8; 2021/03717-0; 2019/25943-1.

01.012 Effect of a Synthetic Curcuminoid on H₂O₂-Damaged Raw 264.7 Cells and Involvement of the Keap1/Nrf2/ARE Pathway. Ananias CF¹, Duarte ALS¹, Cajazeiras GI¹, Silva ME², Magalhães EP², Duque BR², Bezerra VM², Costa MDR², Pinheiro KS¹, Fernandes MO¹, Araújo JR¹, Santos HS¹, Martins AMC¹, Sampaio TL¹, Menezes RRPPB¹ ¹UFC, Faculty of Pharmacy, Dentistry and Nursing, Dept Clinical and Toxicological Analysis, Brazil; ²UFC, Faculty of Pharmacy, Dentistry and Nursing, Post Graduate Program of Pharmaceutical Sciences, Brazil

Curcumin has been extensively studied for its antioxidant properties, acting both as a free radical scavenger and a modulator of cell signaling pathways, particularly the Keap1/Nrf2/ARE axis. However, its clinical application is limited by poor solubility, chemical instability, and low bioavailability at physiological pH. To overcome these limitations, a monocarbonyl analogue was recently synthesized with the aim of developing improved therapeutic molecules. This study aimed to evaluate the cytoprotective effect of the synthetic curcuminoid (1E,4E)-1,5-diphenylpenta-1,4-dien-3-one (DBA) on macrophages subjected to oxidative stress induced by hydrogen peroxide (H₂O₂), and to investigate the involvement of the Keap1/Nrf2/ARE signaling pathway in this effect. RAW 264.7 cells (10⁵/mL) were treated with curcumin (Cur) or DBA for 20 hours, followed by exposure to H₂O₂ (500 µM) for an additional 4 hours. Untreated cells served as the negative control, while cells treated only with H₂O₂ were used for comparison. To assess cytoprotective effects, the MTT reduction assay was performed. Briefly, cells were washed and incubated with 0.25 mg/mL MTT solution for 3 hours. Formazan crystals formed were solubilized in DMSO, and absorbance was measured at 570 nm. Cell viability was expressed as a percentage relative to the control group. Gene expression was analyzed using reverse transcription quantitative polymerase chain reaction (RT-qPCR) with a commercial kit. Total RNA was extracted using TRIzol reagent, and cDNA was synthesized via reverse transcription. Amplification was conducted using a CFX96 Touch Real-Time PCR Detection System (Bio-Rad). The relative expression levels of Nrf2 and HO-1 genes were normalized to GAPDH as the housekeeping gene. All experiments were performed in triplicate (n = 3), and data were analyzed using one-way ANOVA followed by Tukey's post hoc test. As a result, H₂O₂ treatment induced approximately 50% cytotoxicity in macrophages. Both Cur (2.5, 5.0, and 10 µM) and DBA (12.5, 25, and 50 µM) partially prevented this cytotoxicity at all tested concentrations. Regarding gene expression, Cur and DBA treatments reduced the overexpression of Nrf2 and HO-1 induced by H₂O₂, suggesting that their cytoprotective effects involve modulation of the Keap1/Nrf2/ARE signaling pathway. In conclusion, the synthetic curcuminoid (1E,4E)-1,5-diphenylpenta-1,4-dien-3-one exhibited a cytoprotective effect against H₂O₂-induced damage in macrophages, likely through modulation of the Keap1/Nrf2/ARE pathway.

01.013 **Effects of Zinc Sulfate, Sodium-Pyrithione, and TPEN on the Viability of Human Endothelial Cells.** Pinheiro JVS¹, Muscara MN², Barros PR¹, Rodrigues, SF¹ ¹ICB-USP, Vascular Nanopharmacology Laboratory, Dept Pharmacology, São Paulo, Brazil; ²ICB-USP Lab Biochemical Pharmacology of Free Radicals, Inflammation and Pain, Dept Pharmacology, São Paulo, Brazil

Introduction: Thrombus formation and stroke are among the leading causes of cardiovascular morbidity and mortality, and they are often associated with coagulation dysregulation. Zinc (Zn^{2+}), a divalent cation released by activated platelets, plays important roles in cell proliferation, signaling, and gene expression. Variations in extracellular Zn^{2+} levels can positively modulate coagulation pathways and platelet activation within physiological concentrations (10-30 μM ; PMID: 22592845). Despite this critical role of Zn^{2+} in platelet function, the mechanisms by which extracellular Zn^{2+} levels modulate platelet-endothelium interactions and influence endothelial cell (EC) activation remain incompletely understood. Therefore, this study aimed to investigate whether different extracellular concentrations of Zn^{2+} exert a negative regulatory effect on EC viability. **Methods:** This study was conducted using commercially available human umbilical vein endothelial cells (HUVECs; CRL-1730, ATCC, Gaithersburg, MD, USA). HUVECs (passages P4-P7) were treated for 2 hours with increasing concentrations of zinc sulfate (ZnSO_4), as a zinc donor, in the presence of sodium pyrithione ($\text{C}_5\text{H}_4\text{NNaOS}$), a zinc ionophore, or TPEN ($\text{C}_{26}\text{H}_{28}\text{N}_6$), a high-affinity zinc chelator. To determine the non-cytotoxic concentration range for each compound in HUVECs, cell viability was assessed using the MTT assay, with three replicates performed. Tween 20 (0.1%) was used as a positive control. HUVECs were exposed to ZnSO_4 at 10, 30, 50, 70, and 90 μM ; sodium pyrithione at 1.3, 4, 12, 36, 108, and 324 μM ; and TPEN at 10, 15, 20, 30, 50, and 100 μM . Cell viability results are expressed in arbitrary units (a.u.), corresponding to the absorbance values obtained in the MTT assay. Data were analyzed using one-way ANOVA followed by Šidak's multiple comparisons test. **Results:** Exposure to increasing concentrations of ZnSO_4 , sodium pyrithione, or TPEN for 2 hours did not significantly affect HUVEC viability (mean [a.u.]: vehicle control solution = 0.24 ± 0.02 ; ZnSO_4 = 0.65 ± 0.30 ; vehicle control solution = 0.28 ± 0.09 ; sodium pyrithione = 0.26 ± 0.05 ; vehicle control solution = 0.80 ± 0.08 ; TPEN = 0.75 ± 0.05 ; Tween 0.1% = 0.16 ± 0.04) ($P > 0.05$). **Conclusion:** Short-term treatment with zinc sulfate, sodium pyrithione, or TPEN does not affect endothelial cell viability, suggesting the absence of cytotoxicity under the tested conditions. These results enabled the identification of suitable concentration ranges for subsequent mechanistic and functional assays without inducing off-target cytotoxicity. **Funding:** FAPESP (#2024/22073-4, and #2024/02250-9)

01.014 Involvement of the Transient Receptor Potential Ankyrin 1 (TRPA1) in Oxidative and Inflammatory Damage Induced By AOPPs In Microglial Cells. Giovelli M¹, Brum GF², Lovis EP², Montagner TRS³, Machado AK³, Bochi GV^{1,2} ¹UFMS, Dept. of Physiology and Pharmacology, Brazil, ²UFMS, PPG Pharmacology, Brazil, ³Franciscan University, PPG Nanosciences, Brazil

Introduction: The transient receptor potential ankyrin 1 (TRPA1) is an ion channel that acts as a cellular stress sensor. It is expressed in sensory neurons, immune cells, and within the central nervous system (CNS). In microglia, the primary immune cells of the CNS, TRPA1 plays a relevant role by detecting oxidative stress-related products such as reactive oxygen species (ROS) and inflammatory signals. Once activated, it promotes calcium influx and triggers pathways related to neuroinflammation and apoptosis that are associated with the pathophysiology of major depressive disorder (MDD). Advanced oxidation protein products (AOPPs) are markers of protein oxidation and have been associated with various clinical conditions, including MDD, where elevated AOPPs levels contribute to oxidative damage and worsening depressive symptoms. Despite their importance, the relationship between TRPA1 and AOPPs has been poorly explored. Therefore, this study aimed to evaluate whether AOPPs influence TRPA1-mediated effects in microglial cells. **Methods:** For this, BV-2 cells (an immortalized mouse microglial cell line) were cultured and exposed to different concentrations of AOPP-modified mouse serum albumin (AOPP-MSA, 50, 100, 200, 400, and 800 µg/mL). After 24 hours of incubation, parameters such as cell viability, nitric oxide (NO) production, ROS levels, and double-stranded DNA (dsDNA) were assessed. Additionally, we evaluated whether a selective TRPA1 antagonist (HC-030031, 1 µM, for 24 hours of incubation) could inhibit AOPP-induced oxidative damage. **Results:** After 24 hours, the highest concentration (800 µg/mL) significantly reduced cell viability (83.52 ± 3.17 vs. 99.05 ± 4.59 , $p = 0.035$), increased NO production (127.10 ± 1.76 vs. 101.10 ± 2.71 , $p < 0.001$), and dsDNA levels (145.1 ± 9.86 vs. 100.3 ± 1.68 , $p < 0.0001$), compared to the control group. Moreover, 400 µg/mL of AOPP-MSA increased ROS levels (111.3 ± 2.12 vs. 99.26 ± 1.87 , $p < 0.001$) compared to the control group. Co-treated with HC-030031 and AOPP-MSA (800 µg/mL) did not improve cell viability (86.43 ± 1.14 vs. 90.03 ± 1.94 , $p > 0.05$) or prevent NO elevation (131.10 ± 1.16 vs. 127.80 ± 0.60 , $p > 0.05$), compared to the AOPP-MSA group. However, it significantly reduced dsDNA release (123.60 ± 9.03 vs. 73.39 ± 2.18 , $p < 0.0001$), indicating decreased cell death, compared to the AOPP-MSA group. **Conclusion:** This study provides novel evidence that AOPPs elicit oxidative and inflammatory responses in microglial cells, including increased nitric oxide production, impaired cell viability, ROS accumulation, and extracellular dsDNA release. Although TRPA1 antagonism with HC-030031 did not reverse AOPP-induced reductions in viability or NO overproduction, it significantly attenuated dsDNA release, indicating a partial protective effect against AOPP-mediated microglial activation and cell death. **Financial support:** CAPES.

01.015 Evidence of CD39 Upregulation in Mesenteric Microvasculature Following SARS-CoV-2 Infection in a Murine Model. Monteiro MMLV¹, Dutra LS², Carvalho-Barbosa NC², Savio LEB², Coutinho-Silva R², Silva CLM¹ ¹ICB-UFRJ, Biochemical and Molecular Pharmacology Lab., Institute of Biomedical Sciences, Brazil; ²UFRJ, Immunophysiology Lab., Institute of Biophysics Carlos Chagas Filho, Brazil

Introduction: Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is associated with significant **vascular inflammation**, contributing to endothelial dysfunction, thrombosis, and multi-organ damage, including gut complications. Among the key modulators of inflammation during viral infections is purinergic signaling, mediated by extracellular nucleotides (ATP/ADP) and nucleosides (adenosine). In turn, CD39 (Entpd1), which hydrolyzes ATP and ADP into AMP, limits ATP-driven inflammation. In SARS-CoV-2 infection, excessive ATP release may exacerbate vascular inflammation by promoting cytokine storms and endothelial activation. Given the potential involvement of purinergic signaling in COVID-19 pathology, this study aimed to evaluate the expression of purinergic receptors and CD39 in mesenteric vessels during SARS-CoV-2 infection, using a K18-hACE2 transgenic mouse model. **Methods:** K18-hACE2 transgenic humanized mice were intranasally inoculated with 2.5×10^4 PFU of SARS-CoV-2 or saline (MOCK). Animals (MOCK and SARS) received the P2X7 receptor antagonist Brilliant Blue G (BBG; 50 mg/kg intraperitoneally) or saline 24 hours prior to virus inoculation, resulting into 4 groups: MOCK, SARS, MOCK+BBG, and SARS+BBG. Animals were euthanized 7 dpi. **Mesenteric vessels** were isolated, macerated, and total RNA was extracted using the ReliaPrep™ RNA Miniprep System (Promega). Gene expression levels of the purinergic receptors P2X7 and Entpd1 (CD39) were analyzed by RT-PCR using the SYBR® Green qPCR Kit (Promega). For this analysis, the following primers (Applied Biosystems™) were used for murine genes: *P2rx7* (sense, 5'-AATCGGTGTGTTTCCTTTGG-3', antisense, 5'-CCGGGTGACTTTGTTGTCT-3'); *Entpd1* (sense, 5'-AGCTGCCCTTATGGAAGAT-3', antisense, 5'-TCAGTCCCACAGCAATCAAA-3'). Gene expression was calculated by relative quantification using the comparative C_t method ($\Delta\Delta C_t$) with a threshold set at 0.02. As reference gene β -actin was used. **Results:** Preliminary data suggest that SARS-CoV-2 inoculation may increase the relative gene expression of CD39 when comparing MOCK (1.05 ± 0.25 , n=2) and SARS (3.05 ± 0.75 , n=4) groups. BBG treatment had no detectable impact on CD39 expression in either MOCK+BBG (n=2) or SARS+BBG (n=2) groups. No changes in P2X7 gene expression have been observed so far in any of the groups at this early stage. Additional data are required to determine if the disease affects this receptor at the transcriptional level in mesenteric vessels. Ongoing analyses are investigating the roles of the purinergic receptors P2Y2, A2A, and A2B, as well as Caspase-1 and IL-1 β . **Conclusion:** Our preliminary data indicate that SARS-CoV-2 infection may upregulate CD39 gene expression in the mesenteric vasculature. These initial observations underscore a potential role for CD39 in the mesenteric vascular responses to SARS-CoV-2, which may relate to endothelial function and the regulation of local inflammation. **Acknowledgments:** FIOCRUZ (RJ) **Financial support:** CNPq, CAPES, FAPERJ.

01.016 BAG2 Favors Metformin-Induced Reduction in Metabolic Viability in H4 Neuroglioma Cells: Implications for TAU Pathology in Alzheimer's Disease. Duarte GZ, Souza ACS, Carrettiero DC, Almeida MC UFABC, Centro de Ciências Naturais e Humanas, PPG Biosistemas, Brazil

Introduction: Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by the accumulation of hyperphosphorylated TAU protein. Type 2 diabetes mellitus (T2DM) is a known risk factor for AD, and metformin, a first-line antidiabetic drug, has been shown to modulate metabolic and neuroprotective pathways. BAG2, a co-chaperone from the BAG family, promotes the degradation of abnormal TAU via a ubiquitin-independent proteasome pathway. This study evaluated whether metformin affects BAG2-dependent viability responses in human neuroglioma cells. **Methods:** Human H4 neuroglioma cells (a CNS-derived tumor cell line used in neurological research) were used in two variants: wild-type and BAG2 knockdown cells generated via CRISPR interference (CRISPRi). Knockdown validation was performed through immunofluorescence, RT-qPCR (Exp 1), and Western blot. Cells were treated with metformin at 1, 10, 50, or 100 mM, or with 1 μ M camptothecin (CPT) as a positive control. Cell viability was assessed by trypan blue exclusion (exp. 2.1) and metabolic activity by XTT assay (**exp. 2.2**). BAG2 expression after metformin (1 or 10 mM) was evaluated by RT-qPCR (exp. 3). Subcellular BAG2 distribution was analyzed via CellProfiler (**exp. 4**). **Results:** The BAG2 knockdown in CRISPRi cells was confirmed (Exp.1 $t(7) = 8.084$, $p < 0.0001$), validating this model to study the BAG2 protein. Metformin at 1 and 10 mM reduced metabolic activity in BAG2-expressing cells, while BAG2 knockdown cells were unaffected (Exp.2.2: $n = 8$; treatment $F(5,57) = 1302$, $p < 0.0001$; cell type $F(1,57) = 10.10$, $p = 0.0024$), indicating a BAG2-dependent sensitivity. Cell viability remained stable at these doses (Exp.2.1: $n = 6$; two-way ANOVA: treatment $F(5,73) = 114.7$, $p < 0.0001$; cell type $F(1,73) = 10.56$, $p = 0.0018$), reinforcing a specific effect on metabolism. At 50 and 100 mM, both cell types showed significant reductions in viability and metabolism, comparable to CPT. BAG2 mRNA levels were unchanged by treatment (Exp.3: one-way ANOVA: $F = 1.332$, $p = 0.2932$), but its perinuclear localization was reduced, suggesting post-transcriptional regulation (Exp.4: $n = 15$; two-way ANOVA: region $\times$ treatment $F(4,105) = 9.643$, $p < 0.0001$). **Conclusion:** BAG2 enhances cellular sensitivity to metformin-induced metabolic stress in H4 neuroglioma cells. This sensitivity appears to be linked to BAG2's subcellular redistribution rather than transcriptional changes. These findings highlight a potential role for BAG2 in the interplay between metabolic dysfunction and TAU pathology in Alzheimer's disease.

01.017 **Effect of Dietary Supplementation with Chia Oil on Adipose Tissue Remodeling Induced by Caloric Restriction in Obesity.** Pontes-Castro G, Oliveira-Malafaia T, Muniz-Cassuce G, Barja-Fidalgo C, Vargas-Silva S UERJ, Laboratório de Farmacologia Celular e Molecular, Rio de Janeiro/RJ, Brasil

Introduction: Obesity is a chronic disease associated with several health complications, including an increased risk of type 2 diabetes and cardiovascular disease. Excessive accumulation of fat in adipose tissue can lead to metabolic and hormonal dysfunctions. Omega 3 fatty acids have been investigated as a strategy to aid in the treatment of obesity-related comorbidities. Objective: To evaluate the effects of supplementation with chia oil (*Salvia hispanica* L), a source of omega-3 fatty acids, on metabolic parameters after caloric restriction and subsequent weight regain in a murine model of obesity. Methods: Male C57BL/6 mice (30 days old, n = 20 animals/group) were fed a high-fat diet (HFD) for 8 weeks. Then, some of the animals were fed a high-fat diet supplemented with chia oil (1.5% v/v) for 4 weeks. Subsequently, the HFD and HFC groups were subdivided into groups that underwent or did not undergo caloric restriction (-30% of food supply for 4 weeks), followed by weight regain (RG) for another 30 days (HFD+RG and HFC+RG). A control group (CTL) received a standard diet throughout the protocol. Body weight, body composition by magnetic resonance imaging, oxidative metabolism by high-resolution respirometry in skeletal muscle, and plasma concentrations of cholesterol and triglycerides were evaluated. Adipokine gene expression was determined by qRT-PCR. Differences were considered significant for $p < 0.05$. **Results:** The high-fat diet progressively increased body mass (30%) compared to CTL. Chia oil supplementation did not alter weight gain. Caloric restriction reduced body mass (20%) in the HFD and HFC groups. Upon weight regain, both groups increased body mass, but without reaching the maximum values prior to caloric restriction. In oxidative metabolism, the HFD group showed lower carbohydrate oxidation compared to the other groups, while in fatty acid oxidation, the HFC group showed higher values than the HFD and HFD+RG groups ($p < 0.05$). There were no significant differences in the gene expression of leptin and adiponectin or in plasma cholesterol and triglyceride concentrations. **Conclusion:** To date, our results indicate that consumption of a high-fat diet promoted marked weight gain and reduced carbohydrate oxidation. Chia oil supplementation was associated with increased fatty acid oxidation, suggesting improved mitochondrial oxidative capacity in skeletal muscle in a murine model of obesity. **Financial support:** CNPq and FAPERJ.

01.018 **The Role of Ric-8b Protein in Energy Homeostasis of Brown Adipose Tissue.** Pires CVB, Alves JM, Leiria LOS FMRP-USP, Ribeirão Preto, São Paulo, Brazil

Brown adipose tissue (BAT), by dissipating energy in the form of heat, plays a fundamental role in the regulation of energy and lipid metabolism. G protein-coupled receptors (GPCRs) are crucial elements in these processes, integrating hormonal and neural signals necessary for the thermoenergetic control of BAT. The Ric-8b protein acts as a nucleotide exchange factor and molecular chaperone essential for the stability and activation of Gα subunits, ensuring the efficient propagation of intracellular signaling mediated by GPCRs. Dataset analysis from mouse brown fat revealed enriched expression of Ric8b in mature brown adipocytes, which is regulated by environmental temperature. Despite the relevance of GPCR-mediated pathways in adipogenesis and thermogenesis, the specific role of Ric-8b in the metabolic regulation of BAT has not yet been elucidated. In this study, we sought to understand the role of Ric-8b in the thermogenic and metabolic regulation of BAT. All procedures involving mice were approved by the Faculty of Medicine of Ribeirão Preto under protocol number 158/2020. Using the conditional knockout model for Ric-8b in BAT (UCP1⁺/Ric8b^{+/+}) we observed that Ric-8b deletion does not alter body weight, BAT mass, and cold tolerance capacity. However, under thermoneutral conditions, knockout mice of both sexes exhibited elevated fasting glycemia and impaired glucose tolerance compared with controls. qPCR analyses of BAT revealed a reduction in the expression of thermogenic genes (Ucp1 and Prdm16), lipolytic genes (Hsl and Atgl), and the lipogenic marker (Fasn), indicating a broad impairment of metabolic regulatory pathways in BAT. These data suggest that Ric-8b plays a critical role in regulating BAT function and systemic energy metabolism. Key words: GPCR; brown adipose tissue; Ric-8b. **Financial support:** 2024/18873-5/São Paulo Research Foundation (FAPESP)

01.019 **Heme induces oxidative stress and inflammation in gastric epithelial cells via NADPH oxidase activation.** Souza MJ¹, Barcellos-de-Souza P¹, Berger M², Oliveira ACP³, Nicolau LAD³, Figueiredo-Júnior AT⁶, Guimarães JA², Santos CAN⁴, Nasciutti LE⁴, Arruda MA⁵, Moraes JA⁶, Barja-Fidalgo TC¹ ¹UERJ, Lab Cellular and Molecular Pharmacology, Brazil. ²UFRGS, Experimental Research Center, Hospital de Clínicas de Porto Alegre, Brazil. ³UFDPAr, Translational Inflammation and Gastroenterology Lab, Brazil. ⁴UFRJ, Institute of Biomedical Sciences, Brazil. ⁵CNPEM, National Lab Biosciences, Brazil. ⁶UFRJ, REDOX Biology Lab, Brazil

Introduction: Peptic ulcer disease is a common disorder that can result in various complications, such as bleeding, perforation, and gastric outlet obstruction. Rupture of blood vessels leads to erythrocyte lysis, triggering the release of large amounts of free Heme, which interacts directly with the gastric mucosa. Our group previously demonstrated that Heme is a pro-inflammatory and pro-oxidant molecule. Oxidative stress has emerged as one of the major pathogenic factors in the progression of ulcers, directly impairing cellular functions and promoting tissue damage. In this study, we evaluated the effects of Heme on gastric epithelial cell functions. **Methods:** Human gastric epithelial cells (HGE3) were preincubated with diphenyleneiodonium (DPI) (10 μ M), a selective NADPH oxidase inhibitor, and stimulated with Heme (10 μ M). Reactive oxygen species (ROS) production (DCF and MitoSOX probes), protein expression (Western Blotting), and cytokine levels in the culture supernatant were analyzed (ELISA). The activity of nuclear factor kappa B (NF κ B) (Luciferase Assay) and nitric oxide (NO) production (DAF probe) were also evaluated, as well as changes in cell permeability (Dextran-FITC extravasation). Male Swiss mice were pretreated with 100 μ l via oral gavage with one of the following: DPI (10 μ M), Heme (30 μ M), or a combination of DPI and Heme at the same concentrations. One hour after pretreatment, gastric ulcers were induced by oral administration of 50% ethanol (0.5 mL/25 g). The stomachs were photographed, and the areas of gastric lesions were measured. **Results:** *In vitro*, the stimulation of HGE3 with Heme increased total and intracellular ROS production, up to 40% and 100%, respectively, resulting in higher expression of 4-hydroxynonenal, activation of NF κ B, approximately 30%, and increased secretion of TNF- α , IL-1 β , and IL-6, up to 100%. Additionally, Heme stimulated NO production (~40%) through NOS2 (~60%) and increased nitrotyrosine (~40%) expression. Heme also increased paracellular permeability in HGE3 cell monolayers, up to 20%. Heme-induced effects in gastric epithelial cells were mediated by NADPH oxidase, being inhibited significantly by DPI. *In vivo*, free Heme increased hemorrhagic lesions (p<0.001) in ethanol-induced gastric ulcers, while DPI reduced the lesion area. **Conclusion:** In summary, our data demonstrate that Heme induces oxidative stress in gastric epithelial cells by increasing ROS production via NADPH oxidase. These results suggest that the pro-inflammatory and pro-oxidant properties of Heme activate signaling pathways involved in the pathogenesis of gastric diseases. **Financial support:** CAPES, CNPQ and FAPERJ.

01.020 **P2Y6 Receptor Downregulation in Endothelial Cells as a Putative Mechanism for Reduced Intestinal Inflammation in Schistosomiasis.** Oliveira NF, Savio LEB, Silva RC, Silva UFRJ, Lab. Farmacologia Bioquímica e Molecular, Instituto de Ciências Biomédicas, Brazil

Introduction: Schistosomiasis is an intravascular infectious disease characterized by chronic mesenteric inflammation and endothelial dysfunction. These pathological features are linked to cell damage and the release of purinergic agonists, which activate purinergic signaling pathways regulated by ectoenzymes (NTPDases). The P2Y6 receptor (P2Y6R), which is activated by UDP, has been implicated in vascular and non-infectious intestinal inflammation (Stachon et al., 2014; 2022). Previously we showed an increased endothelial expression of NTPDase 2 and 3, which hydrolyses UTP to UDP (Oliveira et al., 2016). However, the role of UDP in mesenteric endothelial cells (MECs) during schistosomiasis remains unclear. Therefore, the aim of this study was to investigate the expression and function of P2Y6R in mesenteric endothelial cells in the context of schistosomiasis.

Methods: Primary cultures of MEC were obtained from control and *S. mansoni*-infected mice (50-70 days p.i.; CEUA UFRJ 124/22) and used for leukocyte adhesion, qRT-PCR and immunocytochemistry (ICC) assays. Confluent MEC were pre-treated with vehicle (basal), P2Y6R selective antagonist (MRS2578 1 μ M), apyrase (2U/L) or ectonucleotidase inhibitor (ARL67156 100 μ M) for 30 min and then stimulated with UDP (100 μ M) for 5h, followed by incubation with freshly isolated monocytes for 30 min for the adhesion assays. For ICC, MECs were incubated overnight with anti-P2Y6 antibody (1:500) followed by incubation with Alexa Fluor 488-conjugated anti-rabbit secondary antibody (1:300, 1h). Coverslips were mounted with 5 μ L of DAPI-containing antifade mounting medium (Vectashield®). Fluorescence imaging was performed using an Olympus IX71 microscope and CellSens software. Quantification of fluorescence intensity was conducted using ImageJ software and expressed in arbitrary units. Data are expressed as mean $\pm$ SEM. **Results and**

Discussion: In the control group UDP increased monocyte adhesion to MEC from 2.6 ± 0.1 (basal) to 5.0 ± 0.4 cells/field ($p < 0.001$, $n = 4$) and its effect was inhibited by MRS2578. However, in the infected group, UDP failed to induce monocyte adhesion ($P = 0.92$ basal vs UDP; $n = 4$) and the NTPDase inhibitor ARL67156 (30 min or 24h) did not change monocyte adhesion, discarding UDP hydrolysis. Corroborating functional data, the RT-qPCR and immunocytochemistry analysis revealed a reduction by 40% of endothelial P2Y6R gene transcription ($p < 0.01$, $n = 3$) and expression ($p < 0.05$, Student's t test, $n = 3$), respectively, in the infected group compared to control. Interestingly, P2Y6R is slowly internalized in the presence of its agonist and fails to undergo rapid recycling after agonist removal from the extracellular environment (Brinson and Harden, 2001). Since EC could release the endogenous agonist UDP, we investigated if its removal could restore receptor signaling. Our data with prolonged (24h) apyrase treatment followed by UDP stimulation did not result in significant changes in monocyte adhesion (from 4.5 ± 0.5 to 5.6 ± 0.5 , $p = 0.3$, $n = 12$). **Conclusion:** Schistosomiasis reduced endothelial P2Y6R expression and function limiting the receptor contribution to mesenteric inflammation. This downregulation could be a host adaptive mechanism to attenuate inflammation and schistosomiasis morbidity.

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01.021 Caveolin-1 Knockdown Disrupts P2Y2/P2X7 Receptors Crosstalk in Schistosomiasis-Primed Mesenteric Endothelial Cells. Oliveira NF, Mainieri NS, Silva CLM UFRJ, Lab. Farmacologia Bioquímica e Molecular, Instituto de Ciências Biomédicas, Brazil

Endothelial damage caused by chronic intravascular schistosomiasis promotes mesenteric inflammation and ATP release, which modulates host immunovascular responses through purinergic P2 receptors. Previously we showed a positive cooperation between endothelial P2Y₂ and P2X7 receptors (P2Y₂R/P2X7R), leading to increased VCAM-1 expression and monocyte adhesion due to an enhanced inflammasome activation and NF-κB signaling during schistosomiasis (Oliveira *et al.*, 2024). Caveolin-1 (Cav-1) plays a critical role in regulating endothelial cell phenotype and multiple signaling pathways, including P2X7R function in macrophages (Sawai *et al.*, 2024). However, the potential contribution of Cav-1 to schistosomiasis-associated endothelial dysfunction remains unclear. Therefore, this study aimed to investigate the role of endothelial Cav-1 in modulating P2Y₂R/P2X7R cooperation in a model of schistosomiasis-induced mesenteric inflammation. **Methods:** Primary cultures of mesenteric endothelial cells (EC) were obtained from control and *Schistosoma mansoni*-infected mice (50-70 days p.i.; CEUA UFRJ 124/22) and used for monocyte adhesion and immunocytochemistry (ICC) assays. Confluent EC were stimulated with vehicle, UTP (100 μM) for 5h and/or with ATP 500 μM (10 min) in the presence or absence of siRNA Cav-1 transfection (SI02731813; 1 pmol) for 48h. For the co-culture assays, after treatments and washing, EC were incubated with freshly isolated monocytes (30 min) and washed. Then four fields/well were imaged to count the number of adhered cells (400X). For ICC double immunofluorescence staining, the EC were sequentially incubated with anti-Cav-1 (sc-53564, 2h, 1:50) and Alexa Fluor 488 anti-mouse (A-11001, 45 min, 1:200) followed by anti-P2X7R (APR-004, overnight, 1:500) and Alexa Fluor 594 anti-rabbit (A-11012, 1h, 1:300). Nuclei were stained with DAPI (1:1000) and cells were imaged using either a fluorescence microscope (Olympus IX71 equipped with CellSens software) or confocal microscope (Zeiss Celldiscover 7, LSM 900), and the fluorescence analysis was performed by ImageJ software (NIH) using Puncta Analyzer plugin for colocalization. Data were expressed as mean and SEM. **Results:** Immunofluorescence confirmed Cav-1 knockdown, showing approximately 70% reduction in expression in both groups. In the control group, Cav-1 knockdown did not alter monocyte adhesion in response to P2Y₂R/P2X7R stimulation ($p = 0.99$, UTP + ATP vs. siRNA Cav-1 + UTP + ATP). However, in the infected group, Cav-1 knockdown significantly inhibited monocyte adhesion induced by P2Y₂R/P2X7R coactivation (from 11.2 ± 1.2 to 5.4 ± 0.3 cells/field; $p < 0.001$, $n = 4$), reducing adhesion to basal levels. Regarding ICC assays, preliminary data revealed an increase from 11.8 ± 2.7 (control group) to 44.2 ± 9.4 punctas in the infected group ($n = 24-36$), suggesting an increased colocalization of endothelial P2X7R and Cav-1 in the caveola during the disease. Ongoing studies are evaluating the putative colocalization of P2Y₂R/P2X7R/Cav-1 in primed EC. **Conclusion:** Endothelial cell priming during schistosomiasis promotes Cav-1-dependent monocyte adhesion in response to P2Y₂R/P2X7R coactivation, implicating this pathway in the pathogenesis of mesenteric inflammation in schistosomiasis. **Acknowledgments:** FIOCRUZ (RJ), CNPq, CAPES, FAPERJ.

01.022 Multidrug Resistance Proteins Regulate Intracellular Cyclic Nucleotide Levels in Prostate Tissue and Cell Lines. Passos GR¹, O'Brien S², D'Ancona CA³, Fregonesi, A³, Antunes E¹, Calebiro D², Monica FZ¹ ¹Unicamp, Dept Pharmacology, Faculty of Medical Sciences,, Brazil ²University of Birmingham, Institute of Metabolism and Systems Research, College of Medical and Dental Science, United Kingdom ³Unicamp, Dept Surgery (Urology), Faculty of Medical Sciences, Brazil

Introduction: Benign prostatic hyperplasia (BPH) is the enlargement of the prostate gland, which can lead to lower urinary tract symptoms. These symptoms are often caused by increased smooth muscle activity and elevated pressure at the bladder outlet. The intracellular levels of cAMP and cGMP are regulated by their synthesis, degradation, and efflux. While phosphodiesterases hydrolysis these cyclic nucleotides, multidrug resistance proteins 4 and 5 (MRP4 and MRP5) actively transport them out of the cytosol. The MRP inhibitor MK571, studied *in vitro* (Bertolotto et al., 2018; de Oliveira et al., 2023; Gomes et al., 2024) and *in vivo* (de Oliveira et al., 2023), reduces smooth muscle reactivity in bladder, prostate, and corpus cavernosum by increasing intracellular cAMP/cGMP. We hypothesize that MRP activity is altered in BPH, and that inhibiting MRPs may reduce prostate overactivity and abnormal cell proliferation. **Methods:** Prostate tissue was obtained from BPH patients undergoing radical prostatectomy. Strips were mounted in a myograph. Concentration-response curves to phenylephrine (PE) were performed with or without MK571 (20 µM), in the presence or absence of nitric oxide synthesis inhibitor L-NAME (100 µM), soluble guanylate cyclase inhibitor ODQ (10 µM), and adenylate cyclase inhibitor SQ 22536 (10 µM). The cAMP and cGMP levels were measured in human stromal myofibroblast (WPMY-1) and BPH (BPH-1) cell lines. A BRET assay was performed with or without MK571, followed by stimulation with a beta-adrenoceptor agonist or nitric oxide donor. Potency and maximal response (Emax) were assessed. Experiments were independently repeated at least three times with two replicates per group. Approved by the UNICAMP ethics committee (88849718.9.0000.5404). **Results:** In isolated prostate from BPH patients, MK571 reduced the PE-induced contractile response by 36% ($p < 0.003$). Co-incubation of MK571 with ODQ, SQ22,536, or L-NAME completely reversed the anticontractile response induced by MK571, restoring it to levels comparable to those of tissues not exposed to MK571. BRET assays showed that MK571 induced a greater increase in cAMP in BPH-1 cells (24.8- and 32.4-fold) than in WPMY-1 cells (2.9- and 11.5-fold), using global and membrane sensors, respectively. MK571 also enhanced the Emax of isoproterenol when measured with the plasma membrane-targeted sensor. Specifically, Emax increased by 22% in WPMY-1 cells and by 40.9% in BPH-1 cells compared to the vehicle control. Additionally, in WPMY-1 cells, MK571 alone and in combination with the NO donor elevated cGMP levels by 4.7-fold and 1.7-fold, respectively, relative to the vehicle. **Conclusion:** These results advance our understanding of MRP4 and MRP5 in regulating cyclic nucleotide levels in prostate tissue and cells, highlighting their role in hyperplasia. The inhibition of these transporters by MK571 reduced smooth muscle hypercontractility and increased intracellular cAMP and cGMP levels in cell lines. **Financial support:** FAPESP 2024/00216-8; 2022/11621-5

01.023 **Caveolin-1 Modulates Monocyte Adhesion Mediated by P2X7 Receptor Activation in Endothelial Cells During Schistosomiasis.** Mainieri NS, Oliveira NF, Silva CLM ICB-UFRJ Biochemical and Molecular Pharmacology Lab., Brazil

Introduction: Chronic schistosomiasis, caused by *Schistosoma mansoni*, leads to persistent mesenteric inflammation and associated morbidity. Intravascular egg deposition triggers the release of DAMPs, such as extracellular ATP, which activate purinergic receptors inducing an inflammatory response and endothelial dysfunction. In this pro-inflammatory context, increased p-Cav-1 followed by Cav-1 degradation has been linked to lung endothelial dysfunction (Marinho, 2023). We have previously shown that mesenteric endothelial P2Y₂ and P2X7 receptors (P2Y₂R/P2X7R) act cooperatively to amplify inflammasome activation and NF-κB signaling, thereby enhancing leukocyte adhesion during schistosomiasis (Oliveira et al., 2024). Based on this, the present study aimed to investigate a potential interaction between endothelial P2X7R and Cav-1 in a model of schistosomiasis-induced mesenteric inflammation. **Methods:** Primary cultures of mesenteric endothelial cells (ECs) were established from control and *S. mansoni*-infected mice (50-70 days post-infection; CEUA UFRJ 124/22). For leukocyte adhesion assay, confluent ECs were stimulated with vehicle or ATP (500 μM, 10 min), with or without prior siRNA Cav-1 transfection (SI02731813; 1 pmol, 48 h). Monocytes were isolated (95% viability) from blood collected via cardiac puncture of control and *S. mansoni*-infected mice and obtained through Ficoll gradient purification (PLUS Ficoll-Paque). After stimulation, cells were washed, and monocytes (10⁴/well) were added for 30 min in DMEM (37°C, 5% CO₂). Then cells were washed and four fields per well were imaged using an Olympus IX71 microscope (400×) with CellSens 1.5 software (Olympus America Inc., USA). Adherent cells were quantified using ImageJ (NIH) (Oliveira et al., 2011). **Results:** Cav-1 knockdown was confirmed by immunocytochemistry assays. P2X7R activation significantly enhanced monocyte adhesion to endothelial cells in both groups (from 2.5 ± 0.19 to 5.4 ± 0.33 cells/field, control; 5.8 ± 0.2 to 11.0 ± 0.54 cells/field; *p* < 0.001, infected). Notably, Cav-1 knockdown did not alter the ATP effect in the control group (*p* = 0.99). However, in the infected group, Cav-1 knockdown significantly reduced ATP-induced monocyte adhesion from 11.0 ± 0.54 to 6.1 ± 0.45 cells/field (*p* < 0.0001), a level comparable to the basal value (5.8 ± 0.2 cells/field). Preliminary data suggest a co-localization of Cav-1 and P2X7R only in the infected group (data not shown). Altogether, our data suggests that P2X7R signaling is modulated by Cav-1 during schistosomiasis. **Conclusion:** Our data suggests that a caveolae-dependent mechanism underlies endothelial P2X7R signaling and leukocyte adhesion during schistosomiasis. **Acknowledgments:** FIOCRUZ (RJ) **Financial support:** CNPq, CAPES, FAPERJ.

01.024 **Plasmatic C3a is Reduced While Cytokines and Matrix Metalloproteinase (MMP)-2 Are Increased in Early-onset Pre-eclampsia** Mello MMB¹, Ramos LVR¹, Palei AC², Santos JETD¹, Cavalli RC³, Sandrim V⁴, Mazzaron M¹ ¹FMRP-USP, Dept Pharmacolgy, Brazil, ²University of Mississippi Medical Center, School of Medicine, Dept Surgery, USA, ³FMRP-USP, Dept Gynecology and Obstetrics, Brazil, ⁴IBB-Unesp, Dept Biophysics and Pharmacology, Brazil.

Introduction: Pre-eclampsia (PE) is a serious gestational complication characterized by high blood pressure and at least one complicated factor that generally occurs at 20th weeks pregnancy. It represents 2 to 8% of the main causes of maternal morbidity and mortality and its causes include: genetic factors, immunological intolerance, deficient placentation, angiogenic imbalance, oxidative stress and inflammation. Endothelial dysfunction in the maternal vascular system plays a significant role in the development of PE. Alterations in the immune system, such as the activation of the complement system and decreased regulatory T cells, contribute to the insufficient immune intolerance. Increased levels and activity of matrix metalloproteinases (MMPs), mainly MMP-2 and -9, play a crucial role in the vascular remodeling and dysfunction of PE. Activation of the complement system, especially the C3a, may increase the protein expression and activity of MMP-2 in arteries with aneurysm or hypertension, thus contributing to vascular dysfunction. The **hypothesis** is that an imbalance occurs between the C3a, inflammatory cytokines and MMP-2 levels in the plasma of PE pregnant women. **Methods:** plasma were collected from pregnant women diagnosed with PE (systolic blood pressure $\geq$ 140 mmHg or diastolic blood pressure $\geq$ 90 mmHg) and healthy pregnant women (control group), who were matched for age, body mass index and ethnicity. Plasma was used to perform enzyme-linked immunosorbent assay (ELISA) to C3a, IL-6, IL-1 β , IL-10, MMP-2 and TIMP-2, and for 2h-incubation of mesenteric arteries from pregnant rats (G19), which were tested in the wire myography, in the presence or absence of C3aR antagonist, SB290157. Vascular contraction was tested in response to the thromboxane analog, U46619, and vascular relaxation was tested in response to acetylcholine. Participants provided written informed consent (TSLE) and the studies are in accordance with the Principles of the Declaration of Helsinki (#4.347.078). **Results:** there was no difference in the plasma levels of C3a, IL-6, IL-1 β , IL-10, MMP-2 and TIMP-2 in the PE pregnant women when compared to the healthy group. However, from PE women, we had patients who were grouped as early-onset PE (before 20 gestational weeks). We then analyzed plasma levels of C3a, cytokines, TIMP-2 and MMP-2 in the early-onset PE compared to controls. Increased plasma levels of IL-6, IL-10, MMP-2 and TIMP-2 were seen in the early-onset PE (*t test*; $p < 0.05$ vs. control), while the C3a levels were decreased (*t test*; $p < 0.05$). In addition, incubation of mesenteric arteries with plasma from early-onset PE patients for 2h seems to increase the contraction response to U46619 (Emax: 5.60 vs 10.9185; preliminary experimental n=1) and to reduce the relaxation in response to acetylcholine (Emax: 90% vs 72.2%; preliminary experimental n=1) and both these effects may be prevented by incubation with SB290157 (Emax contraction: 4.2211 vs 10.9185; Emax relaxation: 83.1% vs 72.2%; preliminary experimental n=1). **Conclusion:** early-onset PE may be related to increased pro-inflammatory cytokines and MMP-2 levels in maternal plasma, while C3a is reduced, thus contributing to vascular dysfunction. **Financial support:** FAPESP, CAPES, CNPq.

01.025 Identification of Intracellular Peptides Present in Adipose Tissues and their Roles in Thermogenic Function. Reckziegel P^{1,2}, Jardim GFR¹, Valdivia LFG², Souza E¹, Moreira RJ¹, Freire PP¹
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Introduction: Brown adipose tissue (BAT) is known for its thermogenic function, primarily due to the expression of mitochondrial uncoupling protein 1 (UCP1). Under specific stimuli, white adipose tissue (WAT) can acquire thermogenic features through the expression of UCP1, a phenomenon known as browning. Activation of BAT and induction of browning are considered promising strategies to combat obesity and related metabolic disorders, as they enhance basal energy expenditure and increase glucose and triglyceride uptake. Intracellular peptides have been shown to modulate several metabolic pathways. The identification and characterization of such peptides in BAT and WAT under thermogenic stimuli may reveal novel biomarkers and therapeutic candidates for obesity and associated diseases. Cold exposure (by adrenergic stimulus) is the primary physiological stimulus for activating thermogenesis, while PPAR γ agonists can also enhance UCP1 expression in adipose tissues. **Methods:** Male C57BL mice were used in this study (CEUA ICB/USP #8874310719 and FCF/USP #652). We performed peptidomic analyses on BAT and WAT samples from mice exposed to room temperature (21 °C) or cold (7 °C), and treated with either vehicle or the PPAR γ agonist pioglitazone (30 mg/kg/day) for 15 days. The effect of some peptides was evaluated by rtPCR with samples of brown and white adipocytes. **Results:** A total of 39 peptides were identified in BAT, 95 in WAT, and 24 were found in common between the two tissues. Among these 24 shared peptides, 12 showed increased abundance in the Cold or Cold+Pio groups compared to Control. These were selected for functional evaluation *in vitro* using primary cultures of brown and white adipocytes. On day 6 of differentiation, adipocytes were exposed for 24 hours to the selected peptides at 1 μ M and 10 μ M, followed by gene expression analysis. Peptide P8 increased the expression of UCP1 and other thermogenic and adipogenic markers, including peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC1 α), carnitine palmitoyl transferase 1A (CPT1A), fatty acid-binding protein 4 (FABP4), and peroxisome proliferator-activated receptor gamma 2 (PPARG2) ($p < 0.05$) in brown adipocytes. In white adipocytes, peptides P1 and P11 upregulated UCP1 expression along with other thermogenesis-related markers ($p < 0.05$). **Conclusion:** In conclusion, this study identified intracellular peptides present in adipose tissues whose levels are modulated by thermogenic stimuli (mainly by cold in comparison to pioglitazone). Furthermore, some of these peptides significantly influenced the expression of thermogenesis-related genes, highlighting their potential as novel targets for therapeutic strategies against obesity and related metabolic conditions. **Financial Support:** PR – FAPESP #2019/25943-1, #2024/02202-4; GFRJ – #2023/02579-8; LFGV – #2021/03717-0; ES and RJM – PRPI/USP #22.1.09345.01.2; PPF – #2024/13521-3

01.026 **Resveratrol in the Pre-Gestational, Gestational and Breastfeeding Phases of Spontaneously Hypertensive Rats (SHR) Reduces Hypertension and Matrix Metalloproteinase (MMP)-2 Activity In The Aortas of Adult Offspring.** Oliveira RR¹; Mello MMB¹; Gomes BQ¹; Dourado TMH^{1,2}; Assis VO¹, Tirapelli CR^{1,2} Castro MM¹ ¹Dept Pharmacology, Ribeirão Preto Medical School; ²Cardiovascular Pharmacology Lab, Ribeirao Preto School of Pharmaceutical Science University of São Paulo (USP)

Background: In hypertension (HT), many vascular changes are associated with the increased activity of matrix metalloproteinases (MMP)-2, which may be activated by oxidative stress. Vascular dysfunction and maladaptive remodeling are improved in the adult male SHR after using antioxidants; however, the effects return when treatment is discontinued. Prevent the occurrence of HT is a better alternative to control the malefic effects in the cardiovascular system. As neonatal periods have significant plasticity, thus being modulated by exogenous environment, the use of resveratrol in these phases may be key for preventing or reducing HT later in life. Treatment with resveratrol during pregnancy of SHR improved endothelial dysfunction and HT in the adult offspring. However, the mechanisms involved in the effects of resveratrol in the vasculature of adult offspring from SHR dams remain to be elucidated. We aimed to assess whether resveratrol given to SHR dams during pre-gestational, gestational and lactation periods reduces increased reactive oxygen species (ROS) and MMP-2 activity in the vasculature, thus reducing hypertrophic remodeling, dysfunction and HT in female and male adult offspring. **Methods:** Wistar Kyoto (WKY) and SHR couples were treated with resveratrol (4g/kg/diet) nearly 14 days before pregnancy. Resveratrol was continued only in females during gestation and lactation, and was ceased. Offspring were analyzed when reached 12 weeks of age. Systolic blood pressure (SBP) was analyzed by tail-cuff plethysmography from 8 until 12 weeks, and aortas were collected to perform gelatin and *in situ* zymography, DHE and nitrotyrosine immunohistochemistry, vascular reactivity and H&E staining. Each litter was considered $n=1$ and 1 to 4 pups from the same litter were pseudo-randomly chosen and used for each sex. Statistical analysis was performed by two-way ANOVA and Tukey post-test or *t*-test, when appropriate ($p<0.05$). **Results:** Treatment with resveratrol decreased SBP and ROS levels in the aortas of adult female SHR offspring ($p<0.05$), although the accentuated aortic MMP-2 activity was decreased in both genders of adult SHR offspring ($p<0.05$). Adult SHR offspring had vascular dysfunction and hypertrophic remodeling, which were attenuated after treating dams with resveratrol ($p<0.05$). On the other hand, adult female WKY offspring that received resveratrol from dams had increased aortic ROS and MMP-2 activity in addition to hypertrophic remodeling and dysfunction ($p<0.05$). **Conclusions:** Treatment with resveratrol in the hypertensive dams in preconception, gestation and lactation improved HT-induced vascular hypertrophy and dysfunction in their adult offspring through decreasing oxidative stress and MMP-2 activity, showing that resveratrol can attenuate the HT in hypertensive adult offsprigs through the treatment of their dams. These effects were opposite in the vasculature of adult female offspring when resveratrol was given to WKY dams. **Financial support:** CAPES, FAPESP, CNPq e FMRP/USP.

01.027 Beyond the Sperm Surface: EPPIN's Distribution and Colocalization with SEMG1 and AKAP4 in Human Spermatozoa. Santos BR¹, Mariani NAP¹, Santos NCM¹, Themer ACF¹, Daroz GA², Martini PV¹, Santos LA², Dias-Melício LA², Teixeira TA³, Hallak J⁴, Santos AGP², Silva EJR¹. ¹IBB-UNESP-Botucatu, ²FMB-UNESP-Botucatu, ³UNIFAP, University Hospital, Division of Urology; ⁴HC-USP, Hospital das Clínicas, Division of Urology

Introduction: Epididymal protease inhibitor (EPPIN) acts as a docking site for multiple protein-protein interactions on the sperm surface. The binding of the seminal plasma protein semenogelin-1 (SEMG1) to EPPIN leads to a temporary inhibition of sperm motility after ejaculation in humans and rodents. Although EPPIN has mainly been seen as a sperm surface protein, recent data from our group revealed its association with structural compartments of the flagellum in mouse spermatozoa. Furthermore, intracellular proteins with key roles in sperm motility, such as A-kinase anchoring protein 4 (AKAP4), co-immunoprecipitated with mouse EPPIN, indicating its involvement in intracellular protein-protein interactions that influence sperm function. However, the subcellular distribution of EPPIN and its colocalization with interacting partners in human spermatozoa are still understudied. Here, we examined EPPIN expression and its colocalization with SEMG1 and AKAP4 in human spermatozoa. **Methods:** Human semen samples were obtained from donors at the *Hospital das Clínicas*, Botucatu Medical School (Ethical Committee: 64342822.9.0000.5411). Spermatozoa were separated by density gradient and processed for either (i) cell fractionation with a Triton X-100 buffer followed by Western blot, or (ii) indirect immunofluorescence assays with anti-EPPIN and anti-SEMG1 or anti-AKAP4 antibodies. EPPIN cellular distribution and colocalization with SEMG1 or AKAP4 were evaluated using epifluorescence and confocal microscopy. Control experiments were performed using primary antibodies pre-adsorbed with recombinant protein or a blocking peptide, or in the absence of primary antibodies. **Results:** EPPIN expression was detected in the Triton-soluble fraction, which contains soluble proteins and membranes, as a ~40 kDa band consistent with dimers. Notably, the same band was also found in the Triton-insoluble fraction, suggesting that EPPIN is present in intracellular compartments, possibly associated with accessory structures of the flagellum. EPPIN immunostaining was mainly observed in the post-acrosomal region (67% of stained cells) and posterior ring (81%) of the sperm head, as well as in the connecting piece (82%), midpiece (71%), and principal piece (55%) of the flagellum. Colocalization of EPPIN with SEMG1 was observed in both the sperm head and flagellum. In contrast, colocalization of EPPIN with AKAP4 was primarily observed on the connecting and principal piece of the flagellum. **Conclusion:** Our results demonstrate an association between EPPIN and intracellular compartments in human spermatozoa, indicating that its distribution extends beyond the sperm surface. The colocalization of EPPIN with SEMG1 and AKAP4 suggests that it may regulate sperm function via protein-protein interactions both on the sperm surface and in intracellular compartments. Our findings offer new insights for developing male contraception strategies targeting EPPIN as a sperm-specific protein. **Financial support:** CAPES, FAPESP, and CNPq.

01.028 **Prospecting for Bioactive Substances in Microorganisms Recovered from Caves of the Parque Estadual Turístico do Alto Ribeira (São Paulo, Brazil).** Rivero BES¹, Sahm BDB^{1,2}, Lopes NP², Costa-Lotufo, LV¹. ¹ICB-USP, ²FCFRP-USP

Caves are unique and unexplored ecosystems, with extreme conditions that limit the distribution of fauna and flora, such as total absence or low level of light, low temperature, high humidity, and low nutrient content. In this adverse environment, microorganisms, which present a high diversity, assume an important role in the cycling of nutrient and organic matter, being determinants in geomorphology. In addition to this intrinsically ecological role, such microorganisms are prolific producers of substances with unique chemical structures with recognized pharmacological potential. In this sense, this study focuses on the prospecting investigation of bacteria presented in cave vermiculations, which may offer new secondary metabolites with therapeutic properties. Vermiculations are common formations in rocky surfaces of caves around the world, generally composed of calcite, associated with quartz, and traces of clay minerals, where bacteria play an important role in the biogeochemical cycle. The macerated material was spread into three different media agar plates - ISP2, SCA and B4, from which the cultured bacteria were picked and isolated. After that, pure strains were grown in liquid media to obtain crude extracts which were tested for anticancer and antibiotic potential. Cytotoxic potential of the extracts was accessed by the MTT assay, at concentrations of 5 and 50 µg/mL under 72h incubation, and subsequently in serial dilution to determine the IC₅₀, against 501mel (metastatic melanoma), HCT-116 (colorectal carcinoma) and MCF-7 (breast adenocarcinoma) cell lines. Antibacterial activity was accessed using disk diffusion technique, against the multidrug-resistant strains *Enterococcus faecium* and *Staphylococcus aureus*. The extracts were applied to paper disks and incubated for 24h. A total of 52 bacteria were recovered from vermiculations, whereas 7 extracts were considered cytotoxic (≥75% inhibition at 50 µg/mL) against 501mel and HCT-116 cells, and 5 extracts against MCF-7. The IC₅₀ ranged from 0.10 to 0.22 µg/mL; 0.11 to 0.26 µg/mL, 0.17 and 0.29 µg/mL, respectively. Antibiotic experiments showed that 9 extracts were able to produce inhibition zones ranging from 0.7 to 1.0 cm against both tested bacteria. In addition, all isolated strains are undergoing molecular identification by 16S rRNA sequencing and the chemical profile of extracts will be investigated through liquid and gas chromatography techniques coupled to mass spectrometry (LC-MS and GC-MS) techniques. This study resulted in the isolation of new species of bacteria with the potential to produce bioactive substances, contributing to the advancement of knowledge in innovation and to the biodiversity of Brazilian caves.

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01.029 **Doxycycline Attenuates Cytokine Responses in a Mouse Model of Lipopolysaccharide-Induced Epididymitis: A Potential Role of Matrix Metalloproteinase Inhibition.** Martini PV¹, Andrade AD¹, Ferreira CT¹, Camargo IA¹, Bizzotto JQ¹, Portela LMF², Kushima H¹, Justulin-Junior LA², Fernandes CJC¹; Silva, EJR¹ ¹Unesp, PPG Biomolecular & Pharmacological Sciences, Brazil; ²Unesp, PPG General & Applied Biology, Brazil

Introduction: Epididymitis is an inflammatory condition of the male excurrent ducts that threatens male fertility, with bacterial infections as the main cause. Epididymitis is characterized by immune cell infiltration, cytokine upregulation, and interstitial fibrosis. Matrix metalloproteinases (MMPs) play a central role in inflammation by controlling extracellular matrix remodeling, cytokine activation, and immune cell infiltration; however, their role in epididymitis remains unclear. Here, we evaluated whether Lipopolysaccharide (LPS)-induced epididymitis alters MMP expression and activity, and whether doxycycline, an antibiotic with broad-spectrum MMP inhibitory activity, modulates MMP activity and cytokine responses. **Methods:** Epididymitis was induced in adult C57BL/6 mice (90 days old; CEUA n°7246220324) by interstitial injection of sterile saline (control) or ultrapure LPS from *E. coli* (50 µg) into the initial segment or cauda epididymidis under general anesthesia. Additional groups were treated with doxycycline (50 mg/kg/day, i.p.) previous to LPS challenge. Mice were euthanized at 6, 24, 72 h, and 7 days post-injection. Tissues were collected for RT-qPCR (*Mmp2*, 7, 9, 13; *Il6*; *Tnf*; *Il1b*) using *Hprt1*, *Rps18*, and *Rplp0* as endogenous controls, zymography (MMP2 and MMP9), and ELISA (MMP2, MMP9 and MMP13) assays. Data were analyzed by Student's t-test or ANOVA, followed by the Tukey test (significance $p < 0.05$). **Results:** In the initial segment, LPS induced an early upregulation of *Mmp13* at 6 h (>208-fold) and a later upregulation of *Mmp9* at 24 h (>1.7-fold), 72 h (>3.4-fold) and 7 days (>19.9-fold) compared to saline control. In the cauda epididymidis, LPS induced a biphasic upregulation of *Mmp13* at 6 h (>18.5-fold) and 7 days (>4.2-fold) and *Mmp7* at 24 h (>4.7-fold) and 7 days (>2.0-fold). LPS further upregulated *Mmp9* at 72 h (>21.1-fold) and 7 days (>8.3-fold), and *Mmp2* (>2.7-fold) at 7 days. Zymography and ELISA analysis confirmed that LPS increased the activity of MMP13 at 6 h in the initial segment and MMP2 and MMP9 at 72 h and 7 days in both epididymal regions. Doxycycline treatment prevented LPS-induced activity of MMP2 and MMP9 in both epididymal regions. Concurrently, doxycycline treatment attenuated LPS-induced upregulation of the cytokines *Il6*, *Tnf* and *Il1b* in the initial segment and cauda epididymis at 72 h (LPS: >3.7-, >4.3-, and >6.2-fold; LPS + doxycycline: >1.6-, >2.0-, >1.8-fold / LPS: >10.5-, >56.7-, and >13.0-fold; LPS + doxycycline: >5.4-, >1.3, and 1.5-fold, respectively) and 7 days (LPS: >30.7-, >12.1-, >21.5-fold; LPS + doxycycline: >1.9, >1.6, 1.8-fold / LPS: >13.5, >14.3, >15.9; LPS + doxycycline: >1.7, >2.2, 1.8-fold, respectively) compared to saline control. **Conclusion:** MMPs exhibit a complex and temporally regulated response in the mouse epididymis in response to LPS, with MMP13 acting as an early and MMP7 and MMP9 as later downstream mediators. Our results highlight that MMP inhibition is a potential therapeutic strategy to mitigate the negative outcomes of epididymitis on male fertility. **Financial support:** FAPESP, CAPES, CNPq.

01.030 **Modulation of NF- κ B by TLR4 Activation in Doxorubicin-Stimulated MCF-7 and MDA-MB-231 Breast Cancer Cells.** Quispe C¹ Buitron Mercado MF², Riera Perez AC³, Concha Escobedo PA⁴, Vallejos Paredes MF⁵, Villanueva-Salas JA⁶, Capiro Carpio JM⁷ ^{1,2,4,5,6}Escuela Profesional Farmacia y Bioquímica, Facultad de Ciencias Farmacéuticas, Bioquímicas y Biotecnológicas de la Universidad Católica de Santa María, Perú. ^{3,7}Escuela Profesional Ingeniería Biotecnológica, Facultad de Ciencias Farmacéuticas, Bioquímicas y Biotecnológicas de la Universidad Católica de Santa María, Perú

Introduction: Breast cancer is the most prevalent type of malignancy among women worldwide. As the first modality of treatment of a malignant primary tumor, neoadjuvant therapy aims to eradicate tumor size reduction and improve overall survival (OS). The neoadjuvant AC-T regimen is based on doxorubicin (DOX)/cyclophosphamide and paclitaxel (PTX). There are reports that cell damage caused by DOX releases mitochondrial DNA, a ligand of Toll-like receptor type 9 (TLR9). On the other hand, DOX is thought to activate the TLR4 receptor by mechanisms such as the release of DAMPs (HMGB1 and HSPs), which bind to TLR4 or by directly interacting with the TLR4/MD-2/CD14 complex, triggering the NF- κ B pathway and the production of inflammatory cytokines (TNF- α , IL-6). Thus, the TLR4/NF- κ B pathway could be involved in mechanisms of cell survival in the face of chemotherapeutic stress. This study evaluated the role of this pathway in promoting viability in two breast cancer cell lines (MCF-7 and MDA-MB-231) treated with DOX. **Objective:** To evaluate the effect of NF- κ B pathway modulation by TLR4 activation in MCF-7 and MDA-MB-231 breast cancer cells in response to doxorubicin treatment, to determine its influence on cellular response to chemotherapy. **Methodology:** MCF-7 and MDA-MB-231 tumor cells were cultured in DMEM medium supplemented with 10% fetal bovine serum, 1% antibiotic and maintained at 37°C and 5%CO₂. Cells were divided into a control group and groups treated for 24 hours with bacterial lipopolysaccharide (LPS 10 μ g/ml in MDA-MB-231 cells; 20 μ g/ml in MCF-7 cells); DOX (0.3 μ M in MDA-MB-231 cells; 0.1 μ M/ml in MCF-7 cells). The control group consisted of treatment with DMEN medium only. After this period, cells were harvested to quantify TLR4 and NF- κ B expression by RT-qPCR. The Student's t-test was used for statistical comparison, and the level of significance was accepted at $p < 0.05$. **Results.** Treatment of MCF-7 and MDA-MB-231 with IC₅₀ of DOX increased TLR4 and NF- κ B gene expression with a statistically significant difference compared to the control group ($p < 0.05$). **Conclusion:** The results suggest that TLR4/NF- κ B pathway might play a key role in promoting breast cancer cell survival in the face of DOX-induced chemotherapeutic stress. Overexpression of TLR4 and NF- κ B in response to the drug could point to a resistance mechanism potentially mediated by this pathway. These findings highlight the importance of TLR4/NF- κ B as therapeutic targets to improve the efficacy of DOX in breast cancer. Future studies should explore strategies to inhibit this pathway and sensitize cells to treatment. **Key words:** TLR4, NF- κ B, doxorubicin, chemotherapy resistance, breast cancer. **Acknowledgment:** This research was funded by the Universidad Católica de Santa María (UCSM) – Perú.

01.031 **Enhanced Neuroprotection by the Glycosylated Chrysin Derivative LQFM280 in an AlCl_3 -Induced Neurodegeneration's Model.** Uchenna N¹, Campos HM¹, Ferreira PYO¹, Okoh VI¹, Costa EA¹, Menegatti R², Ghedini PC¹ ¹UFG, Dept Pharmacology, ²UFG, Faculty of Pharmacy

Introduction: Although chrysin has shown several pharmacological properties, its low oral solubility and bioavailability limit its therapeutic potential. Glycosylation has emerged as a strategy to enhance these characteristics, improving drug stability and tissue targeting. This study aimed to compare the neuroprotective effects of the glycosylated chrysin derivative LQFM280 and its parent compound in an AlCl_3 -induced neurotoxicity model in Swiss mice. **Methods:** Male Swiss mice (25–30 g, n = 15/group) were divided into four groups: Control, AlCl_3 , Chrysin, and LQFM280. Treatments were administered in two phases. In the first, Control and AlCl_3 groups received vehicle (0.9% NaCl, 3% Tween 80, 2% DMSO), while Chrysin and LQFM280 groups received 1 mg/kg of each compound. In the second, AlCl_3 (100 mg/kg, pH 7.4) was administered orally for 45 days, except to the Control group, which received distilled water. Behavioral tests included the Open Field Test (OFT), Chimney Test (CMT), and Step-Down Passive Avoidance Test (SDPAT). After euthanasia, hippocampal tissue was collected for biochemical assays (lipid peroxidation [LPO], catalase [CAT], acetylcholinesterase [AChE]) and molecular analysis of IL-1 β expression. The study was approved by the Animal Ethics Committee (protocol n° 053/2016). Data were analyzed using Shapiro-Wilk for normality, followed by ANOVA (with Welch correction if needed) and appropriate post hoc tests. Significance was set at $p < 0.05$. **Results:** Both LQFM280 and chrysin prevented memory impairment, oxidative stress, and neuroinflammation induced by AlCl_3 . OFT and CMT results showed no significant changes in motor performance ($p > 0.05$). However, SDPAT and biochemical analyses revealed that AlCl_3 significantly reduced memory and CAT activity, while increasing LPO, AChE activity, and IL-1 β levels ($p < 0.05$). Treatment with LQFM280 or chrysin restored memory ($p < 0.0001$), increased CAT activity ($p = 0.001$; $p < 0.0001$), and reduced LPO ($p = 0.0083$; $p < 0.0001$), AChE ($p = 0.0135$; $p < 0.0001$), and IL-1 β expression ($p = 0.0015$; $p < 0.0001$). Comparatively, LQFM280 showed superior protection in most parameters: memory ($p = 0.0046$), CAT ($p = 0.0248$), LPO ($p = 0.0464$), and AChE ($p = 0.0251$). **Conclusion:** LQFM280 and chrysin exhibit neuroprotective effects against AlCl_3 -induced neurotoxicity. Notably, LQFM280 provided greater protection, likely due to improved pharmacokinetics conferred by glycosylation (β -D-glucose tetraacetate). These findings suggest LQFM280 as a promising candidate for further development in neurodegenerative disease research and pharmaceutical applications. **Financial support:** CNPq

01.032 **Identification of MRP/ABCC Transporters Involved in cAMP Efflux from Skeletal Muscle Fibers.** Satori NA and Godinho RO Unifesp-EPM, Dept. of Pharmacology, Div. of Cellular Pharmacology; PPG Pharmacology, São Paulo, Brazil

Introduction: cAMP is a key intracellular second messenger that transduces signals downstream of Gs protein-coupled receptor activation, playing a critical role in cellular communication. Its extracellular presence has been reported in various cell types, including skeletal muscle fibers (Godinho *et al.*, *Br. J. Pharmacol.* 138:995, 2003). The physiological relevance of cAMP efflux lies in its subsequent extracellular metabolism: cAMP is hydrolyzed by ecto-enzymes into adenosine, which modulates skeletal muscle contractility and neuromuscular transmission via activation of adenosine receptors (Duarte *et al.*, *J. Pharmacol. Exp. Ther.* 341:820, 2012). To elucidate the mechanisms underlying cAMP efflux in skeletal muscle, we examined the role of multidrug resistance proteins/ATP-binding cassette (MRP/ABCC) transporters by assessing the impact of their pharmacological inhibition on cAMP release of C2C12 myotubes. **Methods:** Differentiated C2C12 myotubes were preincubated with the non-selective phosphodiesterase (PDE) inhibitor IBMX (0.1 mM) for 40 min, in the presence or absence of the ABCC inhibitors MK-571 (40 μ M, non-selective), reversan (10 μ M; ABCC1-selective) or ceefourin (10 μ M; ABCC4-selective). The cells were then stimulated with the beta adrenoceptor agonist isoproterenol (1 μ M) for 10 or 60 min and the extra- and intracellular cAMP concentrations were quantified, using TR-FRET immunoassay (Lance Ultra cAMP Kit, Perkin Elmer, USA) and expressed as nM $\pm$ S.E.M. Differences between groups were considered significant at $P \leq 0.05$ (ANOVA followed by the Tukey test). **Results:** Activation of beta adrenoceptors induced a transient increase of intracellular cAMP, with a peak of 7-fold at 5 min stimulation, followed by a time-dependent increase in extracellular cAMP. Pre-incubation of MK-571 increased by 67% intracellular cAMP accumulation after 10 min of isoproterenol exposure (cAMP_{intra}, 10 min: isoproterenol = 7.66 ± 0.69 ; +MK-571 = 12.80 ± 1.02 ; n=6), abolishing the parallel increment of extracellular cAMP. A similar response pattern was observed with the ABCC1 inhibitor reversan. In contrast, ABCC4 inhibitor ceefourin only reduced by 29% the isoproterenol-induced cAMP efflux (n=5-6). Notably, pre-treatment of cells with MK-571, reversan or ceefourin did not change the 60 min-stimulation effect of isoproterenol on intracellular cAMP levels. **Conclusion:** Activation of beta adrenoceptors induced a time dependent increase in both intra- and extracellular cAMP levels. The isoproterenol-induced efflux of cAMP was abolished by inhibition of ABCC transporters with MK-571. Since the combined inhibition of PDE with IBMX and ABCC transporter with MK-571 was not able to maintain increased levels of intracellular cAMP, additional mechanisms, such as IBMX-resistant PDE may be involved in the regulation of intracellular cAMP. Finally, considering the well-known expression profiles of ABCC1, ABCC4, and ABCC5 in C2C12 cells, our results indicate that cAMP efflux from skeletal muscle cells is mediated primarily by ABCC1, with a minor contribution of other transporters, such as ABCC4. **Animal Ethics Committee:** CEUA #2384050423 **Financial support:** CAPES, CNPq 310498/2019-8 and Fapesp 23/03689-1

01.033 **Mouse SEMG1 Reduces PKA Phosphorylation Without Changing cAMP Levels in Spermatozoa: A Non-canonical Pathway with Pharmacological Implications.** Santos NCM¹, Mariani NAP¹, Santos BR¹, Fernandes CJC¹, Novero AG², Oliveri LG³, Steeman TJ², Stival C², Luque GM³, Krapf D², Buffone MG³, Silva EJR¹ ¹UNESP, PPG Biomolecular & Pharmacological Sciences, Brazil, ²National University of Rosario, Institute of Molecular and Cellular Biology of Rosario, Rosario, Argentina, ³Institute of Biology and Experimental Medicine, Buenos Aires, Argentina

Introduction: Semenogelin-1 (SEMG1), the major component of the seminal plasma, interacts with ejaculated spermatozoa and temporarily inhibits their motility and capacitation. This event ensures the timely regulation of sperm signaling pathways essential for fertilization, such as cAMP-dependent Protein Kinase A (PKA) activation, which is a marker of capacitation. We previously demonstrated that mouse SEMG1 (mSEMG1) interacts with the sperm-associated protein EPPIN, thereby providing a framework for investigating the underlying mechanisms by which SEMG1 regulates sperm function. Here, we investigated whether recombinant mSEMG1 inhibits cAMP/PKA activation and downstream protein phosphorylation during sperm capacitation via EPPIN binding.

Methods: Spermatozoa were isolated from the cauda epididymis of C57BL/6 mice (90 days old, CEUA n°8952010720) and capacitated *in vitro* in the absence (control) or presence of 20-25 μ M of recombinant full-length mSEMG1 (Q32-G375) or its fragment R98-G375 (mSEMG1^{R98-G375}), which contains the main EPPIN binding site. Additional experiments were performed under the same conditions but in the presence of permeable 8-b-cAMP (500 μ M) and IBMX (100 μ M). The phosphorylation of PKA substrates and tyrosine residues was analyzed by Western Blot. Intracellular cAMP levels were quantified using the cAMP Select ELISA kit. The transient relocalization of the PKA regulatory subunit (PKA-RII) near A-kinase anchoring protein 4 (AKAP4) was assessed in spermatozoa by Laser Scanning Super Resolution Microscopy (LS-SRM). Results were expressed as mean $\pm$ SEM and analyzed by ANOVA, followed by Bonferroni's test; $p < 0.05$ was considered significant. **Results:** mSEMG1 reduced the increase in phospho-PKA substrates ($P = 0.037$) and tyrosine phosphorylation ($P = 0.090$) during sperm capacitation to levels comparable to non-capacitated spermatozoa. Notably, this inhibitory effect was mimicked by mSEMG1^{R98-G375}, suggesting that SEMG1/EPPIN interaction is functionally required. Although mSEMG1 did not affect the capacitation-associated increase in cAMP intracellular concentrations, co-incubation of spermatozoa with 8-b-cAMP/IBMX rescued the reduction in phospho-PKA substrates ($P = 0.04$). LS-SRM revealed that mSEMG1 impaired the redistribution of the PKA-RII subunit along the sperm flagellum during capacitation, retaining PKA in a resting state and preventing the anchoring of PKA-RII to AKAP4. **Conclusions:** Our findings suggest that mSEMG1 uses EPPIN to anchor on the sperm surface and inhibit capacitation by blocking phosphorylation of PKA substrates and tyrosine residues via a cAMP-independent pathway. This inhibition appears to occur by slowing down PKA-RII relocalization, thereby preventing sperm capacitation. These results position SEMG1 as a potential non-canonical pharmacological candidate for male contraception. **Financial support:** CAPES, FAPESP, CNPq, Chang Zuckerberg Initiative (CZI)

01.034 Microglial Alpha 7 Nicotinic Acetylcholine Receptor's: Characterization of immediate response. Bello-Santo, VG; Oiveira, LN; Castro, NG UFRJ, Instituto de Ciências Biomédicas, Laboratório de Farmacologia Molecular, PPGFQM, Brazil.

Nicotinic receptors of the so-called “neuronal” subtypes are also expressed in immune cells and participate in acetylcholine-induced signaling. In microglia, the $\alpha 7$ nicotinic receptor ($\alpha 7$ nAChR) plays an essential role in inflammation by inhibiting the release of pro-inflammatory cytokines, making it an attractive target for drug development in conditions involving neuroinflammation. However, there is a lack of direct evidence supporting an ionotropic function. In fact, some evidence suggests the opposite. Here, we aim to clarify the immediate molecular transduction mechanisms of $\alpha 7$ nAChR and explore its modulation in microglia. Primary cortical microglia were isolated from newborn (P0–P2) Swiss mice. Intracellular calcium fluctuations were assessed by microfluorimetry using the ratiometric and sensitive indicator dye FURA-2-AM. Brief applications of nicotinic agonists and antagonists were applied. A fluorescence assay with α -bungarotoxin–rhodamine (α BGT-Rh) was performed to identify $\alpha 7$ nAChRs in microglia. Microglia incubated with α BGTRh (500 nM) exhibited a mean fluorescence density of 62.6 (4.9 ± 0.9 ; $N = 3$). Pre-incubation with the selective $\alpha 7$ nAChR antagonist methyllycaconitine (MLA) reduced fluorescence by 32.9% (20.6 ± 5.2 ; $N = 3$; 840 cells, RM oneway ANOVA, $P = 0.0001$), confirming receptor expression. Microglia exhibited calcium transients in response to $\alpha 7$ nAChR stimulation (1 mM nicotine + 3 μ M PNU-120596) applied via perfusion, while MLA (10 nM) fully inhibited these responses ($N = 3$; 152 cells). The absence of extracellular Ca^{2+} completely abolished calcium transients in all cells ($N = 1$; 50 cells). Suggesting that intracellular Ca^{2+} increase partially depends on Ca^{2+} influx. These results suggest that $\alpha 7$ nAChR activation triggers calcium transients in microglia that depend at least partly on extracellular calcium, consistent with an ionotropic transduction mechanism.

02.001 **Haloperidol as a Treatment for Wernicke-Korsakoff Syndrome.** Nogueira DL¹, Rocha ME².

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Introduction: Wernicke-Korsakoff syndrome (WK syndrome) has its physiopathology in the Central Nervous System, with its symptomatology divided between Wernicke's encephalopathy and Korsakoff's psychosis. With a quick progression and fulminant aspect, this syndrome is characterized by a chronic deficiency of vitamin B1 (thiamine) and has both acute (Wernicke's encephalopathy) and chronic (Korsakoff's psychosis) stages, with the acute stage including symptoms like horizontal nystagmus, unilateral and convergent strabismus, double vision, mental confusion, disorientation and ataxia. The patient who survives the acute stage starts presenting residual amnesia and confabulation, typical of the chronic stage. The treatment of WK syndrome consists of vitamin B1 IV administration, as an attempt to reverse symptoms. Although, the complete regression of the disease is uncommon if the treatment is delayed, so the residual neurologic symptoms must be treated with an alternative therapeutic action, therefore, Haloperidol, also known by its brand name, Haldol, is an effective option. This study aims in understanding the effectiveness of Haloperidol as a treatment for Wernicke-Korsakoff syndrome.

Methods: This abstract consists of systematic literature review. The search was conducted in the PubMed and SciELO databases using the terms "Haloperidol", "Wernicke-Korsakoff" and "Treatment". The inclusion criteria comprised studies and book chapters published in English or Portuguese in the last five years, addressing the use of Haloperidol in WK syndrome patients. Ten studies were included after full-text analysis. Book chapters were included to complement data from original studies and clinical experiences. This study received no funding. **Results:** Haloperidol is a first-generation typical antipsychotic used worldwide to block dopamine D2 receptors in the brain and exert its antipsychotic action, with a bioavailability from 60% to 70%. The drug reaches its maximum effectiveness when 72% of dopamine receptors are blocked, and the time to reach peak plasma concentration is around 2 to 6 hours after oral administration, 20 minutes after IM administration. As a drug that aims controlling psychosis, mental confusion and other severe behavioral states, symptoms of WK-syndrome, Haloperidol presents highly effective as a treatment for the disease. However, Haloperidol is contraindicated in multiple cases, such as: patients diagnosed with Parkinson's disease, dementia and depression, and presents drug interaction with Lithium, used to treat symptoms of bipolar disorder, which can also be treated with Haloperidol.

Conclusion: Haloperidol may be a beneficial adjunct therapy in treating the neuropsychiatric sequelae of Wernicke-Korsakoff Syndrome, particularly in controlling psychosis and agitation. Despite the limited high-level evidence, the drug shows promise as a symptomatic treatment when thiamine administration fails to fully reverse neurological damage. Further clinical studies are needed to establish its efficacy and safety in this specific population.

02.002 Lisinopril Prevents Stress-Induced Fear Generalization and Memory Impairments in Mice.

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Introduction: In pathological conditions such as post-traumatic stress disorder (PTSD), stress exposure adversely affects defensive behavior and cognitive processes, including conditioned fear responses and recognition memory. The renin-angiotensin system (RAS) has been shown to modulate stress-related behaviors and cognitive functions via actions on central nervous system (CNS) structures. This study investigates how pharmacological modulation of the RAS influences stress-induced behavioral and cognitive impairments in male mice. **Methods:** Male C57BL/6 mice, eight weeks, were randomly assigned to two groups: control and stress-exposed. The stress group was subjected to a stress-recovery protocol (SRS) consisting of 2 hours of restraint in a tube, 20 minutes of forced swim, and exposure to ether until unconsciousness. 7 days later, the animals were subjected to an additional 20 min forced swim. Both groups received daily systemic treatment with either vehicle or Lisinopril (1 mg/kg) for 7 days, administered between stress sessions. 24 h after the final stress protocol, the animals were evaluated using the contextual fear conditioning generalization test (CFC) and the Novel Object Recognition (NOR) test. In the CFC, animals were exposed to an aversive context (Context A, with 3 shocks of 0.85 mA/2s) and a neutral context (Context B, without shocks), with re-exposure to each context for 5 min 24 h later to assess fear generalization. For the NOR test, animals explored two identical objects for 5 min during the acquisition phase. 1h (short-term memory, STM) or 24h (long-term memory, LTM) later, one object was replaced with a new one to evaluate memory performance. **Results:** In the NOR test, control animals exhibited a preference for the novel object [STM: $F(1,34) = 89.3$, $p < 0.05$; LTM: $F(1,36) = 55.4$, $p < 0.05$], whereas SRS animals showed no such discrimination, indicating recognition memory deficits [STM: $F(1,32) = 0.07$, $p > 0.05$; LTM: $F(1,32) = 0.41$, $p > 0.05$]. Lisinopril treatment was effective in preventing memory deficits in the SRS group, with significant results observed in the interaction time when compared to the vehicle group [STM: $F(1,36) = 7.1$, $p < 0.05$; LTM: $F(1,32) = 4.31$, $p < 0.05$]. In the CFC test, both control and SRS groups showed similar levels of freezing when re-exposed to the conditioning context (Context A) [$F(3,58) = 2.1$, $p > 0.05$]. However, when exposed to context B, control animals displayed significantly reduced freezing compared to context A [$F(1,24) = 6.5$, $p < 0.05$], while SRS animals exhibited similar freezing levels across both contexts, suggesting generalized fear [$F(1,34) = 0.35$, $p > 0.05$]. Lisinopril treatment did not affect freezing behavior in Context A for either group [$F(3,29) = 0.17$, $p > 0.05$], but significantly reduced contextual generalization in SRS animals [$F(1,34) = 10.9$, $p < 0.05$]. No such effect was observed in control animals [$F(1,24) = 2.7$, $p > 0.05$]. **Conclusions:** Our findings suggest that Lisinopril can prevent recognition memory deficits and reduce aversive contextual generalization resulting from stress exposure, highlighting a potential role of the RAS in modulating stress-induced cognitive and behavioral alterations. **Animal Research Ethics Committee:** nº 1271/2023. **Financial Support:** CAPES, FAPESP, CNPq and FAEPA

02.003 **Effect of 5-HT1A Receptor Participation in Mediation of the Anxiolytic Effect of Resolvin D5: Evaluation of Sex-Based Differences.** Uada TS, Avanço LC, Zanoveli JM ¹Lab. of Neuropsychopharmacology, Brazil, UFPR

Introduction: Stress can induce physiological changes that facilitate the onset of psychiatric disorders. The serotonergic system is known to be involved in the regulation of emotional behavior and is highly sensitive to the effects of stress, often becoming dysfunctional. Studies have shown that fish oil, rich in omega-3 polyunsaturated fatty acids (ω -3 PUFAs), exerts neuroprotective effects by reducing neuroinflammation and oxidative stress, while also enhancing serotonergic neurotransmission. Research using a DHA-derived pro-resolving lipid mediator, Resolvin D5 (RvD5), has demonstrated anti-inflammatory and anxiolytic-like effects in rats previously subjected to stress or experimentally induced type-1 diabetes mellitus. **Methods:** This study aimed to investigate in rats the involvement of serotonin 1A (5-HT1A) receptors in the likely effects induced by RvD5 treatment, as well as to explore sex-related differences. All procedures were approved by CEUA-BIO/UFPR (#1587). Based in previous study (Bispo matos et al., 2025), animals received the lower and effective dose of RvD5 (1 ng/animal, i.p., in 200 μ l) for 10 consecutive days. Treatments were administered three hours prior to any behavioral procedures. On the 8th day, animals were subjected to the light-dark transition (LDT) test to evaluate the effects of RvD5 under non-stress conditions. On the 9th day, animals underwent a 2-hour acute restraint stress (ARS) protocol, and on the 10th day, they received the 5-HT1A receptor antagonist WAY100635 (i.p.; 0.1 mg/kg) three hours after RvD5 administration. One hour later, animals were tested in the elevated plus maze (EPM). To evaluate the persistence or withdrawal effects of the treatment, animals were subjected to the novelty-suppressed feeding (NSF) test on day 17, one week after the final RvD5 injection. Vaginal smears were collected after each behavioral test to determine the estrous cycle stage of female rats and examine possible behavioral variations across different phases. **Results:** Our results from the LDT and EPM tests demonstrated that preventive RvD5 treatment did not change the anxiety-like responses obtained from LDT but was able to induce an anxiolytic-like effect in stressed male and female rats submitted to the EPM, suggesting the anxiolytic-like effects are stress-dependent. Also, partial reversal of the anxiolytic-like effect induced by RvD5-treated was observed following 5-HT1A receptor blockade, while the antagonist per se had no effect. In the NSF test, long-term anxiolytic-like effects were more prominent in male rats, indicating a sex-dependent difference. Analysis of the estrous cycle is ongoing and will require a larger sample size to obtain statistically significant results. **Conclusion:** These findings support the hypothesis that preventive administration of RvD5 induces anxiolytic effects under acute stress conditions, potentially through modulation of stress-induced neuroinflammatory pathways. The persistence of these effects was observed only in males, suggesting a sex-dependent mechanism. Additionally, the results highlight the involvement of the serotonergic system, particularly 5-HT1A receptors, in the anxiolytic actions of RvD5. **Acknowledgments:** CNPq for the productivity grant (Process No. 307714/2023-3).

02.004 **Copaiba Oil Did Not Induce Significant Changes in the Locomotor Activity of Adult Zebrafish.** Costa CHL¹, Barros Costa G¹, Andrade KC², Abreu LS², Nicole Maciel NMF², Fonteles MV³, Sales M³, Correia MFS³, Franco VMF⁴, Mesquita DS⁵, Honório Júnior JER⁵ ¹Christus University Center, Psychology, Brazil; ²Christus University Center, Biomedicine, Brazil; ³Nursing student at Christus University Center, Brazil; ⁴UFC, Postgraduate in Pharmacology, Brazil; ⁵Christus University Center, Brazil

Introduction: copaiba oil is extracted from the trunks of trees of the genus *Copaifera* L. (Leguminosae), native to tropical regions of Latin America. This oil, traditionally used in folk medicine, is used for its anti-inflammatory and antioxidant properties. To investigate its possible anxiolytic effects on the central nervous system, we used the open field test to assess changes in the locomotor behavior of adult zebrafish (*Danio rerio*). **Methods:** Zebrafish were distributed (approved by CEUA 11/21) equally into four experimental groups (n = 10 per group): a control group and groups treated with 1 mg, 10 mg, and 50 mg of *Copaifera multijuga* oil, administered intraperitoneally. Thirty minutes after administration of the respective doses, the animals underwent an open field test to assess locomotor activity. At the end of the experiment, the data obtained were statistically analyzed using GraphPad Prism 8 software. **Results:** the data were normally distributed but did not meet the criterion of homogeneity of variances. Thus, Welch's ANOVA was applied, which did not indicate statistically significant differences between the groups ($p > 0.05$). The Games-Howell post hoc test also revealed no significant differences between the treated groups and the control. **Conclusion:** *C. multijuga* oil did not impair the animals' locomotion. These results suggest that the substance has no sedative effect and may represent a promising alternative for studies related to disorders such as anxiety and depression. **Keywords:** *Copaifera multijuga*; central nervous system; locomotor activity; zebrafish. **Financial support:** Christus University Center.

02.005 **Evaluation of the Antidepressant Effect of CBD from Cannabis sativa by the Forced Swimming Test in a Chronic Model in Mice.** Costa GB¹, Costa CHL¹, Sousa JDDS²; Pimentel ISB², Dinelly Neto VC², Izidorio JLG², Paixão LDS³, Franco VMF⁴, Mesquita DS⁵, Honório Júnior JER⁵
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Introduction: Depression is a psychiatric disorder characterized by persistent feelings of sadness, hopelessness, and low self-esteem, accompanied by physical and cognitive symptoms. It is estimated that more than 264 million people worldwide suffer from depression, according to the WHO. Refractory depression, in particular, represents a condition in which symptoms persist even after attempts with at least two different antidepressants at adequate doses and for a sufficient period. The present study used an experimental model of chronic depression in mice to evaluate the antidepressant potential of CBD (cannabidiol) from Cannabis sativa in a forced swim test.

Methods: The mice (8 animals in each – CEUA012/24) were divided into the following groups: control (no stress), stress group, stress plus cannabidiol group (1.0 mg CBD, p.o.), and stress plus escitalopram group (10 mg/kg, p.o.). The stress model is induced for 14 days, and in the last 7 days, treatment is applied 1 hour after stress. Thirty minutes after the last treatment, the animals were evaluated by the forced swim test. **Results:** In the test, we observed that the animals induced to stress showed an increase in immobility time compared to the control ($p=0.0002$), and in the animals treated with cannabidiol and escitalopram, there was protection against stress ($p<0.0001$ and $p=0.0009$, respectively. $F(3,29)=11.51$). **Conclusion:** Thus, we can conclude that animals treated with 1.0 mg of CBD had an effect similar to that of escitalopram, reversing the induced depressive behavior. **Keywords:** cannabidiol; mouse; antidepressant activity; central nervous system. **Financial support:** Unichristus

02.006 Neurotoxic Effects of N-Nitrosodimethylamine (NDMA) on oxidative stress markers in the cerebral cortex and hippocampus of prenatally exposed rats

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Introduction: N-Nitrosodimethylamine (NDMA) is a volatile nitrosamine widely present in the environment due to its easy formation from precursors commonly found in food, water, and pharmaceutical products. Classified as a probable human carcinogen, NDMA is known to induce hepatotoxicity and oxidative damage through the generation of reactive oxygen species (ROS). Although its systemic toxicology is well documented, a few studies have addressed the neurotoxic impact of prenatal NDMA exposure. Oxidative stress plays a crucial role in neuronal injury and neurodevelopmental toxicity, particularly through lipid peroxidation (LPO) and disruption of endogenous antioxidant systems such as catalase (CAT) and superoxide dismutase (SOD). The present study aimed to evaluate the effects of gestational NDMA exposure on oxidative stress markers in the cerebral cortex and hippocampus of Wistar rats. **Objective:** Evaluate the effects of NDMA on oxidative stress parameters in the cerebral cortex and hippocampus, using doses of 0.5 mg/kg, 1.0 mg/kg, and 2.0 mg/kg. **Methods:** Pregnant Wistar rats (n=10 per group) were randomly assigned to four experimental groups. The control group received saline solution, while the other groups were treated with NDMA at doses of 0.5, 1.0, and 2.0 mg/kg/day. The treatments were administered orally (by gavage) from gestational day (GD) 0 to 20. On GD 21, the hippocampus and cortex were collected to assess oxidative stress markers, including lipid peroxidation levels, and the activity of endogenous antioxidant defenses (SOD and CAT). The study was approved by the Animal Ethics Committee (CEUA/UEG n°. 009/24). **Results:** In the cortex brain the NDMA 1.0 mg/kg and 2.0 mg/kg treatments decrease the activity of SOD ($p = 0.0243$; $p = 0.0011$) and CAT ($p = 0.0233$; $p = 0.0034$); and increase the LPO levels ($p = 0.0272$; $p = 0.0091$). In the hippocampus all the NDMA doses decrease the activity of SOD ($p = 0.0214$; $p = 0.0103$; $p = 0.0046$) and CAT ($p = 0.0266$; $p = 0.0090$; $p = 0.0050$); and the NDMA 1.0 and 2.0 mg/kg increase the LPO levels ($p = 0.0268$; $p = 0.0003$). **Conclusion:** Gestational exposure to NDMA led to significant alterations in oxidative stress parameters in the cortex and hippocampus, evidenced by reduced activity of the antioxidant enzymes SOD and CAT, as well as increased lipid peroxidation. These findings indicate that NDMA, even at subacute doses, compromises redox homeostasis in the developing central nervous system, which may contribute to permanent neurotoxic effects. Such data reinforces the need to reassess the toxicological risks associated with perinatal exposure to nitrosamines and their possible link to neurodegenerative outcomes. **Financial support:** CAPES, FAPEG and CNPq

02.007 Repeated CBD Treatment Increased Hippocampal Pro-Caspase-1 in Socially Isolated Female Mice. Pedro AP¹, Bilibio JO², Barros ASM², Lisboa SF^{2,3} ¹FFCLRP-USP, Brazil; ²FMRP-USP, Pharmacology PPG, Brazil; ³FCFRP-USP, Brazil

Introduction: The COVID-19 pandemic has had lasting impacts on mental health, particularly due to social isolation (SI). SI has been associated with increased anxiety and depressive disorders, conditions with a higher incidence in women. SI is a stressor linked to emotional, cognitive, and neuroinflammatory impairments that significantly impact the hippocampus. In animal models, SI activates the NLRP3 inflammasome, leading to behavioral changes. Cannabidiol (CBD) has shown anxiolytic and anti-inflammatory effects, possibly involving endocannabinoid (eCB) CB1 receptors, enzymes such as Fatty Acid Amide Hydrolase (FAAH), which metabolize the eCB anandamide, and the NLRP3 pathway. This study investigated the effects of CBD in female mice subjected to SI, focusing on components of the eCB system and NLRP3 inflammasome in the hippocampus.

Methods: Adult CD-1 female mice (9 weeks old) (Ethics committee: 21.1.522.60.3) were housed under either social or isolated conditions. From the 14th to the 33rd day of the experimental protocol, animals received daily injections of CBD (10 mg/Kg/10 mL; i.p.) or vehicle (3% Tween 80 in saline; i.p.). From days 28 to 33, behavioral tests were conducted to assess emotional and cognitive parameters. SI mice displayed depressive-like behavior in the splash test and anxiety-like behavior in the novelty-suppressed feeding test, with the last one being attenuated by CBD. The hippocampal samples from these mice were collected for assessment of CB1, FAAH, and caspase-1 protein levels through Western Blotting. Data were analyzed using two-way ANOVA, considering experimental condition (isolated or grouped) and treatment (vehicle or CBD) as factors. **Results:** Treatment with CBD did not significantly alter the expression of CB1 [Interaction: $F(1, 25)=0,7236$, $p=0,4031$; Condition: $F(1, 25)=0,3543$, $p=0,5571$; Treatment: $F(1, 25)=0,4125$, $p=0,5266$] or FAAH [Interaction: $F(1, 25)=0,5276$, $p=0,4744$; $p > 0.05$; Condition: $F(1, 25)=2,802$, $p=0,1066$; Treatment: $F(1, 25)=0,7019$, $p=0,4101$]. In contrast, CBD treatment increased caspase-1 expression regardless of experimental condition [Interaction: $F(1, 17)=2,657$, $p=0,1215$; Condition: $F(1, 17)=0,02926$, $p=0,8662$; Treatment: $F(1, 17)=8,711$, $p=0,0089$]. **Conclusion:** Our results show that treatment with CBD unexpectedly increased caspase-1 levels in the hippocampus, suggesting that CBD, at the dose used, may inhibit the cleavage of caspase-1 into its active isoform, leading to a compensatory accumulation of the enzyme. Therefore, the observed increase could result from a functional blockade of the inflammasome pathway. Our next steps include assessing cleaved caspase-1, IL-1 β , NLRP3, and ASC protein levels and their gene expression through qRT-PCR to confirm this hypothesis and shed light on involved mechanisms. **Financial support:** FAPESP (2017/19731-6); CAPES.

02.008 Early-Life Reduction of Parvalbumin in the Medial Prefrontal Cortex (mPFC): Behavioral and Electrophysiological Outcomes in Adolescent and Adult Rats. Caceres-Silva GH, Silveira JR, Freitas IS, Guimarães FS, Gomes FV FMRP-USP, Dept. of Pharmacology, Brazil

Introduction: The excitatory-inhibitory (E/I) balance refers to the equilibrium between excitatory and inhibitory neuronal interactions in the central nervous system, an essential dynamic regulation for the temporal control and synchronization of neural activity. Among the neurons involved in maintaining this balance, parvalbumin (PV)-expressing GABAergic interneurons, characterized by their fast-spiking activity, play an important role. The coordinated maturation of PV interneurons delineates critical periods of brain plasticity, marked by heightened sensitivity to environmental stimuli and circuit refinement. Late adolescence coincides with the developmental peak of PV interneurons and represents a vulnerable window in which adverse experiences, such as stress, may disrupt their maturation. Such perturbations can compromise the integrity of E/I balance, potentially contributing to the pathophysiology of neuropsychiatric disorders. Previous studies from our group in rodents indicated that stress exposure during adolescence induces schizophrenia-like changes, including behavioral deficits and dopamine system overdrive in the ventral tegmental area (VTA), which have been associated with functional impairments in PV interneurons in the medial prefrontal cortex (mPFC) and ventral hippocampus (vHip). However, the causal link between PV interneuron dysfunction and these behavioral and electrophysiological abnormalities remains unclear. **Aims:** Here, we evaluate the effects of reducing PV expression in the mPFC of rats during the prepubertal period on behavior and VTA dopamine system activity. **Methods:** Male Sprague-Dawley rats received bilateral stereotaxic injections of lentiviral particles to deliver shRNA targeting the PV gene (*Pvalb*) or a scrambled control at postnatal day 21 (P21). Behavioral tests, including the elevated-plus maze and light-dark box (“anxiety”), social interaction (“sociability”), and novel object recognition (“cognition”) were performed during adolescence (P40-42; n=10 animals/group) or adulthood (P62-64; n=9~12 animals/group). *In vivo* electrophysiological recordings of VTA dopamine neurons were performed in the days following behavioral testing. After electrophysiological recordings, brains were collected for immunofluorescence to assess PV expression levels and confirm the injection sites. This study was approved by the Ethics Committee on the Use of Animals of the Ribeirão Preto Medical School-USP (#155/2018). **Results:** Prepubertal PV knockdown in the mPFC did not induce behavioral alterations or changes in the activity of VTA dopamine neurons, either during adolescence or adulthood. Molecular analyses to verify the reduction in PV expression and confirm injection sites are currently underway. Based on pilot experiments and reference studies using the same shRNA approach, a reduction of approximately 25% in PV expression levels is expected. **Conclusion:** Our findings suggest that early-life PV knockdown in the mPFC does not alter behavior or VTA dopamine system activity. Further studies are needed to evaluate whether this manipulation increases susceptibility to stress, given the prominent role of adverse events during adolescence observed in our previous findings. Moreover, considering the role of the vHip in modulating VTA dopamine neuron activity, it is worth investigating the effects of PV reduction in this region to assess potential behavioral and electrophysiological outcomes. **Financial support:** FAPESP, CNPq, CAPES, and FAEPA

02.009 Prolyl Oligopeptidase (POP) Activity in an Animal Model of Congenital Syndrome Associated with Zika Virus (CZS). Ximenes APX¹, Oyadomari WY¹, Christoff RR², Garcez PP², Hayashi MAF¹

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Flavivirus infections represent a significant challenge to global public health due to their potential to cause severe diseases and negatively impact the quality of life of millions of people. Serological similarities between Zika virus (ZIKV) and dengue virus (DENV) complicate the control and monitoring of infections, particularly due to their similar clinical presentations. ZIKV-induced congenital syndrome (CZS), which gained worldwide attention following the sudden increase in microcephaly cases reported in Brazil during the Olympic Games, has led to several scientific studies and, more recently, highlighting oligopeptidases as promising biomarkers for early diagnosis of impaired brain formation. Flaviviruses can interfere with brain formation and spatial organization of neurons and synapses during neurodevelopment potentially leading to severe neurological complications, which may result in complex clinical manifestations such as cognitive and motor impairments, seizures, behavioral disorders, encephalopathies, and microcephaly. Prolyl oligopeptidase (POP) plays an important role in the maturation and degradation of neuropeptides and oligopeptides, and its activity in the central nervous system (CNS) has been predominantly observed in neuronal populations of the cerebral cortex and the nigrostriatal pathway, where it is known to be involved in neuronal growth, differentiation, and inflammation. Here, we examined POP activity in an animal model of ZIKV infection associated with dysregulated corticogenesis. The animals used in this study were neonatal Swiss mice (postnatal day 0). On postnatal day 0 (P0), the animals received an intracerebroventricular (ICV) or intraperitoneal (IP) injection of Zika virus (ZIKV), dengue virus (DENV), or a control solution (MOCK), brain tissues were collected on postnatal day 7 (P7). We observed a reduction in POP activity associated with congenital syndrome induced by ZIKV isolates, following postnatal injections of different inoculum doses in mouse models. We show here that ZIKV infection reduced POP activity in animals infected via both ICV and IP routes, whereas DENV injection despite its similarities with ZIKV only caused changes via the IP route. Understanding the role of POP and its implications in the regulation of proteins associated with neuronal plasticity and the inflammatory response may help mitigate the neurotoxic effects observed in ZIKV or DENV infections. Given POP's involvement in neurodegenerative and neuropsychiatric disorders, we also propose that assessing POP activity in ZIKV infection, compared to DENV infection which does not cause microcephaly but may lead to neurological complications and can be fatal in some cases may strengthen the potential of this enzyme and other oligopeptidases as biomarkers for the diagnosis of viral pathologies and further elucidate the true biological role of POP. **Keywords:** flavivirus; Zika virus; dengue virus; oligopeptidases; microcephaly. **Financial Support:** FAPESP, CNPq and CAPES. **Ethics:** This study was approved by the Research Ethics Committee of UNIFESP and CEUA [3322141024].

02.010 **Evaluation of the Neuroprotective Effect of Monoterpene 1,8-Cineole Complexed with Beta-Cyclodextrin *in vitro* Model of Parkinson's Disease.** Araújo-Monteiro L¹, Carvalhinho-Lopes PS¹, Soares-Silva B¹, Donzelli NIZ¹, Pereira EGJ¹, Binatto BVCC¹, Ribeiro TF¹, Quintans-Junior LR², Rando DG³, Ribeiro AM¹ ¹Unifesp-Baixada Santista, PPG in Bioproducts and Bioprocesses, Brazil; ²UFS, PPG in Pharmaceutical Sciences, Brazil; ³Unifesp-Diadema, PPG in Pharmaceutical Sciences; Brazil

Introduction: Parkinson's disease (PD) is the second most prevalent degenerative neuropathology worldwide. Although pharmacological treatments can delay progression and/or alleviate symptoms, they can often be associated with severe adverse effects. Natural substances such as terpenes have gained attention as potential chemical models for the development of novel drugs. The monoterpene 1,8-cineole (CIN), found in several plant species including eucalyptus, exhibits antioxidant and anti-inflammatory properties. Moreover, complexation of terpenes with beta-cyclodextrin has been shown to enhance its hydrosolubility and bioavailability, potentially improving its therapeutic efficacy. **Objective:** To evaluate the neuroprotective effects of beta-cyclodextrin complexed 1,8 cineole (B-CIN) using *in silico* pharmacological network analysis and *in vitro* cellular models of PD. **Materials and Methods:** Target fishing method was used to identify potential targets for CIN using the PharmMapper platform. Through network pharmacology, genes related to PD were collected, and overlapping targets were analyzed using a Venn diagram. Human SH-SY5Y neuroblastoma cells, differentiated into a dopaminergic phenotype, were treated with different concentrations of B-CIN (50, 100, 150, 200, 250 and 300 micromolar- μ M) for 24 hours. Cell viability was assessed using the MTT assay, and absorbance was measured at 965nm using a spectrophotometer. All experiments were performed in triplicate with a sample size of three ($n=3$). **Results:** Through target fishing, 100 potential targets for B-CIN interaction were identified, 80 of which are related with PD based on network pharmacology analysis. One-way ANOVA showed that treatment with B-CIN at 50 μ M did not significantly alter cell viability ($103.3 \pm 0.5\%$). However, higher concentrations ($> 100 \mu$ M) significantly reduced viability in a dose-dependent manner, indicating a concentration-dependent cytotoxic effect [$F(7,16) = 961.3$, $p < 0.0001$]. **Conclusion:** The B-CIN complex was associated with 80 protein targets related to PD. These findings contribute to elucidating some of the possible pathways responsible for its neuroprotective effect. Although the complex may have potential cytoprotective effects at low concentrations, while higher concentrations were cytotoxic to SH-SY5Y cells. **Financial support:** CAPES (Process: 88887.111943/2025-00). **Keywords:** 1,8-cineole; cell viability; neuroprotection; network pharmacology; Parkinson's disease.

02.011 **Behavioral and Synaptic Alterations in Offspring Induced by Gestational Valproic Acid Exposure: Sex Differences.** Rodrigues BA, Silva JN, Andreotti DZ, Amorim TL, Kawamoto EM. ICB-USP, Lab of Molecular and Functional Neurobiology, PPG Pharmacology, Brazil

Introduction: Valproic acid (VPA) is a drug used to treat epilepsy. Clinical studies have associated VPA consumption during pregnancy with high risks for the offspring, including developmental delay, cognitive impairments, behavioral deficits, changes in synaptic transmission, and autism. Brain regions such as the hippocampus, cortex and cerebellum are potentially associated with these affected parameters in the offspring. The objective of this study was to investigate possible altered behaviors in the offspring of male and female mice exposed to VPA *in utero*. The study also aimed to verify if this exposure promoted changes in synaptic proteins in the hippocampus, frontal cortex, and cerebellum. **Methods:** Pregnant female mice (C57/BL6) were separated into two groups: a control group and a VPA group. VPA or saline was injected intraperitoneally (300 mg/kg) on embryonic days 10 and 12. At one month old, male and female offspring (n=15) were subjected to behavioral tests: the Elevated Plus Maze (EPM), Social Interaction (SI), and Social Preference (SP) to evaluate anxiety-like behavior and social interaction. After the behavioral tests, the brains of males and females (n=5) were removed for dissection of the brain regions involved in cognition. Total proteins were extracted, and samples were subjected to Western Blotting (WB) to evaluate the expression of synaptophysin and PSD-95. Statistical analysis was performed using a Two-Way ANOVA with Tukey's post-hoc test in GraphPad Prism. This project was approved by CEUA 2945190521. **Results:** In the EPM test, male VPA offspring showed a statistically significant increase in the time spent in the closed arms ($p=0.0247$), a significant reduction in the time spent in the open arms ($p=0.0227$), and a reduction in the number of entries into the open arms ($p=0.0101$). No differences were observed in these parameters for the female offspring, and no differences were found between any of the groups in the number of entries into the closed arms. However, female VPA offspring showed a significant reduction in the frequency of entry into the open arms ($p=0.0152$). Locomotor activity was not assessed in this test. In the SI test, the male VPA group showed a significant reduction in social interaction with an unknown animal ($p<0.001$) but a significant increase in interaction with an object ($p=0.0090$). Female VPA offspring showed a significant increase in interaction with the unknown animal compared to the VPA males ($p=0.0005$). In the SP test, the male VPA group exhibited a significant increase in social interaction with a familiar animal ($p<0.0001$) and in the time spent in the familiar animal's compartment ($p=0.0180$). The female VPA group also showed a significant increase in social interaction with a familiar animal ($p=0.0002$). In the female offspring exposed to VPA, PSD-95 protein expression in the cortex showed a significant increase ($p=0.0242$). No significant differences were observed in the hippocampus and cerebellum between the experimental groups. Synaptophysin protein expression remained unchanged across all evaluated brain regions. **Conclusion:** The results of the behavioral tests suggest that both male and female VPA offspring exhibit anxious-like behavior and a social preference for familiar animals. The WB results suggest a possible increase in excitatory synapses in the frontal cortex of VPA females. It is possible that the cognitive deficits observed in these animals exposed to VPA are a consequence of this synaptic functional dysregulation. Financial support by FAPESP (2022/15514-9) and CAPES.

02.012 **Anticonvulsant and Neuroprotective Potential of Microalgae *Chlorella vulgaris* and *Limnospira platensis* in a Pentylene-tetrazol-Induced Seizure Model.** Cancela LCF^{1,2}, Freitas CM¹², Bentes ILG^{1,2}, Araújo TB¹, Pinto DSV¹, Jesus MS¹, Faustino SMM¹, Nascimento JLM², Oliveira FR^{1,2}
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Introduction: Microalgae are photosynthetic microorganisms considered promising as functional foods due a high nutritional value, rich in proteins, vitamins, minerals, polyunsaturated fatty acids, and fibers, as well as the presence of bioactive compounds with promising pharmacological activity, such as phycocyanin and carotenoids. Among the main species, *Limnospira platensis* and *Chlorella vulgaris* stand out for their antioxidant potential and modulation of neurodegenerative processes and seizure control. Given this profile, those species may not only complement the diet but also exert functional effects, providing benefits beyond basic nutrition. **Objective:** Evaluate the anticonvulsant and neuroprotective effects of microalgae in experimental models of severe seizures in mice. **Methods:** Mice were treated with *L. platensis* and *C. vulgaris* at 100 mg/kg (oral route) for 15 days, and both a untreated control group. Animals were subjected to seizure induction using pentylene-tetrazol (PTZ) 60 mg/kg (intraperitoneal route) in a single dose and observed for 10 minutes. Behavioral parameters such as latency to myoclonic and tonic-clonic seizures and seizure duration were assessed, such as lipid peroxidation in brain samples. The study was approved at UNIFAP Animal Ethics Committee under protocol number 02/2025. Results were expressed as mean $\pm$ standard error of the mean (SEM). Comparisons between the control and treated groups were analyzed using One-Way ANOVA followed by Tukey's post-hoc test. The minimum significance level was set at $p < 0.05$. GraphPad® Prism 8.0 software was used for all analyses. **Results:** *C. vulgaris* and *L. platensis* treated groups showed myoclonic spasm latency of 505 ± 95 s and 530.2 ± 69.80 s, respectively, statistically significant different to the control group (126 ± 24.44 s). Tonic-clonic seizures latency in control group was 146 ± 35.88 s, also showing statistically significant differences when compared to *C. vulgaris* (528 ± 72 s) and *L. platensis* (543.8 ± 56.20 s). A statistically significant reduction in the duration of tonic-clonic seizures was observed in treated groups compared between control (6.2 ± 6.2 s), *C. vulgaris* (4.2 ± 4.2 s) and *L. platensis* (44.6 ± 4.95 s). Lipid peroxidation profile showed higher TBARS levels in the cerebral cortex of control animals compared to the treated groups. The group treated with *C. vulgaris* presented $5.685e-008 \pm 8.053e-009$ MDA/g, and $3.880e-008 \pm 2.599e-009$ MDA/g for the *L. platensis* group, while the control group showed values of $6.829e-008 \pm 7.950e-009$ MDA/g. **Conclusion:** Microalgae species *C. vulgaris* and *L. platensis* demonstrated preventive potential in cerebral oxidative damage and improving anticonvulsant effects in animal models. These findings suggest that such species may represent an accessible natural alternative for the neurodegenerative diseases prevention. **Funding:** CNPq/MCTI FNDCT No. 19/2024

02.013 *In utero* Exposure to Valproic Acid, a Proposed Model of Autism, Enhances Dopamine System Activity in the Ventral Tegmental Area of Adolescent and Adult Rats. Grigório-de-Sant'Ana M, Freitas IS, Souza AJ, Verza FA, Guimarães FS, Gomes FV FMRP-USP, Dept Pharmacology, Post-Graduation Program in Medicine (Neurology), Brazil

Introduction: Autism spectrum disorders (ASD) are a group of neurodevelopmental disorders characterized by impairments in social behavior and the presence of repetitive and stereotyped behaviors. The etiology of ASD is thought to involve both genetic and environmental factors. Prenatal exposure to valproic acid (VPA) is a known environmental risk factor for ASD. Although the pathophysiology of ASD remains unclear, alterations in the midbrain dopamine system have been proposed to underlie core symptoms of ASD. Recently, our group reported increased population activity of dopamine neurons in the ventral tegmental area (VTA) in both male and female adult rats exposed to VPA *in utero*. However, it remains unknown whether such alterations are present at earlier developmental stages or in other dopaminergic regions such as Substantia Nigra (SN). Therefore, we hypothesize that *in utero* exposure to VPA leads to deficits in social and cognition behaviors and increase dopamine system activity in both the VTA and SN in adolescent and adult rats. **Aims:** To evaluate changes in sociability, cognition, and midbrain dopamine system activity in adolescent and adult rats exposed to prenatal VPA. **Methods:** Pregnant Sprague-Dawley rats received intraperitoneal injection of VPA (600 mg/kg) or saline on gestational day 12.5. Adolescent and adult male and female offspring VPA (n=4-7/group) and saline (n=3-7/group) were subjected to social interaction and novel object recognition tests to assess social and cognition behavior. Subsequently, *in vivo* electrophysiological recordings were performed to evaluate the activity of dopamine neurons in the VTA and SN. This study was approved by the Ethics Committee on the Use of Animals of the Ribeirão Preto Medical School-USP (#1337/2024). **Results:** *In utero* VPA exposure reduced social interaction time in adolescent female rats ($t_{15}=3.32$; $p<0.05$) and in both adult male and female rats ($F_{1,24} = 25.41$; $p<0.05$). Cognitive impairments were observed only in adolescent ($t_{15}=2.55$; $p<0.05$) and adult male rats ($F_{1,24}=9.24$; $p<0.05$). Electrophysiological recordings revealed an increased number of spontaneously active VTA dopamine neurons in adolescent male rats ($t_7=4.69$; $p<0.05$) and in adult male and female rats ($F_{1,24} = 39.16$; $p<0.05$). No changes in the activity of dopamine neurons were observed in the SN across age or sex. **Conclusion:** *In utero* VPA exposure induces social impairments in both male and female rats, while cognitive deficits were found only in males. The increased VTA dopamine system activity was observed in both adolescent and adult animals, indicating a possible dysregulation of the VTA dopamine system across developmental stages. **Financial support:** FAPESP, CNPq, CAPES, and FAEPA.

02.014 The Effect of Cannabidiol on Fear Memory Reconsolidation may Involve Neuroactive Steroid Biosynthesis and PPARy Activation. Santos MR¹, Cardoso NC¹, Raymundi AM¹, Guimarães FS², Bertoglio LJ³, Pinna G⁴, Stern CAJ¹ ¹UFPR, Dept Pharmacology, ²FMRP-USP, Dept Pharmacology, ³UFSC, Dept Pharmacology, ⁴University of Illinois Chicago. The Psychiatric Institute, Chicago, USA

Introduction: Neuroactive steroid (NAS) imbalance is linked to post-traumatic stress disorder (PTSD). Impairing fear memory reconsolidation can be an effective treatment for PTSD. Reconsolidation is a retrieval-dependent phase that stabilizes the memory trace and it is impaired by cannabidiol (CBD) treatment. Peroxisome proliferator-activated receptor (PPAR) α isoform activation enhances NAS biosynthesis. Given that CBD activates PPARy, we hypothesized that the reconsolidation-impairing effect of CBD involves PPARy activation and NAS biosynthesis. We aimed to evaluate whether NAS mediates the reconsolidation-impairing effect of CBD in rats. **Methods:** Male and female Wistar rats underwent contextual fear conditioning in Context A. After one day, reconsolidation was induced by a short retrieval in Context A. Independent groups were pre-treated with GW9662 (GW: PPARy antagonist, 1 mg/kg), finasteride (FIN: 5 α -reductase inhibitor, 25, 50, or 75 mg/kg), or vehicle (VEH) i.p. 60 min before retrieval. Immediately after, rats were divided into groups treated with CBD (10 mg/kg, i.p.) or VEH. The effects were assessed 1 day later by reexposure to context A (Test A). Freezing time was evaluated as an index of fear. To assess anxiety and locomotor behavior, independent conditioned rats were treated with FIN50 or VEH i.p. 60 min before being subsequently subjected to an elevated plus maze (EPM) and open field test (OFT), for 5 min in each apparatus. Open arms entries (%OAE) and time (%OAT), number of enclosed arms entries (EAE), and total distance were assessed in the EPM. Central zone entries (CZE) and time (CZT), crossings, and total distance were assessed in the OFT. Data are expressed as mean $\pm$ SEM and were analyzed by Student's t-test, one or two-way, and repeated measures ANOVA, followed by Neumann-Keuls post-hoc. Experiments (n=8–12 rats) were approved by the institutional ethics committee (#1469). **Results:** Treatment with CBD significantly reduced the freezing time in Test A compared to controls (σ : 50 $\pm$ 3.3 vs 66.3 $\pm$ 2.9; ρ : 51.4 $\pm$ 4.6 vs 68.4 $\pm$ 5.8), confirming the reconsolidation-impairing effect. This effect was prevented by both the PPARy antagonist (GW-CBD: σ : 70.7 $\pm$ 6.1; ρ : 81.67 $\pm$ 3.9) and FIN50, but not by FIN25 (σ : 69 $\pm$ 5; ρ : 59.8 $\pm$ 9.6). Males treated with FIN75, but not FIN50 or FIN25, increased freezing time in Test A compared to controls (σ : 79.9 $\pm$ 4 vs 57.6 $\pm$ 4.3), indicating a reconsolidation-enhancing action. In the retrieval session, the male FIN50-VEH group presented higher freezing time than the controls (σ : 86.9 $\pm$ 2.8 vs 64.8 $\pm$ 3.9). In the EPM, FIN50-treated groups displayed reduced EAE, but not %OAE, compared to controls (σ : 4.9 $\pm$ 1.1 vs 9.3 $\pm$ 1; ρ : 4.2 $\pm$ 1 vs 10.5 $\pm$ 1). FIN50 also reduced crossings compared to the control group (σ : 35.4 $\pm$ 6.5 vs 68 $\pm$ 4.8; ρ : 29.5 $\pm$ 10.8 vs 58.9 $\pm$ 3.7), indicating a hypolocomotor effect of FIN50. **Conclusion:** Our results suggest that in both males and females, the CBD reconsolidation-impairing effect occurs via activation of PPARy. Of note, inhibition of 5 α -reductase blocked this effect, implicating NAS as key contributors to CBD pharmacological action. **Funding:** CNPq, Fundação Araucária, FAPESP and NIH RO1AA030292

02.015 **Evaluation of the Anxiolytic Effect of *Lavandula dentata* Essential Oil.**Marco MR, Flores VG, Silva GC, Barcellos ES, Zorzo RR, Veiverberg A, Mattos CB, Betti AH. Feevale University, Institute of Health Sciences, Brazil

Introduction: Aromatherapy is included in the National Policy of Integrative and Complementary Practices in Health within the Brazilian Unified Health System (SUS). The essential oil (EO) of *Lavandula angustifolia* is widely used for the treatment of anxiety; however, the species predominantly cultivated in southern Brazil is *Lavandula dentata*, which lacks scientific evidence supporting its anxiolytic activity. In this context, the aim of this study was to evaluate the anxiolytic and motor effects of *Lavandula dentata* EO in male and female Balb/C mice. **Methods:** Behavioral tests were performed to assess anxiolytic effects (*Elevated Plus maze*), locomotor activity (*Open Field*), and motor coordination (*Rotarod*). The animals were exposed to 10 drops of *Lavandula dentata* EO and *Lavandula angustifolia* EO (positive control) for 15 minutes via inhalation. The elevated plus maze test was conducted one hour after the start of exposure, followed by the open field test and, finally, the rotarod test and were previously approved by Feevale Animal Research Ethical Committee (protocol nº 01.24.131). **Results:** *L. dentata* EO significantly increased both the time spent in the open arms and the number of open arm entries in male mice, suggesting an anxiolytic effect. This effect was not observed in female mice, not even with *L. angustifolia* EO. Motor parameters such as crossings, rearings, groomings, and fecal boli, as well as motor coordination performance, were not altered in either sex. **Conclusion:** The results demonstrated an anxiolytic effect after inhalation of *L. dentata* EO, similar to the effect previously described for the European species *L. angustifolia*. However, this effect was observed only in males, suggesting a sex-dependent response. Further studies are necessary to better understand this difference, reinforcing the importance of including both sexes in pharmacological research. **Financial support:** CAPES and Feevale University

02.016 Assessing Microglial Phenotype Alterations in Rats Exposed to Prenatal Dexamethasone and Acute Stress Kamidai NM, Sbardellotto GE, Munhoz CD USP, PGP Functional and Molecular Biology, Brazil

Introduction: Dexamethasone (DEX), a synthetic glucocorticoid, is widely used in clinical practice for the prophylactic treatment of women at risk of preterm birth between 24 and 34 weeks of gestation (1). However, exposure to a high dose of DEX during gestation may cause a series of psychiatric disturbances, such as anxiety and depression, in adolescents and adults (2). Guided by the two-hit hypothesis, we investigated whether hippocampal microglial cells undergo cellular reprogramming in response to prenatal DEX exposure and exhibit an exacerbated response under acute restraint stress in adulthood. The study evaluates the role of this process in the development of psychiatric disorders in both males and females. **Methods:** Pregnant females received an intraperitoneal (i.p.) injection of either saline or DEX (1 mg/kg) on gestational days (GD) 18–21. After the offspring were born, maternal behavior was assessed on postnatal day 5 (PND 5). The physical and reflex development of male and female offspring from both groups (control [CTL] and DEX) was monitored daily until complete development was achieved. At 60 days of postnatal development (PND 60), the offspring were divided into two groups: stressed (ST) and non-stressed (nST). The stressed group was subjected to 2 hours of acute restraint stress. Ninety minutes after the stress period, the animals were euthanized for subsequent analysis of their microglial phenotype. We performed immunohistochemistry on 40- μ m sections from the hippocampal region, using an anti-Iba1 antibody to analyze microglial density and morphology. Immunofluorescence, confocal microscopy, flow cytometry, and enzyme-linked immunosorbent assay (ELISA) analyses are still pending. **Results:** The physical development analysis (n=15-16/group) revealed that offspring exposed to prenatal DEX had shorter lengths and lower weights on PND 1 compared to the control group. However, reflex development analysis showed no significant differences between the DEX and CTL groups. The maternal behavior analysis (n=5/group) indicated that dams treated with DEX took longer to initiate nursing compared to CTL dams. Although the sample size needs to be increased, data suggest that DEX-treated dams spent more time nest-building, more time outside the nest, and less time actively nursing their pups. Immunohistochemistry data (n=4-5/group) for the Iba1 marker suggest that microglia in the ST, DEX, and DEX+ST groups exhibit a more reactive phenotype, showing an amoeboid shape with retracted processes and a larger cell body. In contrast, the CTL group appears to have cells with a less reactive phenotype, characterized by a small cell body and few cellular processes. **Conclusion:** Our findings suggest that prenatal DEX injection leads to altered maternal behavior, characterized by increased time spent outside the nest and reduced active nursing. This, combined with the physical development data, resulted in lower weight and length in the DEX group compared to the CTL group. Furthermore, DEX injection appears to alter microglial morphology, inducing a more reactive phenotype compared to the CTL group. **Keywords:** Microglia; Dexamethasone; Prenatal Stress. **Funding:** FAPESP, CNPq, CAPES. CEUA 8971050624. **References:** 1. Bereshchenko O, Bruscoli S, Riccardi C. Glucocorticoids, Sex Hormones, and Immunity. *Front Immunol.* 2018 Jun 12;9:1332. doi: 10.3389/fimmu.2018.01332. PMID: 29946321; PMCID: PMC6006719. 2. Purdy IB, Wiley DJ, Smith LM, Howes C, Gawlinski A, Robbins W, Badr LK. Cumulative perinatal steroids: child development of preterm infants. *J Pediatr Nurs.* 2008 Jun;23(3):201-14. doi: 10.1016/j.pedn.2007.09.006. PMID: 18492549; PMCID: PMC2992467.

02.017 Ndel1 Activity in Cultured Neuronal Cells and Effects of Treatment with Drugs and Serum from Patients with Depression and Depressive Bipolar Disorder. De Farias IS¹, Oyadomari WY¹, Fidalgo T², Hayashi MAF¹ ¹Unifesp-EPM, Dept Pharmacology, São Paulo, Brazil; ²Unifesp-EPM, Dept Psychiatry, São Paulo, Brazil

Mood disorders, including major depressive disorder (MDD) and bipolar disorder (BD), are complex, multifactorial conditions influenced by both genetic predisposition and environmental factors. Despite advances in research, diagnosis remains clinical and subjective, with no validated molecular biomarkers to support or guide therapeutic decisions. Moreover, many patients exhibit

partial or no response to standard antidepressant or mood-stabilizing drugs, highlighting the need for novel diagnostic and therapeutic strategies. The classical monoaminergic hypothesis, which attributes depressive symptoms to reduced serotonin, dopamine, and noradrenaline levels, has guided pharmacology for decades. However, it does not fully explain delayed therapeutic responses or treatment resistance, nor does it account for emerging mechanisms such as neuroinflammation, neuropeptidergic signaling, and disrupted neuroplasticity. Recent studies point to neuropeptides, particularly neurotensin, as key modulators of monoaminergic transmission. Ndel1 (NudE Neurodevelopment Protein 1 Like 1), an oligopeptidase highly expressed in neurons, cleaves neuropeptides including neurotensin and interacts with DISC1 and LIS1 to regulate neurite outgrowth and neuronal migration. Decreased Ndel1 activity has been reported in patients with chronic schizophrenia and euthymic BD, along with reduced neurite density and cell viability in neurons exposed to BD serum. Here, we investigated Ndel1 activity in cultured human neuronal cells (SK-N-AS) treated with serum from individuals diagnosed with MDD or depressive episodes of BD. Serum from healthy controls (HC) - individuals with no psychiatric history - was used as a comparator. Additionally, to assess pharmacological modulation, cultures were treated with the antidepressant fluoxetine (10 μ M) added directly to the culture medium for 48 hours. We evaluated cell viability, neurite density, and intracellular Ndel1 activity as indicators of neurotoxicity and neuroprotection. Serum from MDD patients significantly reduced neuronal viability and neurite complexity compared to HC, despite no significant changes in intracellular Ndel1 activity. Fluoxetine treatment also led to reduced neurite density and decreased Ndel1 activity, suggesting a modulatory effect of this drug on intracellular neuropeptidase function. These changes occurred independently of patient serum exposure, indicating that fluoxetine may influence neuronal morphology through mechanisms unrelated to circulating inflammatory factors. Altogether, our findings indicate that peripheral factors associated with mood disorders - including circulating bioactive molecules and pharmacological agents - can alter neuronal structure and enzymatic function. Ndel1 emerges as a potential functional biomarker and a candidate molecular effector involved in the neurobiology of mood disorders. **Keywords:** mental disorder, oligopeptidases, antidepressant. **Financial support:** FAPESP, CAPES, and CNPq.

02.018 Andrographolide Prevents Increases in Hyperlocomotion, GSK3-Beta Levels, 50-kHz Ultrasonic Vocalization Emission and Brain Oxidative Stress in Animal Models of Mania. Kanazawa LKS¹, Radulski DR¹, Pereira GS¹, Nelissen E², Prickaerts J², Schwarting RKW³, Acco A¹, Andreatini R¹

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Introduction: Increased activity of the enzyme glycogen synthase kinase 3-beta (GSK3-beta) is shown to play a pivotal role in the pathophysiology of bipolar disorder (BD). In this line, the inhibition of GSK3-beta has been associated to the antimanic effect of lithium, the standard antimanic drug. GSK3-beta inhibitors block manic-like behaviors, mimicking the effects of lithium. **Methods:** Considering that the inhibition of GSK3-beta activity may display antimanic-like effects, we tested the effects of chronic treatment (3x/week for 21 days) with 0.5 and/or 2.0 mg/kg i.p. andrographolide (ANDRO), a GSK3-beta inhibitor isolated from *Andrographis paniculata*, in animal models of mania: (I) methylphenidate (MPH) and (II) sleep deprivation (SD)-induced hyperlocomotion and the levels of GSK3-beta and its phosphorylated inactive isoform p-Ser⁹-GSK3-beta, in the prefrontal cortex (PFC) and striatum; (III) lisdexamfetamine (LDX)-induced hyperlocomotion, oxidative stress and increases in 50-kHz ultrasonic vocalizations (USVs). Male Swiss mice were used for experiments I and II and male Wistar rats were used for experiment III. The experimental procedures were approved by the Committee of Animal Experimentation of the Federal University of Paraná (CEUA/BIO-UFPR – protocols #733 and #1109), and supported by the Coordination for the Improvement of Higher Education Personnel (CAPES) and by CAPES/NUFFIC (88887.199578/2018-00). **Results:** SD resulted in hyperlocomotion and treatment with lithium ($F_{3,36} = 4.98$, $p < 0.01$), 0.5 mg/kg and 2.0 mg/kg ANDRO (post-hoc LSD, $p < 0.05$; and ($p < 0.01$, respectively) blocked SD-induced hyperlocomotion. SD decreased the p-Ser⁹-GSK3-beta/GSK3-beta ratio in the PFC ($F_{3,35} = 10.08$, post-hoc LSD, $p < 0.001$). Both lithium ($p < 0.001$) and 2.0 mg/kg ANDRO ($p < 0.001$) increased the p-Ser⁹-GSK3-beta/GSK3-beta ratio in the PFC. MPH administration increased locomotor activity compared to the control group and treatment with lithium, 0.5 mg/kg and 2.0 mg/kg ANDRO blocked MPH-induced hyperlocomotion ($F_{3,36} = 20.38$, $p < 0.001$). MPH reduced the p-Ser⁹-GSK3-beta/GSK3-beta ratio in the striatum and both lithium and 2.0 mg/kg ANDRO increased the p-Ser⁹-GSK3-beta/GSK3-beta ratio in the striatum ($F_{5,54} = 5.88$, $p < 0.001$). LDX increased locomotor activity and 50-kHz USVs in rats, which was prevented by chronic treatment with lithium or 2.0 mg/kg ANDRO ($F_{2,37} = 4.42$, $p < 0.001$ and $p < 0.01$, respectively). Lithium and 2.0 mg/kg ANDRO also prevented LDX-induced increases in lipid peroxidation (LPO) in the rat striatum ($F_{2,37} = 13.94$, $p < 0.001$ and $p < 0.05$, respectively). There was a positive correlation between LDX-induced hyperlocomotion and LDX-induced increases in 50-kHz USVs ($r = 0.61$, $p < 0.001$), and with LDX-induced LPO ($r = 0.62$, $p < 0.001$). **Conclusion:** Overall, the results show that chronic treatment with ANDRO prevented manic-like behaviors, increased the p-Ser⁹-GSK3-beta/GSK3-beta ratio and reduced oxidative stress levels. Thus, ANDRO appears to possess antimanic-like effects, and it is a promising agent to be more thoroughly investigated for the management of mania in BD.

02.019 Differential Modulation of the ADAM10-NEGR1-FGFR2 Pathway by Cannabidiol and Escitalopram in a Corticosterone-Induced Stress Model: Sex- and Brain Region-Dependent Effects.

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Introduction: The ADAM10–NEGR1–FGFR2 pathway is involved in neural development and linked to neuropsychiatric disorders, potentially mediating the action of escitalopram (ESC). Due to limitations of conventional antidepressants, interest in cannabidiol (CBD) has increased. This study aimed to investigate whether CBD's antidepressant-like effects under chronic stress involve modulation of the pathway. **Methodology:** Adult male and female CD1 mice (CEUA: 23.1.185.60.9) were treated for 21 days with corticosterone (CORT; 20 mg/kg) to model chronic stress. After 7 days of CORT induction, animals received vehicle, CBD (30 mg/kg), or ESC (30 mg/kg) for 14 days, with CORT. In the final week, behavioral tests assessed locomotion, cognition, anxiety, and depressive-like behaviors, including anhedonia (n = 12–18). Hippocampus and prefrontal cortex were collected for protein analysis of ADAM10/NEGR1/FGFR2 (n = 7). **Results:** Behavioral analyses confirmed the effectiveness of the chronic stress model in inducing changes consistent with depressive- and anxiety-like behaviors, which was also evident by Z Score of emotionality (Males: Interaction: $F(2,79)=4,42$, $p=0,0151$; Stress: $F(1,79)=11,26$, $p=0,0012$; Treatment: $F(2,79)=3,626$, $p=0,0312$; VEH vs CORT, $p<0,001$; Females: Interaction: $F(2,51)=9,821$, $p=0,0002$; Stress: $F(1,51)=0,002720$, $p=0,9586$; Treatment: $F(2,51)=8,868$, $p=0,0005$). In hippocampus of males, ESC increased NEGR1 levels independently of stress (Interaction: $F(2,36)=14,70$, $p<0,0001$; Stress: $F(1,36)=2,745$, $p=0,1062$; Treatment: $F(2,36)=14,11$, $p<0,0001$; VEH vs ESC, $p=0,0014$; CORT vs CORT ESC, $p=0,0380$). CBD induced the same effects in the presence of stress (CORT vs CORT CBD, $p<0,0001$). In the prefrontal cortex, in contrast, ESC reduced NEGR1 levels in stressed males (Interaction: $F(2,36)=5,598$, $p=0,0076$; Stress: $F(1,36)=0,4844$, $p=0,4909$; Treatment: $F(2,36)=1,992$, $p=0,1511$; CORT vs CORT ESC, $p=0,0356$). In females, ESC reduced NEGR1 levels in the hippocampus (Interaction: $F(2,36)=3,367$, $p=0,0457$; Stress: $F(1,36)=0,1933$, $p=0,6628$; Treatment: $F(2,36)=23,42$, $p<0,0001$; CORT vs CORT ESC, $p<0,0001$) in the presence of stress. This was also observed in the cortex (Interaction: $F(2,36)=0,4233$, $p=0,6581$; Stress: $F(1,36)=0,5036$, $p=0,4825$; Treatment: $F(2,36)=0,5011$, $p=0,6100$; VEH vs ESC, $p=0,0016$) in the absence of the stimulus. Additionally, only in stressed females CBD treatment increased cortical FGFR2 levels (Interaction: $F(2,36)=24,33$, $p<0,0001$; Stress: $F(1,36)=20,10$, $p<0,0001$; Treatment: $F(2,36)=23,22$, $p<0,0001$; CORT vs CORT CBD, $p<0,0001$). Stress also significantly increased hippocampal ADAM10 levels in both sexes (Males: Interaction: $F(2,36)=17,10$, $p<0,0001$; Stress: $F(1,36)=6,381$, $p=0,0161$; Treatment: $F(2,36)=38,56$, $p<0,0001$; VEH vs CORT, $p<0,0001$; Females: Interaction: $F(2,36)=1,825$, $p=0,1758$; Stress: $F(1,36)=1,648$, $p=0,2074$; Treatment: $F(2,36)=0,4692$, $p=0,6293$). In males, this was prevented by CBD (CORT vs CORT CBD, $p<0,0001$) and ESC (CORT vs CORT ESC, $p<0,0001$), thus pointing to a possible common mechanism involving modulation of ADAM10 activity. **Conclusion:** CBD and ESC modulate the pathway in a sex, stress and brain region-dependent manner, warranting further studies to determine whether ADAM10 modulation is necessary for CBD's effects. The results underscore the importance of considering biological variables such as sex and stress state when developing more effective and personalized therapeutic strategies for stress-related neuropsychiatric disorders. **Support:** FAPESP (2017/19731-6; 2022/01192-0).

02.021 Hormone Replacement Therapy During Perimenopause: Analysis of Brain Electrical Activity of Female Wistar Rats Treated With 17 β -Estradiol After Surgical Castration. Mattos BG^{1,2}, Souza RD^{1,3}, Teixeira LL^{1,3}, Silveira EP^{1,4}, Hamoy AO⁵, Pinto LC¹, Hamoy M^{3,5}, Lopes DCF^{1,3} ¹UFPA, Lab Neuropatologia Experimental, Hospital Universitário João de Barros Barreto, Brasil, ²FSCM, Pediatria, Brasil; ³UFPA, PPG FARMABIO, Brasil. ⁴FSCMPA, Clínica Médica, Brasil. ⁵UFPA, Lab Farmacologia e Toxicologia de Produtos Naturais, Brasil

Perimenopause is the transition period to menopause, which marks the end of the female reproductive period. During this phase, there is a significant decrease in serum levels of 17 β -estradiol (E2), leading to metabolic, biochemical and cognitive-behavioral impacts, that tends to improve with hormone replacement therapy (HRT) with E2. This study aimed to understand the brain electrical activity in rats submitted to surgical menopause and HRT-treated. Adult female Wistar rats underwent bilateral oophorectomy, or sham surgeries, were treated with E2 (20 μ g/day) or fluoxetine (FXT: 5 mg/kg/day) for 28 days (CEUA n^o: 4532280520). At the end of treatment, the animals were analyzed by electroencephalographic (EEG) evaluations. Our results showed that bilateral oophorectomy induced hormonal deprivation ($H = 39.89$, $p < 0.0001$), due to the reduction in uterine weight (mg) observed in the castrated group (C+V: 0.63 ± 0.35) and those that received FXT (C+FXT: 0.72 ± 0.15) compared to the intact group (NC+V: 3.04 ± 1.10 ; $p < 0.001$ for both comparison). The E2 replacement was effective in maintaining uterine weight similar to the intact group (C+E2: 2.94 ± 0.53 ; $p > 0.9999$) and different from the other groups ($p < 0.001$ vs C+V and C+FXT). Our results showed changes in brain electrical activity ($mV^2/Hz \times 10^{-3}$) induced by both surgical castration and treatments. Untreated castrated animals presented lower total wave power ($[F_{(3, 26)} = 29.48$, $p < 0.0001]$, C+V: 0.487 ± 0.080 and C+FXT: 0.400 ± 0.041) than NC+V (0.654 ± 0.114 , for both comparisons) and C+E2 (0.707 ± 0.039 , $p < 0.0001$, for both comparisons). No difference was observed between NC+V and C+E2 ($p = 0.5075$). Reduction was observed to theta wave ($F_{(3, 26)} = 47.83$, $p < 0.0001$), which was observed that both C+V (0.132 ± 0.010) and C+E2 (0.180 ± 0.015) showed lower values than the NC+V (0.209 ± 0.022 ; $p < 0.05$, for both comparisons), but C+E2 was efficient in increasing theta wave when compared to C+V ($p < 0.0001$). For the alpha wave ($F_{(3, 26)} = 8.787$, $p = 0.0003$), reduction in the oscillations were shown in C+V (0.041 ± 0.007) and C+FXT (0.037 ± 0.008) in comparison with NC+V (0.055 ± 0.007 , $p < 0.01$, for both comparisons). Furthermore, C+E2 (0.049 ± 0.006) presented greater alpha power compared to C+FXT ($p = 0.0151$), and no difference to NC+V ($p = 0.4052$) or C+V ($p = 0.2237$). Taken together, these data suggesting cognitive, emotional and attention deficit alterations, since the decrease in theta wave power has been related to reduced attention to emotional content in people with severe depression and to cognitive impairment in middle-aged women and individuals with depression; and HRT (E2) restore the electrical activity to basal levels. **Key-words:** Perimenopause; 17 β -estradiol; Hormone replacement therapy; Electroencephalography. **Financial support:** CAPES; FAPESPA.

02.022 **Study of the Activity of 8-Benzylidene Digoxin (BD-8), a New Semi-Synthetic Derivative, on LPS-Induced Oxidative Stress in the Murine Brain.** Fonseca MFR¹, Neves EPFI¹, Silva IA¹, Souza VKB¹, Ferreira PYO², Campos HM², Ghedini PC², Costa RM³, VillarJAFP⁴, Ribeiro AAF⁴, Leite JA¹ ¹UFG, Lab Ensino, Pesquisa e Extensão em Neurociências Aplicadas, Goiânia, Brazil. ²UFG, Lab Farmacologia Bioquímica e Molecular, Goiânia, Brazil. ³UFG, Lab Fisiologia e Farmacologia da Reprodução, Goiânia, Brazil. ⁴UFSJ, Lab Síntese Orgânica e Nanoestruturas, Divinópolis, Minas Gerais, Brazil

Introduction: Oxidative stress involves an increase in reactive species that alter intracellular signaling, leading to dysregulation of the inflammatory response. The inability of antioxidant defense systems to regulate proinflammatory response plays a key role in the development and progression of neurodegenerative diseases. In animal models of neuroinflammation, lipopolysaccharide (LPS) is commonly used to induce oxidative stress. BD-8 is a new semi-synthetic cardiotonic steroid with limited studies on its activity, especially in the brain. It is a promising immunomodulatory molecule, although it remains largely unexplored. Previous studies have demonstrated that BD-8 demonstrated anti-inflammatory activity *in vivo* in a mouse paw edema model induced by carrageenan. Thus, the aim of this work was to evaluate the neuroprotective effect of BD-8 on LPS-induced oxidative stress in the brain cortex and hippocampus of mice. **Methods:** Swiss albino mice with 60 days old were randomly divided into five groups (n=8), each group of mice were treated via intraperitoneal (i.p.) injection with the designated solution according to their respective group: Control (DMSO 1%), LPS (1mg/kg) and 3 doses of BD-8 (0.28, 0.56 and 1.12 mg/kg). One hour after the first treatment (Control and BD-8) the animals were challenged with LPS (LPS group and BD-8) and after 4 hours the animals were euthanized. The hippocampus and brain cortex were dissected for the evaluation of oxidative stress parameters: lipid peroxidation (LPO) levels, using the TBARS method; as well as the activities of the antioxidant enzymes, catalase (CAT) and superoxide dismutase (SOD). All research procedures were approved by the Animal Ethics Committee (CEUA/UFG nº 014/21). **Results:** The biochemical evaluation in the brain cortex CAT and SOD was inhibited in the LPS group when compared to the control group ($p < 0.05$), and the treatments with BD-8 at 0.56 and 1.12 mg/kg doses were able to restore the CAT and SOD enzymatic activity ($p < 0.05$) when compared with LPS group. For the oxidative stress biomarkers, the LPS group presented an increase in LPO levels, when compared with control group ($p < 0.05$), and the treatments with all doses of BD-8 were able to protect against this increase ($p < 0.05$). In the hippocampus, the LPS group showed a decrease in SOD and CAT activities when compared with control group ($p < 0.05$), and the treatments with BD-8 restored the activities when compared to LPS group ($p < 0.05$). For the oxidative stress biomarkers, the LPS group presented an increase in LPO levels, when compared with control group ($p < 0.05$), and the treatments with all doses of BD-8 were able to protect against this increase ($p < 0.05$). **Conclusion:** Our results indicate that BD-8 exhibits neuroprotective activity against LPS-induced oxidative stress in the brain cortex and hippocampus by reducing LPO and enhancing the activity of antioxidant enzymes. However, further studies are needed to better understand the influence of 8-BD on neuroinflammatory processes. **Financial support:** CNPq.

02.023 **Kynurenine Neonatal Exposure: Behavioral Consequences in Adolescent Mice.** Corrêa AES, Bomfim ANL, Cruz BE, Andrade BS, Guimarães MZP, Neves GA UFRJ, Lab Molecular Pharmacology, Brazil

Introduction: Schizophrenia is a chronic neuropsychiatric disorder that affects approximately 1% of the global population. The symptoms, which can be classified as positive, negative, and cognitive symptoms, involve various pathophysiological mechanisms, characterizing a complex and debilitating condition. Its etiology is known to be multifactorial, encompassing both genetic and environmental factors. In this regard, the dysregulation of the kynurenine (KYN) pathway, tryptophan's main degradation route, has been associated with processes such as neonatal and perinatal infections, which are frequently implicated as schizophrenia related risk factors. This association is evident through increased kynurenic acid (KYNA) concentrations in patients' plasma and prefrontal cortex. Therefore, KYN administration in animal models, in order to raise KYNA levels in the brain, has been considered a promising approach to investigate the causal link between the KYN pathway and deficits characteristic of the disorder. However, the time course of behavioral changes induced by this approach, especially during the transition between adolescence and adulthood, remains unknown. Based on these considerations, the present study aims to assess behavioral alterations associated with schizophrenia in adolescent mice exposed to KYN during the neonatal period. **Methods:** Animals received KYN (200 mg/kg; i.p.) or PBS (15 mL/kg; i.p.) twice a day, from the postnatal day (PND) 7 to 16 (CEUA-UFRJ: 120/22). Between PND 28-42, both male and female mice were subjected to a series of behavioural tests related to core symptoms of the disorder, including: spontaneous alternations (SA), social approach task, open field (OF), hyperlocomotion induced by MK-801, splash test, sucrose preference and prepulse inhibition of the acoustic startle response. **Results:** The neonatal KYN exposure did not affect mice's locomotion in a novel environment or the latency to startle response. These behaviors were compromised when a different cohort was evaluated at adulthood (PND >70), showing that KYN neonatal exposure only induces significant schizophrenia related behavioral changes after puberty. Moreover, the KYN group showed a trend level reduction in the percentage of social approach compared to the PBS group ($p = 0.055$), which was not sex related. No changes were observed in aspects like self-care, working memory, sensorimotor gating, hedonic profile, or sensitivity to NMDA antagonists. Furthermore, some relevant differences between the sexes were found. Male mice showed higher total displacement in the OF task when compared to females ($p = 0.019$). In addition, females presented a higher percentage of SA than males ($p = 0.029$). **Conclusion:** In summary, our data suggest that the behavioral alterations caused by the dysregulation of the kynurenine pathway have not yet manifested robustly during adolescence. This characteristic is present in the main neurodevelopmental animal models related to the disorder and may be related to attenuated prodromal symptoms. This time course provides a good translational value for the involvement of early KYN pathway imbalance in the etiology of schizophrenia related disorders. Evaluations of KYNA brain levels in these animals are currently in progress to confirm the post-injection biochemical effects. **Financial support:** CAPES, CNPq, FAPERJ.

02.024 Impact of Cholinergic Blockade on Conditioned and Unconditioned Fear Memory in Middle-aged Adult Male Rats. Savicki LR, De Souza STF, Souza LC, Stingelin RL, Vital MABF UFPR, Lab Central Nervous System, PPG Pharmacology, Dept Pharmacology, Brazil

Introduction: Acetylcholine is crucial for attention and memory stability. Although aging effects on cognition are well known, the role of cholinergic signaling in mnemonic processes during mature adulthood—preceding senescence—is less understood. **Methods:** We examined scopolamine (1 mg/kg, i.p.), a non-selective muscarinic antagonist, on acquisition and persistence of memory in 9-month-old male Wistar rats. Twenty-eight rats were assigned to scopolamine (SCO1, n=14) or vehicle (saline, n=14) groups and underwent two behavioral paradigms (all animals participated in both behavioral tests) (CEUA/UFPR 1315). Scopolamine was administered 30 minutes before the training sessions, and testing occurred 1 hour after training. **Results:** An unpaired Student's t-test indicated that in the elevated plus maze transfer latency test (assessing unconditioned fear memory), scopolamine-treated rats showed impaired acquisition: a significant group difference appeared during training (Day 1; $p=0.012$) and intensified at testing (Day 2; $p=0.0003$). Vehicle rats improved latency from training to test ($p=0.037$), reflecting aversive learning (seeking closed arms faster), whereas the scopolamine group showed no improvement ($p>0.05$). In contextual fear conditioning, rats were exposed to the context for 2 min and then received three mild foot shocks after 1 hour. Memory recall at 24h showed no group difference ($p=0.16$), indicating intact acquisition and consolidation. However, at 48h (late recall), scopolamine rats exhibited significantly reduced freezing compared to controls ($p=0.002$), with a marked decrease from 24h to 48h ($p=0.0002$), suggesting impaired persistence of contextual fear memory. Minute-by-minute analysis at 24h revealed a transient reduction of freezing in the scopolamine group during the fifth minute ($p=0.029$), indicating possible modulation of fear expression timing. **Conclusion:** Scopolamine impairs acquisition of aversive memory linked to innate avoidance of open environments and disrupts the persistence—but not initial acquisition—of conditioned contextual fear memory in mature adult rats. These findings underscore the ongoing importance of cholinergic integrity at this life stage for memory maintenance in conditioned contexts and for acquisition in innate contexts. The temporal pattern suggests cholinergic blockade predominantly affects late phases of emotional memory stabilization, while early aversive learning remains relatively resistant. This supports the concept that middle age is a critical neurobiological transition period, where subtle cholinergic dysfunction can induce cognitive deficits that precede and potentially predict worsening with aging. **Funding:** Supported by Coordination for the Improvement of Higher Education Personnel (CAPES), Brazil

02.025 **Effects of Combining Chronic Stress Exposure with Repeated Corticosterone Administration in Female Mice.** Alves MV, Santos LSP, Cruz BE, Silva, ACF, Barros, RP, Neves, GA UFRJ, Lab Molecular Pharmacology, Brazil

Introduction: The manifestations of Major Depressive Disorder (MDD) include depressed mood, decreased interest in previously pleasurable activities, thoughts of death, among others. Chronic stress is recognized as a risk factor for developing MDD, becoming a significant problem in modern society due to its health impact and associated economic costs. Models involving chronic stress or alterations in the HPA axis are commonly used to model the depressive phenotype in rodents. Additionally, women have a 50% higher likelihood of developing depression compared to men. However, despite acknowledging differences in biology between sexes, there has been a history of excluding females from preclinical studies. Therefore, the aim of this project is to evaluate behavioral changes related to the depressive phenotype induced by a protocol that combines exposure to chronic unpredictable mild stress (CUMS) with repeated corticosterone administration. **Methods:** Adult female Swiss mice (PND >60) were divided into six groups: vehicle (DMSO 3%, polysorbate 80 3% in NaCl 0,9% s.c. daily), corticosterone (20 or 40 mg/kg s.c. daily), CUMS (vehicle s.c. daily and exposure to CUMS), and two combined models (corticosterone s.c. daily and exposure to CUMS) (CEUA CCS/UFRJ 058/23). The stressors of CUMS included interventions such as cycle disruptions, restraint, stroboscopic light, cage tilting, removal or soaking of the bedding material, and water or food deprivation. After 21 days of intervention, the anxious and depressive-like phenotype was assessed using the following behavioral tests: sucrose preference, social approach, open field, forced swimming, elevated plus maze, tail suspension, and splash test. For data analysis, one-sample t-tests were performed to compare the results with theoretical means of 50% in the sucrose preference and social approach. To evaluate the effect of stress, two-way ANOVAs were conducted, using intervention (non-stressed animals or CUMS) and treatment (vehicle or corticosterone) as independent factors in the open field, forced swimming, elevated plus maze, tail suspension, and splash test. **Results:** The results showed that non-stressed animals exposed to 40 mg/kg of corticosterone showed reduced sucrose preference, while those exposed to 20 mg/kg exhibited reduced social preference. Additionally, animals exposed to CUMS showed alterations related to anxious and depressive phenotype, including reduced exploration of the center of the open field (non-stressed animals: $21,94 \pm 0,887$; CUMS: $18,57 \pm 0,93$; $p < 0,023$), increased immobility in the forced swimming test (non-stressed animals: $143,61 \pm 9,13$; CUMS: $184,66 \pm 9,71$; $p < 0,003$), and a tendency towards decreased open arm exploration in the elevated plus maze. Increased grooming behavior was also observed in animals exposed to CUMS (non-stressed animals: $104,11 \pm 7,38$; CUMS: $158,61 \pm 7,86$; $p < 0,001$.), as well as in those treated with higher doses of corticosterone, independently of stress exposure (vehicle: $130,44 \pm 7,56$; cort20: $108,58 \pm 9,18$; cort40: $155,06 \pm 10,99$; $p = 0,007$). **Conclusion:** These findings indicate that the CUMS protocol increases anxiety and induces depressive-like behaviors independent of corticosterone treatment, with repeated injections potentially acting as an additional stressor. **Financial support:** CAPES, CNPq, FAPERJ.

02.026 Effects of non-Selective Muscarinic Inhibition on short-Term Recognition and Spatial Memory in Middle-Aged Male Rats. Stingelin, RL, De Souza, STF, Souza, LC, Saviscki, LR, Vital, MABF. UFPR, Lab Central Nervous System, PPG Pharmacology, Dept Pharmacology, Brazil

Introduction: Acetylcholine is a fundamental neurotransmitter involved in cognitive processes such as attention, learning, and memory consolidation. It plays a key role in modulating synaptic plasticity and the stability of neural networks. Aging causes gradual changes in cholinergic signaling, which impairs cognitive performance. However, the role of this signaling in mature adult rats — a stage preceding senescence and characterized by initial neurobiological changes — is still not fully understood. In this context, scopolamine, a non-selective muscarinic antagonist that blocks cholinergic receptors, is widely used to induce cognitive deficits in experimental models, simulating aspects of cholinergic decline. **Methods:** This study investigated the effects of intraperitoneal administration of scopolamine (1 mg/kg) on the acquisition phase of short-term memory in the object recognition (episodic memory and object recognition), object location (spatial recognition memory), and Y-maze (spontaneous alternation — working memory and spatial navigation) tests. A total of 28 nine-month-old male rats (~500g) were used. The same animals participated in all behavioral tests, being divided into two groups: SCO1 (received scopolamine, n=14) and VEH (received the vehicle — 0.9% saline, n=14). Scopolamine (1 mg/kg, i.p.) was administered 30 minutes before the tests (approved by CEUA/UFPR 1315). The time spent in the new arm (Y-maze), exploration of the familiar and displaced object (object location), and exploration of the familiar and novel object (object recognition) were measured using a stopwatch. Results are expressed as mean $\pm$ SEM. An independent Student's t-test was applied for statistical comparisons. **Results:** Y-maze test showed a statistically significant difference between groups ($p=0.0028$), indicating that cholinergic inhibition affects working memory and spatial navigation in middle-aged rats. This suggests that cholinergic signaling remains involved in the encoding of short-term memory at this stage. No significant effects were found in the discrimination index of the object location test ($p=0.3505$) or the object recognition test ($p=0.4703$). **Conclusion:** Although scopolamine did not induce cognitive impairments in these latter tests, the findings are still relevant, as they suggest that in middle age, animals may no longer depend as heavily on cholinergic projections to acquire new information. This could indicate the onset of neuronal reorganization to compensate for natural neurodegeneration associated with aging. Therefore, further molecular analyses are needed to support this hypothesis. **Funding:** This study was supported by the Coordination for the Improvement of Higher Education Personnel (CAPES), Brazil.

02.027 Anti-Inflammatory Potential of Baricitinib in Cellular Models of Neuroinflammation in Astrocytes and PC12 Cells. Duarte ALS¹, Bandeira SMA², Sales ISL², Costa Júnior DC², Rebouças MO³, Araújo JR¹, Costa GC¹, Ananias CF¹, Fernandes MO¹, Pinheiro KS¹, Lima PJS⁴, Sampaio TL⁵, Sousa FCF⁶ ¹UFC, Faculty of Pharmacy, Dentistry and Nursing, Dept Clinical and Toxicological Analysis, Brazil, ²UFC, Dept Pharmacology, PPG in Pharmacology, ³ICC, Ceará Cancer Institute, ⁴UFC, Graduate Program in Translational Medicine, ⁵UFC, Dept Pharmacy, ⁶UFC, Dept Physiology and Pharmacology, Faculty of Medicine

Mental disorders are syndromes characterized by significant alterations in cognition, emotional regulation, and behavior, resulting from dysfunctions in psychological and biological processes. Among the mechanisms under investigation, the neuroinflammation hypothesis has gained prominence, suggesting that inflammatory responses within the central nervous system may disrupt homeostasis and contribute to cognitive deficits, anxiety, and depression. In this context, there is growing interest in anti-inflammatory agents with neuroprotective properties. In this context, we decided to investigate the anti-inflammatory effects of baricitinib (BARI), a selective Janus kinase (JAK) inhibitor, since, as far as it is known, its effects on central nervous system cells are still poorly understood. The neuroinflammation was induced using lipopolysaccharide (LPS, 50 µg/mL) in PC12 cells and astrocyte cultures. Cells were divided into three groups: (1) LPS-treated group, (2) LPS + BARI group, treated with different concentrations of BARI (1.25 µM, 0.63 µM, and 0.31 µM), and (3) control group, without treatment. Levels of the pro-inflammatory cytokines TNF-α and IL-1β were measured in cell culture supernatants using a commercial ELISA kit. For statistical analysis, ANOVA with Tukey's post-test was used, and the results were expressed as mean ± standard error of the mean (p < 0.05). In astrocytes, LPS treatment increased TNF-α levels by 47.51% compared to control (Control: 1.81 ± 0.37; LPS: 2.67 ± 0.49), confirming the inflammatory effect. Treatment with BARI at 1.25 µM (B1) reduced TNF-α levels by 12.98% (1.95 ± 0.12), whereas 0.63 µM (B2) did not reverse the increase (4.00 ± 0.87). In PC12 cells, LPS induced a 98.25% increase in TNF-α levels (Control: 1.06 ± 0.09; LPS: 2.10 ± 0.15); BARI treatment reduced levels by 31.55% (B1) and 26.22% (B2). Regarding IL-1β in astrocytes, LPS increased cytokine levels by 84.21% (Control: 32.55 ± 2.05; LPS: 59.96 ± 15.54); BARI at 1.25 µM significantly reduced these levels by 53.80% (32.89 ± 4.17), while the intermediate concentration had minimal effect (55.10 ± 3.71). In PC12 cells, IL-1β levels remained stable across all experimental groups. In conclusion, baricitinib exhibited a more pronounced anti-inflammatory effect in astrocytes, particularly by reducing IL-1β levels. In PC12 cells, BARI effectively reduced TNF-α levels, although no significant impact on IL-1β was observed. These findings support the potential of BARI as a modulator of neuroinflammation, with variable efficacy depending on the cytokine and cell type. The authors thank CNPq for their financial support.

02.028 Association Between Advanced Oxidation Protein Products (AOPPs) And Anxiety-Like Behavior in a Progressive Multiple Sclerosis Experimental Autoimmune Encephalomyelitis (PMS-EAE) Model In Mice. Dalmolin L¹, Rodrigues P², Frare JM³, Ruviano NA³, Peres DS², Bochi GV², Trevisan G^{2,3} ¹UFSM, Medical Student, Brazil; ²UFSM, Graduate Program in Pharmacology, Brazil; ³UFSM, Graduate Program in Biological Sciences, Brazil **Introduction:** Multiple sclerosis (MS) is a neurodegenerative disease that affects the central nervous system (CNS) generating neuropathic pain often accomplished with anxiety. Primary progressive MS (PPMS) is the most disabling clinical form, and the patients present an intense neurodegenerative process. The development of anxiety-like behaviors in patients and experimental animals with PMS was related to the neuroinflammation process and increased production of reactive species. Advanced oxidation protein products (AOPPs) are oxidized compounds generated via NADPH oxidase (Nox) and myeloperoxidase (MPO) pathways, and their accumulation in plasma has been associated with clinical disability in MS patients. This study aimed to investigate the AOPPs role in anxiety-like behavior in a model of progressive multiple sclerosis experimental autoimmune encephalomyelitis (PMS-EAE) in mice. **Methods:** To assess this, female mice C57BL/6J (20–30g) were used to induce PMSEAE (Protocol #9746010620/2020). Clinical score, weight and rotarod test were evaluated before induction (baseline) on days 7th, 10th, and 14th post-immunization. We assessed nest building, open field and elevated plus-maze (EPM) tests 13 days postimmunization. Animals were euthanized at 14 days post-immunization; then, AOPPs levels, Nox and MPO activity were measured in the prefrontal cortex and hippocampus. Data were expressed as mean ± SEM and analyzed using two-way ANOVA, Student's ttest, or Mann–Whitney test. Principal component analysis (PCA) was used to explore multivariate associations. Results with $p < 0.05$ were considered significant. **Results:** The clinical score increased 14th post-immunization without changes in weight and mobility. PMS-EAE mice exhibited spontaneous nociception and anxiety-like behavior in the open field test, with reduced time spent in the center and increased time in the periphery, along with decreased average speed, rearing and sniffing in the PMSEAE group compared to the control group, indicating altered exploratory activity. In the EPM test, PMS-EAE mice exhibited a reduction in the time in open arms and open-arms entries, showed an increased anxiety index, and reduced closed arms returns, head dipping, and SAP parameter compared to control animals. Additionally, nest-building capacity was reduced 14 days post-immunization in the PMS-EAE group compared to the control group. Regarding biochemical analysis, AOPPs levels, NADPH oxidase, and MPO activities were increased in the prefrontal cortex and hippocampus of PMS-EAE mice compared to control group, suggesting oxidative stress involvement in the observed behavioral changes. **Conclusion:** Our findings demonstrate that the concentration of AOPPs was increased in CNS structures concomitantly with changes in anxiety-like behavior. Along with the levels of AOPPs, the NADPH oxidase and MPO activity were also increased in the prefrontal cortex and hippocampal 14 days post-immunization of the PMS-EAE. Therefore, we indicated the association between anxiety-like behavior and CNS oxidative damage in the PMS-EAE model.

02.029 **Sex-Specific Neurobiological Signatures of Adaptive vs. Maladaptive Fear.** Novelli MLT^{1,2}, Wegener G¹, Joca S¹, Iglesias LP¹ ¹Aarhus University, Health Graduate School, Denmark; ²USP, School of Pharmaceutical Sciences of Ribeirão Preto, Brazil

Introduction: Post-Traumatic Stress Disorder (PTSD) is developed after exposure to a traumatic event and is characterized by strong psychological and physiological responses to trauma-related cues, being considered as a prototypical disorder of fear learning dysregulation. Despite PTSD being more common in women than in men, little is known about how sex differences influence fear learning. **Methods:** Male and female 9-week-old C57BL/6J mice underwent contextual fear conditioning (CFC) with low-intensity (LI, 3×0.45 mA) or high-intensity (HI, 3×0.85 mA) foot shocks, and evaluated during test, generalization (gen), extinction (ext) and extinction test (ext test) each 24h apart. Levels of blood corticosterone were quantified by ELISA while hippocampal levels of FKBP51, GR and CREB were analysed by western blot assays. Data were analyzed via t-tests, ANOVA or Mann Whitney (MW) for nonparametric data. **Results:** In males HI conditioning increased freezing during gen, ext and ext test in comparison with LI conditioning [Phase: $F(2.530,29.52)=38.11$, $p<0.0001$; Condition: $F(1,12)=7.124$, $p=0.0204$; Interaction: $F(3,35)=0.6233$, $p=0.6047$; Test $p=0.2504$; Gen $p=0.0395$; Ext $p=0.0329$; Ext test $p=0.0272$]. However, in females no differences were observed between the HI and LI conditioning groups [Phase: $F(2.400,28.80)=45$, $p<0.0001$; Condition: $F(1,12)=3.762$, $p=0.0763$; Interaction: $F(3,36)=1.955$, $p=0.1381$; Test $p=0.0287$; Gen $p=0.5524$; Ext $p=0.1544$; Ext test $p=0.4017$]. No group differences between HI and LI conditioning were seen in the female for corticosterone [$t=0,5190$, $df=11$, $p=0,6140$], in CREB levels in males [$t=0,9706$, $df=12$, $p=0,3509$] and females [$t=0,4616$, $df=8$, $p=0,6567$] and in FKBP51 levels in males [$t=1,024$, $df=12$, $p=0,3259$]. While the levels of FKBP51 in females [$t=2,221$, $df=8$, $p=0,0571$] and GR levels in males [$t=1,794$, $df=12$, $p=0,0980$] seem decreased in the HI conditioning group. The levels of corticosterone in males [MW test, Two-tailed, $p=0,0480$] were higher in the HI conditioning group, and the levels of GR in females [$t=2,734$, $df=8$, $p=0,0257$] were lower in the HI conditioning group. **Conclusion:** Conditioning with the HI induced generalization and impaired extinction resembling PTSD features in males but not in females, characterizing in males a maladaptive fear. This was accompanied by dysregulations within the Hypothalamic-Pituitary-Adrenal (HPA) axis and corticosterone signaling pathways, specifically the higher corticosterone and lower GR levels. Different intensities of conditioning induced behavioral and neuroendocrine responses in a sex-specific manner. This project was funded by the European Union. Iglesias LP is supported by the Marie Curie Postdoctoral fellowship (grant: HORIZON-MSCA-2022-PF-01-01, ID:101110693). This study was partially financed by the International Brain Research Organization (IBRO) and by the USP International Cooperation Office (project: 1915/2024). The project has the approval of the Danish Council for Animal Experimentation under the number 2023-15-0201-01523.

02.030 **Increased Matrix Metalloproteinase-9 Activity in the Ventral Hippocampus Induced by Adolescent Stress: A Potential Target for the Prevention of Stress-Induced Abnormalities.** Andres ESS, Freitas IS, Santos-silva T, Guimaraes FS, Gomes FV FMRP-USP, Dept Pharmacology, São Paulo, Brazil

Introduction: Adolescent stress is a major risk factor for psychiatric disorders. We previously found that stress during adolescence induces anxiety-like behavior, sociability deficits, and cognitive impairments, associated with disruptions in parvalbumin-positive (PV+) GABAergic interneurons and their perineuronal nets (PNNs), a specialized extracellular matrix, in the ventral hippocampus (vHip). PNN integrity is partly regulated by matrix metalloproteinases (MMPs), including MMP-9.

Aim: We investigated developmental changes in MMP-9 activity in the vHip, its modulation by adolescent stress, and whether MMP inhibition via doxycycline or MMP-9 knockdown prevent stress-induced behavioral and molecular alterations. **Methods:** Male Sprague Dawley rats had MMP-9 activity assessed via gel zymography at postnatal days (PND) 31, 41, 51, 75, 85, and 110 (n=5/group). To evaluate adolescent stress effects, rats were exposed to repeated footshock and restraint from PND31–40. MMP-9 activity was measured at PND41, 51, or 75 (n=5–6/group). Behavioral tests (elevated plus-maze, social interaction [SI], novel object recognition [NOR]) were conducted on PND47–49 in stressed and naïve rats (n=16/group). Levels of MMP-9 and TIMP-1, an endogenous MMP-9 inhibitor, in the vHip were assessed via Western blot and RT-qPCR. To test the protective effect of doxycycline (2 mg/mL in drinking water, PND31–40), a pan-MMP inhibitor, animals were behaviorally tested on PND47–49 and brains were collected on PND51 for in situ zymography and PV+/PNN immunofluorescence (n=7–8/group). For MMP-9 knockdown, siRNA was stereotactically injected into the vHip at PND27. Rats were stressed from PND31–40 and behaviorally tested at PND47–49 (n=4–5/group). This study was approved by the Ethics Committee on Animal Use of the Ribeirão Preto Medical School, University of São Paulo (CEUA/FMRP-USP #248/2019). **Results:** MMP-9 activity decreased from PND41 onward when compared to PND31 ($p<0.05$ for all). Adolescent stress increased MMP-9 activity at PND41 and 51 ($p=0.04$; $p=0.03$). Stressed rats showed reduced SI time ($p=0.0001$) and NOR discrimination index ($p=0.0001$). MMP-9 expression was unchanged in Western blot and RT-qPCR, but TIMP-1 was downregulated ($p=0.0081$), suggesting increased MMP-9 enzymatic activity may be related to a reduced inhibition. Doxycycline reversed deficits in social interaction and novel object discrimination induced by adolescent stress. Doxycycline also attenuated the increased MMP activity and the decreases in the number of PV+ and PNN+ cells in the vHip induced by stress. In the siRNA experiment, stressed rats without knockdown exhibited reduced SI time ($p<0.05$) and NOR index ($p<0.05$), while MMP-9 knockdown prevented these deficits ($p<0.01$). **Conclusion:** MMP-9 activity in the vHip decreases during development but is upregulated by adolescent stress. Both doxycycline and vHip MMP-9 knockdown prevented stress-induced behavioral and molecular alterations, highlighting MMP-9 as a key mediator of stress vulnerability during adolescence and a potential therapeutic target.

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02.031 Early Intestinal Oxidative Stress Triggered by Intranasal MPTP in the Parkinson's Disease

Model. Gomes J, Severo ER1, Evangelista HC, Rosa SG, Pinton S Unipampa, Uruguaiana, RS, Brazil

Introduction: Patients with Parkinson's disease (PD) frequently exhibit non-motor symptoms related to gastrointestinal (GI) dysfunction, which often precede the onset of motor symptoms. PD is characterized by the progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta, leading to a range of motor and non-motor manifestations. Studies have shown that intraperitoneal administration of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) induces both intestinal and neural alterations through the gut-brain-microbiota axis, ultimately contributing to PD-related neurodegeneration. These effects are often associated with the central role of oxidative stress in dopaminergic neuronal loss. MPTP can be administered through alternative routes, among which olfactory vectorization stands out as a particularly effective method for inducing the PD model at the central nervous system level. Nevertheless, the peripheral effects of intranasal (i.n.) MPTP administration remain to be thoroughly investigated. Accordingly, this study aims to investigate the effects of i.n. MPTP administration on oxidative stress in intestinal tissues, with the goal of linking oxidative damage and impaired antioxidant responses to the prodromal stage of PD. **Materials and Methods:** Twenty-five Wistar rats female were used in this study, and the experimental protocol was approved by the Animal Use Ethics Committee (CEUA 003/2024). At 90 days of age, the animals received i.n. administrations of 10 μ L of either MPTP or saline in each nostril (1 mg/nostril). The rats were randomly assigned to two groups (N = 12–13): (i) Control (i.n. saline) and (ii) MPTP (i.n. MPTP) (day 0). On day 15, the jejunum and colon were dissected following ice-cold saline clean. This timeline is well established in the literature, as it consistently induces oxidative damage in brain tissues. Catalase (CAT) activity, non-protein thiol (NPSH) content, and reactive oxygen species (ROS) levels were quantified and analyzed using unpaired t test. **Results:** Our results revealed a significant reduction in catalase (CAT) activity in both the jejunum ($p = 0.0044$) and colon ($p = 0.0079$) of the MPTP group compared to controls. While non-protein thiol (NPSH) content in the jejunum did not differ significantly, a significant increase was observed in the colon of MPTP-treated animals ($p = 0.0178$). Additionally, reactive oxygen species (ROS) levels were significantly elevated in both the jejunum ($p < 0.0001$) and colon ($p = 0.0006$) in the MPTP group. The results reinforce the bidirectional nature of the microbiota-gut-brain axis, showing that disruptions in gut microbiota can impact brain function and vice versa. This study shows for the first time that i.n. MPTP impairs intestinal antioxidant defenses and increases ROS, suggesting the gut's involvement in early Parkinson's disease via the microbiota-gut-brain axis. **Conclusion:** The i.n. MPTP administration induces oxidative stress in the gut, revealing its susceptibility to neurotoxic damage and underscoring the role of the microbiota-gut-brain axis in early PD. This model serves as a valuable tool for investigating gut-related mechanisms and therapeutic strategies in early PD. **Financial support:** Capes, CNPq, Carrefour and Unipampa. **Key-words:** Microbiota-gut-brain axis, Intestinal damage and Prodromal Parkinson's.

02.032 **Involvement of the Hypothalamic-Pituitary-Adrenal (HPA) Axis in the Behavioral, Cardiovascular, and Neuroendocrine Effects Evoked by Social Defeat in Rats.** Suzuki GMF¹, Belém-Filho IJA¹, Busnardo C², Paz HS², Corrêa FMA¹. ¹FMRP-USP, Dept Pharmacology, Lab Cardiovascular Neuropharmacology, Ribeirão Preto, Brazil. ²FCFAR-Unesp-Araraquara Dept Drugs and Medicines, Araraquara, Brazil

Introduction: The social defeat model induces defensive behaviors in submissive rats, triggering behavioral, autonomic, and endocrine responses, notably involving activation of the HPA axis. This axis plays a central role in coordinating stress-related responses by regulating the release of glucocorticoids—cortisol in humans and corticosterone in rodents. This study aims to investigate the role of the HPA axis in the modulation of cardiovascular, neuroendocrine, and behavioral responses to chronic social stress. **Methods:** The experimental procedures were approved by the Animal Experimentation Ethics Committee of the USP (process nº 1352/2024). Male Wistar rats were treated with prednisolone (5 mg/kg; i.p.) or vehicle for 14 days to elucidate the involvement of the HPA axis in the response to social stress. Following treatment, rats underwent the social defeat protocol for seven consecutive days. Behavioral tests (Splash Test, Open Field, Novel Object Recognition and Forced Swimming), cardiovascular (blood pressure and heart rate), and neuroendocrine (corticosterone and ACTH levels) changes were assessed to evaluate the effects of intermittent social stress induced by resident-intruder model. **Results:** Two-way ANOVA was used for most analyses, including mean arterial pressure (MAP) and heart rate (HR) over time, with repeated measures for the time factor. Behavioral responses were evaluated using one-way ANOVA. When statistical significance was found, the Fisher's post hoc test was used. Animals subjected to social stress and treated with vehicle showed reduced grooming time ($F_{1,64} = 28,55$; $p < 0,0001$), indicating an anhedonic-like behavior. Treatment with prednisolone did not reverse this effect ($F_{1,63} = 0,05321$; $p = 0,8183$), suggesting it was ineffective in mitigating stress-induced anhedonia. Prednisolone elicited anxiety-like behavior in the open field test, as stressed animals treated with the synthetic glucocorticoid explored the center zone less than stressed animals treated with vehicle ($F_{3,60} = 2,891$; $p < 0,01$). In the Novel Object Recognition test, social stress impaired long-term memory performance ($F_{16,39} = 1,975$; $p = 0,0420$), and prednisolone did not prevent this deficit, indicating a lack of efficacy in mitigating stress-induced memory impairment. On the other hand, in the forced swim test, prednisolone reduced immobility time in stressed animals compared to the stressed vehicle group ($F_{1,39} = 19,94$; $p < 0,0001$), suggesting an antidepressant-like effect. Finally, the treatment did not produce significant changes in mean arterial pressure or heart rate. **Conclusion:** The results of the negative modulation of the HPA axis using prednisolone suggest the involvement of the HPA axis in specific behavioral alterations induced by intermittent social stress, such as depressive-like behavior, while having no impact on stress-induced cardiovascular responses. **FUNDING:** FAPESP grants number (2021/10014-5 and 2023/03044-0), CNPq (305464/2019- 1), CAPES and FAEPA.

02.033 Cannabidiol Attenuates Neurotoxic Effects of Anhydroecgonine Methyl Ester (AEME), a Crack Pyrolysis Product, in Hippocampal Astrocytes: Role of CB1 and TRPV1 Receptors Patrocínio TB, Silva SC, Leitão SGR Unifal, PPG Biociências aplicadas à Saúde, Brasil

Anhydroecgonine methyl ester (AEME), the main pyrolysis product of crack cocaine, has been shown to be neurotoxic. However, its effects on glial cells remain poorly understood. Considering that astrocytes are glial cells essential for maintaining the homeostasis of the tripartite synapse, this study aimed to determine whether AEME's cytotoxic effects also affect this cell type, and whether treatment with cannabidiol (CBD) can modulate this response. In addition, we evaluated whether concomitant treatment with antagonists of two potential target receptors of CBD (CB1 and TRPV1) would interfere with this effect. To this end, hippocampal astrocytes were isolated from primary cultures obtained from neonatal rats. To assess cell viability after exposure to different treatments, hippocampal astrocytes were cultured in 96-well plates (1×10^5 cells/well) and exposed for 2.5 hours to combinations of the following treatments: CBD (10 μ M), Rimonabant (RIMO, CB1 antagonist, 1 μ M), Capsazepine (CAPS, TRPV1 antagonist, 30 μ M), and AEME (0.1 mM). All procedures were reviewed and approved by the Animal Research Ethics Committee of UNIFAL (protocol 0032/2023). Cell viability was assessed using the MTT assay. Exposure to AEME significantly reduced cell viability compared to the control group ($99.99 \pm 2.52\%$ vs. $8.06 \pm 0.42\%$, $p < 0.001$). Interestingly, incubation with CBD alone also reduced the percentage of viable cells ($99.99 \pm 2.52\%$ vs. $66.75 \pm 4.43\%$, $p < 0.001$), as did isolated treatments with RIMO ($99.99 \pm 2.52\%$ vs. $77.62 \pm 4.94\%$, $p < 0.001$) and CAPS ($99.99 \pm 2.52\%$ vs. $89.02 \pm 1.44\%$, $p < 0.001$). Co-incubation with CBD partially attenuated the cytotoxic effect of AEME, resulting in a significant increase in cell viability compared to the group exposed to AEME alone ($8.06 \pm 0.42\%$ vs. $21.00 \pm 1.05\%$, $p < 0.01$). However, concomitant treatments with RIMO and CAPS did not interfere with the protective effect of CBD. These findings reinforce the cytotoxic potential of AEME and also demonstrate the modest efficacy of CBD as a modulator of this toxicity. Nevertheless, the results indicate that CB1 and TRPV1 receptors do not appear to mediate the protective effects of CBD under these experimental conditions.

02.034 **Analysis of the Anticonvulsant and Neuroprotective Potential of Carotenoid-Enriched Extracts from Amazonian Microalgae in an Acute Pentylentetrazol-Induced Seizure Model.** Bentes ILG¹, Cancela LCF^{1,2}, Freitas CM^{1,2}, Araújo TB¹, Pinto DSV¹, Jesus MS¹, Faustino SMM¹, Nascimento JLM², Oliveira FR^{1,2} ¹Unifap, Depto de Ciências Biológicas e da Saúde, Curso de Farmácia, Brazil ²UFPA, Graduate Program in Pharmacology and Biochemistry, Brazil

Introduction: Microalgae are fast-growing, photosynthetic microorganisms that can be cultivated with relative ease, with their biomass recognized for its therapeutic and nutritional potential. Species such as *Chlorella vulgaris*, *Scenedesmus ecoris*, and *Limnospira platensis* exhibit high antioxidant potential, partly attributed to carotenoids with neuroprotective activity. In the present study, these species were evaluated for their anticonvulsant and neuroprotective properties in a murine model of severe seizures. **Methods:** Swiss mice (n=6 per group) were treated orally with *C. vulgaris*, *L. platensis*, and *S. ecoris* at a dose of 100 mg/kg for 15 days. A control group received saline solution. Subsequently, seizures were induced with a single intraperitoneal injection of pentylentetrazol (PTZ, 60 mg/kg), and animals were monitored for 10 minutes. Behavioral assessments included the latency to onset of myoclonic and tonic-clonic seizures, duration of convulsive episodes, and lipid peroxidation levels in brain tissue. The experimental protocol was approved by the Animal Use Ethics Committee of UNIFAP (protocol nº. 02/2025). Data were expressed as mean±standard error of the mean (SEM), and group comparisons were made using One-Way ANOVA followed by Tukey's post hoc test. Statistical significance was set at p<0.05, and analyses were conducted using GraphPad® Prism 8.0. **Results:** No statistically significant difference was found in the total carotenoid content between *C. vulgaris* (501.5±6.52 mg EβC/g) and *S. ecoris* (451.5±21.56 mg EβC/g); however, *L. platensis* (121.5±11.54 mg EβC/g) differed from both species. The latency to myoclonic spasm in the control group was 56±9.96 s, significantly shorter than that observed for *C. vulgaris* (172.6±25.08 s) and *L. platensis* (234.3±40.36 s), but comparable to *S. ecoris* (162.5±19.54 s). Groups treated with *C. vulgaris*, *L. platensis*, and *S. ecoris* showed tonic-clonic seizure latencies of 571.6±28.44 s, 574.3±22.6 s, and 285±63.79 s, respectively. Latencies for *C. vulgaris* and *L. platensis* were significantly longer than in the control group (243.9± 63.05 s). There was a statistically significant reduction in the duration of tonic-clonic seizures in the *C. vulgaris* (3.56±4.2 s), *L. platensis* (4.1±2.08 s), and *S. ecoris* (17.17±3.92 s) groups compared to the control group (34.86±3.98 s). **Conclusion:** *C. vulgaris* and *L. platensis* demonstrated protective effects against cerebral oxidative stress and enhanced anticonvulsant activity, whereas *S. ecoris* exhibited only minor effects on the evaluated parameters. The total carotenoid content analysis suggests that additional bioactive compounds within the extracts may contribute to the observed neuroprotective action. This is supported by the finding that *L. platensis*, despite containing lower carotenoid concentrations, produced positive in vivo results, while *S. ecoris*, with higher carotenoid content, showed no significant differences from the control group. These microalgae may be promising natural candidates for preventing neurodegenerative diseases. **Funding:** CNPq/MCTI FNDCT nº 19/2024

02.035 **Impact of Pilocarpine on Angiotensin I-Converting Enzyme (ACE) Activity in Transgenic Rats with Aberrant Neurodevelopment.** Avila F, Basso L, Nani J, Janisset N, Cruz F, Oyadomari W, Hayashi M Unifesp-EPM, Lab Molecular and Cellular Neuropharmacology, Dept Pharmacology, São Paulo, Brazil

Introduction: Angiotensin I-converting enzyme (ACE) is an oligopeptidase that converts angiotensin I into hypertensive angiotensin II, playing a key role in the renin-angiotensin system (RAS), associated with systemic blood pressure regulation and inflammation. Recently, RAS functions in the central nervous system (CNS) have gained attention. Increased ACE activity has been linked to cognitive decline in neurodegenerative and psychiatric disorders. Angiotensin II is pro-inflammatory, modulates neurotransmitters such as acetylcholine and dopamine, and acts as a neuromodulator in the CNS with implications in epilepsy. Pilocarpine, a muscarinic agonist, induces seizures via excessive cholinergic stimulation and is used to study cognitive deficits from neurodegeneration caused by status epilepticus (SE). Pilocarpine administration results in spatial disorganization of neurons, and the tgDISC1 transgenic model, which overexpresses the full-length human DISC1, is valid for psychiatric disorder studies and shows changes in dopaminergic homeostasis. These animals also present neuronal disorganization and deficits in long-term memory and attention, which can be reversed by intranasal dopamine administration. **Objective:** Investigate ACE activity in tgDISC1 rats compared to wild-type animals following pilocarpine-induced seizures. **Methods:** Male WT and tgDISC1 rats ($n = 20$; 3 months old) were divided into a pilocarpine-treated group, which received scopolamine 30 min before pilocarpine to induce seizures, and a mock-treated control group, which received the same treatments except pilocarpine. After pilocarpine injection, animals were observed for 30 min and seizure severity assessed on the Racine scale, from stage 1 (facial automatisms) to stage 5 (rearing then falling). SE was defined as continuous motor seizure at stages 4 or 5, after which convulsions were stopped by diazepam; 12 h later animals were euthanized for collection of cortex, hippocampus, striatum, nucleus accumbens and cerebellum. ACE activity was measured by fluorimetry using a quenched substrate (Abz-Phe-Arg-Lys(DNP)-Pro-OH), in the presence or absence of the inhibitor captopril. ANOVA, t-test, Spearman with $p < 0.05$ was used for analyses. **Results:** Based on Racine scale, there was no difference in SE-related behaviors between WT and tgDISC1 rats. After pilocarpine, ACE activity increased in the hippocampus ($p = 0.004$) and decreased in the cerebellum ($p = 0.036$) of tgDISC1 rats, compared to the respective mock group. ACE activity in WT rats was not modulated by pilocarpine. **Conclusion:** The hippocampus, a key structure for memory and cognition, exhibited increased ACE activity in tgDISC1 rats following pilocarpine-induced seizures, suggesting a potential involvement of central ACE in epileptic states. The decrease in cerebellar ACE activity further supports the notion of strain- and region-specific modulation of ACE, warranting additional studies to elucidate the role of central RAS components in SE pathophysiology. **Keywords:** tgDISC1, ACE, oligopeptidase, epilepsy.

02.036 **SARS-CoV-2 Spike Protein Causes Behavioral and Neuro-Oxidative Changes Differently in Male and Female Mice.** Willrich CH, Cury BJ, Longo B, de Amorim GES, Machado GM, de Oliveira IM, Queiroz LY, Casimiro LM, Santos MR, Cimarosti HI, Prediger RDS, da Silva L UFSC, Dept Pharmacology, Florianópolis, Santa Catarina, Brazil

Introduction: Long COVID is characterized by the persistence of symptoms for months or even years beyond the initial phase of infection. Neurological dysfunctions, such as cognitive impairment and anosmia—collectively referred to as "brain fog"—are among the most frequently reported complaints. Although symptoms manifest differently in males and females during both the early and late stages of the disease, the underlying mechanisms remain poorly understood, as well as the sexual differences. In addition to direct damage caused by the virus or its proteins to the nervous system, inflammatory and oxidative insults at the gastrointestinal tract may also contribute to their neurological effects. Therefore, this study aimed to assess the Spike systemic administration impacts on behavioral and neuro-oxidative parameters, as well as the role of intestinal damage and the presence of sexual dimorphism in these effects. **Methods:** Female and male C57BL/6 mice were assigned to three experimental groups: active Spike (Ac-S; 10 µg, s.c.), denatured Spike (Dn-S; 10 µg, s.c.), and vehicle control (Vei; 100 µL PBS, s.c.). Acute-phase behavioral testing began three days post-injection (dpi) with the open field test (OFT), followed by the novel object recognition (NOR) test, Y-maze, and olfactory discrimination (OD) test. The same behavioral tests were repeated at 30 dpi to assess long-term effects. On day 35 dpi, animals were euthanized, and brain and intestinal tissues were collected for biochemical analyses, including: reduced glutathione (GSH), malondialdehyde (MDA), lipopolysaccharide (LPS), total reactive oxygen species (ROS), and the activities of N-acetylglucosaminidase (NAG) and myeloperoxidase (MPO). Ethics approval: CEUA 8965220424 / UFSC. **Results:** During the acute phase, female, but not males, mice showed a hypo locomotor phenotype, as evidenced in the OFT by decreased distance traveled, reduced line crossings, increased immobility time, and reduced mobility time. Olfactory function remained unchanged in both sexes at all time points. In the late phase, male mice in the Ac-S group exhibited memory impairment. Despite increased GSH levels, female brain tissue exhibited signs of oxidative damage, including elevated MDA levels and increased MPO and NAG activity. Moreover, increased ROS levels were found in the intestine suggesting enteric injury, which was accompanied by elevated serum LPS. Similarly, males exhibited oxidative brain damage, reflected by increased MDA and ROS amount, and MPO activity. **Conclusion:** This study found that the Spike protein affected males and females differently in terms of cognitive function, exploratory activity, and locomotion in mice. Females exhibited a hypolocomotor phenotype in the acute phase, indicating an early effect, something not found in males; however, in females late damage may be associated with a disruption in the gut-brain axis, particularly intestinal barrier impairment, which has been linked to increased levels of LPS in the brain. Nevertheless, cognitive decline, through the RO test, was observed late only in males, which may be related to the neuro-oxidative consequences of the viral protein. Funding: FAPESC.

02.037 **Effects of Live and Inactivated *Bifidobacterium lactis* on Cerebral NPSH Levels Induced by MSG in Wistar Rats.** Fontoura EA, Arena RVP, Comis-Neto AA, Rodrigues ES, Gomes J, Rodrigues BG, Pinton S, Rosa SG Unipampa – Uruguaiiana, RS, Brazil

Introduction: Monosodium glutamate (MSG), a common flavor enhancer, is linked to metabolic diseases like obesity when consumed in excess. Obesity is a chronic pathology disrupting homeostasis, characterized by high body fat from deregulated diet and inactivity, leading to several comorbidities (cardiopathies, neuropathies, diabetes) and contributing to metabolic syndrome (MS). Animal models are crucial to investigate these mechanisms and therapies. MSG-induced MS in neonatal rodents is a well-accepted model, causing neuronal destruction in the CNS and systemic metabolic dysfunctions. Probiotics show promise in preventing and attenuating metabolic imbalance. This study aimed to evaluate the effects of live and heat-inactivated *Bifidobacterium lactis* on NPSH levels of different brain structures of Wistar rats injected with MSG. **Methods:** Newly-born male Wistar rats were divided into six groups: Control (CON); Live *Bifidobacterium lactis* (BLV); Inactivated *Bifidobacterium lactis* (BLM); MSG (MSG); MSG + Live *Bifidobacterium lactis* (GBLV); and MSG + Inactivated *Bifidobacterium lactis* (GBLM), with n between 7 and 9 per group. Controls received 0.9% saline while the MSG group received MSG (4 g/kg, s.c.) from PND 5-14. From PND 25-53, animals were daily gavaged with live or inactivated *Bifidobacterium lactis* (1×10^9 CFU/rat) or 1 mL/rat saline. At PND 54, animals were euthanized, and cortex, hippocampus, and hypothalamus were collected for non-protein thiols (NPSH) evaluation. All procedures were approved by CEUA/UNIPAMPA (nº 019/2024). Results (Mean $\pm$ SEM) were analyzed by one-way ANOVA with Tukey's post-hoc test. Differences with $p < 0.05$ values are considered statistically significant. **Results:** MSG induced a decrease of NPSH levels in the hypothalamus of rats when compared to the control group. In contrast, live and inactive *Bifidobacterium lactis* were effective against this decrease when compared to MSG group. In addition, no significant differences were detected in NPSH levels in the cortex and hippocampus of rats among groups. Non-protein thiols are crucial for antioxidant defense, suggesting MSG impacted this region's redox status and subsequent modulation by *Bifidobacterium lactis*. **Conclusion:** This study demonstrated that neonatal MSG administration induced a significant and specific effect on an important cerebral antioxidant defense in Wistar rats, evaluated by NPSH levels. Moreover, Live and inactive *Bifidobacterium lactis* effectively attenuated this imbalance, suggesting protective potential. These findings reinforce the gut-brain axis's role in modulating cerebral oxidative stress and position *Bifidobacterium lactis* as a promising therapeutic alternative for MSG-induced metabolic conditions.

02.038 Cannabidiol Attenuates General Neurotoxicity and Paclitaxel-Induced Cognitive Impairment (Chemobrain) in a Sex- and Treatment Regimen-Dependent Manner. Pereira ACMP¹, Queiroz ABWV², Vatanabe IP¹, Barros ASM², Meyer E¹, Lisboa SF¹ ¹FCFRP-USP, Brazil, ²FMRP-USP, Brazil

Introduction: Chemobrain is a condition in which chemotherapy patients experience decreased cognitive performance. Symptoms may be transient but can persist long-term, and no treatment is currently available. This study evaluated the effects of CBD on paclitaxel (PTX)-induced chemobrain. **Methods:** male and female C57BL/6 mice received six doses of PTX (20 mg/kg) or vehicle over two weeks; 24 hours after the final PTX injection, mice received CBD (1 or 10 mg/kg) or vehicle. Next, behavioral tests were performed, including the Open-Field (OFT), Novel Object Recognition (NOR)/Location(NOR), Y-Maze (YM), Puzzle-box (PB), Nest building (NB), and Von-Frey (VF). At the end, mice were euthanized for further ex vivo analysis of the hippocampus (Western Blot-WB, Immunofluorescence-IF). *In vitro*, undifferentiated or differentiated SH-SY5Y cells were treated with PTX. Cell viability was assessed by MTT, neurite length was measured by microscopy, and reactive oxygen species (ROS) formation was measured by the DCFH-DA assay. The project was approved by the local CEUA (23.1.223.60.8). **Results:** Among surviving mice, PTX did not alter the main exploratory parameters of the OFT (total distance and center activity; $p > 0.05$). Also, PTX impaired the cognitive performance in NOL ($F_{(2,85)} = 9.143$; $p = 0.0003$), NOR-ST ($F_{(2,85)} = 17.03$, $p < 0.0001$), NOR-LT ($F_{(2,85)} = 12.91$, $p < 0.0001$), Y-M ($F_{(2,85)} = 4.860$; $p = 0.01$), and PB (male KW = 13.13, $p = 0.0013$; female: KW = 14.96, $p = 0.0006$). There was no effect in the NB ($p > 0.05$). Notably, CBD co-treatment with PTX reduced cognitive impairments and peripheral sensibility changes compared to PTX. However, general toxicity parameters (food/water intake, spleen index, weight gain, survival) did not improve with CBD. Although female mice also exhibited cognitive impairment, they were more resistant than males to general toxicity parameters. Since males were more susceptible, we chose them to further analyze possible pathophysiological changes ex vivo first. Levels of SIRT-1 and Synaptophysin protein expression in WB were decreased in PTX-treated animals (SIRT: $t = 2.824$, $df = 14$, $p = 0.0135$; Syn: $t = 2.351$, $df = 14$, $p = 0.0339$), while the cleaved/pro IL-1 β proportion increased ($t = 2.296$, $df = 14$, $p = 0.0376$). All PTX-induced changes were attenuated in CBD-treated animals. No changes were detected in Catalase or Caspases 1 and 3 expressions. IF revealed that PTX increased GFAP and IBA-1 expression differentially according to the area (CA1, CA2/3, Dentate Gyrus), while NeuN expression tended to decrease; CBD attenuated these changes. Of note, the beneficial effects of CBD were observed when given 24 hours after PTX; when administered simultaneously, or 24 hours after at higher doses, it increased PTX toxicity, evidencing a pharmacological interaction, which was also observed *in vitro*. **Conclusion:** These results suggest that long-term neuroplasticity can have a role in PTX-induced chemobrain, and CBD treatment could improve this condition, depending on the administration interval. Both PTX and CBD effects were sex-dependent. **Financial support:** FAPESP, CAPES.

02.039 Antidyskinetic Effects of Cannabidiol and Cannabigerol in Parkinsonian Rats: Role of 5-HT1A Receptors. Mattos BA¹, Nascimento GC², Crippa JA³, Hallak J³, Raisman-Vozari R⁴, Michel PP⁴, Guimaraes FS⁵, Del Bel EA² ¹FMRP-USP, Dept Physiology, Ribeirao Preto, Brazil; ²FORP-USP, Dept Basic and Oral Biology, Ribeirao Preto, Brazil; ³FMRP-USP, Dept Neuroscience and Behavior, Medical School of Ribeirao Preto; ⁴ICM- CNRS-Inserm, Paris Brain Institute, Sorbonne Université, Hôpital Pitié Salpêtrière, Paris, France; ⁵FMRP-USP, Dept Pharmacology, Brazil

Cannabigerol (CBG) and Cannabidiol (CBD) are phytocannabinoids with neuroprotective, anti-inflammatory, antioxidant, and anti-excitotoxic properties. Recent evidence suggests their potential as therapeutic agents to prevent or attenuate L-3,4-dihydroxyphenylalanine (L-DOPA)-induced dyskinesia (LID) in Parkinson's disease (PD). This study aimed to evaluate the antidyskinetic effects of CBG and CBD, administered separately or in combination, in a rat model of PD. Precisely, we tested whether treatment with CBD (10 or 30mg/kg) or CBG (1, 5, or 10mg/kg) given separately or in combination, could have antidyskinetic effects. To this aim, Male Wistar-Hannover rats received unilateral 6-hydroxydopamine (6-OHDA) lesions to produce a hemiparkinsonian model of dopaminergic degeneration (Animal Research Ethics Committee nº 2021.1.723.58.7). After one week of daily treatment with L-DOPA (10mg/kg/daily; s.c.) animals develop severe axial, limb, and orofacial abnormal involuntary movements (AIMs). Then, rats were treated with CBG and CBD, i.p., separately, or in combination 30 minutes before L-DOPA treatment, for 2 weeks. We established that 30mg/kg CBD and 5mg/kg CBG were effective in limiting LID development when given separately. CBD and CBG, both at 10 mg/kg had no inhibitory effect. Notably, co-administration of subeffective doses (CBD 10 mg/kg + CBG 5 or 10 mg/kg) produced a synergistic reduction in dyskinesia. Importantly, none of the treatments interfered with L-DOPA's antiparkinsonian motor effects, as demonstrated by the forepaw adjusting step test. To explore the underlying mechanisms, we used the selective 5-HT1A receptor antagonist WAY100635 (0.5 mg/kg, i.p.), administered during the final week of treatment. This reversed the antidyskinetic effects of CBD alone and CBD/CBG co-treatments, indicating that antidyskinetic effects occurred in this paradigm through activation of 5HT1A receptors. Additionally, the immediate early gene Fos-B was also found to be increased in the striatum of lesioned animals treated with L-DOPA, and CBG and CBD prevented this effect. In conclusion, CBG and CBD attenuate L-DOPA-induced dyskinesia via 5-HT1A receptor activation and suppression of striatal FosB expression, without compromising motor function. These findings support the potential of phytocannabinoid-based strategies for managing LID in PD.

02.040 Microglial Involvement in Adolescent Stress-Induced Behavioral Impairments and Deficits in Parvalbumin Interneurons and their Perineuronal Nets in Mice. Hadera V¹, Bobotis B², Landwehr A², Guimarães FS¹, Tremblay ME², Gomes FV¹ ¹FMRP-USP, Dept Pharmacology, Brazil ²University of Victoria, Medical Sciences Building, Canada

Introduction: Studies suggest that trauma during adolescence, a critical period of neurodevelopment, increases the risk of psychiatric disorders. Microglia, the resident immune cells of the brain, play a fundamental role in the response to trauma and may contribute to long-lasting stress-induced alterations. Our previous work has shown that adolescent stress induces behavioral deficits and alters parvalbumin (PV) interneurons and their perineuronal nets (PNNs) in the ventral hippocampus (vHip) in adulthood. **Objective:** To evaluate whether systemic administration of minocycline, which attenuates microglial activity, or intra-vHip infusion of clodronate disodium (CDS), which depletes microglia, could reverse stress-induced impairments during adolescence. **Methods:** Male C57BL/6J mice (n=8/group) were subjected to 10 days of foot-shock stress during adolescence [postnatal days (PND) 31 to 40]. One day later, they received either 10 days of treatment with minocycline (30 mg/kg/day, i.p.) or a single intra-vHip infusion of CDS. Behavioral tests (social interaction and novel object recognition test) and molecular analyses (quantification of PV+, PNN+, PV+/PNN+ cells, as well as number and morphology of Iba-1+ microglia) were performed in adulthood (>PND62). This study was approved by the Institutional Ethics Committee (FMRP 49/2021). **Results:** Adolescent stress led to reduced social interaction in adulthood. This impairment was attenuated by minocycline treatment. Two-way ANOVA indicated an effect of stress [$F(1,28)=125.7$; $p<0.05$], treatment [$F(1,28)=12.80$; $p<0.05$], and an interaction between these factors [$F(1,28)=85.74$; $p<0.05$]. In the novel object recognition test, animals exposed to stress during adolescence showed reduced discrimination between novel and familiar objects in adulthood. This deficit was attenuated by minocycline administered after stress. Two-way ANOVA revealed effects of stress [$F(1,28)=347.4$; $p<0.05$], treatment [$F(1,28)=58.29$; $p<0.05$], and interaction [$F(1,28)=190.5$; $p<0.05$]. Adolescent stress reduced the number of PV+ cells [$F(1,28)=7.04$; $p<0.05$], PNN+ cells [$F(1,28)=6.09$; $p<0.05$], and PV+/PNN+ cells [$F(1,28)=7.02$; $p<0.05$], with all these reductions being attenuated by minocycline (with respective treatment and interaction effects). Adolescent stress and minocycline treatment did not significantly alter the number of microglial cells, NND (nearest neighbor distance), or the Spacing Index. However, changes were found in cell area ($H=9.53$; $p<0.05$), circularity ($H=9.63$; $p<0.05$), and the number of branches ($H=10.44$; $p<0.05$), without changes in skeleton length. Dunn's post hoc test indicated that microglial cells from stressed animals treated with minocycline showed smaller cell area and greater circularity compared to controls ($p<0.05$ vs. naïve + vehicle). Moreover, microglia from naïve animals treated with minocycline displayed fewer branches (Dunn's post hoc test; $p<0.05$ vs. naïve + vehicle). Additionally, adolescent stress reduced social interaction in adulthood, which was attenuated by CDS. Two-way ANOVA indicated effects of stress [$F(1,28)=68.73$], treatment [$F(1,28)=42.29$], and interaction [$F(1,28)=108.4$]. In the novel object recognition test, the deficit was attenuated by CDS [stress: $F(1,28)=30.80$; treatment: $F(1,28)=68.0$; interaction: $F(1,28)=41.52$]. Stress reduced PV+ cells [$F(1,28)=8.31$], PNN+ cells [$F(1,28)=4.81$], and PV+/PNN+ cells [$F(1,28)=4.84$], which were mitigated by CDS (with significant treatment and interaction effects). Adolescent stress and CDS did not significantly alter the number of microglial cells, NND, or the Spacing Index. No changes were observed in cell area, but alterations were found in circularity ($H=45.75$; $p<0.05$), number of branches ($H=45.75$; $p<0.05$), and skeleton length ($H=16.36$; $p<0.05$), all of which were attenuated by CDS. **Conclusion:** Our findings indicate that adolescent stress induces long-lasting behavioral and cellular alterations, particularly affecting PV interneurons, their perineuronal nets (PNNs), and microglial morphology in the ventral hippocampus (vHip). Systemic minocycline treatment or local microglial depletion after stress mitigated these effects, highlighting microglia as a potential target for the treatment of stress-related psychiatric disorders. **Funding:** FAPESP, CNPq, CAPES and FAEPa.

02.041 **Evaluation of the Potential Neuroprotective Effects of Klotho Pre-Treatment in Primary Neuronal and Cerebellar Glial Cell Cultures Exposed to Ferrous Sulfate (FeSO₄) and Staurosporine (STS).** Santiago TCP, Kawamoto, EM ICB-USP

Introduction: Klotho protein is correlated with numerous beneficial effects on the organism, such as increased longevity that has been observed in transgenic mouse models that overexpress klotho, it is interesting to mention other models that downregulated klotho expression have been characterized as an efficient model of premature aging. In the central nervous system (CNS), klotho has been studied by our group as a potential neuroprotective agent against neuronal damage, including oxidative stress and/or apoptosis. Klotho protein is expressed in different regions of the brain, and is also found in the cerebellum, which is an important region for motor control, that requires further investigation to establish and clarify its relationship with klotho. Based on this, the purpose of this work was to evaluate the potential neuroprotective effect of recombinant klotho (rklotho) in primary mixed culture from the cerebellum mice (C57BL/6) against toxic insults such as exposure to Ferrous Sulfate (FeSO₄) and Staurosporine (STS). **Methods:** After the growth period with the conditions determined according to Thangnipon et al. (1983), a characterization of the culture was carried out using immunofluorescence. Next, one group was subjected to pre-treatment with rKlotho 35 (R&D Systems) at concentrations ranging from 0.1 nM to 5 nM for 4 h or 6h, using a previously established protocol. In parallel, a second group was used to standardize the toxic compounds, and a third group was used to verify cell viability and cytotoxicity (MTT and LDH assays, respectively) in the culture of cells pretreated with rklotho against insults with FeSO₄ and STS. Quantitative analysis of cell types from the primary mixed culture of cerebellum, obtained through the Immunofluorescence assay, was performed using the ImageJ software (National Institute of Health, USA). The results of the MTT and LDH assays were analyzed using the GraphPad Prism 8 program. Statistical differences were analyzed by Student's t-test or one-way ANOVA followed by Tukey's post-test, and a significant difference between groups was characterized by the value of $p < 0.05$. All graphs were represented as mean values $\pm$ standard error of the mean. The work was carried out with the necessary approvals by CEUA (1520020720). **Results:** After analyzing the data, it was first verified that the culture of mixed cells from the cerebellum of C57BL/6 mice is composed of approximately 74.92% neurons, 18.53% astrocytes and 13% microglia. Cells treated with rklotho showed higher cell viability compared to cells treated exclusively with FeSO₄ ($p = 0,0061$) or STS ($p < 0,0001$). And then, we observed an increase in cell viability and a significant decrease in cytotoxicity in the group pre-treated with rKlotho for 4 h followed by receiving FeSO₄ ($p = 0,0282$), or STS ($p < 0,0001$) for 24h when compared to the control group. **Conclusion:** Pre-treatment with rklotho protected cells from neuronal damage and apoptosis, improving cell viability and reducing toxicity from the addition of FeSO₄ and STS to the mixed cerebellar cell culture medium, corroborating the group's investigations related to neuroprotective role of the klotho protein.

02.042 Unveiling Dexamethasone's impact on adolescent male and female rat neurodevelopment

Sbardellotto GE, Kamidai N, Munhoz CD ICB-USP, Dept Pharmacology

Introduction: Glucocorticoids (GCs), such as cortisol and corticosterone, are essential steroid hormones in the stress response, acting through GR and MR receptors in the brain to influence mood, cognition, and development. Dexamethasone (DEXA), a synthetic GCs, is the gold standard treatment for pregnant women at risk of preterm birth. However, its prenatal exposure may impair neurodevelopment and increase vulnerability to psychiatric disorders. Stress activates the HPA axis, leading to increased cortisol levels, which can impair memory and learning. DEXA may reduce neurogenesis in the hippocampus. Despite the risks, it remains vital to prevent prematurity complications. Understanding its effects can guide policies for monitoring individuals who are exposed. **Objective:** This project aims to investigate the effects of prenatal dexamethasone exposure on anxiety-like behavior using the EPM test during adolescence - a critical phase of brain maturation - along with associated neurobiological changes. The study analyzed GR expression and evaluated neurogenesis through double labeling with BrdU and DCX in hippocampal sections from each experimental group. **Material and Methods:** Pregnant females were administered DEXA (i.p., 1 mg/kg) or vehicle (0.9% saline) between days 18-21 of gestation (GD18-21). The pups were divided into experimental groups based on sex and the condition to which they were subjected during the prenatal period. On postnatal days 22, 24, and 26, animals received an i.p. injection of BrDU (bromodeoxyuridine, 100 mg/kg) to analyze neurogenesis. On PND30 (considered crucial for neurodevelopment and a period that marks the beginning of adolescence), the animals were subjected to the Elevated Plus Maze (EPM) test to analyze anxiety-like behavior. Ninety minutes after the test, the animals were euthanized by transcardiac perfusion. **Results:** Dams for both groups (DEXA and CTL) exhibited a similar weight gain until gestational day 18. The DEXA group presented a mean gain of $4.312 \text{ g} \pm 0.60$ between days GD10-14 and $8.267 \text{ g} \pm 0.65$ between days GD15-18, while the CTL group had a mean gain of $3.33 \text{ g} \pm 1.18$ and $9.61 \text{ g} \pm 3.55$ in the same periods. From GD19 onwards, a significant difference was observed in mean weight gain ($p < 0.005$). The CTL group maintained a mean gain of $7.55 \text{ g} \pm 3.27$, while the DEXA group presented a weight loss of $-1.91 \text{ g} \pm 1.20$. Therefore, DEXA treatment appears to significantly influence maternal weight gain in the late stages of pregnancy, indicating a possible metabolic effect in these dams. A significant difference in the offspring weight was observed between the CTL and DEXA groups, both at birth – $6.89 \text{ g} \pm 0.35$ in the CTL group versus $5.62 \text{ g} \pm 0.52$ in the DEXA group – and throughout the nursing period (up to PND21). For the EPM test, there was no difference between the CTL and DEXA groups for male animals. However, among females, antenatal DEXA exposure was associated with a reduction in the percentage of entries into the open arm (63.38% in the CTL group vs. 48.82% in the DEXA group, $p < 0.05$) and an increase in the anxiety index (0.35 in the CTL group vs. 0.48 in the DEXA group, $p < 0.05$) in the adolescent offspring. These findings indicate that the antenatal DEXA treatment increased anxiety-type behavior exclusively in females. **Financial support:** FAPESP (2024/14414-6). **References:** (1) BERESHCHENKO, O.; BRUSCOLI, S.; RICCARDI, C. Glucocorticoids, sex hormones, and immunity. *Frontiers in Immunology*, v. 9, 2018.; (2) ROBERTS, D.; DALZIEL, S. R. Antenatal corticosteroids for accelerating fetal lung maturation for women at risk of preterm birth. In: ROBERTS, D. (ed.). *Cochrane Database of Systematic Reviews*. Chichester, UK: John Wiley & Sons, Ltd, 2006. DOI: 10.1002/14651858.CD004454.pub2. (3) LAUTARESCU, A.; CRAIG, M. C.; GLOVER, V. Prenatal stress: Effects on fetal and child brain development. In: [S.l.: s.n.], 2020. p. 17-40. DOI: 10.1016/bs.irm.2019.11.002.; (4) CAMPOS-CARDOSO, R. et al. The resilience of adolescent male rats to acute stress-induced delayed anxiety is age-related and glucocorticoid release-dependent. *Neuropharmacology*, v. 226, p. 109385, 2023.

02.043 **Neurobiological Alterations Induced by Fipronil in *Nauphoeta cinerea* Cockroaches.** Silva AB¹, Souza ABG¹, Araujo SOM¹, Rosa ME¹, Oliveira IN², Talone SC², Hyslop S², Vinadé L¹, Dal Belo CA^{1,2,3} ¹Unipampa-São Gabriel, ²Unicamp, ³Unifesp-Osasco

Introduction: Fipronil (FPL) is a highly effective, broad-spectrum insecticide used against arthropod pests. However, its indiscriminate use can lead to environmental accumulation and deleterious effects in non-target insects, even at sublethal doses. **Objectives:** The aim of this work was to examine the effects of sublethal doses of FPL (0.001, 0.01, 0.1 and 1 µg/g) on neurochemical and neurophysiological parameters in the lobster cockroach *Nauphoeta cinerea*. **Methods:** Cockroaches obtained from local suppliers were housed in polyamide plastic cages on a wood shavings substrate with access to water and rodent chow (Purina®). For use, the cockroaches were cryoanesthetized at -5 °C (5-8 min) and then injected with FPL or saline solution (0.9% NaCl; control), 10 min before the assays. The cytotoxicity of FPL to central nervous system neurons was assessed using the MTT assay. After treatment with FPL, cockroaches were decapitated and the brain was removed, homogenized in 0.1 M phosphate buffer, pH 6.8, and centrifuged (600 g, 10 min, 4 °C). An aliquot of the supernatant (30 mg protein/mL) was incubated with 50 µL of MTT solution (0.5 mg/mL) for 2 h to allow formazan formation and the resulting absorbance was measured at 540 nm in an ELISA reader. For electrophysiological measurements of the mechanosensory system, cockroaches were fixed ventral side up with entomological needles to a cork board, and one of the metathoracic legs was connected via two Ag/AgCl electrodes to a Neuron Spiker Box three-stage bioamplifier (Backyard Brains, Ann Arbor, MI, USA). The electric signals were band-pass filtered between 300–1300Hz and amplified 900x. Spontaneous compound action potentials (SCAP) were analyzed using Win WCP software (John Dempster, University of Strathclyde, Scotland). The results were expressed as the mean ± SEM and were compared statistically using one or two-way ANOVA followed by the Dunnett or Tukey post-hoc tests, with p<0.05 indicating significance. All data analyses were done using Prism software v.7.0 (GraphPad Inc., San Diego, CA, USA). **Results:** SCAP recordings in saline-injected cockroaches resulted in 143±7 events with an amplitude of 0.7±0.07 V (n=10) after 1 h. The lowest dose of FPL (0.001 µg/g) increased (to 188±7; n=10; p<0.05) and then decreased (to 109±8; n=10; p<0.05) the number of SCAPs. There was also a significant increase in the SCAP amplitude (to 1.13±0.07 V; n=10; p<0.05) compared to the saline controls. Higher doses of FPL significantly increased the number, but not the amplitude, of SCAPs (n=10 per dose). FPL dose-dependently reduced the viability of brain cells (% reductions of 20±3, 30±2, 40±8 and 50±10 for doses of 0.001, 0.01, 0.1 and 1 µg/g, respectively; n=10 each; p<0.05 to p<0.001). **Conclusions:** FPL significantly altered the neurophysiological activity and reduced the brain cell viability in *N. cinerea* cockroaches, even at low sublethal doses. These findings highlight the neurotoxicity of FPL on non-target insects and its potential environmental risks.

02.044 **Study of the Effects of the Exposure to Uremic Serum on Healthy Astrocytes.** Custodio GT, de Sá LL, Andreotti DZ, Lima TAS, Kawamoto EM. ICB-USP, Dept Pharmacology

Introduction: The Chronic Kidney Disease (CKD) can lead to cognitive impairment, compromising the central nervous system (CNS). The reasons for this may be the uremic toxins, result of the accumulation of organic waste in the blood, which are excreted through the kidneys in healthy individuals. To better understand this relationship between CKD and the central nervous system, cultures of healthy astrocytes from rats and humans were exposed to uremic serum from patients with CKD. Methodology: Neonatal rats (0-4 days) astrocytes and human astrocytes derived from induced pluripotent stem cells (iPSCs), that were generated through samples of tooth pulp, were used (protocol number CEUA 7018050724 and CEP CAAE 67426623.0.0000.5467) and exposed to the three different types of serum. In order to analyze cytotoxicity, LDH assays were performed with rat astrocytes. For all the assays, GraphPad Prism was used to perform one-way ANOVA followed by Bonferroni post-hoc analysis and the group means were compared with the control mean in the three cases: (I) healthy serum, and (II) pre- and (III) post-hemodialysis serum from patients with CKD stage V. Outliers were excluded and a significant difference was considered when $p < 0.05$. A student's t-test was also performed to identify significant differences that were not identified before.

Results: In the LDH assay, rat astrocytes exposed to treatment with uremic serum from patients before hemodialysis at a concentration of 10% showed a cytotoxic effect compared to the control group [$F(5,41)=11.31$, $p < 0.0001$]. It was also observed that the concentrations of 5% ($p < 0.01$) and 10% ($p < 0.0001$) are the ones that induce more cell death, while lower concentrations do not do it so significantly. The exposure to uremic serum from patients after hemodialysis ($F(5,19)=9.917$, $p < 0.01$ for 5% concentration vs. control and $p < 0.0001$ for 10% concentration vs. control) and to serum from healthy volunteers [$F(5,23)=2.809$, $p < 0.05$] obtained through the student's t-test for the 10% concentration vs. control demonstrated, through the LDH, smaller differences in cytotoxicity between the control group and the other groups with different serum concentrations. **Conclusion:** Regarding the LDH results, the pre-hemodialysis serum appears to be more cytotoxic for the cells. Considering the standard deviation, both LDH graphs, from the treatment with post-hemodialysis serum and with healthy serum, showed great similarity, which may indicate a positive result of dialysis as a therapy for patients affected by CKD. **Financial support:** CAPES, CNPq e FAPESP (process number 2023/15619-8)

02.045 Adolescent Exposure to a Synthetic Cannabinoid Affects the late Rewarding Response to the Same Drug? Moutinho IC, Corrêa EAS, Lemos LC, Freire LC, Souza INO, Neves GA UFRJ, Lab Molecular Pharmacology, Brazil

Introduction: Adolescence is a phase of many physiological, emotional, and social changes. Thus, young individuals are more susceptible to drug abuse. Synthetic cannabinoids, created to replicate and/or enhance the effects of phytocannabinoids, recently reached the streets, and their abuse, especially among young people, became a public health concern. Although synthetic cannabinoids have not been widely studied yet, a relationship between their use and adolescents' hospitalizations, acute kidney injuries, and possible psychosomatic disorders has already been described as later consequences. In addition, early drug abuse is associated with an increased risk of substance use disorders in adulthood. Therefore, this study aims to investigate behavioral changes related to reward in mice exposed to a synthetic cannabinoid in adolescence, aiming to identify potential long-term consequences. **Methods:** We used male Swiss mice from postnatal day (PND) 28 onwards, which were exposed to WIN 55,212-2 (2 mg/kg i.p.), a synthetic cannabinoid, or vehicle (DMSO 3% in saline 2 mL/100 g i.p.) once daily for 10 days (CEUA-UFRJ: 065/24). In adulthood (PND>70), we performed the conditioned place preference (CPP) task, which assesses preference or aversion for the compartment paired with the drug to analyze changes in the reward response. The test is performed in an acrylic apparatus divided into three compartments. The central compartment has two doors that provide access to the side compartments with different patterns and textures on the floor and walls. The experiment had three phases. In the first phase (preconditioning), the animals were allowed to move freely around the apparatus for another 15 minutes to become familiar with the apparatus. The second phase (conditioning) lasted eight days and consisted of daily sessions. In those sessions, the mice were administered WIN (0.5 mg/kg i.p.) or vehicle immediately before being placed in the apparatus. Each solution was paired with a particular compartment, which varied between animals. The animals received WIN in one day, and vehicle on the next day, totaling four days of administration of the drug and four of the vehicle. The control group received vehicle only. During this phase, the development of locomotor sensitization to the drug was evaluated by recording the total distance travelled in the conditioned compartment after the drug. In the last phase (postconditioning), the animals were placed in the apparatus for 15 minutes of free movement. Time in each compartment was recorded to evaluate the development of CPP. **Results:** During the conditioning phase, no motor sensitization was observed after WIN administration. Moreover, no preference or aversion to WIN was observed in any experimental group during the postconditioning. **Conclusion:** Overall, these results suggest WIN exposure in adolescence did not induce persistent changes in reward-related behavior into adulthood. Additional studies are needed to better understand the potential long-term consequences of synthetic cannabinoid exposure during this critical period of development. **Financial support:** CAPES, CNPq, FAPERJ.

02.046 **Preliminary Assessment of Mice Impulsive Behavior in the Delay Discounting Task.** Scafi H¹, Kondo T², Gogola J², Dalagrana H², Rosa E³, Pradella G³, Garlet QI^{1,2,3}. ¹UFPR, Graduate Program in Pharmacology, Brazil. ²UFPR, Course of Pharmacy, Brazil. ³UFPR, Biomedical Science Course, Brazil.

Impulsivity is the inability to inhibit the initiation of an unplanned action or behavior in response to environmental and/or internal factors, without a cost-benefit assessment of the consequences. Maladaptive impulsivity becomes pathological and is observed in several neuropsychiatric disorders, such as Attention Deficit Hyperactivity Disorder, Obsessive-Compulsive Disorder, Binge Eating Disorder, and Substance Abuse. The aim of this study was to evaluate innate impulsive behavior or impulsivity under the influence of lisdexamfetamine (LDX) in male and female Swiss, C57BL/6J, and BALB/c mice using the Delayed Discounting Task (DDT). The DDT was conducted in a T-maze with guillotine doors at the entrance and 10 cm before the end of each arm. The protocol consisted of 6 phases: habituation, discrimination between high reward (HRA; 200 μ L of 10% sucrose) and low reward (LRA; 50 μ L of 10% sucrose), reward choice test, and a new test after LDX administration (10 mg/kg, i.p.). In the tests, the animals were placed in the central arm of the maze and had to choose between immediate LRA or delayed HRA (30 seconds) in 5 trials. All procedures were approved by the Ethics Committee of UFPR (n^o 1610). There was no significant difference between strains ($p = 0.8848$, $F(2, 32) = 0.1228$, $\eta^2 = 0.0076$), sex ($p = 0.9371$, $F(1, 32) = 0.0063$, $\eta^2 = 0.0002$) or their interaction in relation to the choice of HRA (two-way ANOVA). In the LDX test, there were also no differences between strains ($p = 0.6776$, $F(2, 32) = 0.3940$, $\eta^2 = 0.0235$) or sex ($p = 0.4284$, $F(1, 32) = 0.6435$, $\eta^2 = 0.0192$) (two-way ANOVA). Regarding task completion time, no differences were found between strains ($p = 0.7808$, $F(2, 32) = 0.2494$, $\eta^2 = 0.0144$), sex ($p = 0.3328$, $F(1, 32) = 0.9668$, $\eta^2 = 0.0278$) or their interaction (two-way ANOVA). However, in LDX-treated animals, there was a difference between sex and strain, where BALB/c males took longer to complete the task compared to C57BL/6J and Swiss males ($p = 0.0104$, $F(2, 32) = 5.287$, two-way ANOVA, $\eta^2 = 0.2276$). When the data were regrouped by treatment (with and without LDX), keeping strain and gender as factors, a reduction in HRA choices was observed in the LDX group compared to the naïve group ($p = 0.0148$, $W = -219.0$, Wilcoxon signed-rank test, $n = 38$, two-tailed). As an initial assessment, we observed some differences between strains and the effect of LDX on impulse choice. Our results are preliminary and, so far, indicate that LDX enhance impulsive-like behavior in male and female mice of different strains. **Funding support:** CAPES, PIBIC-UFPR. **Keywords:** Swiss, C57BL/6J, BALB/c, impulsivity, lisdexamfetamine.

02.047 **Differential Effects of Probiotic and Paraprobiotic *Lactobacillus casei* on Intestinal ROS in a rat model of Alzheimer's Disease.** Severo ER, Gomes J, Meus SS, Riella D, Comis AA, Rosa, SG, Pinton S Unipampa

Introduction: Alzheimer's disease (AD) is the most prevalent form of dementia, characterized not only by progressive cognitive decline, including memory impairment, executive dysfunction, and language deficits, but also by motor symptoms (such as gait disturbances and bradykinesia) and non-motor symptoms, including sleep disturbances, mood disorders, gastrointestinal dysfunction, and autonomic dysregulation, all of which significantly impact quality of life. Emerging evidence suggests a connection between AD and gastrointestinal dysfunctions via the gut-brain axis. The intracerebroventricular (ICV) streptozotocin (STZ) model mimics sporadic AD by inducing oxidative stress and neuroinflammation, leading to the excessive production of reactive oxygen and nitrogen species (ROS/RNS) and synaptic dysfunction. *Lactobacillus casei*, in both probiotic (viable) and paraprobiotic (non-viable) forms, has shown therapeutic potential due to its ability to modulate inflammation and promote gut health. This study evaluated the effects of *L. casei* on ROS modulation in an STZ-induced AD rat model. **Materials and Methods:** Fifty female Wistar rats (110–120 days old) were used. On day 0, animals received bilateral ICV injections of saline or STZ (3 mg/3 μ L/kg) to induce the AD model, according to a protocol approved by the ethics committee (n°024/2024). They were randomly divided into four groups: (i) Control, (ii) STZ, (iii) STZ+Probiotic, and (iv) STZ+Paraprobiotic. After three days of postoperative recovery, treatments began on day 4. Daily oral administrations were performed for 14 days using *L. casei* as a probiotic (1×10^9 CFU/kg), a paraprobiotic (100 mg/kg, equivalent to 1×10^9 CFU/kg, heat-inactivated), or saline. At the end of the treatment, rats were euthanized, and intestinal tissues (jejunum and colon) were dissected and rinsed with ice-cold saline. ROS levels were quantified and analyzed using one-way ANOVA. **Results:** There was a significant increase in ROS levels in the STZ group in both the jejunum ($p = 0.0321$) and colon ($p = 0.0291$) compared to the control. Similarly, the STZ+Probiotic group showed increased ROS in the jejunum ($p = 0.0018$) and colon ($p = 0.0001$) versus control, indicating that the probiotic treatment failed to prevent STZ-induced ROS elevation. In contrast, the STZ+Paraprobiotic group exhibited a significant reduction in ROS levels in both the jejunum ($p = 0.0472$) and colon ($p = 0.0149$) compared to the STZ group. These findings suggest that STZ increases ROS in intestinal tissues, likely through mechanisms involving the microbiota-gut-brain axis and ICV-induced damage. Moreover, the differential responses between probiotic and paraprobiotic treatments highlight the superior protective effect of paraprobiotics in reducing ROS levels in both intestinal regions. **Conclusion:** ICV-STZ administration induces elevated ROS levels in the jejunum and colon. Probiotic treatment was insufficient to counteract this increase, whereas paraprobiotic supplementation significantly reduced ROS accumulation in both tissues. These findings underscore the greater antioxidative efficacy of paraprobiotics compared to probiotics in this model of intestinal oxidative injury related to the microbiota-gut-brain axis. Future research should investigate additional oxidative stress markers to further elucidate the underlying mechanisms. **Acknowledgment:** Capes, CNPQ, Unipampa and Carrefour. **Key-words:** Paraprobiotics; Alzheimer Disease; Gut-brain axis

02.048 **Neonatal Exposure to Kynurenine: Behavioral Changes Related to Schizophrenia.** Bomfim ANL, Cruz BE, Faria ACC, Nunes LED, Andrade BS, Guimarães MZP, Neves GA UFRJ, Lab Molecular Pharmacology, Brazil

Introduction: Schizophrenia is a neurodevelopmental disorder that affects approximately 21 million people worldwide. As a multifactorial disorder, its development can be influenced by different insults in the early stages of life. Among these, infections occurring during critical periods, such as prenatal and neonatal, can lead to abnormal central nervous system development, acting as a risk factor for this disorder. However, the mechanisms involved in this association are not clear. In this sense, studies demonstrate a strong correlation between early infections, neuroinflammation, and an imbalance in the kynurenine pathway (KP). This is the primary degradation pathway of the essential amino acid tryptophan, generating kynurenine (KYN) and other neuroactive metabolites, such as kynurenic acid (KYNA). Therefore, the objective of this study was to standardize a model of neonatal insult induced by KYN in our experimental conditions, focusing on behavioral changes related to schizophrenia symptoms. **Methods:** For this purpose, KYN (200 mg/kg i.p.) or PBS (15 mL/kg i.p.) were administered twice a day to Swiss mice from postnatal day (PND) 7 to 16 (CEUA-UFRJ 120/22). From PND 70 onwards, these animals were subjected to a series of behavioral tasks: spontaneous alternations, exposure to the open field, novel object recognition, splash test, social approach, prepulse inhibition of the acoustic startle response (PPI), sucrose preference, and MK-801-induced hyperlocomotion. **Results:** The results showed differences between sexes, where, regardless of exposure to KYN, females presented were more anxious in the open field (higher permanence in the periphery of the apparatus, $p = 0.047$), greater sucrose preference ($p = 0.045$), and lower startle response ($p = 0.028$) compared to males. Furthermore, neonatal exposure to KYN induced schizophrenia-related behaviors in mice, characterized by hyperlocomotion in a novel environment ($p = 0.004$), increased latency ($p = 0.024$), and decreased amplitude ($p = 0.043$) of the startle reflex. **Conclusion:** These data corroborate the causal relationship between the imbalance in the KP and some behavioral changes related to schizophrenia. Moreover, our results indicate that KP contributes to the etiology of at least part of the symptoms presented by schizophrenia patients. **Acknowledgments:** FAPERJ, CAPES, CNPq.

02.049 **Modulating Effects of Acai Extract on Neuroinflammation after Ischemic Stroke: Evaluation of Astrocytic Reactivity.** Teixeira LL^{1,2}; Ferreira LO²; Rodrigues JCM; Pinto LC²; Rogez HLG^{1,3}; Lopes DCF^{1,2} ¹ICB-UFPA, Graduate Program in Pharmacology and Biochemistry, ²UFPA, Lab Experimental Neuropathology, João de Barros Barreto University Hospital. ³UFPA, Center for the Valorization of Bioactive Compounds from the Amazon

Introduction: Inflammation has protective and deleterious effects on the central nervous system (CNS), mediated mainly by glial cells (astrocytes and microglia). This response is part of the defense system, and it is essential, however, in neurological diseases, as in ischemic stroke (IS), this inflammatory response is exacerbated and deleterious, and it can result in irreversible damage such as neuronal death, causing motor damage; and the extent of damage depends on the origin, intensity, and duration of the inflammatory process. Thus, the search for new therapeutic tools in the Amazon biome has been the focus of several studies, and products derived from *Euterpe oleracea* Mart. (EO), popularly known as açai, are considered rich source of bioactive compounds, such as anthocyanins, which act as anti-inflammatory and antioxidant properties. Our goal was used the clarified extract of açai as pharmacological tool for ischemic stroke treatment, in order to evaluate its effect on the neuroinflammatory response by quantifying reactive astrocytes in a murine model of stroke. **Methods:** For this, 22 male Wistar rats (10-12 weeks old, 300 ± 20 g) from the Central Animal Facility of the Evandro Chagas Institute (CEUA no. 1225030320; ICB/UFPA) were subjected to middle cerebral artery occlusion (MCAO) and treated with EO extract for 14 days. At the end of the experiment, the inflammatory response was assessed through histological analysis, by quantifying astrocytes labeled with anti-GFAP antibody in the penumbra regions of the somatosensory cortex and striatum ipsilateral to the lesion. Statistical analysis was performed using two-way analysis of variance (ANOVA) and Tukey's test for multiple comparisons. **Results:** No statistical difference was observed in the quantification of GFAP+ cells between the control groups (somatosensory cortex: sham [2.66±1.76] vs. sham+EO [3.87±3.04], $p > 0.98$; striatum: sham [3.33±1.35] vs. sham+EO [3.37±1.95], $p > 0.99$). In contrast, the group that underwent surgery and did not receive treatment (MCAO) showed a significant increase in astrocyte quantification for both regions compared to the control groups (somatosensory cortex: MCAO [23.89±6.90], $p < 0.05$ vs. sham and vs. sham+EO; striatum: MCAO [26.50±4.28], $p < 0.0001$, for all comparisons), suggesting an exacerbated and deleterious inflammatory response. Additionally, the group that underwent surgery and received treatment (MCAO+EO) had a similar number of astrocytes to the control groups in the somatosensory cortex (MCAO+EO [9.97±6.32], $p > 0.05$ vs. sham and vs. sham+EO), and striatum (MCAO+EO [9.85±4.59], $p > 0.05$ vs. sham and vs. sham+EO). Surprisingly, animals in the MCAO+EO group showed a significant decrease in astrocytic reactivity when compared to the MCAO group for both regions studied (MCAO vs. MCAO+EO, $p < 0.001$ for somatosensory cortex and striatum). **Conclusion:** In conclusion, the administration of *Euterpe oleracea* Mart. attenuated the inflammatory response after cerebral ischemia by inhibiting astrocytic reactivity. **Financial support:** CAPES

02.050 The Role of Cingulate Cortex Orexin-1 Receptors in the Expression and Organization of Innate Fear Carvalho MLC¹, Oliveira GVM¹, Hernandez PM², Coimbra NC², Almada RC¹ ¹FCLAs-UNESP-Assis, Dept Biological Sciences, Lab Neurobiology and Neurobiotechnology, ²FMRP-USP, Ribeirão Preto, Dept Pharmacology, Lab Neuroanatomy and Neuropsychobiology, Postgraduate Program in Medicine (Neurology), Brazil

Introduction: The ability to distinguish between aversive, neutral, and appetitive stimuli is crucial for survival. Fear and anxiety are fundamental adaptive responses, and the neurobiology of fear has been extensively studied. The orexinergic system, with orexin produced in hypothalamic subnuclei and connected to brain structures such as the anterior cingulate cortex (ACC), has garnered increasing interest. The ACC plays a critical role in the organization and expression of fear. Recent studies have linked the orexinergic system to the modulation of fear, panic, and anxiety, but its specific role within the ACC remains unclear. Therefore, this study aimed to investigate the role of orexinergic mechanisms in the Cg1 subregion of the ACC during the organization and expression of innate fear responses. Specifically, we examined the effects of selective blockade of orexin type 1 receptors (Orx1R) in the Cg1 of mice subjected to the beetle mania task (BMT). **Methods:** All experimental procedures were approved by the Animal Experimentation Ethics Committee of São Paulo State University (UNESP) (protocol no. 001/2024). C57BL/6 mice (*Mus musculus*, Rodentia, Muridae), 8–14 weeks of age, were housed under controlled conditions (12-h light/dark cycle, 22 ± 1°C, food and water available ad libitum). Experiments were conducted during the light phase (9 a.m. to 5 p.m.). Animals underwent stereotaxic surgery for unilateral implantation of a guide cannula into the Cg1. Five days after surgery, mice were assigned to experimental groups: (i) control (20% DMSO) or (ii) SB-334867 (3, 30, or 300 nM in 0.1 µl per side). Treatments were administered immediately before the beetle mania task (BMT) to assess their effects on the expression of innate fear. The BMT consisted of two successive five-minute sessions: (i) habituation to the arena, followed by (ii) a test session in which a robotic beetle was introduced. Behavioral responses, including rearing, crossings, interaction, tolerance, freezing, and escape, were recorded and analyzed. **RESULTS:** During the habituation phase, no statistically significant differences were observed among groups in the number of crossings ($F_{3,32} = 0.481$, $p = 0.697$) or rearing ($F_{3,32} = 0.688$, $p = 0.565$). In the BMT test phase, when confronted with the robotic beetle, no significant differences were found in contacts between the beetle and mice ($F_{3,32} = 1.11$, $p = 0.356$), avoidance behaviors ($F_{3,32} = 0.270$, $p = 0.846$), or close following of the robotic beetle ($F_{3,31} = 0.677$, $p = 0.572$). However, the highest dose of SB-334867 (300 nM) significantly increased the tolerance response ($F_{3,32} = 4.596$, $p = 0.0087$). **Conclusion:** Selective blockade of Orx1R in the Cg1 significantly increased tolerance to the robotic beetle, suggesting that orexinergic mechanisms in this region, mediated by Orx1R, are fundamental in modulating behavioral responses associated with innate fear. These findings highlight the orexinergic system as a promising therapeutic target and may guide the development of novel interventions for psychiatric disorders characterized by maladaptive fear processing. **Funding:** FAPESP grant number (2018/03898-1), and CAPES (88887.940007/2024-00).

02.051 *In Vitro* Effects of Exposure to Anhydroecgonine Methyl Ester (Byproduct of Crack/Cocaine) on the Viability, Morphology, and Activity of Hippocampal Astrocytes. Silva SC, Santos SO, Patrocínio TB, Leitão SGR, UNIFAL, Programa de PósGraduação em Biociências Aplicadas à Saúde, Brasil

For decades, drug abuse has been causing globally impactful complications for public health. Crack, being a relatively low-cost drug, has become increasingly accessible, especially to economically disadvantaged groups. It is a fact that crack use affects cognition; however, it is not entirely clear how the drug's main metabolite Anhydroecgonine methyl ester (AEME) affects brain function. Considering the critical events of neurotransmission, astrocytes (glial cells) have been regarded as potential targets of crack, since they play a fundamental role in synaptic function. In this context, the present study investigated the effects of *in vitro* treatment with AEME on: 1) cell viability, and 2) cell morphology, through the expression of glial fibrillary acidic protein (GFAP), 3) intracellular calcium concentrations, and 4) lactate release by cultured hippocampal astrocytes. In order to achieve these goals, primary astrocyte cultures were established from fresh hippocampal tissue collected from neonatal rats (2 to 4 days old). The cells were incubated under basal conditions and exposed to the metabolite AEME (0.1 mM) for 2 hours. All procedures were reviewed and approved by the Animal Research Ethics Committee of UNIFAL (protocol 0032/2023). The cell viability results showed that incubation with AEME produced a significant cytotoxic effect ($p < 0.001$). Incubation with AEME also significantly increased GFAP expression ($p < 0.05$), indicating an increase in cell area (astrocyte reactivity). Intracellular calcium concentrations were also significantly elevated in cells exposed to AEME ($p < 0.05$). However, AEME did not induce any changes in lactate release to the extracellular medium under the same experimental conditions. Together, these data confirm the cytotoxic potential of AEME in astrocytes and indicate that calcium may mediate, at least in part, the intracellular effects resulting from the metabolite exposure. Furthermore, the data suggest that incubation with AEME possibly does not affect lactate-mediated glia-neuron signaling. Keywords: hippocampus, synapse, calcium, GFAP, lactate Funding: CAPES and CNPq (Process: 406727/2021-0)

02.052 Early Exposure to a Synthetic Cannabinoid Effects on Anxiety and Depressive-Related Behaviors. Freire LC, Moutinho IE, Côrrea AES, Lemos LC, Souza IO, Neves GA UFRJ, Lab Molecular Pharmacology, Brazil

Adolescence is a critical period for brain development, which individuals become more susceptible to drug abuse, including cannabinoids. Synthetic cannabinoids were initially developed to study the endocannabinoid system but have become a public health concern due to their increasing abuse, particularly among adolescents. While numerous studies have focused on delta9-tetrahydrocannabinol (delta9-THC), only a few have investigated the consequences of synthetic cannabinoid exposure during adolescence in animal models. This study aimed to investigate whether juvenile exposure to the synthetic cannabinoid WIN 55,212-2 (WIN) induces long-lasting changes in anxiety and depressive-like behavior. Male Swiss mice (n = 30) were exposed to WIN (2 mg/kg/day, i.p. daily) or vehicle (DMSO 3% in NaCl 0,9%) for 10 consecutive days starting at postnatal day (PND) 28 (CEUA/UFRJ 065/24). A single dose was used and based on previous studies reporting behavioral effects in rodents at levels associated with cannabinoid dependence. Animals were weighed every other day during treatment to follow their development. In adulthood (PND > 70), the following behavioral tests were conducted: 1) splash test (n = 14), an index of self-care and anxious behavior; 2) sucrose preference (n = 12), assessing hedonic profile; and 3) elevated plus maze (n = 10), which assesses the animals anxiety level. Data were analyzed using two-way RM ANOVA and Student's t test (GraphPad Prism 8.0), and results are expressed as mean $\pm$ SEM. Our results showed no significant differences between groups in body weight gain during WIN exposure (p = 0,628). Moreover, WIN did not affect self-care behavior (p = 0,875), sucrose preference (p = 0,483), or change the anxiety level of animals in the long term (p = 0,661). These findings suggest that juvenile exposure to WIN does not induce persistent alterations in anxiety, self-care, or hedonic behavior. Additional studies are required to better understand the potential consequences of synthetic cannabinoid exposure during the critical developmental period of adolescence. **Financial support:** CAPES, CNPq, FAPERJ.

02.053 **Effects of the Prebiotic Inulin on Behavioral Changes in a Model of Autism Spectrum Disorder (ASD) In Adolescent Rats.** Amorin BM¹, Bertotti G², Haddad NM², Longo B¹, Silva LM¹
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Introduction: Autism Spectrum Disorder (ASD) is a neurodevelopmental disorder with varied manifestations touching the social, communicative, and behavioral spheres of the individual. The prevalence of ASD has significantly increased in recent years, which, to some extent, could be attributed to greater diagnostic precision and the development of more effective screening instruments. Although the pathophysiology of the disorder has not been fully elucidated, the impact of genetic and environmental factors on its etiology is recognized. There is also evidence that patients with ASD frequently present with gastrointestinal disturbances and alterations in gut microbiota as comorbidities, whose manifestation could be correlated with a contributing aggravation of the condition, via the gut-brain axis. In this context, therapeutic strategies aimed at modulating the gut microbiota, such as the use of prebiotics, have gained prominence. The present study aimed to evaluate the effects of inulin on behavioral parameters in an animal model of ASD induced by prenatal exposure to valproic acid (VPA). **Methods:** To induce an autistic phenotype in the offspring, pregnant Wistar rats received a single intraperitoneal injection of 600 mg/kg valproic acid (VPA) or saline solution on gestational day 12.5 (GD 12.5). On postnatal day 23 (PND 23), after weaning, the pups were divided into four experimental groups: Control (exposed to saline solution); Inulin (exposed to saline solution and treated with 5% inulin); VPA (exposed to VPA); and VPA + Inulin (exposed to VPA and treated with 5% inulin). Inulin was provided daily in the drinking water until PND 60. Subsequently, on PND 60, the animals were subjected to open-field and three-chamber social tests to evaluate the effects of prebiotic intervention on neurobehavioral parameters. Moreover, statistical analyses were performed using GraphPad Prism software, and the data were analyzed using two-way ANOVA followed by Bonferroni's post hoc test for multiple comparisons. CEUA/UNIVALI, protocol 001/21p2. **Results:** In females, VPA reduced the number of crossings by 50% (47.5 ± 2.8 compared to 94.0 ± 4.3 in controls) in the open-field test ($p < 0.05$). Inulin treatment (VPA + Inulin) partially reversed this effect (79.2 ± 5.2 crossings; $p < 0.05$). Rearings also decreased in VPA females (15.3 ± 1.2) compared to controls (26.5 ± 1.4 ; $p < 0.05$). In males, inulin alone reduced crossings (71.1 ± 3.7) compared to controls (91.7 ± 3.2 ; $p < 0.001$), but had no effect in the VPA + Inulin group. In the three-chamber test, VPA-exposed females exhibited more social interactions (22.3 ± 1.1) than those in the VPA + Inulin group (17.7 ± 0.9), in a non-expected manner ($p < 0.05$). Inulin-treated males showed a higher number of entries (24.3 ± 0.8) compared to the control group (21.7 ± 0.9 ; $p < 0.05$). **Conclusions:** VPA induced autistic-like signs in the offspring, according to the behavioral parameters analyzed. Young females exposed to VPA exhibited significant deficits in locomotor activity in the open-field test, which were partially prevented by inulin administration. Interestingly, VPA-exposed males and females also showed an increase in social interactions in the three-chamber test, with the therapeutic effects of inulin differing between sexes. The findings suggest that the isolated use of this prebiotic is not entirely effective in reducing ASD signs, showing variations according to the animal's sex. **Financial support:** CAPES.

02.054 *Baccharis dracunculifolia* Extract Induces Anxiolytic-Like Behavior and Enhances Brain Antioxidant Activity in Male Rats. Souza RML¹, Batista MAC², Silva AO¹, Moura SAL³, Cardoso LM², Oliveira LB¹, Ceron CS² ¹UNIFAL, Dept Food and Medicine, PPG Pharmaceutical Sciences, Brazil, ²UFOP, Research Center in Biological Sciences, PPG Cipharma, Brazil, ³UFOP, Dept Biological Sciences, PPG Cipharma, Brazil

Introduction: Anxiety disorders affect millions of people worldwide and are often associated with increased oxidative stress in the central nervous system (CNS). Although pharmacological treatments are available, their adverse effects and potential for dependence represent important limitations. In this context, medicinal plants have gained attention as promising therapeutic alternatives. Studies indicate that *Baccharis dracunculifolia*, in addition to other properties, exerts neuroprotective effects by reducing oxidative stress markers in the brain, thereby contributing to the attenuation of oxidative damage in this region. Therefore, this study aimed to investigate, for the first time, the anxiolytic and antioxidant effects of chronic intragastric administration of *B. dracunculifolia* extract at a dose of 50 mg/kg (BD 50) in healthy male Wistar rats. **Methods:** Twenty-nine animals were randomly assigned to three groups and treated orally once daily for 17 days with distilled water (vehicle, n = 9), BD 50 (n = 10), or fluoxetine 10 mg/kg (positive control, n = 10). Behavioral tests, including the open field test (OFT), elevated T-maze (ETM), and light-dark transition test (LDT), were conducted on the 15th day of treatment. On day 18, the animals were euthanized with ketamine (300 mg/kg) and xylazine (30 mg/kg), and brains were collected for oxidative analyses: superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and thiobarbituric acid-reactive substances (TBARS). **Results:** In the behavioral evaluation, chronic treatment with BD 50 significantly reduced avoidance 1 latency in the ETM compared to the vehicle (p = 0.0231), similarly to the positive control, indicating an anxiolytic effect. However, unlike the positive control, it did not increase escape latency (p > 0.05). In the OFT, BD 50 increased locomotor activity (p = 0.0275), the number of entries (p < 0.0001), and the time spent in the central area (p = 0.0004), reinforcing the anxiolytic effect, as also observed with the positive control. In the LDT, no significant differences were found in the number of transitions or the time spent in the light compartment between groups (p > 0.05). Regarding oxidative parameters, BD 50 significantly increased the activity of the antioxidant enzymes SOD (p = 0.0263) and CAT (p = 0.0452), while GSH and TBARS levels remained unchanged compared to the vehicle group (p > 0.05). **Conclusion:** In conclusion, these findings support the potential of *B. dracunculifolia* as a therapeutic agent with anxiolytic and antioxidant properties in the CNS. This experimental protocol was approved by the Ethics Committee on Animal Use of the Federal University of Alfenas (0014/2023). **Financial support:** Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, 302076/2022-0), Fundação de Amparo à Pesquisa do Estado de Minas Gerais, Brazil (FAPEMIG, APQ-00946-23), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES, Financial Code 001) and Samarco Mineração S.A. (Samarco - UFOP agreement #23109.002909/2022-11).

02.055 Effects of Maternal Melatonin Deficiency on Klotho Protein Expression in the Cerebellum of Male and Female Rat Offspring. Silva, JRM¹, Aliaga ALB², Lima PH², Tida-Oliveira CH², Andreotti, DZ¹, Sandoval, MRL², Cipolla-Neto J³, Amaral FG⁴, Abdalla FMF², Afeche SC², Kawamoto, EM¹ ¹ICB-USP, Lab Molecular and Functional Neurobiology; ²IB, Lab Pharmacology; ³ICB-USP, Pineal Gland Neurobiology Lab; ⁴Unifesp, Dept Physiology

Melatonin is a hormone synthesized by the pineal gland at night, thus acting as a temporal synchronizer of the physiology and behavior of animals. Studies have shown that maternal melatonin deficiency during pregnancy and lactation can lead to alterations in neural development and result in long-term negative health outcomes. The Klotho protein is expressed primarily in the kidney, choroid plexus, and cerebellum, and exists in both membrane-bound and soluble forms. The membrane-bound form is cleaved by proteases, releasing a soluble form that is found in blood, urine, and cerebrospinal fluid. The Klotho protein has anti-aging properties. In humans, Klotho levels decline with age and in several age-related diseases, such as diabetes, Alzheimer's disease, and other conditions. Conversely, overexpression of the Klotho gene has been shown to extend lifespan in mice, improve cognitive function, and play a suppressive role in cancers. The aim of this study was to investigate the role of maternal melatonin in the expression of Klotho protein in the cerebellum of adult male and female rat offspring in which gestation and lactation occurred under hypomelatoninemia conditions. Klotho protein expression was evaluated in the cerebellum of male and female offspring during the light and dark periods. Three experimental groups were made: 12 maternal rats were pinealectomized (PINX), 6 maternal rats were pinealectomized and had melatonin replacement (PINX + MEL) and 6 maternal rats were used as control (CTR). Melatonin was replaced in the drinking water during the dark period for the PINX + MEL group, after surgery and during gestation and lactation, at a dose of 0.1 mg/kg of body weight, being adjusted weekly. Mating was performed one month after surgery. Ninety-day-old male and female offspring were euthanized at ZT2 (2 h after the onset of the light period) and at ZT14 (2 h after the onset of the dark period). Klotho protein concentration in cerebellar tissue was measured using Klotho ELISA kit (R&D Systems), according to the manufacturer's instructions (protocol nº 9805191021). The absence of maternal pineal melatonin during gestation and lactation (PINX) induced an increase in Klotho protein expression in the cerebellum of males and a reduction in females at ZT2 when compared to the control group. On the other hand, melatonin hormone replacement (PINX+MEL) reversed the alterations induced by maternal melatonin deficiency in males, but not in females. At ZT14, there was no difference for males and females between the different groups. Our results suggest that the absence of maternal melatonin during pregnancy and lactation influences the expression of Klotho protein in the cerebellum of male and female offspring in adulthood in a sex- and time-of-day-dependent manner. These findings indicate that melatonin is involved in the fetal programming of cerebellar function, which may have implications for cognitive performance. Support from CAPES and FAPESP 9805191021

02.056 Activation of GPER Promotes Functional Recovery and Modulates Neuronal Degeneration Following Transient Focal Cerebral Ischemia in Male Rats. Jucá PM1 Munhoz CD LaNEFI-ICB-USP, Neuroendocrinepharmacology and immunomodulation Lab, Dpt of Pharmacology

Introduction: Stroke is among the leading causes of death and disability worldwide, with limited therapeutic options for promoting neuroprotection and functional recovery (Feigin, VL. The Lancet Neurol., v. 20, p. 795, 2021). There are gender differences in the incidence and progression of the disease, with males being more affected and having a worse prognosis than females (Roy-O ´Reilly, M. Endocrinology, v. 159, p. 3120, 2018). In this context, estrogen (E2) has been shown to have a neuroprotective effect in numerous pathologies (McEwen, BS. Endocrine Reviews, v. 20, p. 279, 1999). Several studies have explored the role of the G protein-coupled estrogen receptor (GPER) in significant conditions since its activation generates rapid and non-feminizing responses. And G1, selective GPER agonist, has emerged as a useful pharmacological tool to investigate the effects of GPER activation. However, its role in post-ischemic recovery remains poorly understood. This study aimed to evaluate the effects of GPER activation on behavioral outcomes and neuronal degeneration following transient middle cerebral artery occlusion (tMCAO) in rats. **Methods:** To this end, male Wistar rats (300 ± 30 g) were subjected to tMCAO for 30 minutes and subsequently treated with G1 (30 µg/kg, subcutaneously), a selective GPER agonist, or vehicle (DMSO) once daily for 7 consecutive days (n = 8 animals per group). Neurological, motor, and cognitive functions were assessed throughout the treatment period. After 7 days of treatment and behavioral testing, animals were euthanized, and brain tissue was collected for histological analysis. Neuronal degeneration was assessed using Fluoro-Jade C (FJC) staining. **Results:** G1 treatment significantly increased the survival rate (p = 0.0075), improved neurological scores (p = 0.0294), and reduced motor deficits in the beam walking test from the third day of treatment. Furthermore, G1 appeared to rescue spatial memory in the novel object recognition task. In the short-term memory test, treated animals exhibited a higher discrimination index than vehicles (p = 0.0405). However, a reduction was observed when the object ´s location changed (p = 0.0012), indicating altered spatial recognition. This alteration likely reflects integrity of the dorsal hippocampus, although it is important to note the complex interplay with the prefrontal cortex, which also contributes to spatial orientation. Qualitative FJC analysis suggested that G1 may influence hippocampal neuronal degeneration dynamics, potentially delaying or redirecting the cell death process. **Conclusion:** These findings support a neuroprotective and functionally beneficial role of GPER activation after cerebral ischemia. G1 treatment promoted survival, improved behavioral recovery, and may modulate neuronal death, highlighting GPER as a potential therapeutic target in ischemic stroke in males. Ongoing investigations aim to clarify the molecular mechanisms involved in cognitive and motor recovery after G1 treatment post-stroke. **Financial support information:** This work was supported by research grants from FAPESP (2022/08767-8), CNPq and CAPES.

02.057 Antioxidant and Anticholinesterase Activities of a Semisynthetic Derivative of (–)-Myrtenol as a Potential Therapeutic in Alzheimer’s Disease. Souza RP¹, Freitas MM², Fernandes GS², Oliveira MYCM¹, Pimentel VD³, Nobre TA⁴, Nascimento MLLB⁴, Furtado PVOS⁵, Feitosa CM⁵, Castro e Sousa JM⁴, Viana AFSC⁶, Kutyla M⁷, Trytek M⁷, Ferreira PMP², Oliveira AP¹ ¹UFPI, Lab Cardiovascular Pharmacology (Lafac), Brazil; ²UFPI, Lab Experimental Cancerology (LabCancer), Brazil; ³UFPI, Lab Inflammation Pharmacology (LAFIN), Brazil; ⁴UFPI, Lab Toxicological Genetics (Lapgenic), Brazil; ⁵UFPI, Lab Natural Products and Experimental Neurochemistry (Lpnnex), Brazil; ⁶UEMA, Campus Santa Inês, Brazil; ⁷Maria Curie-Skłodowska University, Lublin, Poland

Introduction: Alzheimer’s disease (AD) is a chronic neurodegenerative disorder marked by memory loss, cognitive decline, and neuroinflammation. Its pathogenesis involves β -amyloid accumulation, tau hyperphosphorylation, and neuronal death. Natural compounds such as (–)-myrtenol have demonstrated neuroprotective potential (Aktary et al., 2025). **Objective:** This study aimed to evaluate the antioxidant, chemopreventive, cytotoxic, and anticholinesterase properties of a semisynthetic (–)-myrtenol derivative (D-Myrt). **Method:** D-Myrt was obtained via fungal biotransformation (Lublin, Poland). Molecular docking was conducted using AutoDock Vina and BIOVIA Discovery Studio against AChE and NMDA receptors. *In vitro* cytotoxicity was tested on U-87 glioblastoma cells via MTT assay (Mosmann, 1983). Hemolysis was assessed using 5% sheep erythrocyte agar (Sæbø et al., 2023), and acute toxicity in *Artemia salina* nauplii (Meyer et al., 1982). Antioxidant modulation was tested in *Saccharomyces cerevisiae* mutants under H₂O₂ stress (Oliveira et al., 2014). AChE inhibition was measured spectrophotometrically. All tests were performed with $n = 3$, in triplicate. Data were analyzed using one-way ANOVA with Student-Newman-Keuls post hoc ($\alpha = 0.05$). **Results:** D-Myrt showed higher binding affinity for AChE ($\Delta G = -95.9 \pm 4.4$ kcal/mol) and NMDA receptors ($\Delta G = -61.0 \pm 4.2$ kcal/mol) compared to reference drugs rivastigmine (-69.9 ± 3.0 kcal/mol) and memantine (-45.16 ± 1.43 kcal/mol). It exhibited no acute toxicity in *A. salina* ($LC_{50} > 1000$ μ M), but selectively inhibited U-87 cell viability ($IC_{50} = 6.63$ μ M; 95% CI: 4.67–9.31 μ M). No hemolysis was observed, indicating preserved membrane integrity. D-Myrt effectively mitigated H₂O₂-induced oxidative stress in all *S. cerevisiae* strains at 6.25–800 μ M, with no pro-oxidant effects. AChE inhibition was confirmed ($IC_{50} = 61.6$ μ M; 95% CI: 52.71–70.45 μ M). **Conclusion:** D-Myrt displayed dual-target activity (AChE/NMDA receptors), antioxidant efficacy, and selective cytotoxicity, supporting its potential as a therapeutic candidate for AD. Further *in vivo* and pharmacokinetic studies are needed to confirm its clinical utility. **Funding:** FAPEPI, CAPES, CNPq. **References:** Aktary, N. et al. (2025). *Front. Pharmacol.*, 16, 1601712. <https://doi.org/10.3389/fphar.2025.1601712> Meyer, B. N. et al. (1982). *Planta Med.*, 45(5), 31–34. <https://doi.org/10.1055/s-2007-971236> Mosmann, T. (1983). *J. Immunol. Methods*, 65(1-2), 55–63. [https://doi.org/10.1016/0022-1759\(83\)90303-4](https://doi.org/10.1016/0022-1759(83)90303-4) Oliveira, G. L. S. et al. (2014). *Afr. J. Pharm. Pharmacol.*, 8(5), 136–147. <https://doi.org/10.5897/AJPP2013.3836> Sæbø, I. P. et al. (2023). *Int. J. Mol. Sci.*, 24(3), 2914. <https://doi.org/10.3390/ijms24032914>

02.058 **Redox Imbalance and Rescue: Very Long Chain Fatty Acids Drive Neuroinflammation in X-ALD, Reversed by Monomethyl Fumarate.** Pôças P¹, Gomes A¹, Mendes F², Moraes JA¹ ¹UFRJ, Lab. of Redox Biology, Brazil, ²UFRJ, Lab. of Cell Signaling and Mechanobiology, Brazil

Introduction AND OBJECTIVES: X-linked Adrenoleukodystrophy (X-ALD) is a genetic disorder marked by the accumulation of very long-chain fatty acids (VLCFAs), leading to neuroinflammation and progressive demyelination. Microglia, the resident immune cells of the central nervous system, play a central role in this inflammatory response. Monomethyl fumarate (MMF), known for antioxidant and anti-inflammatory properties, is a promising candidate for the treatment of neuroinflammation and oxidative stress in X-ALD. This study aimed to assess the cytotoxic impact of VLCFAs on microglial cells and to evaluate MMF's potential to counteract this effect through its redox-modulating properties. **Methods:** Primary microglial cells were isolated from cerebral cortices of neonatal male Swiss mice (CEUA 015/24). Animals were housed in groups of five per cage with free access to food and water, under a 12 h light/dark cycle, with controlled temperature and humidity. Mixed glial cultures were maintained at 37°C with 5% CO₂, and after 14 days, microglia were collected and cultured for two weeks in DMEM/F-12 with 10% fetal bovine serum, 1% antibiotics and 1% glutamine. For cell viability assays (n=3), microglial cells were seeded in 96-well plates and exposed for 24h to VLCFAs - tetracosanoic acid (C24:0 - 30, 300 and 3000 nM) and hexacosanoic acid (C26:0 - 500, 5000 and 50000 nM) - either alone or co-administered with MMF (100 µM). VLCFAs were solubilized in ethanol and MMF in DMSO, diluted to ≤0.1% in medium. MTT (0.5 mg/mL) was added and incubated for 4 h at 37°C; absorbance was measured at 570 nm. To evaluate oxidative stress (n=3), mitochondrial reactive oxygen species (ROS) and peroxynitrite (ONOO⁻) were assessed using MitoSOX™ Red (5 µM) and aminophenyl fluorescein (APF; 5 µM), respectively. Cells were seeded in black-walled 96-well plates and probes were incubated for 1 h at 37°C in the dark. Fluorescence was measured (MitoSOX: 580 nm; APF: 515 nm) for 2 h. Background fluorescence was subtracted, and values were normalized to untreated cells. Comparisons between groups were performed using ANOVA one-way test, followed by Bonferroni post-test. Statistical evaluation was performed using GraphPad Prism software version 7.0. **Results:** Exposure to increasing concentrations of VLCFAs, within pathological range reported in X-ALD patients, led to a concentration-dependent decrease in microglial cells viability (20 - 35% ±10 SEM). Co-administration of MMF significantly attenuated VLCFA-induced cytotoxicity, restoring cell viability to near-baseline levels. Consistent with these findings, VLCFA induced marked increases in mitochondrial ROS (2 - 2.5 fold change ±0.2 SEM) and ONOO⁻ production (2 fold change ±0.1 SEM), both significantly suppressed by MMF co-treatment, demonstrating its potent antioxidant and neuroprotective effects in this model. **Conclusion:** Collectively, these findings show that VLCFAs induce significant cytotoxicity and oxidative stress in microglia, reflecting key pathological features of X-ALD. MMF exhibits robust cytoprotective and antioxidant effects, counteracting VLCFA-induced damage. Overall, the data support MMF's therapeutic potential as a modulator of neuroinflammation in X-ALD and encourage further clinical investigation. **Financial Support:** FAPERJ, Capes and CNPq.

02.059 Nicotine-Induced Conditioned Place Preference: Different Responses Between Female and Male Mice. Lemos LC, Corrêa AE, Costa LF, Nunes LED, Souza INO, Neves GA UFRJ, Lab Molecular Pharmacology, Brazil

Smoking is a global concern that affects public health and society. Smoking is intrinsically linked to nicotine addiction, a chemical compound found in the leaves of *N. tabacum*. When consumed, nicotine interacts with nicotinic acetylcholine receptors, increasing the release of excitatory neurotransmitters and dopamine in the mesolimbic area, inducing a reward response. These effects are related to its abuse potential. Epidemiological data indicate differences between men and women related to smoking habits. However, the differences in the response to nicotine between sexes are not fully clarified. In this work, the conditioned place preference test (CPP) was used to investigate differences in the response to nicotine between males and females. Adult Swiss mice (PND > 70) were housed in groups of 3-5 mice/cage at the Animal Experimentation Unit of ICB/UFRJ, at $23 \pm 2^\circ\text{C}$ and humidity at $60 \pm 5\%$, under a 12-hour light-dark cycle (lights on at 6:00 a.m.) (CEUA CCS/UFRJ 113/23). All animals were evaluated in the nicotine-induced CPP protocol as follows. During the preconditioning session, animals remained in the CPP apparatus for 15 min to establish their basal preference for one of the compartments. This information is required for using a biased protocol. The conditioning phase consisted of two daily sessions of 30 min each, for seven consecutive days, when animals were conditioned with nicotine (0.05 mg/kg, 0.1 mg/kg, or 0.5 mg/kg s.c.; $n = 11-12$ females and $13-14$ males/group) or received only vehicle (NaCl 0.9%, $n = 9$ females and 10 males/group). Finally, the postconditioning session lasted for 15 min and aimed to evaluate the development of a conditioned preference or avoidance to the compartment associated with the drug. All sessions were videotaped, and the permanence time in each compartment apparatus was recorded by a researcher blind to the experimental group. The effects of nicotine withdrawal on mice locomotion were assessed by recording the total number of compartment entries in the post-conditioning session. Data were analyzed using Student's t-test for paired samples comparing data from the pre- vs. post-conditioning sessions (GraphPad Prism v.8 software). Results showed a significant preference for the nicotine 0.1 mg/kg paired compartment in both males ($40.9 \pm 2.0\%$ vs. $52.4 \pm 3.8\%$, $p = 0.023$) and females ($44.9 \pm 0.6\%$ vs. $53.4 \pm 2.7\%$, $p = 0.006$). Nicotine at 0.5 mg/kg and 0.05 mg/kg doses did not induce preference or aversion to the paired compartment. During the post-conditioning session, females exposed to nicotine 0.05 mg/kg (40.7 ± 5.9 entries vs. 65.0 ± 4.9 entries, $p = 0.009$) and 0.1 mg/kg (46.2 ± 3.3 entries vs. 61.1 ± 3.4 entries, $p < 0.001$) exhibited hyperlocomotion. In comparison, males exposed to nicotine 0.5 mg/kg expressed a decrease in the number of compartment entries (37.6 ± 2.8 entries vs. 29.6 ± 3.8 entries, $p = 0.047$). In summary, our data shows that some nicotine-induced effects, especially those related to nicotine withdrawal, may vary according to sex. **Financial support:** CAPES, FAPERJ, CNPq.

02.060 Acute Administration of Fluoxetine Reverses Stress-Induced Deficits in Contextual Fear Extinction in Rats. Franzoni ACM, Moraes-Neto TB, Belem-Filho IJA Furtado ASJ, Bertacchini GL, Resstel LBM FMRP-USP, Dept Pharmacology

Introduction: In threatening situations, the organism responds through behavioral and physiological reactions aimed at confronting the threat. These responses are strongly associated with aversive memories. The Contextual Fear Conditioning (CFC) model is used to study the association between neutral and aversive stimuli, forming conditioned responses that can be extinguished. In disorders such as Post-Traumatic Stress Disorder (PTSD), there are deficits in both the formation and extinction of these memories. Fluoxetine (FLX), a selective serotonin reuptake inhibitor (SSRI), is employed in the treatment of PTSD, promoting the extinction of aversive memories. The stress-re-stress (SRS) model is used to examine the effects of continuous stress on fear memory extinction.

Methods: Male Wistar rats (240-280 g) were divided into a control group and a stress group (SRS). The SRS group was subjected to a stress protocol consisting of 2 h of restraint in a tube, 20 min of forced swimming and ether exposure until fainting. After 7 days, the animals were exposed to an additional 20 min of forced swimming. Both groups were then exposed to a context across three sessions: conditioning (3 electric shocks to the paws; 0.65 mA/1 s), extinction (30 min of re-exposure without shocks), and testing (10 min of re-exposure without shocks). A polyethylene catheter was implanted in the femoral artery to record cardiovascular parameters. On the day of testing, 24 h after catheter implantation, freezing and autonomic responses, such as mean arterial pressure (MAP) and heart rate (HR) were evaluated during the test session. The FLX (20 mg/kg) was administered 30 min prior to the extinction session. **Results:** No significant differences were observed in the conditioning ($F(12, 225) = 0.33$; $p > 0.05$) or extinction sessions ($F(27, 450) = 1.51$; $p > 0.05$) ($n=12-14$). However, during the test session, the SRS group exhibited increased freezing responses ($F(3, 45) = 4.8$; $p < 0.05$), along with heightened MAP ($F(42, 630) = 4.72$; $p < 0.05$) and HR ($F(42, 630) = 12.15$; $p < 0.05$), compared to the control group. Additionally, FLX treatment reduced these increases, decreasing the freezing response ($p < 0.05$) and attenuating the elevation in MAP ($F(3, 45) = 10.1$; $p < 0.05$) and HR ($F(3, 45) = 22.36$; $p < 0.05$). Conversely, FLX did not induce significant changes in the control animals. **Conclusion:** The results indicate that the extinction deficit in contextual fear, induced by stress, is reduced through acute FLX administration before the extinction session. **Financial support:** CAPES, CNPq and FAPESP. Animal Research Ethics Committee: Protocol N° 1233/2023R1.

02.061 **Hemodynamic and Histological Alterations Caused by Venom of the Coralsnake (*Micrurus spixii*) in Anesthetized Rats.** Ramos JS^{1,2}, Macedo TCS^{1,2}, Takabayashi LA^{1,2}, Matos-Silva H³, Silva Júnior NJ², Hyslop S¹. ¹FCM-UNICAMP, Depto de Farmacologia, PPG Farmacologia, Brazil; ²PUC-GO, PPG em Ciências Ambientais e Saúde, Brazil; ³NUPMA-UNIRV-Goiânia, Núcleo de Pesquisa da Faculdade de Medicina, Brazil

Introduction: Coralsnake (*Micrurus* spp.) venoms can cause cardiovascular alterations in experimental animals. The venom of the Amazonian coralsnake *Micrurus spixii* causes blockade in vertebrate isolated neuromuscular preparations *in vitro* that is mediated by α -neurotoxins (three-finger toxins, 3FTx) and presynaptically-active phospholipases A₂ (PLA₂). In contrast, the cardiovascular actions of this venom have not been investigated. In this work, we examined the hemodynamic and systemic histological alterations caused by *M. spixii* venom in anesthetized rats and assessed some of the mediators involved. **Methods:** Male Wistar rats were anesthetized with isoflurane and cannulated for hemodynamic measurements, with the parameters being monitored continuously (LabChart software v.7). Venom was dissolved in 0.9% NaCl and injected i.v. 4-Aminopyridine (a voltage-gated K⁺ channel (K_v) blocker; 3.5 mg/kg), atropine (a non-selective muscarinic receptor antagonist; 2 mg/kg), and L-NAME (a non-selective nitric oxide (NO) synthase inhibitor; 20 mg/kg) (Sigma-Aldrich) were injected i.v. in 100 μ l of 0.9% NaCl 15-20 min before venom. At the end of the experiments, the lungs, heart and kidneys were collected, fixed in formalin and processed for histological analysis. The experimental protocols were approved by an institutional Committee for Ethics in Animal Use (CEUA/UNICAMP, protocol no. 6260-1/2023). The data were expressed as the mean $\pm$ SD and statistical comparisons were done using one-way ANOVA followed by the Tukey test, with $p < 0,05$ indicating significance. All data analyses were done using Prism software v.5 (GraphPad). **Results:** A venom dose of 0.3 mg/kg (n=3) caused minimal hemodynamic and cardiac alterations, whereas doses of 1 mg/kg and 3 mg/kg caused irreversible hypotension and death in 15.1 $\pm$ 2.0 min (n=5) and 10.7 $\pm$ 2.2 min (n=5), respectively (no significant difference). These doses also caused ventricular arrhythmias, progressive bradycardia, and bradypnea. The hypotension with 1 mg/kg was biphasic: from 84.2 $\pm$ 9.5 mmHg, to 45.5 $\pm$ 5.5 mmHg after 5 min, and partial recovery to 65.1 $\pm$ 14.9 mmHg after 10 min (n=5 each) and then hypotension to death. Histological analysis indicated variable degrees of cardiac, pulmonary and renal edema, hyperemia and hemorrhage, but no tissue necrosis. Pretreatment with L-NAME and atropine abolished the secondary recovery of hypotension, with death occurring in 11.6 $\pm$ 3.5 min and 8.6 $\pm$ 1.5 min (n=5 each), respectively, without affecting the ECG alterations. Aminopyridine slightly delayed but did not prevent death (survival time: 15.0 $\pm$ 1.5 min, n=5). Overall, the antagonists did not attenuate the cardiovascular alterations. **Conclusions:** *Micrurus spixii* venom caused marked hypotension and was lethal to anesthetized rats at doses ≥ 1 mg/kg, in addition to causing tissue damage. The hypotension was not attenuated by blocking NO synthesis, muscarinic receptors, or K_v channel activation.

02.062 Preclinical Evaluation of the Therapeutic Potential of Doxycycline in Male Rats of the MAM Schizophrenia Model. Dassow PZ, Ferrarezi ESM, Mendes V, Toscano LF, Vargas L, Salum C UFABC, Post-Graduation Program in Neuroscience and Cognition, Brazil

Schizophrenia is a disorder that affects approximately 0.3% to 0.7% of the population, characterized by positive and negative symptoms, as well as cognitive deficits. One of the most current hypotheses considers schizophrenia as a neurodevelopmental disorder, with evidence of neuroinflammation. Existing treatment is based on the dopaminergic hyperfunction hypothesis and is capable of attenuating positive symptoms, but has limited effects on negative or cognitive symptoms. Based on the neuroinflammation hypothesis, a widely used animal model of schizophrenia involves the use of Methylazoxymethanol (MAM), which presents behavioral, neurochemical, and structural alterations resembling those observed in human schizophrenia, such as dopaminergic, glutamatergic, and interneuron dysfunctions. The present study aimed to conduct a preclinical behavioral, biochemical, and neuroinflammatory evaluation of acute and chronic treatment with doxycycline (DOX) at a dose of 40 mg/kg administered via intraperitoneal injection (5 ml/kg) in male rats of the schizophrenia model based on neurodevelopmental impairment induced by the mitotoxin MAM. Pregnant rats received a dose of MAM (25 mg/kg) or saline via intraperitoneal injection (1 ml/kg) on gestational day 17 to generate MAM model and control group rats, and the offspring underwent a 13-day treatment with DOX or saline. All rats were subjected to three tests that assess behaviors related to positive, negative, and cognitive symptoms: the prepulse inhibition (PPI) test, the social interaction test, and hyperlocomotion in the open field. The acute test was performed one hour after the first DOX injection, and the chronic test occurred 24 hours after the final dose. On the second day of testing, hyperlocomotion was induced by the psychostimulant MK-801 (0.05 mg/kg) administered via subcutaneous injection (1 ml/kg). Results from the acute tests show that MAM model rats exhibited significant impairments in proximity and duration of proximity in the social interaction test compared to the control group. The results also indicate that treatment with DOX reversed such impairments. In the PPI and hyperlocomotion tests, the data indicate a reduction in startle response and decreased locomotion in DOX-treated rats. In the chronic test, MAM model rats exhibited increased locomotion in the open field compared to the control group. Treatment with DOX in MAM model rats significantly increased prepulse inhibition compared to untreated MAM model rats. These findings suggest that treatment with DOX may be a therapeutic approach to reduce behavioral alterations, particularly those related to the negative and cognitive symptoms of schizophrenia. Funding: UFABC. Approval from Animal Research Ethics Committee of UFABC: CEUA n° 7691231024.

02.063 **Dorsal Hippocampal Orexin 1 Receptors Modulate the Expression of Contextual Fear Conditioning in Rats.** Rodrigues JS, Santos L B, Rossato GO, Bertacchini GL, Morars-Neto TB, Resstel LBM FMRP-USP, Dept Pharmacology, Ribeirão Preto, São Paulo Brazil

Introduction: The dorsal hippocampus (DH) is a limbic system structure involved in emotional processing, learning, memory and autonomic modulation. DH plays a critical role in the expression of conditioned emotional responses (CER), characterized by freezing behavior, increased mean arterial pressure (MAP) and heart rate (HR), and decreased cutaneous temperature (CT). The orexin system consists of neurons located in the lateral hypothalamus, which produce the neuropeptides orexin A and orexin B. These neuropeptides act on two specific receptors: OX1R and OX2R. The projections from these neurons are widely distributed throughout the central nervous system, including the DH. Alterations in orexinergic signaling have been linked to various disorders, including substance use disorders, anxiety, and depression. **Methods:** Male Sprague-Dawley rats (240-280g) were implanted with guide cannulas bilaterally into the DH. Six days after surgery, the animals were subjected to a conditioning protocol consisting of 3 inescapable footshocks (0.85 mA/2s) delivered at random intervals between 20 and 60 s. Twenty-four hours later, a polyethylene catheter was implanted in the femoral artery to record cardiovascular parameters. On the test day, 24 h after catheter implantation, behavioral and autonomic responses, including MAP, HR and CT were recorded for 10 min. Microinjections of 0.2 μ L of the SB334867, an OX1R antagonist (0.1 nmol, 1 nmol and 10 nmol), the TCS OX2 29, an OX2R antagonist (10 nmol) or vehicle were administered into the DH 10 min before testing. **Results:** The antagonism of OX1R receptors (1 nmol and 10 nmol; $n=7$) significantly reduced freezing behavior ($F(3, 31) = 15.13$; $p < 0.05$), whereas the dose of 0.1 nmol ($p > 0.05$; $n=10$) did not produce a significant effect. Additionally, OX1R antagonism decreased the increases in MAP ($F(42, 434) = 3.41$; $p < 0.05$) and HR ($F(42, 434) = 4.09$; $p < 0.05$), and attenuated the drop in CT ($F(42, 434) = 5.2$; $p < 0.05$) compared to the vehicle group ($n=11$). Conversely, antagonism of OX2R receptors (10 pmol) did not induce significant changes in any of the measured parameters, including freezing behavior ($t=1.9$; $p > 0.05$), MAP ($p > 0.05$), HR ($p > 0.5$), or CT ($p > 0.5$). **Conclusion:** The results indicate that DH OX1R receptors are involved in the modulation of CER, whereas OX2R receptors do not exert a significant influence on these responses. These findings reinforce the role of the DH in regulating CER, including the purinergic system via OX1R receptors, as an integral part of the neurochemical interaction within this structure. Animal Research Ethics Committee: nº 1358/2024 **Financial support:** CAPES, CNPq and FAEPA.

02.064 **Sex vs Stress: Their Role in a Mice Model of Depression.** Silva ACF, Barros RP, Cardoso AR, Souza RV, Neves, GA UFRJ, Lab Molecular Pharmacology, Brazil

Introduction: Major depression is a multilayered mental disorder that can compromise one's quality of life and affects approximately 300 million people worldwide. It is known that exposure to stress is related to the etiology of this disorder. Consequently, animal models that explore this relationship are a useful tool in research. However, most preclinical studies only include males, even though mood disorders such as depression and anxiety affect twice as many women as men. Therefore, the aim of this study is to assess the effects of a Chronic Unpredictable Mild Stress (CUMS) mouse model, while evaluating and comparing the differences and similarities between the sexes.

Methods: Adult Swiss mice of both sexes were divided into groups that were exposed to a series of environmental stressors for 4, 6, or 8 weeks, or control groups (n=10-16 per group). Five stressors were used per week, for five days a week, including strobe light, white noise (90dB), food or water deprivation, restraint, overnight lighting, predator odor exposure, cage tilting (45°), wet or no bedding. All animals were kept at 23 ± 2 °C on a 12h light-dark cycle, with water and food ad libitum except when stress was applicable. After each period of stress, the animals underwent behavioral tests that evaluate depressed and anxious-like phenotypes, including sucrose preference, social approach, open-field, and forced swimming tests (CEUA/UFRJ 100/19). Two-way analysis of variance (ANOVA) was used to compare data between experimental groups, using sex (male or female) and stress (control or CUMS) as independent factors, followed by Tukey's post-hoc test. GraphPad Prism software (GraphPad® v. 8.0.2) and Sigma Stat v. 11 (Jandel Scientific Corporation®) were used. **Results:** Our data showed that male mice exhibited a higher preference for sucrose than females (4 weeks: $p=0,004$; 6 weeks: $p=0,035$; 8 weeks: $p=0,003$). In addition, female mice showed reduced immobility time in the forced swimming test compared to males (4 weeks: $p<0,001$; 6 weeks: $p<0,001$). Considering the stress factor, female mice were more susceptible to the anxiogenic effects of the stress protocol, since they exhibited reduced exploration of the center of the open field apparatus after 6 ($p=0,020$) and 8 weeks ($p=0,003$) of stress exposure. Moreover, in the 8 weeks CUMS groups, stress-exposure increased immobility time in forced swimming in both sexes ($p=0,012$), successfully inducing a depressive phenotype. **Conclusion:** In general, male and female mice responded differently to different periods of CUMS exposure, highlighting the importance of including both sexes in research and recognizing the key role the sex factor plays in various protocols. Acknowledgments: FAPERJ, CAPES, CNPq.

02.065 Neuroprotective Effects of Environmental Enrichment in a PTZ-Induced Seizure Model.

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Epilepsy is a chronic disease characterized by spontaneous and recurrent epileptic crises and affects around 50 million people throughout the world. Approximately 30 to 40% of patients are resistant to existing drugs, underscoring the need for research on new therapeutic approaches, such as environmental enrichment (EE). EE may benefit epileptic patients being used in a combined therapy style with the commonly used pharmacological treatment. However, information on whether the moment of intervention use affects its efficacy is not widely elucidated, hence the need for this research. Also, comparisons of EE with existing anti-epileptic drugs are scarce. This study aimed to investigate, in two different moments, whether EE can positively impact crisis severity and latency. Male Wistar rats were randomly assigned to six experimental groups: (1) EE before epileptic seizure induction (EEB); (2) EE during seizure induction (EED); (3) negative control group without enrichment (C); (4) EE combined with dexamethasone treatment at 1 mg/kg (DX1); (5) EE combined with dexamethasone treatment at 2 mg/kg (DX2); and (6) EE combined with saline treatment (placebo group, S). The EE groups had bigger housing, nesting materials, wooden and calcium structures, and “toys”, as well as a hammock for rest or hiding. The EEB group was enriched for 30 days before the start of seizure induction. Statistical analyses were performed using SPSS (v.18). Group comparisons were conducted using the Kruskal-Wallis test, followed by Dunn's post hoc test with Bonferroni correction for multiple comparisons. All animals were subjected to a pentylenetetrazole (PTZ)-induced seizure model over 14 days. PTZ was administered on alternating days, totaling seven injections. This study was approved by the Animal Research Ethics Committee (CEUA) under the number 44316. Following each administration, animals were monitored for 20 minutes to assess seizure severity, based on the Racine scale, and to determine the latency to seizure onset. Preliminary results obtained revealed that, on the 4th day of PTZ administration, the latency for the onset of seizures was greater in the EED group compared to the EEB group ($p=0.017$). Also, on the 6th day of PTZ administration, latency was longer in the EED group compared to both the EEB group ($p<0.01$) and C group ($p=0.044$). There were no differences between other groups or at other moments. Regarding the severity of the seizures, a protective effect was observed in the last two days of PTZ administration. On the 6th ($p=0.026$) and 7th days ($p=0.018$), the EED group had fewer severe crises than the C group. Current findings indicate that EE exerted a beneficial effect during seizure induction, reducing seizure severity and increasing latency to onset.

02.066 **Involvement of Glutamatergic and Nitrergic System in Memory Processes Deficits and Aversion Induced by Stress.** Solovi SR, Valera Sanchez CC, Bertacchini GL, Resstel LBM Dept Pharmacology, FMRP-USP, Ribeirão Preto, São Paulo, Brazil

Introduction: A consolidation and evocation of aversive memories are fundamental for the survival of organisms, involving several brain structures and a complex interaction between neurotransmitters. However, the exacerbation of reactions associated with these memories leads to the development of psychiatric disorders, such as post-traumatic stress disorder (PTSD). Thus, the aim of this study was to evaluate the involvement of the glutamatergic and nitrergic system in the consequences of stress on the extinction process of aversive memory in mice. **Methods:** Eight-week-old C57BL/6 mice were divided into a control group and a stressed group. The stressed group was subjected to a stress protocol consisting of 20 minutes of restraint in a tube, 20 minutes of forced swimming, and exposure to ether until fainting. After 7 days, the animals underwent 20 minutes of forced swimming. The animals of both groups were exposed to a context in three sessions: conditioning (three electric shocks on the paws; 0.85 mA/2 s), extinction (21 minutes of re-exposure without shocks), and testing (5 minutes of re-exposure without shocks). Thirty minutes before the extinction session, the animals received 0.2 mg/kg, 0.05 mg/kg, 0.02 mg/kg and 0.01 mg/kg of MK-801 (NMDA antagonist), 5 mg/kg and 15 mg/kg of 7-NI (selective inhibitor of nNOS) systemically or vehicle. **Results:** In the test session, stressed animals vehicles (n= 14) exhibited a deficit in the extinction process, characterized by longer freezing times compared to control animals vehicles (n=16). The doses of 0.01 mg/kg (n=16), 0.02 mg/kg (n=16), 0.05 mg/kg (n= 17) of MK801 in the stressed group had no effects on the percentage of freezing ($p>0.05$) on the test session, when compared to the vehicle stressed group. However, the dose of 0.2 mg/kg of MK801 (n=10) reduced the percentage of freezing ($P<0.05$) on the stressed group when compared to the vehicle group (n=14) upon test session. About the group that received 7-NI, in the test session, stressed animals vehicles (n= 17) exhibited a deficit in the extinction process, compared to control animals vehicles (n= 24). The dose of 5 mg/kg (n= 9) in the stressed group had no effects on the percentage of freezing ($p>0.05$) on the test session, when compared to the vehicle stressed group. However, the dose of 15 mg/kg (n= 12) reduced the percentage of freezing ($P<0.05$) on the stressed group when compared to the vehicle group upon test session. **Conclusion:** Our results demonstrate experimentally that the administration of MK-801, an NMDA receptor antagonist, at doses of 0.05 and 0.2 mg/kg, and 7-NI, a nNOS inhibitor, at a dose of 15 mg/kg, were able to reverse deficits in the extinction of contextual aversive memory in a PTSD model. Therefore, these results indicate a possible involvement of the NMDA/NO receptor pathway in the extinction deficits of aversive memory in animals subjected to a stress model. **Financial support:** CAPES, FAPESP, CNPq and FAEPA.

02.067 **Kynurenine Neonatal Exposure: Deficits in Mice Development.** Cruz BE¹, Corrêa AES², Bomfim ANL¹, Andrade BS¹, Guimarães MZP², Neves GA¹ ¹UFRJ, Lab Molecular Pharmacology, Postgraduate Program in Pharmacology and Medicinal Chemistry, Brazil ²UFRJ, Lab Molecular Pharmacology, Brazil

Introduction: The kynurenine (KYN) pathway is a major degradation pathway for tryptophan, leading to the synthesis of several neuroactive compounds. Among them, kynurenic acid is an antagonist of N-methyl-D-aspartate and α -7 nicotinic acetylcholine receptors, believed to have neuroprotective activity. This pathway is upregulated by inflammatory cytokines, such as those produced after a viral infection, which appear to be involved in the etiology of several neurodevelopmental disorders, including schizophrenia. However, the immediate effects of increased kynurenine levels in the neonatal period remain unknown. The presented study aims to identify outcomes of kynurenine exposure in neural and motor development in Swiss mice.

Methods: All the animals were kept at the Animal Experimentation Unit, in a twelve-hour light-dark cycle, and each litter was housed separately depending on the treatment received. The animals received KYN (200 mg/kg i.p.) or PBS (15 mL/100 g i.p.) twice a day from postnatal day (PND) 7 to 16 (CEUAUFRJ: 120/22). During this period, developmental milestones were monitored, including the opening of eyes and ears, fur development, and the emergence of the righting reflex. Animals' weight and mortality were also recorded. Moreover, mouse motor development was evaluated through the hind limb suspension test at PND 12 and the wire hanging test at PND 17. The data on motor performance were analyzed by a two-way ANOVA (SigmaPlot), with sex and exposure as independent factors and the latency to fall as the dependent variable, followed by a Tukey post hoc test. The sample number of animals was expressed by the average of each male and female in the litters ($n = 5$), and the data was expressed as mean $\pm$ standard error of the mean, with significance was considered at $p < 0,05$. **Results:** KYN did not compromise mice's weight gain nor increase their neonatal mortality rate. No differences were found between animals exposed to KYN and PBS in terms of developmental milestones or the wire hanging test. However, in the hind limb suspension test, the KYN group showed a significant reduction in time to fall compared to controls in both sexes, indicating a decrease in muscle strength (PBS male: $32 \pm 8,792$ s; KYN male: $18,2 \pm 5,238$ s; PBS female: $38,2 \pm 9,589$ s; KYN female: $17,2 \pm 3,611$ s; $p = 0,031$). **Conclusion:** In summary, our data indicate that exposure to KYN may delay mice's motor development, suggesting that neonatal KYN administration is a relevant model of neurodevelopmental insult. Analyses of animals' brain tissue, looking for changes in cholinergic neurotransmission, are underway. **Financial support:** CAPES, FAPERJ, CNPq.

02.068 **Voltage-Gated Sodium Channel Modulation by Mexiletine Analogues.** Rosado-Santos PL, Bello-Santos VG, Castro NG ICB-UFRJ, Lab Farmacologia Molecular, PPGFQM, Brazil

Introduction: Voltage-gated sodium channels (VGSC) are essential for the rapid conduction of information in the CNS and also in non-neural cell excitability. They are important therapeutic targets of pharmacological modulators, and their abnormal functioning generates conditions known as “channelopathies”. Modulation of VGSC function must be extremely delicate, as even small changes in the activation, inactivation, or refractoriness of these ion channels can lead to major physiological effects. Conditions such as cardiac arrhythmias, neuropathic pain, and specific myopathies may arise from channelopathies. Due to the subtlety required to appropriately modulate VGSC according to clinical demand, the availability of a wide range of modulating drugs becomes crucial. Mexiletine is an orally active VGSC inhibitor whose specific properties are useful in controlling certain types of ventricular arrhythmias, but which has also been proposed for use in chronic pain and neurological conditions. In collaboration with groups from FIOCRUZ, we are investigating novel compounds structurally related to mexiletine. **Methods:** We used the patch-clamp technique to characterize the effects of compounds on the amplitude and voltage-dependent kinetics of VGSC currents in GH3 cells, a rat pituitary cell line that expresses the main subtypes of neuronal VGSCs, primarily Nav1.3. **Results:** We have identified two novel potent and state-dependent VGSC inhibitors, which showed IC₅₀s of 28.3 and 6.1 microM for tonic block. At near-IC₅₀ concentrations, these compounds prolonged channel inactivation (1.4x and 2.4x the time constant of recovery) and shifted the voltage-dependence of inactivation to more negative potentials by 13.5 ± 1.9 mV and 10.9 ± 1.9 mV, respectively, without much effect in VGSC activation. Accordingly, these compounds showed marked frequency-dependent block. **Conclusion:** These effects are similar to those previously reported for mexiletine, which is no longer marketed in Brazil. If the novel compounds show favourable pharmacokinetic properties comparable to those of mexiletine, they may become new candidate therapeutic agents.

02.069 **Klotho Silencing Decreases AMPA Receptor Expression in Mixed Cerebellar Culture.** dos Santos GRO¹, Kawamoto EM¹ ICB-USP, Functional and Molecular Biology Post-Graduation Program, Brazil

Introduction: Klotho protein is involved in aging and various processes in the central nervous system (CNS), including the protection of cerebellar granule cells. Glutamatergic signaling is essential for synaptic plasticity, since N-methyl-D-aspartate (NMDA) and α -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors are involved in long-term potentiation (LTP), long-term depression (LTD), and the maturation of cerebellar granule cells. Additionally, Klotho could influence the expression of AMPA receptor in the cerebellum. This study aimed to investigate possible alterations in the protein levels of AMPA and NMDA receptors in Klotho-silenced cerebellar cells. **Methods:** Mixed cerebellar cultures were prepared from neonatal Wistar rats (8 days postnatal). The cells were cultured in minimum essential medium supplemented with 10% fetal bovine serum. On day 8, the cells were transfected with small interfering RNA targeting Klotho (siRNA-Klotho) at concentrations of 10, 100 and 200 pmol using Lipofectamine RNAi max. Protein extraction was performed 48 hours later using RIPA buffer containing protease and phosphatase inhibitors. Cytotoxicity of siRNA-Klotho transfection was assessed by lactate dehydrogenase (LDH) release assay. Western blotting was performed to quantify the protein levels of soluble Klotho, GluN1 subunit (NMDA), and GluA1 subunit (AMPA), using beta-actin as a loading control. On day 9, immunofluorescence labeling for MAP-2, GFAP and IBA-1 was performed to identify the cell types present in the culture. Data were analyzed using two-way ANOVA with Tukey's multiple comparisons ($F(DFn, DFd)$) and Student's t-test (t, df). The project is approved by the Animal Research Ethical Committee (1677130524). **Results**

The results indicated that transfection with siRNA-Klotho at 100 pmol significantly reduced the soluble Klotho protein levels by 40% ($t=3,422, df=16; p = 0,0035$), but not at concentrations of 10 and 200 pmol. The cytotoxicity assay showed no significant difference when compared to control. Klotho silencing did not alter the protein levels of the NMDA receptor, but it resulted in a 55% reduction in the protein levels of AMPA receptor ($t=2.093, df=9; p=0.0330$). Immunofluorescence analysis showed that the mixed cerebellar culture is composed of 67% neurons, 22.5% microglia, and 10.5% astrocytes. **Conclusion:** These findings suggest that Klotho silencing decreases AMPA receptor protein levels in mixed cerebellar culture, which may impact the maturation of cerebellar granule cells. Future experiments will explore whether β -estradiol exerts a protective modulatory effect on the expression of AMPA receptor in Klotho-silenced cells. **Financial support:** The project is supported by the São Paulo Research Foundation (FAPESP) (grant number 2024/07231-2).

02.070 Chronic Social Stress in C57BL Female Mice Impairs Cognition, but Not Memory or Social Behavior. Covre LHH^{1,2}, Munhoz CD¹ ¹USP-ICB, Lab of Neuroendocrine Pharmacology and Immunomodulation, Brazil, ²USP, PPG Functional and Molecular Biology, Brazil

Introduction: Depressive disorders have become one of the leading causes of health and economic burden worldwide, with rising numbers, especially after the COVID-19 pandemic. Among the various findings in depression, a significant aspect is the hyperactivity of the HPA axis, resulting in increased glucocorticoid (GC) secretion. It has been shown that, in chronic stress, GCs have deleterious and cytotoxic effects on the central nervous system (CNS), particularly the hippocampus and pre-frontal cortex. In mouse models, different protocols of chronic stress are used to study depressive-like behavior and cognitive impairment, but most published studies are conducted in male animals. **Objectives:** This project aims to assess the impact of 28 days of social isolation on depressive-like behavior, social behavior, and cognitive functions in C57BL female mice. **Methods:** C57BL female mice (CEUA 3979091121) were submitted to 28 days of chronic isolation stress in their home cages with free access to food and water. On day 28, the behavioral tests started with two different series: the first, with the sucrose preference (n=5-6), novelty preference (n=12), splash (n=10), and tail suspension (n=12) tests; the second, with the T-maze (n=11-12) and passive avoidance (n=11) tests. After the behavioral tests, animals underwent euthanasia. The experiments were analyzed using the t-test, two-way ANOVA with the Tukey post-hoc test, or repeated measures two-way ANOVA. **Results:** In the sucrose preference test, stressed mice showed a loss of sucrose preference compared to control mice (Mean=41.92±5.078 Control vs Stress, $p<0.0001$). In the social novelty test, no difference was observed between the groups in either direct or indirect interactions. In the tail suspension test, stressed animals showed less mobility time than control mice (Mean=90.30±20.40 Control vs Stress, $p=0.0002$). In the splash test, stressed mice showed a decreased time spent grooming compared to controls (Mean=43.31±12.77, Control vs. Stress, $p=0.0033$). In the T-maze test, stressed mice showed a lower percentage of alternation hits than control mice ($t=2.359$; $p=0.0281$), but no differences in the mean latency ($t=0.3974$; $p=0.6951$). In the passive avoidance test, no difference between groups was observed ($t=0.0006612$; $p=0.9995$). **Conclusions:** Chronically socially isolated C57BL female mice exhibited classic depressive-like behaviors, including anhedonia and despair. Additionally, stressed mice displayed some cognitive impairment, without any influence on memory processing or social behavior. **Financial support:** This study was financed by Conselho Nacional de Desenvolvimento Científico e Tecnológico, Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001 and Fundação de Amparo à Pesquisa do Estado de São Paulo.

02.071 **Molecular Mechanisms Underlying Photobiomodulation-Induced Neuroplasticity: Implications for Psychiatric Therapeutics.** Schons T^{1,3}, Bortoluzzi L^{1,2}, Colombo R², Viscardi LH², Padilha AT², Agostini JF², da Cunha SB², Rosa AR^{1,3,4} ¹IBCS-UFRGS, Postgraduate Program in Biological Sciences: Pharmacology and Therapeutics, ²UCS, Caxias do Sul, Brazil. ³HCPA, Lab Molecular Psychiatry, 4UFRGS, Postgraduate Program in Psychiatry and Behavioral Sciences, Faculty of Medicine, Porto Alegre, Brazil

Introduction: Photobiomodulation (PBM) with low-level red and near-infrared light has shown therapeutic potential in modulating neural activity and promoting neuroplasticity. Although clinical and pre-clinical studies suggest benefits in neurological and psychiatric conditions, the molecular mechanisms underlying PBM-induced plasticity in the central nervous system (CNS) remain incompletely understood. This study aimed to identify molecular targets and signaling pathways modulated by PBM in CNS structures. **Methods:** A comprehensive review of original articles (2013–2023) was conducted using PubMed, using MeSH terms related to PBM, neuroplasticity, and CNS proteins in *Rattus norvegicus*. Exclusion criteria included reviews, *in vitro* studies, and articles without molecular outcome data. From 1,660 initial records, a curated list of PBM-responsive proteins was compiled. The identified proteins were analyzed using STRING and Cytoscape to construct PPI (protein–protein interaction) networks and perform pathway enrichment analyses (KEGG and Reactome databases). **Results:** PBM modulated key proteins, including BDNF, CREB, GAP-43, AKT1, and MAPK1, forming central nodes in interconnected networks. These proteins were significantly associated with MAPK/ERK, PI3K/AKT, and JAK/STAT signaling pathways — all of which play critical roles in neurogenesis, synaptic remodeling, and inflammation control. Cluster analysis revealed modules with high functional coherence and strong overlap with targets previously implicated in the pathophysiology of depression and schizophrenia. Notably, TrkB and mTOR emerged as pharmacologically actionable nodes, reinforcing the translational relevance of PBM-induced molecular changes. **Conclusion:** This study demonstrates that PBM modulates conserved signaling cascades involved in CNS plasticity, providing a molecular basis for its potential therapeutic use in psychiatry. The integration of proteomics and bioinformatics highlights targets that may inform pharmacological innovation or adjunctive interventions aiming to restore neural adaptability and resilience in mental disorders.

02.072 **Esterification of Geraniol to Geranyl Acetate Enhances its Therapeutic Potential in Adult Zebrafish.** Bastos LM¹, Gallas-Lopes M¹, Rosa GO¹, Patelli-Alves A¹, Portela SM¹, Costa PM³, Herrmann AP¹, Piato A^{1,2} ¹UFRGS, Porto Alegre, PPG Farmacologia e Terapêutica, Brazil; ²UFRGS, Porto Alegre, PPG Neurociências, Brazil; ³UFCSA, Porto Alegre, Brazil

Introduction: Geraniol (Ger) is a terpenoid identified in certain essential oils and has demonstrated anxiolytic properties in both rodents and fish studies. Esterification of molecules is an important pharmacological strategy known to enhance bioavailability and potentially increase drugs' affinity for receptors. Therefore, this study aimed to evaluate the behavioral effects of acute exposure to Ger and its esterified form, Geranyl Acetate (GerAc), in adult zebrafish (*Danio rerio*). **Methods:** 214 adult zebrafish, with similar proportions of males and females, were used in two experiments (Ger and GerAc experiments). Fish were assigned to the experimental groups (n=23-28) 1% DMSO and three concentrations of Ger or AcGer (4, 40, and 400 μ M) following block randomization to counterbalance baseline characteristics (sex, home tank, and day of testing). On the test day, animals were individually exposed to the treatment solutions for 10 minutes and then immediately after allocated to the novel tank test, where fish were recorded for 6 minutes. Analyses were performed using the ANY-Maze Software by researchers blinded to the treatment groups, and the following outcomes were assessed: time and entries in the upper zone, time and entries in the bottom zone, distance traveled, swimming speed, number and time of immobile episodes. Data were analyzed using Kruskal-Wallis/Dunn and One-Way ANOVA/Tukey tests, according to data distribution. Experiments were pre-registered in <https://osf.io/8d9qt/> and <https://osf.io/pt26k/>. All procedures were approved by CEUA/UFRGS no. 36307. **Results:** Ger did not alter any behavioral outcomes. However, exposure to 40 and 400 μ M GerAc increased the time spent in the upper zone (p=0.001 and p=0.0004, respectively) and decreased the time spent in the bottom zone (p=0.009 and p=0.002, respectively). Additionally, 400 μ M GerAc increased the number of entries in the upper zone (p=0.007). Neither Ger nor GerAc altered locomotor outcomes at any tested concentration. **Conclusion:** zebrafish naturally exhibit geotaxis, a behavior characterized by seeking safety in lower zones when exposed to novel environments. Therefore, the increase in fish height in the water column without locomotor alterations after exposure to GerAc suggests an anxiolytic effect, which was not seen with Ger exposure. These findings indicate that esterification enhances the therapeutic potential of Ger. However, the underlying mechanism — whether due to pharmacokinetic or pharmacodynamic changes — remains unclear and warrants further investigation. **Financial support:** CAPES, CNPq, and PROPESQ at UFRGS.

02.073 **Effect of TNFR1 Deletion on Different Types of Memory in Mice with LPS-Induced Neuroinflammation.** Lima TAS, de Sá LL, Andreotti DZ, Kawamoto EM. ICB-USP, Dept Physiology and Pharmacology

Introduction: In the central nervous system, tumor necrosis factor receptor type 1 (TNFR1) not only mediates pro-inflammatory signaling but also regulates synaptic plasticity, which is often impaired under neuroinflammatory conditions. However, it remains unclear whether inhibiting TNFR1 signaling yields exclusively beneficial effects. Using a mouse strain with complete TNFR1 deletion, we investigated whether loss of TNFR1 protects against cognitive impairments induced by neuroinflammation. **Methods:** Eighteen wild-type (WT) and homozygous *Tnfr1*^{tm1Mak} knockout (TNFR1^{-/-}) male mice (8–12 weeks old) were housed in micro-isolators at the Animal Facility of the Department of Pharmacology of the Institute of Biomedical Sciences (USP). The animals were divided into four groups: (a) WT SAL (n=3); (b) WT LPS (n=4); (c) TNFR1^{-/-} SAL (n=5); and (d) TNFR1^{-/-} LPS (n=6). LPS (1 mg/kg) or sterile saline was administered intraperitoneally once daily for three consecutive days. Body weight was recorded over seven days. Behavioral tests began 24 hours after the final injection, in this order: reference memory in the T-maze (dpi 1–2), object location and recognition memory (dpi 3–4), and fear conditioning (dpi 6–8). Animals were euthanized 2 hours after the last test. IL-1 β concentrations in the hippocampus were measured using ELISA assay. Data were analyzed by two-way ANOVA, followed by Tukey's post-hoc test for interactions and Bonferroni correction for simple effects. Results are presented as mean $\pm$ SEM. All procedures were approved by the Animal Ethics Committee (CEUA) under protocol number 1345200524. **Results:** Body weight was reduced in LPS-treated groups compared to controls [interaction: $F(18, 84) = 44.41$, $p < 0.0001$], during both the three days of administration (percent change in body weight at the third injection: WT SAL -1.867 ± 0.203 ; WT LPS -15.693 ± 0.640 ; KO SAL -1.146 ± 0.762 ; KO LPS -15.445 ± 0.609) and in the subsequent four days ($p < 0.05$). Reference memory and both object location and recognition memory tests showed no significant differences among groups. In contextual fear conditioning, the TNFR1^{-/-} LPS group (7.450 ± 3.184) froze significantly less than the WT LPS group [23.700 ± 3.894 ; genotype: $F(1, 14) = 6.664$, $p = 0.0217$; $p < 0.05$]. During cued fear memory test, following habituation and five conditioned tones, freezing was also reduced in TNFR1^{-/-} LPS group (21.533 ± 5.485) versus WT LPS [43.425 ± 6.627 ; interaction: $F(1, 14) = 6.395$, $p = 0.0241$; $p < 0.05$]. Hippocampal IL-1 β increased significantly in WT LPS mice (41.046 ± 6.196) compared to WT SAL [8.157 ± 4.452 ; interaction: $F(1, 14) = 12.97$, $p = 0.0029$; $p < 0.05$], whereas no changes were observed in TNFR1^{-/-} groups. **Conclusion:** Systemic LPS induced up to 15% weight loss by day 3 but not affect reference, object location or recognition memory. Although LPS alone did not impair cognition, TNFR1 deletion under neuroinflammatory conditions led to deficits in both contextual and cued fear memory. The absence of hippocampal IL-1 β elevation in TNFR1^{-/-} mice suggests TNFR1-dependent modulation of cytokine induction and fear memory processes.

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02.074 Ouabain modulates BDNF expression in lung and brain regions in a murine model of mixed asthma. Ribeiro MR¹, Santos AS¹, Leite JA², Alves VF¹, Melhado IVS¹, Lima TAS¹, Suaiden AS¹, Lima LS¹, Viana DZA¹, Oliveira MA¹, Kawamoto EM¹, Tavares-de-lima W¹, Scavone C¹ ¹ICB-USP, Dept. of Pharmacology, São Paulo Brazil. ²ICB-UFG, Dept. of Pharmacology, Goiás, Brazil

Brain-derived neurotrophic factor (BDNF), a protein highly expressed in neurons and glial cells, plays key roles in neural development, survival and plasticity. Previous studies have shown that BDNF is involved in the pathophysiology of eosinophilic asthma (Watanabe et al., AJRCMB 2015; 53:844); however, its role in mixed asthma remains unknown. Asthma is a chronic inflammatory lung disease characterized by epithelial damage, immune cell infiltration, with no known cure. In the mixed asthma phenotype, both eosinophils and neutrophils contribute to the inflammatory response, making the disease more complex and challenging to treat. Ouabain (OUA), a potent Na⁺,K⁺-ATPase inhibitor, has been investigated for its neuromodulatory and immunomodulatory properties. It has been shown to attenuate allergic airway inflammation by reducing granulocyte infiltration into the bronchoalveolar lavage fluid (Galvão et al., 2022). **Methods:** Female Balb/c mice were sensitized with ovalbumin (OVA) and Complete Freund's Adjuvant (subcutaneous, Day 0). OVA challenges (intranasal) were administered on days 21, 22, and 23. The OVA+OUA group received ouabain (0.56 mg/kg, i.p.) one hour before each challenge. Experiments were conducted on day 24. Control mice received PBS or OUA via the same route. Lung inflammation was assessed microscopically by quantifying cells recovered from bronchoalveolar lavage (BAL). BDNF levels were measured using an ELISA kit. Data are expressed as mean±SEM and analyzed using the Student's t-test or one-way ANOVA (GraphPad10). **Results:** BAL fluid from OVA-challenged mice showed a significant increase in total cells (n=5) ($\times 10^4$ /mL: 116±12 P<0.0001), neutrophils (54±4, P<0.0001), and eosinophils (59±8, P<0.0001). Total lung cell recruitment was also increased. In lung tissue, BDNF levels (pg/mL) were elevated in groups treated with ouabain: OUA vs PBS (312±203, P<0.0003) and OVA vs OVA+OUA (274.8±617.6, P<0.0001) (N=5-7). In the frontal cortex, OUA treatment decreased BDNF levels in the OVA+OUA group compared to PBS (313±575, P<0.02) (N=4-6). No significant differences in BDNF levels were observed in the hippocampus. **Conclusion:** This murine model of mixed asthma reflects the main pathological features described in the literature. OUA treatment significantly increased BDNF levels in the lungs of asthmatic and non-asthmatic animals. Our preliminary results indicate that OUA treatment in asthmatic animals reduced BDNF expression in the frontal cortex, with no changes observed in the hippocampus. Further studies must explore the difference between peripheral and central effects of OUA in the nervous system in the presence of an inflammatory disease. **Financial support:** FAPESP 2021/12906-0, FAPESP 2024/06058-5, FAPESP 2016/07427-8, CAPES, and CNPq.

02.075 Perillyl Alcohol Decreases the Frequency and Severity of Convulsive-Like Behavior in the Adult Zebrafish Model of Acute Seizures. Pereira ACM^{1,2}, Sánchez-Ortíz BL^{2,3}, Melo EL², Hage-Melim LIS^{1,4}; Carvalho JCT^{1,2} ¹UEAP, Pharmaceutical Sciences Post-Graduation Program, Biological and Health Sciences Department; ²UEAP, Drugs Research Laboratory, Biological and Health Sciences Department; ³National Autonomous University of Mexico Natural Products Pharmacology Laboratory, Pharmacy Department, Chemistry Faculty, University City; ⁴UEAP, Laboratory of Medicinal and Pharmaceutical Chemistry (PharMedChem), Biological and Health Sciences Department, State University of Amapá

This research aimed to assess the effect of perillyl alcohol (PA) on convulsive behavior *in vivo* using adult zebrafish (*Danio rerio*, both sexes). The seizures were induced with pentylenetetrazole (PTZ) intraperitoneally at 170 mg/kg, and diazepam (DZP) was used as the control anticonvulsant (2 mg/kg, oral); PA was tested at 10, 50, and 100 mg/kg orally. The groups had ten animals per group (total $n = 60$), observed for 10 minutes after seizure induction. We manually appraised typical seizure phenotypes for quantification and used an animal tracking software (Toxtrac) to assess the motor parameters. Next, we sought to find a mechanism of action for PA anticonvulsant activity *in silico* using a structure-based activity prediction server and molecular docking. The results show that PTZ induced seizure-like behavior in all untreated animals with hyperlocomotion episodes, seizure itself, posture loss, and immobility. DZP inhibited the seizures in all animals of the positive control group. PA, in turn, inhibited the occurrence of seizures in a dose-dependent manner, with frequencies of 90%, 70%, and 40% (for 10, 50, and 100 mg/kg, respectively). The PA treatments also decreased several seizure endpoints in a dose-dependent manner. Also, the difference of the group treated with highest dose of PA was statistically significant compared with the negative control group for all the endpoints assessed ($p < 0.05$, Kruskal-Wallis). The *in silico* analyses suggested that PA can affect the GABAergic system, which might be involved in its anticonvulsant activity, but other mechanisms cannot be ruled out. Overall, our results suggest an anticonvulsant potential in perillyl alcohol.

03. Psychopharmacology

03.001 **The Neurobehavioral Role of Leptin in a Murine Model of Chronic Stress.** Sampaio LL, Albernaz KA, Munhoz CD ICB-USP, Dept Pharmacology, Neuroendocrinepharmacology and Immunomodulation Lab, PPG Functional and Molecular Biology, Brazil

Introduction: Stress, while essential for adaptation and survival, can become detrimental when it becomes chronic and intense. The stress response is primarily mediated by the hypothalamic-pituitary-adrenal (HPA) axis, which is responsible for the release of glucocorticoids (GCs). Depression, often associated with HPA axis dysfunction, affects approximately 5% of the global population. Several hypotheses have been proposed to explain its pathophysiology, including the monoamine theory and the brain-derived neurotrophic factor (BDNF) hypothesis. Leptin, a hormone produced by adipose tissue and involved in energy regulation, has also been suggested as a modulator of depression. This hormone acts on the HPA axis, influencing GC release, and plays a role in neurogenesis and neuronal plasticity. Evidence indicates that leptin deficiency exacerbates the harmful effects of chronic stress, promoting depressive-like behaviors. The present study aimed to evaluate the role of leptin in modulating memory, behavior, and neuroprotection in mice subjected to chronic predictable stress (CPS), as well as its influence on the activation and regulation of the HPA axis. **Methods:** Wild-type (WT) and leptin-deficient (ob/ob) C57BL/6 mice were maintained under controlled conditions. After acclimatization, animals were divided into control (n = 6–8) and stressed groups (n = 6–8), subjected to CPS, and evaluated in behavioral tests for depressive-like behavior and memory. Data were analyzed by two-way ANOVA with Tukey and Fisher post hoc tests ($p < 0.05$). **Results:** The ob/ob mice exhibited higher body weights from the onset of the stress protocol (WT: $25,88 \pm 0,8$ g; ob/ob: $49,28 \pm 1,7$ g; $p < 0,0001$), with no additional changes induced by stress ($p = 0,1880$). Serum corticosterone (CORT) levels were higher in ob/ob animals (WT: $7,137 \pm 5,297$ ng/mL; ob/ob: $12,31 \pm 1,164$ ng/mL; $p = 0,0338$) regardless of stress exposure. In terms of locomotor activity, ob/ob mice exhibited reduced movement compared to WT mice (WT: $12,9 \pm 5,24$ m; ob/ob: $5,61 \pm 3,17$ m; $p < 0.0001$), although stress increased their general mobility ($p = 0,0017$). In the social interaction test, stressed WT mice showed reduced interest in novel social stimuli (control: $78,73 \pm 6,78$ s; stress: $62,67 \pm 17,48$ s; $p = 0,0090$), whereas ob/ob mice did not exhibit social deficits but displayed reduced mobility ($p < 0.0001$). Regarding anhedonic behavior, ob/ob mice exhibited increased grooming (WT: $32,4 \pm 13,75$ s; ob/ob: $56,4 \pm 2,44$ s; $p = 0,0007$), with no significant changes induced by stress in the tail suspension test ($p = 0,1760$). In memory evaluation, ob/ob mice exhibited increased latency to descend from the platform in the inhibitory avoidance test (WT: $228,1 \pm 69,17$ s; ob/ob: $302,7 \pm 71,22$ s; $p = 0,048$), with a slight impairment in long-term aversive memory due to stress ($p = 0,0159$). **Conclusion:** These findings suggest that the absence of leptin increases behavioral vulnerability and blunts stress responsiveness, indicating altered dynamics of the stress response. Altogether, these results reinforce the key role of leptin in the proper regulation of stress responses, influencing anhedonic behavior and social interaction. CEUA: 3979091121. **Financial support:** CAPES, CNPq and FAPESP.

03.002 **The Role of Adenosinergic Signaling in the Antidepressant-like Effects of Cannabidiol and Potential Molecular Mechanisms Involved.** Silveira JR¹, Sales AJ¹, Ciruela F², Guimarães FS¹ ¹FMRP-USP, Dept. of Pharmacology, Brazil; ²University of Barcelona, Institute of Neurosciences, Dept. of Pharmacology and Experimental Therapeutics, Spain

Introduction: Cannabidiol (CBD), the main non-psychotomimetic constituent of the *Cannabis sativa* plant, has shown antidepressant-like effects in animal models. The mechanisms underlying this effect are not fully elucidated. *In vivo* and *in vitro* results demonstrate that CBD allosterically modulates adenosinergic receptors (AR) and inhibits the adenosine transporter ENT-1. Studies indicate that the adenosinergic system is implicated in the pathophysiology of psychiatric disorders and represents a potential therapeutic target for the development of new drugs. **Aims:** The aim of this study was to investigate the potential molecular mechanisms associated with involvement of the adenosinergic system in the antidepressant-like effect of CBD. **Methods:** 8-week-old male C57BL/6 mice (n=8-10 animals/group) were pretreated intraperitoneally with the adenosine receptor antagonists SCH-58261 (A2AR antagonist) or CPT (A1R antagonist), followed by intraperitoneal administration of CBD or dipyridamole (ENT-1 inhibitor). Subsequently, animals were subjected to the open field test (OFT) and forced swimming test (FST). This study was approved by the Ethics Committee on the Use of Animals of the Ribeirão Preto Medical School-USP (#1156/2023). For molecular analyses, HEK293T cells were transiently transfected and subjected to the NanoBIT assay to evaluate CBD's effects on A2AR signaling and heteromerization. Additionally, confocal microscopy was used to monitor extracellular adenosine levels via the adenosine biosensor GRABAdo1.3. **Results:** Acute treatment with CBD promoted an antidepressant-like effect in the FST ($F_{(3, 28)} = 4,028$; $p < 0,05$). Pre-treatment with SCH-58261 blocked this effect ($F_{(3, 28)} = 4,028$; $p > 0,05$), while CPT had no influence. Similarly, pre-treatment with SCH-58261 also prevented the antidepressant-like effect of dipyridamole ($H=8,173$; $p < 0,05$). In the NanoBIT assay, CBD, in the presence of CB1 ($F_{(4, 25)} = 2,992$; $p < 0,05$) or A1 ($F_{(4, 25)} = 4,482$; $p < 0,05$) receptors, potentiated the effect of the agonist CGS on A2AR activation. Furthermore, CBD reduced the number of A2AR-A1R ($F_{(3, 8)} = 5,624$; $p < 0,05$), A2AR-CB1R ($F_{(3, 19)} = 3,162$; $p < 0,05$), and A2AR-D2R ($F_{(3, 20)} = 4,214$; $p < 0,05$) heteromers after 2 or 16 hours of incubation. In confocal microscopy assays with the adenosine biosensor GRABAdo1.3, CBD modulated extracellular adenosine levels similarly to other ENT-1 inhibitors (AUC H= 29,26; $p < 0,0001$). **Conclusion:** Our findings suggest that A2AR, but not A1R, activation is involved in the antidepressant-like effect of CBD in the FST. Moreover, CBD modulates adenosinergic signaling by enhancing A2AR activation in the presence of A1R and CB1R, altering receptor heteromerization, and modulating extracellular adenosine levels, supporting our behavioral results. **Financial support:** FAPESP, CNPq, CAPES and FAEPA.

03.003 **Epigenetic Regulation by HAT p300/CBP is Temporally Controlled by the Endocannabinoid System During Fear Memory Reconsolidation.** Bergmann MF, Raymundi AM, Stern CAJ Dept Pharmacology, UFPR, Curitiba, PR, Brazil

Introduction: Impairing fear memory reconsolidation has been suggested as treatment for posttraumatic stress disorder. Reconsolidation is a retrieval-dependent phase lasting around 6 h and recruits brain areas such as the prelimbic cortex (PL). Cannabinoid type-1 receptor (CB₁) located in PL underlies the phytocannabinoids effects on reconsolidation. Reconsolidation also relies on epigenetic mechanisms like histone acetylation, which is regulated by histone acetyltransferases like HAT p300/CBP and has been suggested to be modified by CB₁ receptor activation. Previously, we showed that intra-PL infusion of C646 50 ng, a HAT p300/CBP inhibitor, immediately or 1h after retrieval impaired fear memory reconsolidation. Thus, we hypothesized that an interplay between CB₁ receptors and histone acetylation underlies fear memory reconsolidation in the PL cortex. We aimed to investigate the interaction between HAT p300/CBP and the endocannabinoid system (ECS) in the PL during reconsolidation. **Methods:** Male Wistar rats with guide-cannulas aiming the PL underwent contextual fear conditioning. After 1 day, to induce reconsolidation, they underwent a short retrieval session. Immediately or 1h later, rats received intra-PL infusions of sub-effective concentrations of AM251 (CB₁ receptor antagonist; 50 pmol/0.2 µL/side), URB597 (fatty acid amide hydrolase inhibitor; 10 pmol/0.2µL/side) or vehicle (VEH). After 5 min or 1h, they received C646 (effective or sub-effective concentrations: 50 ng or 5 ng/0.2µL/side) or VEH in the PL. To evaluate the treatment effects, animals were exposed to Test A after 24 h. The percentage of freezing time was evaluated and analyzed by repeated-measures ANOVA, followed by Newman-Keuls post-hoc test. Results are expressed as mean ± SEM. All procedures were approved by the local ethics committee (#1570). **Results:** Rats treated with C646 50 ng immediately or 1h after retrieval exhibited significantly less freezing compared to controls in Test A (0h-0h: 44.6±3.6 vs 65.4±4.6; 0h-1h: 41.7±6.6 vs 62.2±4.8; 1h-1h: 39.5±5.2 vs 67.4±3.8). Immediately after retrieval, the CB₁ blockade with AM251, infused 5 min before C646, did not change the C646 effect. Administering AM251 1h before C646 (0h-1h: 53.8±5.9) partially abolished the C646 effect, showing no significant difference compared to the C646 (0h-1h: 41.7± 6.6) or control group (0h-1h: 62.2 ± 4.8). However, 1h after retrieval, the CB₁ blockade 5 min before C646 administration, completely prevented the C646 effect (1h-1h: 69.6 ± 6.4), with freezing levels comparable to the control group (1h-1h: 67.4±3.8), suggesting that CB1 receptor activation 1h after retrieval is involved in the HAT p300/CBP inhibiting effect on reconsolidation. Furthermore, 1h post-retrieval, co-infusion of sub-effective C646 (5 ng) and URB597 (10 pmol) significantly reduced freezing behavior compared to control (1h-1h: 17.5±7.2 vs 41.19±5.6), suggesting an interplay between AEA and HAT p300/CBP in promoting a reconsolidation impairing effect. **Conclusion:** Our results suggest time-dependent involvement of AEA and CB₁ receptor in the reconsolidation-impairing effects of HAT p300/CBP inhibition in the PL, being preferentially recruited 1h after retrieval. Thus, supporting ECS and epigenetic interplay in fear memory reconsolidation. **Funding:** CAPES and CNPq.

03.004 Role of the Globus Pallidus Endocannabinoid System in the Expression of Defensive Responses in Mice with a Depressive-Like Phenotype. Coelho-Silva WC, Coimbra NC FMRP-USP, Ribeirão Preto, São Paulo, Brazil

Introduction: In recent years, the endocannabinoid system has been extensively studied as a potential pharmacological target for the treatment of psychiatric disorders, given the high density of cannabinoid receptors in key brain regions. The external globus pallidus (GP), a nucleus of the basal nuclei, is traditionally associated with motor functions and is densely populated by CB1 receptors pre-synaptically located on GABAergic neurons. While its role in movement disorders is well established, its contribution to emotional regulation remains underexplored. Notably, the globus pallidus actively participates in circuits of fear and motivation, influencing learning of aversive stimulus-related and reward-based responses. The combined use of URB597 (a FAAH inhibitor) and AM251 (a CB1 receptor antagonist) in stressed animal models allows the investigation of CB1 receptor-mediated modulation of stress, fear, and anxiety. This study aimed to investigate the effects of endocannabinoid modulation in the globus pallidus on defensive responses in a murine model of stress-induced depression. **Methods:** Male C57BL/6 mice were subjected to a 16-day chronic stress protocol (combining restraint stress and social defeat). On day 17, depressive-like behavior was confirmed using the Splash Test (ST) and the Forced Swim Test (FST). From days 22 to 24, animals were exposed to a risk assessment (RA) paradigm involving direct confrontation by a venomous snake (*Bothrops jararaca*) in a custom-built apparatus. Defensive behaviors were video-recorded and scored over 5 minutes. For analysis, Risk Assessment Frequency and Time were calculated as the sum of specific behaviors: defensive alertness, stretched attend posture, and flat-back approach. Stressed animals exhibiting depressive-like phenotypes were microinjected with AM251 (0.1 nmol) followed, 5min later, by URB597 (1 nmol) into the globus pallidus. Naïve animals received vehicle injections. All animals were then tested in the RA paradigm with a real snake (*Bothrops jararaca*). **Results:** Chronic stress induced anhedonia in the splash test ($t = 2.536$, $df = 18$, $p = 0.0207$, $n = 10$) and increased immobility in the FST ($t = 2.858$, $df = 18$, $p = 0.0104$, $n = 10$), confirming the depressive-like phenotype. Risk assessment behaviors (frequency and time) were not significantly altered by URB597 + AM251 treatment (Risk Assessment Frequency: $p = 0.9975$; Time: $p = 0.5107$; $N = 10$). Panic-like freezing and escape behaviors showed a trend toward reduction in the drug-treated group (escape: $p = 0.1933$; freezing: $p = 0.7928$; $N = 10$ per group). **Discussion:** These findings suggest that the anxiolytic-like effects of URB597 are modulated by CB1 receptor activity, but not its panicolytic-like effects. This aligns with previous reports showing that coadministration of AM251 blocks the antiaversive effects of URB597, supporting a CB1-dependent mechanism of its anxiolytic-like effects. **Conclusion:** CB1 receptor blockade in pallidum impaired anxiety-induced defensive responses but not panic-like reactions decreasing caused by endocannabinoid modulation in the globus pallidus in animals with a depressive-like phenotype. These findings suggest the involvement of alternative pharmacological mechanisms, such as CB2 or TRPV1, or a selective effect on fear-reactivity circuits while preserving evaluative behaviors. The globus pallidus emerges as a promising target for therapeutic intervention in comorbid anxiety and depression disorders.

03.005 **Evaluation of Parvalbumin Interneurons and Perineuronal Nets Across Brain Regions in Relation to Motor and Cognitive Effects Induced by Typical Antipsychotics.** Ponciano, R¹, Issy AC², Gomes FV³, Del-Bel EA^{1,2} ¹USP-RP, Dept Physiology, Brazil; ²USP-RP, Dept Basic and Oral Biology, Brazil; ³USP-RP, Dept Pharmacology, Brazil

Introduction: Parvalbumin-positive GABAergic interneurons (PVIs) form local inhibitory circuits and are regulated by perineuronal nets (PNNs), specialized components of the extracellular matrix that support synaptic plasticity and neuronal stability. Disruptions in PVIs and PNNs have been associated with schizophrenia. Typical antipsychotics, such as haloperidol (HAL), alleviate positive symptoms of schizophrenia but can induce tardive dyskinesia (TD), characterized by involuntary orofacial movements—vacuous chewing movements (VCMs) in rodents—and cognitive impairment. While the involvement of PVIs in other motor disorders is well established, their specific role, along with that of PNNs, in the pathophysiology of TD remains poorly understood. **Aims:** This study aimed to elucidate the role of PVIs and PNNs in the emergence of HAL induced VCMs and cognitive impairments in rodents. **Methodology:** Male Swiss mice (8 weeks old, n=12/group) were treated for 21 days with HAL (3mg/kg), a classical TD model, clozapine (CLO - 15mg/kg), an atypical antipsychotic used as a negative control or vehicle (VEH). Behavioral assessments were performed before treatment and on day 22, 24 hours after the final dose. Evaluations included VCMs (quantified as mouth openings with or without tongue protrusion and mandibular tremors), memory performance via the novel object recognition (NOR) test, and locomotor activity during object exploration. Following the last behavioral test, animals received an additional dose of the assigned drug and were euthanized 90 minutes later. Brains were collected for immunofluorescence of PV/PNN and PV/FosB expression in the prefrontal cortex, motor cortex, dorsolateral striatum, and dorsal hippocampus. Behavioral data were analyzed using two-way ANOVA; histological data with one-way ANOVA, both followed by Tukey's posthoc test ($p < 0.05$). All analyses were performed using GraphPad Prism10. The study was approved by the Animal Use Ethics Committee (protocol no. 2023.1.669.58.4). **Results:** Our findings show that HAL induces VCMs ($28,41 \pm 5,63$ to VEH and $58,66 \pm 8,80$ to HAL). A two-way ANOVA showed effect of time ($F(1, 33) = 42,81$; $p < 0,0001$), but not of treatment ($p = 0,1252$), however there is an interaction between these variables ($F(2, 33) = 10,63$; $p = 0,0003$). Moreover, HAL produces a marginal impairment in long-term memory ($p = 0.06$), without significantly affecting locomotor activity, suggesting a primarily cognitive rather than motor deficit. CLO had no effect on any of the parameters analyzed. Immunofluorescence revealed a marked increase in FosB expression in the dorsolateral striatum following HAL treatment, compared to VEH ($p = 0,0011$) and to CLO ($p = 0,0017$). One way ANOVA showed effect of treatment ($F(2, 13) = 14.68$, $p = 0,0005$); however, no significant changes were observed in PV/FosB colocalization across groups. Additionally, neither antipsychotic altered PV/PNNs expression in the brain regions examined. **Conclusion:** Under the conditions tested, HAL-induced behavioral alterations are not associated with changes in PV/PNNs expression. Moreover, neither HAL nor CLO modulates PV or PNN expression. **Financial support:** National Council for Scientific and Technological Development (CNPq).

03.006 **Two-Step Pharmacological Treatment with Roflumilast and Cannabidiol Prevents Fear Return After a Reminder Stress.** Sohn JMB¹, Daneluz DM¹, Satori NA², Godinho RO², Stern CAJ¹
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Introduction: Fear memory undergoes reconsolidation following brief retrieval or extinction through prolonged reexposure to the conditioned context. The transition between reconsolidation and extinction involves an intermediate phase known as limbo, during which memory is insensitive to amnesic agents. The mechanisms underpinning limbo remain poorly understood. Previously, we showed that roflumilast, a phosphodiesterase 4 inhibitor, switches the memory fate from reconsolidation to extinction. Therefore, we hypothesized that PDE4 may underlie the limbo phase. We aimed to investigate whether inhibiting PDE4, an enzyme that degrades cAMP, could modulate limbo and influence the susceptibility of fear memories to amnesic agents such as cannabidiol (CBD), a phytocannabinoid with therapeutic potential for memory-related disorders such as posttraumatic stress disorder (PTSD). **Methods:** Male Wistar rats underwent contextual fear conditioning, followed by 3 min reexposure to the conditioned context to induce reconsolidation (retrieval 1) on the next day. Immediately after, they received roflumilast (ROF, 0.1 mg/kg) or vehicle (VEH) i.p. After 24 h, animals were subjected to the retrieval 2 for 10 (limbo phase; experiment 1) or 30 min (extinction session; experiment 2) and then received CBD (10 mg/kg) or VEH i.p. Freezing behavior percentage was used as an index of fear memory retention and assessed during subsequent tests: Test A1 (1 day post-treatment), Test A2 (10 days later), and Test A3 (reinstatement test via a reminder shock in a novel context). In experiment 3, we evaluated cAMP levels in blood and fear-related brain regions in samples of naive animals obtained 90 min after ROF or VEH treatment. Data was assessed and analyzed by two-way repeated-measures ANOVA, followed by Newman-Keuls post-hoc test, or Student's t test. UFPR Ethics Committee Approval number: 1636. **Results:** In experiment 1, ROF-treated groups significantly expressed less freezing in Tests A1 (VEH-VEH: 50±7; ROF-VEH: 25±7; ROF-CBD: 27±7) and A2 (VEH-VEH: 40±7; ROF-VEH: 11±3; ROF-CBD: 14±5) compared to the control. During Test A3, VEH-VEH and ROF-VEH groups reinstated the fear memory, confirming that ROF treatment after retrieval 1 facilitates fear extinction in a subsequent 10 min retrieval 2 session. However, the ROF-CBD group expressed less freezing behavior during Test A3 than the control (VEH-VEH: 36±6; ROF-CBD: 9±3), suggesting a lack of fear reinstatement and a reconsolidation impairment. No effects were observed in the VEH-CBD group, confirming the limbo phase in retrieval 2. In experiment 2, the ROF-VEH group expressed enhanced extinction in Test A2 compared to control. In experiment 3, ROF treatment increased cAMP levels in plasma (VEH: 113±11; ROF: 180±11), and in the dorsal hippocampus (VEH: 16±2; ROF: 35±9), implicated in extinction learning, while decreasing cAMP in the amygdala (VEH: 35±4; ROF: 23±3), a key region for fear expression. **Conclusion:** The results suggest that PDE4 inhibition promotes fear memory extinction during the limbo phase and enhances the efficacy of CBD in disrupting fear memory reconsolidation. These data support the potential of PDE4 inhibitors as adjuncts to pharmacotherapies targeting maladaptive fear memories, particularly in disorders such as PTSD. **Financial support:** CAPES, CNPq, Fundação Araucária, FAPESP.

03.007 **Modeling the Psychocardiac Impact of Social Stress: Behavioral, Cardiovascular, Electrocardiographic, and Endocrine Responses and The Role of Oxytocin Receptors.** Belém-Filho IJAB¹, Suzuki GMF¹, Giati LO², Machado LS³, Santos LB¹, Busnardo C², Corrêa FMA¹ ¹FMRP-USP, Dept Pharmacology; ²FCFAR-Unesp, Dept Pharmaceuticals and Medicines, ³UFPA

Introduction: Social stress, including conflicts, violence, and bullying, is a major contributor to psychiatric disorders such as depression. This type of stress is linked to depressive-like behaviors, cardiovascular changes, and hypothalamic-pituitary-adrenal (HPA) axis dysregulation. Oxytocin (OT), a neuropeptide involved in social bonding and antistress responses, may play a modulatory role. We hypothesized that short-term social defeat induces behavioral, autonomic, and neuroendocrine alterations, with the oxytocinergic system playing a key role. **Methods:** All experimental protocols were approved by the local ethics committee (process 1352/2024). Male rats were exposed to the social defeat for 7 days. After, behavioral evaluated on: splash test, open field, and novel object recognition test. After behavioral testing, animals were implanted with femoral artery cannulas for cardiovascular monitoring (rest and during exposure to the aggressor) or ECG recording. Plasma corticosterone was measured. Animals were treated daily with: L-368,899 (3 µg/kg), carbetocin (3 ng/kg or 3 µg/kg), or vehicle. **Results:** Social stress induced clear anhedonic behavior, demonstrated by a significant reduction in grooming time and increased latency to initiate grooming in the splash test [$F(1,51)=10.54$; $p<0.05$]. Locomotor activity and anxiety-like behavior remained unchanged in the open field test [$F(1, 62) = 2.438$; $p>0.05$]. However, deficits in learning and memory were evident in the novel object recognition test, affecting both short-term [$F(1, 54) = 4,797$; $p<0.05$] and long-term [$F(2, 54) = 5.042$; $p<0.05$] memory phases. Cardiovascular analysis showed a marked increase in basal heart rate among stressed animals [$F(3, 47) = 9,946$; $p<0.05$], suggesting sympathetic overactivation, although autonomic reactivity to the known aggressor remained unaltered. Treatment with the OTR antagonist L-368,899 worsened behavioral and cardiovascular outcomes compared to stress alone, without evidence of additive or synergistic effects, suggesting possible floor and ceiling effects. At the high dose (3 µg/kg) carbetocin completely prevented all behavioral and autonomic effects of social stress, reversing anhedonia and preventing learning and memory deficits, without influencing locomotion or inducing anxiolytic/anxiogenic responses. About ECG analysis treatment with the OTR antagonist L-368,899 in stressed rats further exacerbated these issues, leading to a significant increase in the QT interval ($p<0.05$). This suggests that blocking the oxytocin system worsens the arrhythmogenic effects of social stress. In contrast, treatment with the OTR agonist carbetocin successfully prevented this increase in the QT interval ($p>0.05$). Social stress induced a paradoxical hypoactivation of the HPA axis, as evidenced by significantly reduced plasma corticosterone levels [$F(1, 20) = 25,91$; $p<0.05$]. Neither L-368,899 nor carbetocin prevented this endocrine suppression ($p>0.05$). **Conclusion:** Short-term social defeat stress over 7 days induced depressive-like behaviors, neuroendocrine suppression (HPA hypoactivity), and significant cardiovascular alterations, including elevated basal heart rate changes. OTR antagonism worsened behavioral and ECG outcomes, while high-dose OTR agonism with carbetocin prevented behavioral and heart rate changes but failed to correct neuroendocrine suppression. These findings underscore the relevance of the OT-OTR system and point to a possible link between psychosocial stress and cardiovascular risk. **FUNDING:** CNPq, FAPESP and FAEPA-HCFMRP

03.008 **Lithium Microdose Enhanced Object and Spatial Memory in Male Rats.** De Souza STF, Kanazawa LKS, Saviski LR, Stingelin RL, Ferreira J, Pereira GC, Andreatini R, Vital MABF UFPR, Lab Central Nervous System, PPG Pharmacology, Dept Pharmacology, Brazil

Introduction: Lithium carbonate (Li_2CO_3) is a potent mood stabilizer used in the treatment of bipolar disorder. In addition, it has shown pro-cognitive potential effect at subtherapeutic doses and exerts its action through the modulation of intracellular second messengers with subsequent alteration of complex and interconnected intracellular enzyme cascades. One target is the protein kinase glycogen synthase kinase (GSK-3 β) which is an enzyme involved in the regulation of synaptic plasticity and neuronal viability, whose hyperactivity has been associated with cognitive dysfunction, neuropsychiatric, and neurodegenerative disorders. Direct and indirect inhibition of GSK-3 β by lithium leads to improvements in impaired cognition and neuroprotection. **Methods:** This study investigated the behavioral effects of microdoses of Li_2CO_3 on memory, anxiety, and locomotion in male Wistar rats (aged 3 months). Animals (n = 10–11 per group) received daily intraperitoneal injections of either 0.9% saline (control) or Li_2CO_3 at doses of 0.05, 0.1, or 0.25 mg/kg for 24 consecutive days. Starting on day 21, animals underwent a behavioral test battery to assess locomotion and anxiety (open field test), spatial recognition memory (object location test), episodic and object recognition memory (novel object recognition test), and spatial working memory (Y-maze spontaneous alternation) (CEUA/UFPR 1618). **Results:** One-way ANOVA followed by Tukey's post hoc test revealed that, among the doses tested, only 0.1 mg/kg of Li_2CO_3 significantly improved episodic and object recognition memory ($F(3,37) = 3.00$; $p = 0.0391$) and spontaneous alternation performance in the Y-maze ($F(3,41) = 3.09$; $p = 0.0381$). No significant changes were observed in anxiety-related parameters (center crossings, time in center, freezing time) or locomotor indices (distance traveled and average speed). Moreover, no significant effect was found in the object location test. Samples from the hippocampus and prefrontal cortex were collected for further analysis. **Conclusion:** Taken together, these findings demonstrate that a dose approximately 200 to 1000 times lower than the therapeutic range enhanced object recognition and spatial working memory, without affecting anxiety-related behaviors. These results support further investigation into lithium's neuroprotective potential for the prevention of neurodegenerative diseases such as Alzheimer's, as well as its ethical application in individuals with normal but suboptimal cognitive performance. **Funding:** This study was supported by the Coordination for the Improvement of Higher Education Personnel (CAPES), Brazil.

03.009 Effects of Chronic Oral Administration of Cannabidiol on The Gut Microbiota Profile in Mice. Higa S¹, Hassib LC.¹, Ferreira F², Guimarães FS¹ ¹USP, PPG Pharmacology, Brazil ²FIOCRUZ, Brazil

Introduction: Cannabidiol (CBD) is widely recognized for its various effects, including anxiolytic, antidepressant, and antipsychotic effects, which could reflect an indirect increase of the endocannabinoid anandamide. In clinical settings, the suggested therapeutic doses are generally high (>200 mg/day). However, there are several reports of anxiolytic effects using low doses of CBD. Although a placebo effect likely plays an important role in these reports, the question has been raised whether they might also involve modulation of the gut microbiota, since CBD has recently been shown to also possess antimicrobial properties. The objective of this project was to investigate the effects of different doses of CBD, administered chronically via oral gavage in mice, on the host's microbiota profile and its relation to stereotyped behavior. **Methods:** Male and female C57BL/6-WT mice (CEUA 1234/2023) were divided into five groups (n = 11 or 12): a SHAM control group without any type of manipulation; a control group treated with the vehicle oil used to dissolve CBD; and three treatment groups receiving CBD at doses of 3, 10, and 30 mg/kg. The treatment was carried out in three weeks (21 days) via oral gavage. At the end of the treatment period, mice were subjected to the Marble Burying Test to analyze stereotyped behavior. Additionally, fecal samples were collected for 16S sequencing. The characterization of microorganisms was performed using the SILVA database. **Results:** In the Marble Burying Test, the intermediate dose group, 10 mg/Kg dose, (Male: 6,36 ± 1,4; Female: 7,81 ± 1,3) buried fewer marbles compared to the low (Male: 12 ± 1,3; Female: 13,8 ± 2,4; p = 0,018) and high (Male: 13,5 ± 1,6; Female: 11,5 ± 2,3; p = 0,033) dose groups (3 and 30 mg/kg) in both sexes, indicating a reduction in stereotyped behavior, (ANOVA, F₍₄₎ = 3,877, p<0.05). These results suggest that chronic administration of low doses of CBD may modulate stereotyped behavior in mice in a dose-dependent manner. To investigate if this effect could involve the selection of specific microorganisms promoted by the 10 mg/kg dose, fecal sample 16S sequencing was performed (5 or 6 samples of each group). The Linear Discriminant Analysis (LDA) Effect Size showed that CBD treatment at all doses increased the abundance of the genera *Akkermansia*, *Bifidobacterium*, *Ruminococcus* and *Butyricimonas* compared to the Vehicle and Sham groups (P < 0.05 e Log LDA > 2). This increase was more pronounced at the lower doses (3 and 10 mg/kg). These genera are known producers of short-chain fatty acids, which have demonstrated antidepressant and anxiolytic effects in various animal models, potentially helping to explain the behavioral outcomes observed and the reports in the literature. We also analyzed blood samples of these animals (male: n = 3 per group; female: n = 6 per group), collected right after the Marble Burying test, by high-performance liquid chromatography (HPLC) in order to detect cannabinoids. No CBD was detected in these samples. Moreover, there was no difference between groups regarding the plasma levels of the endocannabinoid anandamide in both male and female samples, indicating that the observed behavioral effect in the Marble Burying test is not due to the inhibition of anandamide metabolism. **Conclusion:** In summary, the results indicate that low doses of CBD can modulate the gut microbiota, favoring the growth of *Akkermansia*, *Bifidobacterium*, and *Butyricimonas*. These microbial groups have been previously associated with anxiolytic and antidepressant effects and may be involved in the behavioral changes observed in the Marble Burying Test at the 10 mg/kg dose. **Financial support:** FAPESP (2024/03720-9, 2023/16182-2, 2017/24304-0)

03.010 **Effects of a Single Dose of Ayahuasca or Dextroketa mine on the Menstrual Cycle and Premenstrual Symptoms: A Randomized, Double-Blind Study.** Bellucci T¹, Gomes CC¹, Maekawa RM¹, Hallak JEC^{1,2}, Santos RG^{1,2} FMRP-USP, Dept Neuroscience and Behavior; ²INCT-TM, National Institute for Translational Medicine

Introduction: The menstrual cycle is a natural physiological process in the female body, consisting of two primary phases: the follicular and luteal. During the luteal phase, many women may experience premenstrual syndrome (PMS), which can include symptoms such as breast tenderness, bloating, headaches, mood swings, depression, anxiety, anger, and irritability. In Brazil, it is estimated that approximately 17% of women suffer from Premenstrual Dysphoric Disorder (PMDD), a more severe form of PMS that can profoundly impact personal, social, familial, and professional domains, often resulting in distress comparable to that associated with major depressive disorder, panic disorder, and post-traumatic stress disorder (PTSD). Current treatment options for PMDD include selective serotonin reuptake inhibitors (SSRIs) and oral contraceptives. However, there is a pressing need for alternative therapeutic approaches due to potential side effects and varying degrees of effectiveness in certain individuals. In recent years, psychedelics such as psilocybin, LSD, and N,N-dimethyltryptamine (a compound present in ayahuasca) have gained attention as potential treatments for various psychiatric conditions. Emerging research indicates that these substances may exert anti-inflammatory and neuroprotective effects and may be beneficial in addressing anxiety, depression, pain, and other health disorders. Additionally, ketamine, a dissociative anesthetic, has also been investigated in the context of depression, anxiety, and pain. Despite the promising findings, there remains a significant gap in research specifically examining the effects of these substances on the menstrual cycle and the symptoms associated with PMS and PMDD. Therefore, it is essential to conduct controlled studies to further explore these potential effects. **Methods:** The current study aims to evaluate these aspects by administering, in the follicular phase, an oral dose of ayahuasca (1ml/kg) or ketamine (2mg/kg) in a randomized and double-blind trial with 20 women with regular menstrual cycles to assess PMS and PMDD symptoms. This investigation utilizes the Premenstrual Symptom Tracking Instrument (PSST) as the main outcome, applied at baseline and at 7, 14, 21, and 28 days after the intervention. **Results:** In our preliminary results, five volunteers (mean age $\pm$ SD: 26.8 $\pm$ 5 years; mean BMI $\pm$ SD: 24.99 $\pm$ 2.1 kg/m²) showed high scores in PSST at baseline (mean score $\pm$ SEM 27.86 $\pm$ 5.1) which were reduced in all follow-up points (mean score $\pm$ SEM: 14.21 $\pm$ 3.7; 16.5 $\pm$ 2.2; 19.23 $\pm$ 2.7; 14.9 $\pm$ 4.6), and remaining below baseline values. Treatments were well tolerated, and there were no serious adverse reactions. However, the sample is small and the study is still blinded, so we cannot make any conclusions yet. **Conclusion:** In conclusion, ayahuasca and ketamine were well tolerated by the volunteers and seem to reduce PMS symptoms over 28 days.

03.011 **Automated vs. Manual Immobility Analysis: Fluoxetine Effects on a Rat Forced Swim Test Model.** Fonseca RD¹, Almeida FB¹, Silva IAG¹, Battú GS², Flores CD³, Nin MS⁴, Barros HMT⁴
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Introduction: Advancements in preclinical understanding of psychiatric disorders critically rely on animal behavior analysis. However, traditional methods are often constrained by observer bias and the time-consuming nature of manual assessment, while commercial automated systems present significant financial barriers. In order to address these limitations, computational ethology offers a cutting-edge paradigm, applying computer vision and machine learning algorithms for automated and scalable behavioral phenotyping. Within this framework, the present study aimed to comprehensively characterize the effect of fluoxetine (FLX) on depressive-like behavior observed in Wistar rats during the Forced Swim Test (FST). **Methods:** Data from 48 Wistar rats (males and females) were collected from a strain specifically bred for a high immobility phenotype in the FST. All animals were subjected to a FST training session and then were treated with either saline or FLX (1, 10 or 20 mg/kg), with injections administered at 23.5 h, 5 h, and 1 h before the FST test session. Five-minute videos from the test sessions were analyzed. Manual immobility scores were obtained by a trained observer using Behavsoft® software. For automated analysis, a DeepLabCut-based deep learning model was trained to track the animal's nose, head, and ears. Immobility was defined when the head point remained below a displacement of 5 pixels/frame. Data were statistically analyzed using repeated measures ANOVA to investigate the interaction among analysis methods (manual vs. automated), experimental subgroups, and sex. **Results:** Shapiro-Wilk test indicated that immobility times were not normally distributed ($W = 0.79$, $p < 0.001$). Immobility times from both analysis methods showed a strong and significant positive correlation (Spearman's $\rho = 0.85$, $p < 0.001$), indicating good agreement. However, a significant main effect of analysis method was observed [$F(1,40) = 10.97$, $p = 0.002$, $\eta^2 = 0.215$], indicating overall differences between them. Furthermore, a significant interaction between analysis method and treatment was found [$F(3,40) = 5.15$, $p = 0.004$, $\eta^2 = 0.279$]. Post hoc comparisons with Bonferroni correction revealed a significant reduction in immobility time at the highest FLX dose compared to control in the manual analysis ($p < 0.05$), a difference not observed with the automated method ($p = 0.177$). Additionally, at the highest dosage, there was a significant difference between the two analysis methods ($p < 0.001$). No significant interaction with sex was identified. **Conclusion:** This study demonstrates that different behavioral analysis methods significantly influence the detection of phenotypic effects in preclinical animal models. The findings highlight the critical importance of methodological choice in behavioral neuroscience and psychopharmacology to achieve more reliable results. **Ethics declarations:** All experimental procedures with animals were approved by UFCSPA's Animal Research Ethics Committee (CEUA/UFCSPA 213/2022). **Financial support:** CAPES.

03.012 **Effects of Subchronic Treatment and Withdrawal of Cannabigerol on Anxiety-like Behaviors Related to Acute Stress in Female Wistar Rats.** Hirabayashi MH, Storgatto FJ, Zanolini JM UFPR, Dept Pharmacology, Brazil

Introduction: Acute and chronic stress are major contributing factors to the development of anxiety disorders. It is well known that exposure to stress can dysregulate key systems involved in emotional regulation within specific limbic brain regions. Although antidepressants are the first-line treatment, a considerable portion of patients exhibit inadequate responses, emphasizing the need for more effective and personalized therapeutic approaches. In that context, recent studies suggest that Cannabigerol (CBG), a non-psychoactive cannabinoid, has shown potential as an alternative treatment for anxiety. Whereas such disorders are more prevalent in women, female animals are often underrepresented in research due to the hormonal complexity of the estrous cycle. Thus, this study aims to evaluate anxiety-like behaviors in female rats exposed to acute stress following subchronic treatment with different doses of CBG. Additionally, a withdrawal effect will be assessed, along with behavioral differences across hormonal phases. **Methods:** For 2 consecutive days, individual 5-minute handling sessions were conducted per animal. On the third day, half of the animals underwent the Acute Restraint Stress (ARS) protocol (S group) for 120 min, whereas the other half was not exposed to stress (NS group). After 7 days of continuous intraperitoneal treatment with CBG (3 or 30 mg/kg), fluoxetine (FLX; 10 mg/kg, positive control) or vehicle, all animals were subjected to the Elevated Plus Maze (EPM) test to assess the potential anxiolytic-like effects of CBG. Following a 3-day treatment interruption, the animals underwent the Light-Dark Transition (LDT) test to evaluate whether there would be any lasting or rebound effects from the CBG treatment. At the end of each test, individual vaginal smears were collected for estrous cycle identification. Proestrus and estrus phases (PE) were classified as one, as were the diestrus and metestrus phases (MD). All procedures were approved by the Animal Use Ethics Committee (#1620). **Results:** In both the EPM and LDT tests, S animals presented an increase in anxiety-like behavior compared to NS animals, without changes in exploratory activity. Treatment with both CBG and FLX prevented the anxiety-like response induced by the ARS in the EPM. However, in the LDT, only CBG (30 mg/kg) and FLX produced a lasting anxiolytic-like effect. In both tests, exploratory activity remained unchanged across all groups. Regarding the estrous cycle, in both the EPM and LDT, PE animals exhibited reduced anxiety-like behavior compared to those in the MD group. However, this was only shown in NS animals and FLX-treated S animals. These findings suggest that the anxiolytic effect of CBG in S animals is independent of the estrous cycle phase. **Conclusion:** Subchronic treatment with CBG effectively reduced anxiety-like behaviors in previously S female animals and induced a lasting anxiolytic response, without evidence of rebound effect post-treatment withdrawal. Also, CBG appeared to counteract anxiety-like behavior during the MD phases of the estrous cycle, indicating that its effects are independent of hormonal fluctuations associated with the cycle. **Acknowledgments:** CNPq for the productivity grant (Process No. 307714/2023-3).

03.013 **Neuronal Activity of the Periaqueductal Gray in Response to Reward-Associated Cues: An Investigative Analysis.** Gouvea IN, Costa BY, Camarini R ICB-USP, Dept Pharmacology, Lab Neurochemistry and Behavioral Pharmacology, Brazil

Introduction: The response to a rewarding stimulus is fundamentally associated with learning processes and the ability to respond to predictive cues. In this form of learning, an initially neutral stimulus is repeatedly paired with a stimulus that elicits a reward-related response. Recent studies suggest the role of Periaqueductal Gray (PAG) in processing reward-related stimuli and motivation for reward-seeking, primarily the l/vIPAG. **Methods:** Adult male C57BL/6 mice were tested using the Conditional Place Preference (CPP) protocol to evaluate reward-associated learning, using Ethanol, Morphine, and Social Interaction as positive stimuli. After conditioning, immunofluorescence analysis was used to evaluate activity in the PAG, staining to detect protein expression. The analysis utilized c-Fos, an immediate early gene commonly recognized as a marker of neuronal activation. **Results:** Exposure to the drug-paired chamber in the CPP test resulted in a significant increase in c-Fos expression along the rostral, intermediate, and caudal portions of the lPAG in the groups conditioned with Ethanol ($p < 0.001$, $p < 0.001$, $p = 0.023$), Morphine ($p < 0.001$, $p < 0.001$, $p < 0.001$), and Social Interaction ($p = 0.027$, $p < 0.001$, $p = 0.021$) compared to control measurements. Notably, c-Fos expression peaked 90 minutes post-conditioning, indicating heightened neuronal activity associated with reward processing. **Conclusion:** The results show a significant increase in the expression of c-Fos in the lPAG in the different groups. This suggests that the region may be involved in the associative learning processes underlying conditioning, reinforcing the idea that the PAG plays a role in the motivational response to reward, whether induced by a drug of abuse or a social stimulus. It is worth noting that the control groups did not show an increase in c-Fos expression, including both the control group exposed to the CPP protocol and the control group exposed only to the environment.

03.014 Investigation of the Involvement of Cannabinoid Receptors CB1 and CB2 in the Mediation of Resolvin D5 Effects on Anxiety-Like Responses: A Comparative Study Between Sexes. Avanço LC, Uada TS, Zanolini JM UFPR, Dept Pharmacology

Introduction: Stress-related anxiety negatively affects brain regions, leading to dysregulation of key systems responsible for emotional regulation. Fish oil supplementation has gained popularity, and several studies have highlighted its benefits, including enhanced emotional balance, reduced neuroinflammation, and restored homeostasis. Among its active components, Resolvin D5 (RvD5)—a pro-resolving lipid mediator derived from docosahexaenoic acid—has demonstrated neuroprotective and anxiolytic-like effects in previous studies from our laboratory. Importantly and of interest to this study, evidence also suggests potential interactions between RvD5 and the endocannabinoid system. Therefore, we aimed to investigate the effects of RvD5 on anxiety-like behavior under stress and non-stress conditions. Given there are sex-based differences in sensitivity to stress and anxiety response, both male and female animals were included to account hormonal variation. Additionally, we examined the potential involvement of cannabinoid CB1 and CB2 receptors in mediating the effects induced by RvD5. **Methods:** Animals received a daily intraperitoneal injection of RvD5 (1 ng/animal in 200 µl) for 10 consecutive days. On day 8, the light-dark transition (LDT) test was performed to assess effects under non-stressed conditions. On day 9, animals were subjected to 2-hour restraint stress. On day 10, at least 3 hours after RvD5 administration, animals received intraperitoneal injections of either AM630 (CB2 antagonist) or AM251 (CB1 antagonist). One hour later, the elevated plus maze (EPM) test was conducted. To evaluate the persistence or withdrawal of RvD5 effects, the novelty-suppressed feeding (NSF) test was performed one week after treatment ended (day 17). Vaginal lavages were collected after each behavioral test to determine the estrous cycle stage and assess behavioral variation across phases. **Results:** In the LDT test, no significant differences were observed between control and RvD5-treated groups in either male or female rats, suggesting that RvD5 does not exert detectable anxiolytic-like effects under non-stressed conditions. However, in the EPM test, RvD5 induced a clear anxiolytic-like effect in both sexes. This effect was reversed by CB1 and CB2 antagonists in females. In males, only the CB2 antagonist reversed the anxiolytic-like effect of RvD5, while CB1 antagonist administration yielded inconclusive results—likely due to an insufficient sample size—highlighting the need for further investigation. In the NSF test, only male rats treated with RvD5 exhibited anxiolytic-like behavior, whereas this effect was not observed in females, further supporting a sex-dependent response to treatment. The analyses of the groups of females separated by estrous cycles (metaestrus/diestrus and proestrus/estrus) are still being carried out. **Conclusion:** These preliminary findings suggest that RvD5 holds therapeutic potential as an anxiolytic agent under stress-related conditions. Its efficacy appears to be modulated by both sex and behavioral context, possibly through interactions with cannabinoid receptors. Further research is required to elucidate the underlying mechanisms and confirm these initial observations.

03.015 Standardization of a Depression Model for Assessing the Antidepressant Potential of Bufotenine in Rats. Oliveira PS, Queiroz KS, Paulo MEFV, Gonçalves TCP, Nencioni ALA IB, Lab. of Pharmacology, Brazil

Introduction: Depression is a severe and debilitating psychiatric disorder affecting approximately 280 million people worldwide, characterized by persistent mood changes, anhedonia, apathy, and, in more severe cases, suicidal thoughts or behaviors. Despite the widespread use of antidepressants, current treatments often lack a combination of high efficacy, rapid onset, and a favorable safety profile. To study depression in research, the Chronic Unpredictable Mild Stress (CUMS) model is widely used and well validated for inducing depressive-like phenotypes in rats. This model involves exposing animals to a variety of mild, unpredictable stressors over an extended period, effectively simulating the chronic stress that contributes to depression in humans. As a result, it reproduces typical behavioral and physiological characteristics of the disorder. Given the limitations of conventional antidepressants, recent interest has focused on psychedelic agents as promising alternatives for patients with treatment-resistant depression and anxiety. Bufotenine, a compound found in seeds of *Anadenanthera* spp, has emerged as a potential therapeutic candidate. Although it is an isomer of psilocin, bufotenine does not produce significant psychotropic effects. Its action as an agonist of the 5-HT_{2A} receptor, which plays a key role in mood regulation, positions it as a promising candidate for novel antidepressant development. **Methods:** Male Wistar rats were subjected to a chronic unpredictable mild stress (CUMS) protocol for six weeks. From the fourth week onwards, the animals received a daily oral treatment via gavage, either with saline (1ml/kg, stress group) or fluoxetine (3 mg/kg, fluoxetine group). A control group of animals was not subjected to any stress or treatment for six weeks. At the end of the CUMS protocol, the rats underwent a series of behavioral tests to assess their depressive level including the Sucrose Preference Test (SPT), the Forced Swim Test (FST), and the Open Field Test (OFT). Body weight was monitored throughout the experimental period. At the end of the protocol, biological samples were collected for further analysis. **Results:** In terms of body weight, both the stress and fluoxetine groups exhibited significantly lower weight gain than the control group ($p < 0.0001$). In the SPT, the stress group showed a decreased preference for sucrose while the fluoxetine group exhibited a tendency to prefer sucrose. No statistically significant differences were observed between the groups in the FST. In the OFT, significant differences were found among the stress, fluoxetine, and control groups for the parameters evaluated: reduction in grooming time, number of rearing and time spent in the central area were all highly significant compared to the control group. **Conclusion:** The results demonstrate that we have developed an efficient CUMS model, capable of effectively inducing a depressive-like phenotype in rats. Fluoxetine, used as a positive control, was able to reverse the depressive effects in the animals. With a well-established model in place, we will proceed to test bufotenine, comparing its results with fluoxetine in order to evaluate the antidepressant effect of bufotenine. **Financial support:** Fundação Butantan

03.016 **Effects of TNFR1 Deletion on the Behavior of Female Mice.** Neves MCV, Lima TAS, Kawamoto, EM ICB-USP, Lab Molecular and Functional Neurobiology Dept Pharmacology

Introduction: Tumor necrosis factor receptor 1 (TNFR1) is primarily associated with the regulation of neuroinflammatory processes; however, some studies also suggest its involvement in regulating neuroplasticity under basal, non-inflammatory conditions. The estrous cycle of female mice and its hormonal fluctuations lead to changes in memory and anxiety-related behaviors. Although its effects have already been characterized in males, the impact of TNFR1 deletion on the behavior of female mice and its interaction with the estrous cycle has not yet been described. Our study aimed to investigate the potential effects of TNFR1 signaling on motivational behavior, anxiety-like behavior, and memory according to the estrous cycle phase. **Methods:** Twenty-eight wild-type (WT) and TNFR1 knockout (TNFR1 KO) female mice (8–12 weeks old) were used, divided into six experimental groups: (a) WT diestrus; (b) WT proestrus; (c) WT estrus; (d) TNFR1 KO diestrus; (e) TNFR1 KO proestrus; and (f) TNFR1 KO estrus. The estrous cycle was determined via vaginal smears taken two hours before testing. Behavioral tests included the open field test followed by novelty-seeking, spontaneous alternation, and the elevated plus maze. Two-way ANOVA and repeated-measures two-way ANOVA were conducted, followed by post-hoc analysis using Tukey's test for interactions and Bonferroni correction for main effects. All procedures were approved by the Animal Ethics Committee (CEUA) under protocol number 1345200524. **Results:** The open field test showed a significant difference in the distance traveled over the time intervals [$F(2.019, 32.30) = 42.31$, $p < 0.0001$]. In this regard, in the WT genotype, a significant difference was found between the 0–5 minute and 20–25 minute intervals ($p < 0.0001$); in the TNFR1 KO genotype, a difference was also found between the 0–5 minute and 20–25 minute intervals ($p = 0.0123$). The observed decrease in distance traveled over time intervals indicates habituation to the apparatus. No significant differences were found for genotype or estrous cycle stage regarding the time spent in the center of the apparatus (an indicator of anxiety-like behavior levels). The novelty-seeking test showed no significant differences for cycle or genotype in the time spent exploring the object, that is, the amount of time the animals spent sniffing and directly interacting with the object. The spontaneous alternation test showed no significant differences for cycle or genotype in the number of alternations, indicating no changes in working memory between groups. Finally, in the elevated plus maze test, there were no significant differences for genotype or cycle in the time spent in the open arms of the maze. **Discussion:** The data obtained so far, with the current sample size, did not indicate any impact of TNFR1 deletion on mouse behavior, nor was any interaction between hormonal variations of the estrous cycle and TNFR1 deletion observed.

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03.017 **Effects of Muscimol Administration in the Dorsal Hippocampus of Wistar Rats on the Strength and Specificity of Contextual Memory During its Recall.** Castro JC, Quintiliano dos Santos AC, Vido Lopes L, Glória VTL, Boscariol Lourenço WT, Fornari RV UFABC, Centro de Matemática, Computação e Cognição

Introduction: Memory consolidation is a gradual and dynamic process that involves coordinated activity across multiple brain regions, particularly along the hippocampal–neocortical axis. Recent literature suggests that distinct engrams can be formed and reorganized over time, with the hippocampus playing a central role in the encoding and early retrieval of memories. According to the Trace Transformation Theory (TTT), these changes reflect a transformation in the quality of the memory, shifting from detailed, context-specific representations to more generalized. Furthermore, studies in rats in the contextual fear conditioning (CFC) task show that stronger training induces generalization in remote memory tests, while in recent memory tests the animals still discriminate. On the other hand, weaker training creates specific memory traces for a longer period. Studies in our laboratory have shown that animals subjected to strong CFC training (1.0 mA) presented discrimination of contextual memory after two days, a time during which memory is still hippocampus-dependent. **Objectives:** This study aimed to replicate and extend findings on the role of the dorsal hippocampus (dHPC) in the evocation of a recent (2 days) contextual memory, as well as to evaluate the specificity of this memory. To this end, we temporarily inactivated the dHPC with the GABA_A agonist muscimol before testing and the animals were tested in the training and novel contexts. **Methods:** In Experiment 1, adult male Wistar rats (CEUA-UFABC #6893221018) were implanted with bilateral cannulas in the dHPC. After seven days, they were trained with three footshocks (1.0 mA, 1 s). Two days later, they were infused with muscimol (MUSC, n = 4) or saline (VEH, n = 4) and tested, fifteen minutes later, in the training context. In Experiment 2, Wistar Hannover rats were used (MUSC, n = 7; VEH, n = 6). The protocol was similar, but the test included both novel and training contexts. On Day 3, animals were re-exposed to both contexts in a drug-free retest. In both experiments, freezing behavior was measured as an index of fear memory. **Results:** In Experiment 1, muscimol-treated rats showed significantly lower freezing than controls during the test [$t = -4.13$, $p = 0.006$; MUSC: 17.6%, SD = 18.4; VEH: 60.3%, SD = 9.56], indicating reduced memory strength. In Experiment 2, no treatment effect was found in the test. However, during the drug-free retest, there was a significant Context × Treatment interaction [Two-way ANOVA, $p < 0.05$] only the muscimol group discriminated between contexts. The generalization index (freezing in novel / total freezing) further supported this, showing a significant Group × Session interaction [Two-way ANOVA, $p = 0.026$, followed by Tukey test]. **Conclusions:** In Experiment 1, dHPC inactivation impaired the strength of recent memory, during the retrieval. In Experiment 2, pre-test inactivation did not affect immediate performance but preserved specificity in a later session, possibly by preventing extinction. These results suggest that the dHPC is involved not only in memory strength but also in contextual specificity and extinction processes. Funding: PIBIC/CNPq 03/2023.

03.018 **Z-Score Standardization for the Characterization of Resilient and Non-Resilient Female Mice in a Chronic Restraint Stress Model.** Pereira GJS^{2,3}, Schneider GK³, Lovis EP^{3,4}, Zanchet EM^{1,3,4}, Bochi GV^{1,3,4}. ¹UFSM, Dept Physiology and Pharmacology, Brazil, ²UFSM, Faculty of Pharmacy, Brazil, ³UFSM, Lab. of The Study of Depression – LEDep, Brazil, ⁴UFSM, PPG Pharmacology, Brazil

Introduction: The study of major depressive disorder (MDD) and pharmacological strategies for its treatment requires refined animal models to capture parameters that reflect human symptoms. Z-score analysis is relevant as it normalizes independent variables with different scales into a single "emotionality scale". It is calculated by subtracting the control mean from an individual's raw value and dividing by the control standard deviation. **Methods:** This study aimed to apply the Z-score to characterize female mice resilient and non-resilient to chronic restraint stress (CRS) and to validate behavioral tests composing the Z-emotionality: tail suspension test (TST), splash test (ST), open field test (OFT), and elevated plus maze (EPM). Female Swiss mice (6–8 weeks), approved by the Animal Ethics Committee of UFSM (CEUA no. 3124060323/2023), underwent CRS for 6 hours daily over 28 days. Body weight was recorded weekly for 4 weeks, and behavioral tests were performed 24 hours after the last CRS session. Subsequently, the Z-emotionality was calculated considering the individual Z-score calculated for each test. Based on the Z-emotionality, the animals were grouped as follows: (1) The control group was defined as having Z-emotionality values within $\pm$ half a standard deviation (SD) from the mean of all animals that were not subjected to CRS; (2) The resilient group had the same range of Z-emotionality mean and SD as the control group; (3) The non-resilient group included animals with Z-emotionality greater than 1.5 times the SD of the control group. **Results:** The mean $\pm$ SD of the Z-emotionality for the control group was -0.0007 ± 0.472 . The weekly measurements of body weight showed that only the control group experienced significant weight gain (Week 2: control vs. non-resilient: $p = 0.015$; control vs. resilient: $p = 0.024$; resilient vs. non-resilient: $p = 0.947$. Week 3: control vs. non-resilient: $p < 0.001$; control vs. resilient: $p < 0.001$; resilient vs. non-resilient: $p = 0.979$. Week 4: control vs. non-resilient: $p < 0.001$; control vs. resilient: $p < 0.001$; resilient vs. non-resilient: $p = 0.961$). Pearson's correlation showed Z-TST and Z-OFT positively influenced analysis, with moderate ($r = 0.4902$; $p = 0.0046$) and strong ($r = 0.7414$; $p < 0.0001$) correlations to the Z-emotionality, respectively. No significant correlations were observed for Z-EPM and Z-ST. Analysis using Z-TST + Z-OFT distinguished properly resilience after CRS (control vs. non-resilient: $p = 0.0141$; control vs. resilient: $p = 0.3232$; resilient vs. non-resilient: $p < 0.0001$), while Z-EPM + Z-ST identified only anxious phenotype (control vs. non-resilient: $p < 0.0001$; control vs. resilient: $p = 0.5257$; resilient vs. non-resilient: $p = 0.1624$). **Conclusion:** These findings suggest that TST and OFT are statistically reliable to compose the Z-emotionality and classify female mice resilience to CRS. The Z-score may play a crucial role in characterizing depressive-like behavior and resilience, given the symptom variability of MDD among individuals this analysis contributes to a most specified future studies with antidepressants entailing more accuracy to the pharmacological treatment.

03.019 **Evaluation of Anxiety- And Depression-like Behaviors in Tumor-Free Mice Treated with Paclitaxel: Potential Neuroprotective Effects of Cannabidiol** Queiroz ABWV¹, Pereira ACM², Lisboa SFS² ¹FMRP-USP, Neuropsychopharmacology Lab, Brazil; ²FCFRP-USP, PPG Pharmaceutical Science

Introduction: Chemotherapy has dramatically improved cancer survival, but it often leads to neurological comorbidities. Cognitive deficits (especially in memory, learning, and spatial skills) are well documented in humans and animals. In contrast, affective disturbances remain understudied with inconsistent findings. Clinical studies rarely control tumor presence, limiting causal conclusions, since the disease is a confounding factor itself. Animal studies are few and conflicting, highlighting the need for better models. Here, we evaluated anxiety- and depression-like behaviors in tumor-free mice treated with paclitaxel (PTX). Cannabidiol (CBD), known for neuroprotective effects against neuroinflammation and senescence, was tested as a preventive treatment.

Methods: Female C57BL/6 mice (7 weeks old) were housed in groups of 3-4 animals per cage under standard laboratory conditions (22 ± 2°C, 12 h light/dark cycle), with food and water available ad libitum. All procedures were approved by the Animal Research Ethics Committee (CEUA nº 23.1.223.60.8). Mice received 20 mg/kg PTX intraperitoneally (i.p.) on alternating days (three doses/week) for two consecutive weeks, with two rest days between weeks. One experimental group also received 1 mg/kg CBD i.p. on the day following each PTX dose. Control groups received the respective vehicles. Behavioral tests were conducted 48 hours after the final PTX injection. Anxiety-like behavior was assessed using the open field and light/dark box tests; depression-like behavior was evaluated using the splash test and tail suspension test. **Results:** In the open field (n = 5-12), ANOVA indicated a significant effect on locomotor activity ($F_{(2, 25)} = 10,69$; $p = 0,0004$) and a tendency in center time ($F_{(2, 25)} = 2,711$; $p = 0,0860$). PTX group showed a significant decrease in locomotor activity when compared to vehicle (Tukey's test; $p = 0,0005$), with no CBD effect (Tukey's test: $p = 0,6369$). No changes were found in center distance ratios. In the light/dark box (n = 10-18), although no differences were found in total time, ANOVA showed a significant effect in the number of entries in the light part of the box ($F_{(2, 42)} = 6,639$; $p = 0,0031$) and risk assessment behavior ($F_{(2, 42)} = 4,600$; $p = 0,0156$). The PTX group presented fewer entries in the light compartment (Tukey's test: $p = 0,0031$), but no CBD effect (Tukey's test: $p = 0,8102$). PTX also induced a reduction in risk assessment behavior (Tukey's test: $p = 0,0146$), with no influence by CBD (Tukey's test: $p = 0,8246$). In the splash test (n = 10-17), there was a tendency to increase grooming latency by PTX (ANOVA: $F_{(2, 41)} = 3,054$; $p = 0,0580$), with no change in grooming events or duration. No effects were observed in the tail suspension test ($p > 0,05$). **Conclusion:** PTX reducing locomotor activity and exploratory behaviors may reflect sickness behavior. Although PTX-induced decreased entries in the light/dark box and center time in the open field may reflect an anxious-like phenotype, these findings should be interpreted cautiously as the absence of changes in total time in the light zone and reduced risk assessment behavior, along with reduced locomotor activity, could point to a confounding effect of hypolocomotion. Moreover, no evidence of depression-like changes was observed. These findings highlight a predominant effect of PTX on locomotor and exploratory behavior in female mice, with limited evidence of influencing anxiety or depressive-like effects under the present experimental conditions. Further investigation is necessary to assess PTX's ability to alter mood, and CBD's protective effects against PTX, which was not evident in the current experimental protocol. **Financial support:** FAPESP and CAPES.

03.020 **Effects of *Lactiplantibacillus plantarum* 286 and *L. plantarum* 81 on Ethanol-Induced Reinstatement in Conditioned Place Preference Associated with Sleep Deprivation in Mice.** Figuera YM, Silva KSO, Resende GR, Tamura EK UESC, Chronobiology Research Group, Ilhéus, BA

Introduction: Ethanol dependence remains a significant global health issue. Recent studies emphasize the role of the microbiota-gut-brain axis in neuropsychiatric conditions, including substance use disorders (Moloney et al., 2014). Additionally, sleep-wake cycle disruptions have been associated with altered activity in reward-related brain areas, contributing to addictive behaviors (Nechifor et al., 2020). Psychobiotics such as *Lactiplantibacillus plantarum* 286 (Lp286),

isolated from cocoa (*Theobroma cacao*), and *L. plantarum* 81 (Lp81), from cupuaçu (*Theobroma grandiflorum*), have shown promising results in mood modulation in mice (Barros-Santos et al., 2020). This study aimed to investigate the effects of Lp286 and Lp81 on ethanol-induced conditioned place preference (CPP), with or without sleep deprivation, in Swiss mice. **Methods:** Forty male Swiss mice were divided into four groups (n=10) and treated daily in the morning (8–9:00h) via gavage with vehicle (0.85% saline + 15% skim milk), Lp286, or Lp81 (10^9 CFU). The CPP paradigm followed Cata-Preta et al. (2018), using ethanol (1.8 g/kg, i.p.) during conditioning. Mice underwent one day of pre-conditioning, followed by conditioning (ethanol or saline on alternate days), and two post-conditioning tests (post 4 and post 8). Lp286 and Lp81 treatments occurred only during the conditioning phase. Sleep deprivation was induced by the gentle handling method for 6 continuous hours (Castillo-Ruiz & Nunez, 2011), targeting both REM and slow-wave sleep. Control animals were kept in the same room, undisturbed. Behavioral data were recorded and analyzed using Anymaze® software. **Results:** Animals showed increased time in the ethanol-paired compartment after conditioning, confirming successful CPP induction. However, in the final post-exposure phase, no significant preference was observed in the sleep-deprived groups (Vehicle-ETA Deprived, Lp286-ETA Deprived, Lp81-ETA Deprived), suggesting reduced reinstatement under these conditions. Additionally, a reduction in total distance traveled was noted from post 4 to post 8, likely due to habituation. No significant differences were found in the number of entries into the compartments. **Conclusion:** The data suggest that Lp286 and Lp81 may modulate ethanol-induced reward behavior under conditions of sleep deprivation, indicating potential psychobiotic properties. However, their effects on preventing reinstatement remain inconclusive, warranting further investigation into their mechanisms and therapeutic relevance in alcohol dependence. Funding: YMF and GRR were undergraduate fellows, and KSOS was a graduate fellow, all funded by FAPESB (Fundação de Amparo à Pesquisa do Estado da Bahia). Ethical Approval: All experimental procedures were approved by the Institutional Animal Care and Use Committee (CEUA-UESC; protocol nº026/23).

03.021 Effects of Aripiprazole on Social and Locomotor Behavior in Adult Zebrafish Acutely Exposed to Ketamine. Patelli-Alves A, Becker SZ, Gallas-Lopes M, Bruck SM, Müller DV, Bastos LM, Stahlhofer-Buss T, Piato A, Herrmann AP. UFRGS, Pharmacology Dept

Introduction: Schizophrenia is a heterogeneous neurodevelopmental disorder marked by positive, negative, and cognitive symptoms. Although its pathophysiology remains unclear, studies implicate dopaminergic hyperactivity in subcortical circuits and NMDA receptor hypofunction in GABAergic interneurons. Ketamine, an NMDA antagonist, is widely used to model schizophrenia-related behaviors in animals. In rodents, ketamine reliably induces social withdrawal, a core negative symptom. However, its effects, and their modulation by antipsychotics are less consistent in zebrafish (*Danio rerio*). Therefore this study aimed to assess whether aripiprazole, a third-generation antipsychotic, counteracts ketamine-induced social and locomotor alterations in adult zebrafish assayed in the social preference test (SPT). **Methods:** 128 wild-type male and female (62 males, 66 females) adult zebrafish were randomly allocated to the experimental groups (n=16): control (DMSO 0.94%) or aripiprazole at 0.1, 0.5 or 2.5 mg/L, and exposed individually for 20 minutes. Immediately after, they were exposed to either water or ketamine 40 mg/L for another 20 minutes. Animals were then individually placed in the SPT tank for 7 minutes. The test was recorded and the time spent in the zone close to the stimulus shoal (interaction zone) and locomotor parameters (crossings, total distance and percent distance in the interaction zone) were quantified using ANY-Maze® software by blinded evaluators. Data were analyzed by two-way ANOVA followed by Bonferroni post hoc test. Ethical committee approval #44680, CEUA-UFRGS. **Results:** Acute ketamine exposure significantly reduced the time spent in the interaction zone ($F(1, 120) = 434.5$; $p < 0.0001$) and the percent distance traveled in the interaction zone ($F(1, 120) = 452.7$; $p < 0.0001$). Additionally, it increased the number of zone crossings ($F(1, 120) = 214.0$; $p < 0.0001$) and the total distance traveled ($F(1, 120) = 58.58$; $p < 0.0001$). These findings indicate that ketamine significantly impairs social preference, mimicking schizophrenia-related phenotypes. An interaction between ketamine and aripiprazole was observed, with aripiprazole reversing the ketamine-induced increases in total distance traveled ($F(3, 120) = 11.70$; $p < 0.0001$) and zone crossings ($F(3, 120) = 6.308$; $p = 0.0005$). However, aripiprazole unexpectedly potentiated ketamine's effects on social behavior by further decreasing both the time spent in the interaction zone ($F(3, 120) = 4.548$; $p = 0.0047$) and the percent of distance in the interaction zone ($F(3, 120) = 3.820$; $p = 0.0118$). **Conclusion:** Ketamine impaired social behavior in adult zebrafish, modeling negative symptoms of schizophrenia. Aripiprazole reversed ketamine-induced hyperlocomotion, but potentiated the social deficits. This dual effect may relate to its complex receptor profile, as it acts not only as a D2 partial agonist but also on serotonergic and other dopaminergic receptors. These broad interactions may underlie its limited efficacy in restoring social behavior, reflecting clinical findings, where antipsychotics treats positive symptoms more effectively but shows limited efficacy in negative symptoms. **Financial support:** CAPES.

03.022 Effect of Lisdexamfetamine on the Reactivity Behavior of Mice in the Looming Test across Sex and Lineages. Rosa EB, Kondo TA, Pradella GR, Gogola JV, Dalagrana H, Garlet QI UFPR, Biological Sciences Sector, Dept Pharmacology, Brazil

Psychiatric disorders often present symptoms related to exacerbated emotional reactivity. In this context, the Looming Test is a behavioral tool used to assess animals' responses to threatening visual stimuli, simulating an imminent attack by triggering the innate fear of aerial predators. The expected response to the Looming stimulus includes survival-oriented behaviors, primarily associated with fear or anxiety, such as freezing or escape to a refuge, allowing the investigation of individual differences in the neurobiological basis of reactivity to stress-inducing situations. Lisdexamfetamine (LDX) has been investigated in mice as a potential pharmacological tool to induce impulsive-like behavior, which may lead to alterations in emotional reactivity. This project aims to evaluate the effect of LDX on mice behavioral response to threat, when subjected to the Looming Test. A total of 38 mice were used: C57BL/6J (n=18: 10F/ 8M), Swiss (n=10: 5F/ 5M), and Balb/c (n=10: 5F/ 5M)—and maintained under controlled conditions (temperature, light/dark cycle, and *ad libitum* access to food and water). The animals were subjected to the Looming Test, which takes place in an acrylic box (36 cm x 30 cm x 29 cm) equipped with a ceiling-mounted projector that displays a rapidly expanding visual stimulus (a black disk that grows to the full size of the screen in 0.3 seconds, repeated 15 times in sequence), simulating an approaching predator, and then, observed for 45 seconds. The box also contains a semi-open structure that serves as a refuge. The test was conducted 30 minutes after intraperitoneal administration of LDX 10 mg/kg. All procedures were approved by the UFPR Ethics Committee (#1610). Statistical analyses were performed using RStudio and GraphPad Prism, evaluating three behavioral parameters: latency to escape to the refuge, time spent inside the refuge, and freezing time. Non-parametric analyses were conducted using the Scheirer-Ray-Hare test, and parametric analyses with two-way ANOVA, both followed by Bonferroni *post-hoc* tests. Only results with $p < 0.05$ were considered statistically significant. Regarding freezing time, there were differences between sexes ($F(1)=7.80$, $p=0.0087$). Also, there was a significant effect of strain on latency to refuge ($H(2)=16.41$, $p=0.00027$) and for time spent in the refuge, ($H(2)=10.69$, $p=0.0047$). However, the post-hoc analysis did not detect which specific groups differed, likely due to the low statistical power. Thus, the difficulty in identifying clear differences may be attributed to the limited sample size. We detected sexual dimorphism and lineage differences in mice reactivity following LDX administration. We also propose LDX as a pharmacological enhancer of reactivity in mice subjected to the Looming Test, particularly in males. These findings may contribute to a deeper understanding of pathological reactivity in mice across lineages, leading to the validation of the Looming Test in our research group as a tool to investigate reactivity in mice by emulating conditions characterized by maladaptive fear. Moreover, future experiments are scheduled to be conducted with larger sample sizes to increase statistical power.

Funding support: UFPR Keywords: reactivity, impulsivity, mice, Lisdexamfetamine

03.023 **Psychogenic Profile of Early Life Trauma Induced by Single Prolonged Stress in an Experimental Model of Post-Traumatic Stress Disorder.** Silveira EP^{1,2}, Teixeira LL^{1,3}, Andrade AS¹, Pinto LC¹, Hamoy M⁴, Lopes DCF¹ ¹UFPA, Lab Neuropatologia Experimental, Hospital Universitário João de Barros Barreto, Brasil, ²FSCMPA, Programa de Residência Médica em Clínica Médica, Brasil, ³UFPA, Programa de Pós-graduação em Farmacologia e Bioquímica, Brasil, ⁴ UFPA, Lab Farmacologia e Toxicologia de Produtos Naturais, Instituto de Ciências Biológicas, Brasil

Introduction: Stressful events in childhood are a risk factor for post-traumatic stress disorder (PTSD), and is commonly associated with anxiety, depression and cognitive impairment. Our work aimed to elucidate the psychogenic profile in PTSD using the single prolonged stress (SPS) model in prepubescent rats. **Methods:** Thirty male Wistar rats (3 weeks old) (CEUA/UFPA no. 3254260123) were divided into three groups: (a) control group, free from stress; (b) SPS group, submitted to SPS on the 1st day of the experiment; and (c) SPS + reinforcement group, subjected to SPS on the 1st and 5th day. The SPS consists in a sequential exposure to 2 hours of restraint in a containment tube; forced swimming for 20 minutes; and anesthesia with 3% isoflurane. SPS was delivered together with an acoustic and an olfactory stimuli to create stress situational reminders. On the 8th day, all SPS groups were re-exposed to stimuli, and their behavior was assessed. After the experiments, hippocampal cell nuclei were counted in the CA1, CA3, hilum and dentate gyrus regions of the hippocampus. **Results:** SPS-associated cues did not trigger fear behavior, however trauma reinforcement reduced immobile time when rats were exposed to stimuli : 43.62 ± 10.69 control: 92.20 58.84, $p = 0.025$), with no differences from the SPS group (SPS + reinforcement vs. SPS: 68.81 ± 31.88, $p = 0.335$). In the open field test, the SPS traveled a shorter distance compared to control (SPS + reinforcement: 27.44 ± 6.19 control: 35.65 ± 5.58, $p = 0.009$), without differing from the SPS group (vs. SPS: 44.33 ± 34.61, $p = 0.147$). The group had a lower mean speed and did not differ from the SPS group (SPS + reinforcement: 0.091 ± 0.020 vs. control: 0.118 0.018, $p = 0.030$; vs. SPS: 0.147 ± 0.115, $p = 0.464$). The SPS group spent more time in the center of the apparatus it (SPS: 159.8 129.6 vs. control: 19.49 ± 9.687, $p = 0.001$), with no differences from the SPS + reinforcement group: 30.35 ± 14.17, $p = 0,134$). Neither SPS alone nor its reinforcement induced trauma-cue avoidance response or short-term memory impairment. We did not report any difference between the control group and the SPS group in the CA1 region (control: 6.16 ± 0.849; vs. SPS: 6.62 ± 0.937, $p = 0.707$). The same pattern was observed for the CA3 (control: 6.20 ± 1.126; vs. SPS: 7.98 ± 1.933, $p = 0.147$), the hilus (control: 9.04 ± 1.447; vs. SPS: 8.04 ± 1.278, $p = 0.578$), and the dentate gyrus (control: 12.23 ± 1.594; vs. SPS: 14.20 ± 2.719, $p = 0.285$). Similarly, the SPS group did not differ from the SPS + reinforcement group in the CA1 (SPS vs. SPS + reinforcement: 6.99 ± 0.912, $p = 0.794$), the CA3 (SPS vs. SPS + reinforcement: 6.83 ± 0.899, $p = 0.416$), the hilus (SPS vs. SPS + reinforcement: 9.06 ± 1.869, $p = 0.563$) and the dentate gyrus SPS vs. SPS + reinforcement: 13.10 ± 1.212, $p = 0.654$). **Conclusions:** A single childhood trauma does not necessarily result in PTSD or mixed anxiety-depressive disorders and cognitive impairment, since we did not observe behavioral and histological changes, however reinforcement of SPS can generate hyperreactivity to situational reminders and reduce locomotor activity.

03.024 **Acute Effects of Cannabigerol on Anxiety-Like Behavior Associated with Acute Restraint Stress in Male and Female Rats.** Storgatto FJ, Hirabayashi MH, Zanolini JM UFPR, Dept Pharmacology, Curitiba, Paraná, Brazil

Introduction: Anxiety is an anticipatory emotional state characterized by heightened vigilance and has adaptive value in humans. However, severe or chronic stressors can induce pathological anxiety, triggering physiological and behavioral changes that impair well-being. Moreover, it is important to consider the higher prevalence of anxiety in women and the lack of studies on anxiolytics focused on female subjects. Potential new therapeutic approaches include the medical use of compounds found in *Cannabis sativa*. Some phytocannabinoids, such as Cannabigerol (CBG), still require further investigation regarding behavioral effects and potential sex-dependent differences. This study aims to evaluate whether CBG, a non-psychoactive phytocannabinoid, can attenuate behavioral symptoms in male and female Wistar rats exposed to Acute Restraint Stress (ARS), and assess any differences according to the estrous cycle. **Methods:** After 2 days of handling, animals were subjected to a 2-hour ARS session, while non-ARS controls remained in home cages. Afterwards, animals received intraperitoneal injections of CBG (3 mg/kg or 30 mg/kg), vehicle (VEH), or Fluoxetine (FLX; 10 mg/kg), used as a positive control. To evaluate acute CBG effects, all animals were tested in the Open Field Test (OFT) for 5 minutes the next day. After each test, vaginal smears were collected, and estrous cycle phases were determined via vaginal cytology as described by Goldman et al. (2007). Phases were grouped into Proestrus-Estrus (PE) and Metestrus-Diestrus (MD). Behavioral responses were tracked using ANY-maze software, and statistical analysis was performed with GraphPad Prism. All procedures were conducted in accordance with the guidelines and regulations of the Animal Research Ethics Committee of UFPR (CEUA-BIO UFPR #1620). **Results:** Both male and female VEH-ARS animals showed significantly reduced center exploration compared to non-ARS controls, indicating a pronounced anxiety-like response. In male rats, acute CBG administration did not significantly alter center exploration. However, in female rats, both doses of CBG increased center exploration, suggesting an anxiolytic-like effect. Conversely, FLX had no effect on either sex. Furthermore, no significant differences were observed between the PE and MD cycle phases for any measured parameter. **Conclusion:** Our data replicate findings showing that ARS functions as an anxiety-inducing factor. Acute treatment with CBG at both doses reversed anxiety-like behavior in female rats, potentially offering a more effective short-term alternative for managing anxiety compared to conventional anxiolytics such as FLX. This effect does not appear to depend on the estrous cycle. Despite these promising results, further studies are needed to confirm the sex-dependent therapeutic effects of CBG through additional preclinical and clinical research. The study of phytocannabinoids may contribute to the development of more effective and inclusive therapies for stress-related anxiety. References: Goldman JM, Murr AS, Cooper RL. The rodent estrous cycle: characterization of vaginal cytology and its utility in toxicological studies. *Birth Defects Res B Dev Reprod Toxicol.* 2007 Apr;80(2):84–97. doi:10.1002/bdrb.20106. Acknowledgments: CNPq for the productivity grant (Process No. 307714/2023-3).

03.025 Modulating the Reconsolidation of Generalized Fear Memories: A Preclinical Model of Combined Pharmacological Intervention Soares LA^{1,2}, Gazarini L³, Guimaraes FS⁴, Bertoglio LJ^{1,2}

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Introduction: Traumatic memories, such as in PTSD, are more intense, persistent, and resistant to modulation than typical aversive memories. While most aversive memories become labile upon retrieval and thus susceptible to pharmacological modification, traumatic memories often remain stable, preventing reconsolidation. This resistance limits the effectiveness of therapies that rely on temporary memory lability for lasting change. A two-stage pharmacological intervention may offer a promising way to overcome this limitation. **Objective:** This study investigated whether systemic administration of D-cycloserine (DCS; 15 mg/kg, i.p.) potentiates the effects of clonidine (CLO; 0.3 mg/kg, i.p.) on the reconsolidation of intense and generalized fear memories in rats. We also evaluated whether NMDA receptor blockade through intra-nucleus reuniens infusion of ifenprodil (IFE; 0.25 nmol/ μ l) interferes with these effects. **Methods:** Ninety-three male Wistar rats underwent contextual fear conditioning using 1.3 mA footshocks, which reliably induced a traumatic memory. Before the recovery session, animals received DCS or vehicle (VEH) and, immediately after, were treated with CLO or VEH (i.p.: n = 10 for VEH/VEH, VEH/CLO, and DSC/CLO; n = 11 for DSC/VEH). Intra-reunions: n = 8 for VEH/PBS/VEH, DSC/PBS/VEH, DSC/PBS/CLO, and VEH/PBS/CLO; n = 6 for VEH/IFE/VEH; n = 7 for DCS/IFE/VEH and DSC/IFE/CLO). Freezing behavior was quantified during retrieval and test sessions in conditioned (A1/A2) and neutral (B1/B2) contexts. Data were analyzed using mixed-design ANOVA followed by Newman-Keuls post hoc tests ($p < 0.05$). **Results:** As previously observed with 1.3 mA footshocks, rats exhibited generalized fear responses characterized by high levels of freezing ($76 \pm 11\%$) and lack of context discrimination. These memories proved resistant to destabilization. CLO alone did not affect reconsolidation under these conditions. However, when retrieval was preceded by DCS administration, CLO significantly reduced freezing in trials A1/A2 ($p \leq 0.00003$) and B1/B2 ($p \leq 0.002$), suggesting that DCS induced memory lability, thus allowing CLO to effectively block reconsolidation. NMDA receptor blockade in the nucleus reuniens with IFE did not significantly alter freezing in context A, although a significant reduction in reactivation freezing was observed for A1/A2 ($p \leq 0.0001$). In context B, the DCS/IFE/CLO group showed a significant increase in freezing compared to the VEH/IFE/CLO control group. Furthermore, significant differences were observed between sessions B1/B2 in most groups, except for VEH/IFE/CLO, suggesting that IFE did not completely prevent reconsolidation blocking in this condition. **Conclusion:** DCS facilitates the destabilization of traumatic memories, allowing CLO to attenuate their reconsolidation. NMDA receptor blockade in the nucleus reuniens did not interfere with these effects, suggesting that reconsolidation modulation may occur independently of this thalamic region. Differences between treatment groups highlight the complex nature of the mechanisms involved in traumatic memory reconsolidation. **Financial support:** UFMS, FAPESP, and FAPESC. CEUA/UFSC N^o 7819200422.

03.026 Lipopolysaccharide Preconditioning Disrupts the Behavioral and Inflammatory Response to Restraint Stress in Male Mice. Lovis EP¹, Pereira GC¹, Viero FT², Arboit F³, Becker G⁴, Zanchet EM¹, Trevisan G¹, Oliveira SM⁴, Pillat MM¹, Bochi GV¹. ¹UFSM, PPG Pharmacology, Brazil. ²USP, Institute of Chemistry, Brazil. ³UFSM, PPG Veterinary Medicine, Brazil, ⁴UFSM, PPG Biological Sciences: Toxicological Biochemistry, Brazil

Introduction: Major depressive disorder (MDD) is a complex neuropsychiatric disorder influenced by multiple factors. Chronic stress can lead to maladaptive brain changes that may trigger immune hyperactivation, contributing to MDD pathophysiology. While inflammation is well established in MDD, the effects of inflammatory preconditioning in animals subsequently exposed to chronic stress remain unclear. This study aimed to investigate the impact of inflammatory preconditioning with lipopolysaccharide (LPS) on behavioral, peripheral, and central inflammatory alterations in male Swiss mice subjected to chronic restraint stress (CRS). **Methods:** Forty male Swiss mice (25-30 g; 6-8 weeks old) were approved (UFSM Animal Care and Use Committee, 3601271021/2021) and randomly assigned to 4 groups: vehicle + control (VEH+CON), VEH+CRS, LPS+CON, and LPS+CRS. A single intraperitoneal injection of LPS (830 µg/Kg) or VEH (saline, 10 mL/Kg) was administered 24 hours before CRS. CRS was performed 6 hours/day for 28 days. Behavioral tests, including open field test (OFT), elevated plus maze (EPM), forced swim test (FST), and tail suspension test (TST), were conducted 24 hours after the last CRS. Euthanasia was executed 24 hours after the final behavioral tests to collect brain structures, for central inflammatory analysis using an ELISA kit, and blood samples for peripheral inflammatory assessment through flow cytometry. **Results:** In the OFT, the LPS+CRS showed a reduction in rearing compared to VEH+CON ($p<0.0001$), VEH+CRS ($p=0.0011$), and LPS+CON ($p<0.0001$). Additionally, LPS+CRS ($p=0.0052$) reduced the grooming time compared to VEH+CON, whereas VEH+CRS was compared to VEH+CON ($p=0.0002$) and LPS+CON ($p=0.0274$). The number of crossings, average speed, and total distance were only reduced in LPS+CRS versus VEH+CRS ($p=<0.0001$, $p=0.0100$, $p=0.0098$). In the EPM, LPS+CRS spent less time in open arms and more time in closed arms than VEH+CON ($p=0.0031$ and $p=0.0455$), VEH+CRS ($p=0.0172$ and $p=0.0033$), and LPS+CON ($p=0.0002$ and $p=0.0019$). This group also displayed a reduced number of head-dipping and an increase in anxiety index compared to VEH+CON ($p=0.0022$ and $p=0.0465$) and LPS+VEH ($p=0.0020$ and $p=0.0196$). Emotionality Z-score indicated depressive-like behavior in LPS+CRS versus VEH+CON ($p=0.0020$), VEH+CRS ($p=0.0201$), and LPS+CON ($p=0.0106$). Inflammatory peripheral parameters showed a trend toward elevated IL-17 levels in LPS+CRS ($p=0.0751$) and a significant increase in VEH+CRS ($p=0.0130$) compared to VEH+CON, without altering IL-2, IL-4, IL-6, IL-10, TNF- α , or IFN- γ . Inflammatory central parameters demonstrated reduced IL-10 levels in the prefrontal cortex (PFC) of the LPS+CRS ($p=0.0159$) compared to VEH+CON, with no changes in IL-6 or TNF- α in either PFC or hippocampus. **Conclusion:** These findings suggest that inflammatory preconditioning significantly contributes to behaviors phenotypically associated with MDD. Furthermore, the results indicate that these behavioral alterations are related to an imbalance in the central immune response. Further investigations are needed to elucidate the underlying mechanisms in this MDD model. **Financial support:** FAPERGS.

03.027 The CB1 Receptor Negatively Modulates the Recent Reconsolidation of Long-Term Spatial Memory. Queiroz R, da Silva WC Unicentro Neuropsychopharmacology Lab

Introduction: Reconsolidation is the process by which consolidated memories, become temporarily labile upon retrieval, allowing for modifications. The endocannabinoid system plays an important role in various aspects of memory and learning and is considered a powerful system in the modulation of many processes. It has been reported that this system influences both the acquisition and consolidation of spatial memories, but its role in the reconsolidation of these memories remains controversial. This study aimed to investigate the role of cannabinoid receptors CB1 and CB2 in the reconsolidation of long-term spatial memory in rats. **Materials and Methods:** Male Wistar rats were implanted bilaterally with stainless cannulae under deep anesthesia by stereotaxic procedures in the dorsal hippocampus (DH) (CA1 region). After recovery, the animals were trained in the Morris Water Maze (five daily sessions) to consolidate spatial memory. A retrieval test was conducted 24h after training, followed by bilateral infusions of pharmacological agents immediately after the test. The drugs included Anandamide (AEA) (CB1/CB2 agonist), Oleamide (CB1 agonist), HU 308 (CB2 agonist), AM4113 (CB1 antagonist), SR141716A (CB1 inverse agonist), COR170 (CB2 inverse agonist), Lactacystin (proteasome inhibitor), and Rapamycin (protein synthesis inhibition). Memory retention was assessed in tests performed 24h, 5 and 10 days post-infusion. **Results:** AEA in doses of 10 and 100 nmol/side impaired late memory reconsolidation (5 and 10 days post-first-test; $F(4, 50) = 8.197$ and 10.09 , respectively), but did not affect recent memory (24h post-first-test, $F(4, 50) = 1.693$). This effect was blocked by co-infusion of anandamide and AM4113 (5 and 10 days post-first-test; $F(3, 33) = 11.51$ and 6.517 , respectively). The amnesic effect of AEA on spatial memory reconsolidation is mimicked by activation of receptor CB1 (24h, 5 and 10 days post-first-test; $F(2, 18) = 10.78$, 18.65 and 16.52 , respectively). The amnesic effect of CB1 receptor agonist on spatial memory reconsolidation is blocked by proteasome inhibition (24h, 5 and 10 days post-first-test; $F(3, 37) = 5.253$, 13.79 and 10.16 , respectively). The amnesic effect of protein synthesis inhibition on spatial memory reconsolidation is blocked by inhibiting the CB1 receptor (24h, 5 and 10 days post-first-test; $F(3, 41) = 15.91$, 13.03 and 6.315 , respectively). **Discussion:** Our results show that AEA and oleamide given in the DH immediately after a probe performed 24h post-training impairs spatial memory retention during subsequent probe tests carried out 24 hours (only oleamide), 5 days and 10 days later. CB1 receptor is coupled to an inhibitory G protein, which, once activated, converges to a decrease in the synthesis rate of new proteins, which implies a consequent impairment in long-term memory retention. Corroborating with this, we show that the amnesic effect of CB1 receptor activation on spatial memory reconsolidation disappears if there is no protein degradation. Furthermore, we show that the amnesic effect caused by inhibition of protein synthesis during spatial memory reconsolidation can be blocked by the inverse activation of CB1 receptor, implying this receptor participates in the protein turnover process regulation. **Funding:** This work was supported by CAPES and non-governmental organization AEPAPA (Association for Research, Studies and Support for Alzheimer's Patients).

03.028 **Pubertal Treatment Of N-Acetyl-L-Cysteine or Brilliant Blue G Prevents Behavioral Deficits and Inflammatory Cytokines in Male Rats of Schizophrenia MAM Model.** Toscano LF¹, Vargas L¹, Ribeiro DE², Ulrich H², Salum C¹ ¹UFABC, Post-Graduation Program in Neuroscience and Cognition, Brazil. ²IQ-USP, Dept Biochemistry, Institute of Chemistry

Introduction: The hypothesis of impaired neurodevelopment in schizophrenia has led to the use of animal models based on interventions in the perinatal phase. Among them, MAM consists of the injection of the antimitotic methylazoxymethanol acetate (MAM) on the seventeenth day of gestation in Wistar rats, whose offspring present behavioral and neurochemical impairments in adulthood, such as increased locomotion in the arena, sensitivity to psychostimulants, reduced social interaction (SI) and deficit in the pre-attentional pre-pulse inhibition (PPI) test. Recent studies point to the role of oxidative stress and inflammation in the pathophysiology of this disorder. Our recent results demonstrated that acute or chronic treatment in adulthood with the antioxidant glutathione precursor, N-Acetyl-L-Cysteine (NAC), reversed the behavioral deficits of the MAM model. Purinergic receptors play an important role in neuroinflammation, especially P2X7R. Thus, the use of P2X7R antagonists, such as Brilliant Blue G (BBG), may be effective in investigating schizophrenia. The aim of this study is to evaluate whether acute or chronic treatment with NAC or BBG in the pubertal phase can reduce behavioral deficits and inflammatory cytokines in male rats of the MAM model. **Methods:** All procedures were approved by CEUA nº 1121171221. Wistar rats approximately 90 days old were used for mating. The rats received treatment with MAM (25 mg/kg, ip) or saline from the 17th day of gestation. After weaning, they were separated into six groups (Sa/Sa, MAM/Sa, Sa/NAC, MAM/NAC, Sa/BBG, MAM/BBG), for this study only male rats were used, N=12 per group. At PN45, the offspring received an injection of NAC (250 mg/kg, ip), BBG (50 mg/kg) or saline and one hour later were subjected to behavioral tests (acute evaluation), locomotion in the arena, SI and PPI. They were treated with daily doses of respective treatment for 15 days and 24h after the last treatment (chronic evaluation), the rats were again subjected to the same tests. Blood and brain tissue were collected for the cytokine evaluation. The statistical analysis was developed using two-way ANOVA and Tuckey's post hoc test. Considered statistically significant differences at $p < 0.05$.

Results: NAC and BBG treatments significantly reduced the acoustic startle response on PPI test during chronic evaluation in male rats. Social interaction deficits were observed in MAM-treated rats, with reductions in proximity and active interaction behaviors, alongside increased self-grooming. These effects were reversed by NAC and BBG treatment. Cytokine analysis revealed MAM-treated rats showed altered hippocampal and serum cytokine levels. Notably, a general effect of NAC increasing anti-inflammatory IL-10 in the hippocampus was identified. Pearson's Correlation analyses indicated positive correlation between IL-10 and SI, and between TNF- α and startle responses. **Conclusion:** Taken together, this data indicates the MAM model presents inflammatory alterations in agreement with what is observed in patients with schizophrenia. Also, NAC or BBG treatment during puberty can reduce some of the behavioral deficits of the MAM model, indicating a preventive effect. Founding: UFABC, CAPES, FAPESP.

03.029 Ayahuasca Reduces Stress-Induced Deficits In The Process Of Contextual Conditioned Fear Extinction In Male Rats. Furtado ASJ¹, dos Santos, RG², Resstel, LBM¹ ¹FMRP-USP, Pharmacology Dept, Lab. of Neuropharmacology, PPG Pharmacology, Brazil ²HC-FMRP-USP, Neuroscience and Behaviour Dept, Lab. of Psychopharmacology

Introduction: Ayahuasca is a beverage used for ritualistic purposes, prepared from the decoction of two plants: *Banisteriopsis caapi* and *Psychotria viridis*. It has been suggested that ayahuasca may facilitate the extinction of conditioned fear and impair the reconsolidation of aversive memories. Stress is a well-established factor that worsens the extinction of conditioned fear, as it can evoke the generalization of the aversive stimulus. However, little is known about the effects of ayahuasca on the extinction of contextual fear memories and how stress influences this process, including the generalization of aversive contexts. In this context, animal models of stress, such as the Single Prolonged Stress (SPS), are valuable tools for investigating impairments in the extinction and generalization of aversive memories, particularly those associated with the contextual fear conditioning (CFC) model. **Methods:** Male Wistar rats (240–280 g) were divided into a control group and a stress group (SRS). The SRS group was subjected to a stress protocol consisting of 2 h of restraint in a tube, 20 min of forced swimming and ether exposure until fainting. After 7 days, the animals underwent an additional 20 min forced swim. Both groups were then exposed to a aversive context (Context A) across three sessions: conditioning (3 electric footshocks; 0.65 mA/1 s), extinction (30 min without shocks) and testing (5 min without shocks). During the test session, an additional exposition was conducted in a non-aversive context (Context B) for generalization assessment. Oral administration of ayahuasca at a dose of 0.3 mg/kg was given either 1 hour before or immediately after the extinction session. Ethics committee approval: 1373/2024. **Results:** The administration of ayahuasca before the extinction session increased freezing behavior during extinction in SRS animals (n=8) compared to SRS vehicle treated group (n=7) and control animals (n=6) ($F(15, 115) = 3.74$; $p < 0.05$). No differences were observed in control animals treated with ayahuasca (n=6) ($p > 0.05$). Additionally, SRS animals showed generalization and impaired extinction during the test session, which were not affected by ayahuasca ($p > 0.05$). When ayahuasca was administered after the extinction session, in SRS group (n=7) the generalization ($F(3,58) = 2.345$, $p = 0.0823$; Tukey's test $p < 0.05$) and extinction deficit ($F(3, 25) = 4.171$, $p < 0.05$; Bonferroni's test $p < 0.05$) observed in SRS vehicles treated animals were reversed (n=5). No changes were observed in control ayahuasca group (n=5) compared with control vehicle group (n=5) ($p > 0.05$). **Conclusion:** Our findings suggest that ayahuasca increases the aversiveness associated with stress during fear conditioning extinction when administered before the extinction session. In contrast, it produces an antiaversive effect on fear generalization and extinction deficits when administered immediately after the extinction session. **Financial support:** Capes/CNPq.

03.030 Primary Cortical Neurons from Maternal Immune Activation Animal Model Presents Disruptions on Mitochondrial Function, Dendritic Arborization and Synaptic Events: Relevance to Schizophrenia Pathophysiology. Santos ASD^{1,2}, Araujo VC^{1,2}, Ribeiro MR^{1,2}, Pérez MMM², Hernández DM², Madrigal JLM², Scavone C¹, Leza JC² ¹ICB-USP, São Paulo, Brasil ² University Complutense de Madrid, Medical School, Madrid, Spain

Maternal immune activation (MIA) induced by polyinosinic:polycytidylic acid [Poly(I:C)] is a widely used animal model that reproduces both behavioral and biochemical features of schizophrenia. Nevertheless, the neuronal alterations elicited by Poly(I:C) and the detailed characterization of neurons in this context are still not well defined. In this study, we established primary cortical neuron cultures derived from rat embryos prenatally exposed to Poly(I:C) and examined their neuronal phenotype along with mitochondrial function. Furthermore, we evaluated the susceptibility of these neurons to rotenone, a mitochondrial complex I inhibitor that has been reported to induce schizophrenia-like traits *in vivo*. Pregnant rats received either saline (S) or Poly(I:C) (4 mg/kg; P) at embryonic day 14 (E14), and embryos were removed at E19 to prepare primary cortical cultures. After 14 days *in vitro*, neurons were treated for 24h with vehicle (V; 0.01%Dimethyl Sufoxide) or rotenone (R, 1.35 nM), resulting in four experimental groups (SV, SR, PV, PR; n=4 per group). Molecular and morphological assays were performed to evaluate: i) cytotoxicity by CytotoxGlo luminescence; ii) mitochondrial superoxide production by Mitosox DeepRed fluorescence; iii) expression of proteins related to mitochondrial dynamics (Opa1, pDRP1, TOM40) and mitochondrial complex 1 function, NDUFS2, both by electrophoresis; iv) dendritic number and length by immunocytochemistry and v) synaptic occurrence by immunocytochemistry. Data is expressed by mean± SEM (SV=100%) and were analysed by ANOVA Two Way with Tukey posthoc. It was considered statistically significant $p \leq 0.05$. We saw that Rotenone treatment did not alter neuronal viability. However, mitochondrial superoxide levels decreased in SR neurons ($85.7 \pm 3.23\%$; $p=0.05$) and increased in PV ($141.7 \pm 12.0\%$; $p=0.005$) and PR neurons ($127.7 \pm 14.8\%$; $p=0.04$). Mitochondrial fusion markers were elevated, with Opa1 upregulated in PV ($231.9 \pm 42.4\%$; $p=0.02$) and PR ($178.5 \pm 19.6\%$; $p=0.01$), and TOM40 increased in SR ($160.4 \pm 17.6\%$; $p=0.06$) and PV ($124.0 \pm 9.4\%$; $p=0.05$). We did not found changes on pDRP1 levels. Otherwise, NDUFS2 levels were decreased in SR ($216.2 \pm 15.9\text{pM}$; $p=0.05$) but increased in PV ($151.6 \pm 14.4\text{pM}$; $p=0.002$) and PR ($142.5 \pm 11.3\text{pM}$; $p=0.03$). Morphological analysis revealed that SR (5.0 ± 0.41 ; $p=0.006$), PV (5.42 ± 0.5 ; $p=0.001$), and PR (4.1 ± 0.5 ; $p=0.001$) neurons displayed reduced dendritic number and shorter dendrites (SR: $30.2 \pm 1.8\text{mm}$ $p=0.05$; PV: $28.55 \pm 2.33\text{mm}$ $p=0.03$) compared to controls (number: 10.2 ± 0.8 and length: $35.33 \pm 2\text{mm}$). In addition, we saw impairment in synaptic function by Pearson Correlation in SR (0.352 ± 0.016 ; $p=0.02$) and PV (0.31 ± 0.014 ; $p<0.0001$) in relation to control (0.41 ± 0.015). However, the PR group showed a recovery (0.377 ± 0.01) in synaptic occurrence in relation to PV. In conclusion, cortical neurons derived from Poly(I:C)-induced MIA offspring exhibit mitochondrial dysfunction and impaired dendritic development and synaptic function, suggesting deficient neurodevelopmental processes relevant to schizophrenia pathophysiology.

This work was approved by Animals Ethical Use Committee from University Complutense de Madrid 159.0/2024 and funded by FAPESP 2023/14145-3 and 2023-01789-9; Ministerio de Ciencia, Innovación y Universidades (Gobierno de España): PID2021-123871OB-I00 y PID2022-137932NB-I00

03.031 Standardization of a Depression Model for Assessing the Antidepressant Potential of Bufotenine in Rats. Oliveira PS¹, Queiroz KS¹, Paulo MEFV¹, Gonçalves TCP¹, Nencioni ALA¹
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Introduction: Depression is a severe and debilitating psychiatric disorder affecting approximately 280 million people worldwide, characterized by persistent mood changes, anhedonia, apathy, and, in more severe cases, suicidal thoughts or behaviors. Despite the widespread use of antidepressants, current treatments often lack a combination of high efficacy, rapid onset, and a favorable safety profile. To study depression in research, the Chronic Unpredictable Mild Stress (CUMS) model is widely used and well validated for inducing depressive-like phenotypes in rats. This model involves exposing animals to a variety of mild, unpredictable stressors over an extended period, effectively simulating the chronic stress that contributes to depression in humans. As a result, it reproduces typical behavioral and physiological characteristics of the disorder. Given the limitations of conventional antidepressants, recent interest has focused on psychedelic agents as promising alternatives for patients with treatment-resistant depression and anxiety. Bufotenine, a compound found in seeds of *Anadenanthera* spp, has emerged as a potential therapeutic candidate. Although it is an isomer of psilocin, bufotenine does not produce significant psychotropic effects. Its action as an agonist of the 5-HT_{2A} receptor, which plays a key role in mood regulation, positions it as a promising candidate for novel antidepressant development. **Methods:** Male Wistar rats were subjected to a chronic unpredictable mild stress (CUMS) protocol for six weeks. From the fourth week onwards, the animals received a daily oral treatment via gavage, either with saline (1ml/kg, stress group) or fluoxetine (3 mg/kg, fluoxetine group). A control group of animals was not subjected to any stress or treatment for six weeks. At the end of the CUMS protocol, the rats underwent a series of behavioral tests to assess their depressive level including the Sucrose Preference Test (SPT), the Forced Swim Test (FST), and the Open Field Test (OFT). Body weight was monitored throughout the experimental period. At the end of the protocol, biological samples were collected for further analysis. All procedures were approved by the Institutional Animal Care and Use Committee (protocol number 9416180723). **Results:** In terms of body weight, both the stress and fluoxetine groups exhibited significantly lower weight gain than the control group ($p < 0.0001$). In the SPT, the stress group showed a decreased preference for sucrose while the fluoxetine group exhibited a tendency to prefer sucrose. No statistically significant differences were observed between the groups in the FST. In the OFT, significant differences were found among the stress, fluoxetine, and control groups for the parameters evaluated: reduction in grooming time, number of rearing and time spent in the central area were all highly significant compared to the control group. **Conclusion:** The results demonstrate that we have developed an efficient CUMS model, capable of effectively inducing a depressive-like phenotype in rats. Fluoxetine, used as a positive control, was able to reverse the depressive effects in the animals. With a well-established model in place, we will proceed to test bufotenine, comparing its results with fluoxetine in order to evaluate the antidepressant effect of bufotenine. **Financial support:** Fundação Butantan.

04. Inflammation and Immunopharmacology

04.001 **Prenatal Malnutrition Impairs Alveolar Macrophage Function and Increases Infection Susceptibility in Adult Offspring.** Pádua MEB¹, Azevedo GA^{1,2}, Ferreira RR^{2,3}, Giaquinto LRR⁴, Landgraf MA⁴, Landgraf RG¹ ¹Unifesp-Diadema, Dept Pharmaceutical Sciences, Diadema, SP, Brazil; ²UNIFESP, PPG Medicina Translacional, São Paulo, SP, Brazil; ³USP-SP Biotério Central, Brazil; ⁴UNIP-Rangel, Santos, SP, Brazil

Introduction: Maternal food restriction during pregnancy induces morphological and metabolic fetal adaptations and predisposes offspring to metabolic diseases and defective inflammatory responses. This study aimed to evaluate the influence of maternal malnutrition during pregnancy on the development of sepsis in adult Offspring. **Methods:** Pregnant female Wistar rats were subjected to 50% calorie-protein food restriction throughout gestation. At 12 weeks of age, male offspring rats were subjected to cecal ligation and puncture or a sham operation. Six hours later, the animals were euthanized for evaluation of temperature, glycemia, creatinine and urea (urine), cytokines (in serum and lung tissue), and the phagocytic activity of alveolar macrophages incubated with *Saccharomyces cerevisiae* (CEUA-UNIFESP 2268240919). Results: Malnutrition during gestation caused a reduction in fetal birth weight (LBW) compared to the offspring of the control group (normal birth weight - NBW). Hypothermia, hyperglycemia, and elevated levels of creatinine and urea were also observed in both groups subjected to the sepsis protocol. Serum levels of IL-6, IL-1 β , IL-10, TNF- α , and MIP-2 were higher in the LBW group than in the NBW group. The colony-forming unit count in the peritoneal fluid was significantly higher in the LBW group than in the NBW group. The LBW group showed defective phagocytic activity, as the number of yeasts phagocytosed by alveolar macrophages was lower than in the NBW group. **Conclusions:** Low birth weight induced by maternal malnutrition during pregnancy impairs the phagocytic activity of alveolar macrophages and may compromise the immune response, increasing susceptibility to infections. **Financial support:** FAPESP (2024/23135-3, 2024/02740-6), CNPq and CAPES. **Animal Research Ethical Committee:** CEUA-UNIFESP 2268240919

04.002 **Second-Line and Biologic Therapies in Pediatric Chronic Nonbacterial Osteomyelitis: A Systematic Review of Multicenter Cohort Data.** Maraia ME^{1,2}, Lemes FS³, Oliveira CJF², Forte GV³, Miguel CB^{2,3}, Rodrigues WF^{2,3} ¹UFTM, Undergraduate course in Biomedicine, Uberaba, MG, Brazil ²UFTM, Postgraduate Course in Tropical Medicine and Infectious Diseases, Uberaba, MG, Brazil ³Unifimes, Multidisciplinary Lab of Scientific Evidence, Mineiros, GO, Brazil.

Introduction: Chronic nonbacterial osteomyelitis (CNO) and its multifocal variant (CRMO) often require therapeutic escalation beyond nonsteroidal anti-inflammatory drugs (NSAIDs), particularly in refractory cases. Despite increasing use of immunosuppressants and biologics, there is no consensus on the most effective secondline interventions, and treatment approaches remain heterogeneous worldwide. **Objective:** To compare the reported clinical efficacy and remission rates of second-line and biologic therapies in pediatric patients with CNO/CRMO who failed to respond to NSAID monotherapy. **Methods:** A systematic review was conducted across six major databases (PubMed, Embase, Cochrane, Web of Science, SciELO, and Scopus) and gray literature using MeSH terms. Observational studies and case series involving pediatric patients treated with methotrexate, corticosteroids, sulfasalazine, anti-TNF agents, pamidronate, or tocilizumab were included. Data were extracted to a structured Excel spreadsheet and synthesized narratively. As this was a systematic review of secondary data, ethical approval was waived. **Results/Discussion:** Among nine studies comprising over 200 pediatric patients, methotrexate demonstrated complete or partial remission rates between 66% and 70% (Borzutzky et al., 2012; Gamalero et al., 2020). Corticosteroids achieved up to 66.6% remission, especially when used as bridging therapy. Biologic agents such as etanercept, adalimumab, and infliximab showed complete remission in 42–46% of patients, with partial responses in an additional 30–40%. Pamidronate, used in cohorts from Belgium and Denmark, led to significant reductions in inflammatory lesions and imaging scores (e.g., median mRINBO reduction from 5 to 4, $p=0.02$). In contrast, sulfasalazine had inconsistent outcomes, with <20% achieving full remission in some studies. Treatment protocols, including dosing and duration, were variable across cohorts, limiting direct comparability. **Conclusion:** Second-line therapies, particularly methotrexate, corticosteroids, and anti-TNF biologics, are effective options in NSAIDrefractory pediatric CNO/CRMO. Pamidronate offers additional promise, especially in patients with vertebral involvement. However, the lack of standardized regimens and randomized clinical trials underscores the need for individualized approaches and consensus guidelines.

04.003 **Evaluation of the Anti-Inflammatory Effect Of hDPSC in an *in vitro* Model of Neuroinflammation Induced By LPS.** Silva MR¹, VF Gonzaga¹, Kerkis I² ¹Butantan Institute, Lab of Genetics, São Paulo, Brazil ²Cellavita Scientific Research Ltda, Valinhos, São Paulo, Brazil

Introduction: Neurodegenerative diseases are a heterogeneous group of disorders with the fastest-growing incidence worldwide. Despite distinct pathophysiological mechanisms, they share neuroinflammation, a process signaling brain homeostasis loss, as a common feature. Neuroinflammation not only results from but also contributes to neurodegeneration. Current treatments are palliative, unable to modify disease progression. Mesenchymal stem cell (MSC)-based therapies have gained attention for treating neurodegenerative, inflammatory, and oncological diseases due to their regenerative and immunomodulatory properties. Recent studies highlight the potential of MSCs, including human immature dental pulp stem cells (hDPSC), as promising tools for modulating neuroinflammation. **Objectives:** To investigate the anti-inflammatory and immunomodulatory effects of hDPSC in an *in vitro* neuroinflammation model using BV-2 murine microglial cells activated with LPS. **Methods:** BV-2 microglial cells were initially stimulated with 6-hydroxydopamine (6-OHDA) to induce oxidative stress and neurodegeneration, and with lipopolysaccharide (LPS) to induce neuroinflammation. Cell viability was assessed using MTT and Trypan Blue assays. Due to the high cytotoxicity of 6-OHDA (>50% reduction in viability after 24 hours) and its inability to induce cytokine release, only LPS was used for the neuroinflammation model. hDPSC, provided by Cellavita Scientific Research LTDA in collaboration with the Butantan Institute, were co-cultured with LPS-activated BV-2 cells at a 1:1 ratio. After 24 hours, the culture supernatant was collected for quantification of pro- and anti-inflammatory cytokines via Cytometric Bead Array (CBA). Post-treatment viability was reassessed through MTT and Trypan Blue assays. **Results and Conclusions:** 6-OHDA showed high neurotoxicity to BV-2 cells without triggering inflammatory cytokine production, proving unsuitable for modeling neuroinflammation. LPS induced significant cytokine release (notably TNF and IL-6) with limited cytotoxicity, effectively establishing a neuroinflammatory model. Treatment with hDPSC modulated this inflammatory response, reducing pro-inflammatory cytokine levels and restoring microglial morphology from an amoeboid (activated) to a ramified (resting) state. Additionally, cell viability improved after stem cell treatment. These findings, although preliminary, suggest that hDPSC possess notable anti-inflammatory and immunomodulatory properties, supporting their potential therapeutic application in neurodegenerative diseases. **Financial Support:** Cellavita Scientific Research LTDA, Butantan Foundation, and National Council for Scientific and Technological Development (CNPq, Brazil).

04.004 **Dietary Modulation of Zinc Induces Sex-dependent Responses in Inflammatory Pain by CFA in Mice.** Silva, MC¹, Mathias, DO², Lima, LMTR³, Miranda, ALP¹ ¹UFRJ, Lab Estudos em Farmacologia Experimental ²Fiocruz-RJ ³UFRJ, Lab Biotecnologia Farmacêutica e Nutricional

Abstract: Zinc is an essential micronutrient for the human body, playing a fundamental role in various biological processes such as DNA synthesis, immune function, and enzymatic activity (Prasad A S, J Trace Elem Med Biol, 28, 364, 2014). It is involved in antioxidant defense and acts as a cofactor for more than 300 enzymes (Costa M I et al, J Mol Sci, 24, 4822, 2023). Zinc deficiency can lead to significant disorders such as growth retardation and neurological impairments. Experimental studies have shown that zinc deficiency is associated with increased production of inflammatory mediators and amplified nociceptive response, suggesting that zinc supplementation may reduce pain sensitization (Lima C K F et al, AABC, 95, 2023; Matias D O, PhD Thesis, 2023).

Objective: To evaluate the effects of dietary zinc restriction and supplementation on inflammatory and nociceptive parameters in mice subjected to the CFA-induced inflammatory pain model.

Methods: Adult Swiss mice were divided into three groups and submitted to 9 weeks of dietary zinc intervention: control diet (30 mg/kg of Zn), zinc-deficient diet (11 mg/kg of Zn), and zinc-enriched diet (150 mg/kg of Zn) (CEUA/UFRJ 030/24). Baseline behavioral assessments were performed before the administration of the modified diet. Body weight was measured weekly and blood glucose levels every two weeks. CFA (20 µl) was injected into the right hind paw of mice at the beginning of the 6th week to induce inflammation. Pain and inflammation parameters were analyzed for 3 weeks after CFA injection through behavioral tests: mechanical hyperalgesia (Von Frey), thermal heat hyperalgesia (Hargreaves), paw thickness (edema), and open field test. Euthanasia was performed on day 23, and plasma, plantar tissue, sciatic nerve, and spinal cord were collected for biochemical (TNF, MPO, IL-10, IL-6, and IL-1B) and histological analyses.

Results: Dietary zinc modulation affected male and female mice differently. In females, there were no significant changes in weight gain, food intake, paw edema, or pain responses. In males, zinc deficiency reduced body mass gain (**p = 0.0020) and worsened sensitivity to both thermal (*p = 0.0195) and mechanical (***p < 0.0001) pain. On the other hand, supplementation prevented weight loss and partially attenuated mechanical hypernociception but worsened CFA-induced paw edema and did not offer protection against thermal hyperalgesia. Notably, weight variations were not determined by food intake. **Conclusion:** The results suggest that the effects of zinc vary according to sex and inflammatory status, being beneficial only in non-inflammatory contexts. Ongoing histological and biochemical analyses aim to clarify the cellular and molecular mechanisms involved, in order to determine the actual efficacy and potential limitations of prolonged zinc supplementation as a therapeutic strategy in chronic inflammation and associated pain conditions. **Financial support:** CAPES, CNPq, PIBIC/CNPq, FAPERJ.

04.005 **Therapeutic Effects of the Rho-Kinase Inhibitor LASSBio-2065 on Silica-Induced Chronic Inflammation in Mice.** Ferreira GG¹, Ferreira TPT¹, Santana ACC¹, Arantes ACS¹, De Souza LM¹, Martins MA¹, Silva PMR, Pinheiro PMS², Lima LM² ¹IOC-Fiocruz, Lab of Inflammation; ²UFRJ, Lab of Evaluation and Synthesis of Bioactive Substances, RJ, Brazil

Introduction: Silicosis is a form of pneumoconiosis caused by the inhalation of crystalline silica particles; a mineral abundantly found in the environment. Chronic silica exposure triggers a sustained inflammatory response characterized by the recruitment of inflammatory cells, elevated production of pro-inflammatory mediators, fibroblast proliferation and differentiation, and excessive extracellular matrix deposition. These pathological processes culminate in granulomatous pulmonary fibrosis and progressive respiratory dysfunction. The Rho-associated protein kinase (ROCK) signaling pathway plays a critical role in regulating various cellular functions and has emerged as a promising therapeutic target for antifibrotic intervention. In this study, we evaluated the therapeutic potential of LASSBio-2065, a ROCK inhibitor, in a murine model of silica-induced pulmonary fibrosis. **Methods:** Male Swiss-Webster mice were anesthetized and intranasally instilled with crystalline silica particles (10 mg/50 μ L sterile saline). Control animals received saline alone. LASSBio-2065 was administered, subcutaneously, at doses of 10 or 20 mg/kg, once daily from day 21 to day 27 post-silica exposure. Assessments were performed 24 hours after the final treatment. Fasudil, a well-characterized ROCK inhibitor (10 mg/kg), served as a reference compound. Lung structural changes were evaluated using micro-computed tomography (microCT). Histological analyses included Hematoxylin & Eosin (H&E) staining for general morphology, Picrosirius red staining viewed under polarized light for collagen visualization, and quantitative collagen measurement using the Sircol™ assay. All animal procedures were approved by the Animal Ethics Committee of the Oswaldo Cruz Institute/FIOCRUZ (CEUA L-001/19A6). **Results:** MicroCT imaging of silica-exposed mice revealed dense, opaque regions in lung tissue, indicative of fibrosis. Histological examination confirmed the presence of multiple granulomas within the lung parenchyma, accompanied by significant collagen deposition. Therapeutic administration of LASSBio-2065, particularly at the 20 mg/kg dose, significantly reduced non-aerated lung areas while increasing normo-aerated regions. Consistent with imaging findings, LASSBio-2065 treatment also suppressed granuloma formation and collagen accumulation. In contrast, Fasudil, at 10 mg/kg, failed to mitigate silica-induced lung injury and fibrosis. **Conclusion:** LASSBio-2065 demonstrated robust antifibrotic effects in a murine model of silica-induced pulmonary fibrosis, effectively reducing lung inflammation, granuloma formation, and collagen deposition. These findings reinforce the therapeutic relevance of targeting the ROCK1/2 pathway in fibrotic lung diseases and suggest that LASSBio-2065 may represent a promising candidate for the treatment of established pulmonary fibrosis. **Financial Support:** FIOCRUZ, CNPq, FAPERJ, CAPES.

04.006 Cholinergic Modulation Attenuates Lung Inflammation *in vitro* and *in vivo* Experimental Model of Asthma-COPD Overlap. Queiroz VC¹, De Almeida FM¹, Nascimento C¹, Suehiro C², Pinheiro NM¹, Prado MAM¹, Prado VF¹, Tibério IFLC¹, Hamouda AK¹, Prado CM² ¹FM-USP, ²Unifesp

Introduction: Asthma–COPD overlap (ACO) is a complex clinical syndrome that exhibits features of both asthma and chronic obstructive pulmonary disease (COPD). It is characterized by mixed eosinophilic and neutrophilic inflammation, as well as persistent non-reversible airway obstruction. Current therapeutic strategies often fall short in addressing the multifaceted nature of ACO, underscoring the need for novel therapeutic approaches. Acetylcholine, acting through nicotinic acetylcholine receptors (nAChRs), particularly the $\alpha 7$ subtype, has been implicated in protective mechanisms against lung inflammation. **Aims:** i. To investigate whether activation of $\alpha 7$ nAChR modulates IL-13-induced inflammation in A549 lung epithelial cells; ii. To evaluate the impact of cholinergic deficiency on lung inflammation and function in an *in vivo* model of ACO. **Methods:** A549 cells were stimulated with IL-13 and treated with or without PNU-282987, a selective $\alpha 7$ nAChR agonist. Gene and protein expression of inflammatory markers were analyzed. For the *in vivo* study, wild-type (WT) and vesicular acetylcholine transporter knock-down (KD-VACHT) male and female mice were sensitized with ovalbumin on days 1 and 14 via intraperitoneal injection. This was followed by inhalation exposures on days 21, 23, 25, and 27. Porcine pancreatic elastase (PPE) was administered on day 21 to induce ACO-like features. Control animals received saline. Lung function and bronchoalveolar lavage fluid (BALF) cellularity were assessed. **Results:** IL-13 stimulation upregulated $\alpha 7$ nAChR expression in A549 cells. Treatment with PNU-282987 significantly reduced IL-6, TNF- α , and NF- κ B gene expression, as well as IL-6 protein levels, in IL-13-stimulated cells. *In vivo*, ACO induction led to increased respiratory system and tissue elastance in KD-VACHT mice compared to WT controls. Furthermore, only KD-VACHT mice developed pronounced neutrophilic, eosinophilic, and lymphocytic inflammation in the BALF following ACO induction. **Conclusion:** These findings suggest that acetylcholine, likely via activation of nAChRs, exerts a protective role against lung inflammation in the context of ACO. The exaggerated inflammatory response observed in cholinergic-deficient mice highlights the critical role of endogenous cholinergic signaling in regulating pulmonary immune responses. Pharmacological targeting of nAChRs may represent a promising therapeutic strategy for ACO and other chronic airway inflammatory diseases.

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04.007 **Antifungal Action of Rapamycin Might Prolong the Life of Mice with T1DM Infected by *Sporothrix brasiliensis*.** Oliveira MA¹, Albuquerque RC², Migliorini S¹, Tavares YPST¹, Almeida SR², Martins JO¹ ¹FCF-USP, Dept. of Clinical and Toxicological Analyses, Lab. of Immunoendocrinology, Brazil. ²FCF-USP, Dept. of Clinical and Toxicological Analyses, Lab of Mycology, Brazil

Introduction: Type 1 diabetes mellitus (T1DM) is an autoimmune disease that destroys pancreatic β cells, leading to chronic hyperglycemia and impaired immune responses. This condition increases susceptibility to infections, including sporotrichosis caused by *Sporothrix brasiliensis* (Sb), present especially in endemic regions like Rio de Janeiro and São Paulo. Autophagy plays a critical role in host defense and immune regulation. This study aimed to analyze the impact of autophagy modulation with rapamycin (RAPA) on Sb infection in diabetic and non-diabetic mice. **Material and Methods:** T1DM was induced in C57BL/6J mice via intraperitoneal streptozotocin (STZ) injection (CEUA Protocol No. 642). Mice were infected subcutaneously with 10^7 viable yeast cells of *S. brasiliensis* (strain M1168). After 14 days, three doses of RAPA (0.05 mg) were administered by gavage at 5-day intervals. Organs including liver, brain, spleen, lymph nodes, skin lesions, and kidneys were collected for analysis. **Results:** Infected T1DM mice, whether treated with RAPA or not, reached 100% mortality by day 36 post-infection, with deaths starting on day 16. In contrast, infected control (CT) mice showed no mortality during the 35-day period and were euthanized at the end ($p < 0.0001$). MIC (Minimum Inhibitory Concentration) assays showed that RAPA reduced fungal growth by ~89%, indicating antifungal potential. Immune profiling revealed increased monocyte levels in infected mice (CT-infected: 4.7%; T1DM-infected: 6%) compared to uninfected ones (CT: 2.03%; T1DM: 3.1%). Granulocytes were also elevated in infected groups (CT-infected: 49.3%; T1DM-infected: 62.88%) compared to uninfected mice (CT: 17.11%; T1DM: 23.65%) ($p < 0.05$). Conversely, lymphocyte percentages decreased significantly in infected T1DM mice (CT-infected: 45.93%; T1DM-infected: 31.41%) compared to their respective uninfected controls (CT: 80.85%; T1DM: 73.18%) ($p < 0.05$). Analysis of autophagy-related proteins showed a 555.2% increase in p62 expression in the infected T1DM group compared to the infected CT group, both without RAPA treatment, suggesting impaired autophagic flux. **Conclusions:** These results demonstrate that T1DM increases vulnerability to *S. brasiliensis* infection, as shown by elevated mortality, fungal load, and altered immune responses. Although RAPA significantly inhibited fungal growth *in vitro*, it did not prevent mortality *in vivo*, possibly due to impaired immune competence in diabetic mice. The accumulation of p62 points to dysfunctional autophagy, which may contribute to ineffective pathogen clearance. Together, these findings support the need for further investigation into host-directed therapies that can enhance immune and autophagic responses in diabetic settings. **Funding:** FAPESP, CAPES, CNPq **Keywords:** diabetes; infection; sporotrichosis; autophagy; rapamycin

04.008 Enhanced Anti-Inflammatory and Immunomodulatory Action of Geopropolis in Synergism with Low-Dose Dexamethasone in Human Macrophages. Honorio MS, Lopes EC, Almeida JFS, Sforcin JM IBB-Unesp-Botucatu, Programa de Pós-Graduação em Ciências Biológicas, Brazil

The inflammatory response can be triggered by various stimuli, including pathogens, allergens, toxic compounds, or tissue damage, and may be acute or chronic. Given the diversity of cells and mediators involved in inflammation, different therapeutic interventions have been explored. Geopropolis produced by stingless bees has shown promising biological properties, though it has been less studied compared to propolis from Africanized bees. This study evaluated the anti-inflammatory effects of geopropolis alone or combined with lower concentrations of dexamethasone, a conventional anti-inflammatory drug, on THP-1-derived macrophages stimulated by LPS. THP-1 cells were differentiated into macrophages using PMA for 24 h and treated with LPS for 2 h. Then, geopropolis combined with reduced dexamethasone concentrations were added for 24 h. Cell viability was assessed via MTT assay, and pro-inflammatory (TNF- α , IL-1 β , IL-6) and anti-inflammatory (IL-10) cytokines were quantified by sandwich ELISA. Eicosanoids (PGE2 and LTB4) were measured by competitive ELISA, and hydrogen peroxide (H₂O₂) production was evaluated fluorometrically. Cell surface markers (TLR2, CD80 and CD62L) were analyzed by flow cytometry. No cytotoxicity was seen after incubation with geopropolis alone or combined with dexamethasone, which significantly reduced pro-inflammatory cytokines, eicosanoids, and H₂O₂, suggesting anti-inflammatory and antioxidant effects. No differences were seen in TLR2, CD80 and CD62L expression, indicating they were not affected by the treatments. This study demonstrates that geopropolis exerts inhibitory and antioxidant effects in inflammatory conditions, potentially contributing to new therapeutic approaches for treating inflammatory diseases.

04.009 **Evaluation of the Proliferative Potential of *Lavandula dentata* Essential Oil on HaCaT Keratinocytes.** Sousa MBN¹, Purgatto, E², Greco KV³, Moreira V¹ ¹Unifesp-EPM, Dept Pharmacology, Brazil, ²FORC-CEPID-USP, School of Pharmaceutical Sciences, Brazil, ³University College London, United Kingdom

Introduction: Skin regeneration is a complex biological process that can be influenced by conditions such as psoriasis, atopic dermatitis, keloids, and diabetes - many of which are associated with chronic inflammation and fibrotic changes. In recent years, lavender essential oil has drawn growing interest due to its wide range of biological actions, including anti-inflammatory, antioxidant, antiviral, analgesic, and tissue-reparative effects. Although *Lavandula angustifolia* and *Lavandula latifolia* are well-studied among European species, *Lavandula dentata* - acclimatized and cultivated in Brazil - remains relatively uncharacterized. Its distinct composition of terpenes and terpenoids suggests possible synergistic activity, warranting further exploration. **Methods:** To evaluate the regenerative potential of *L. dentata*, HaCaT keratinocytes were cultured in DMEM supplemented with 10% fetal bovine serum, 1% sodium pyruvate, and 1% penicillin/streptomycin, and incubated at 37 °C with 5% CO₂. Cell viability was assessed via mitochondrial activity following 24 h exposure to a *L. dentata* nanoemulsion (NEL) at concentrations ranging from 0.5 to 128 µg/mL (96-well plates, 5×10³ cells/well, N=4). For proliferation analysis, cells were seeded in 24-well plates (1×10⁵ cells/well) and grown to ~90% confluence. A uniform scratch was made into the monolayer, and cells were treated with NEL (0.5–4 µg/mL) in complete DMEM. To specifically assess the effect of NEL on cell migration during regeneration, the same scratch assay protocol was followed, but the concentration of fetal bovine serum (FBS) in the DMEM was reduced from 10% to 1% to limit cell proliferation. In both assays, images were acquired at 0 and 24 h. To confirm the proliferation findings, a BrdU incorporation assay was also performed according to the manufacturer's protocol. Cells were seeded in 96-well plates (4×10⁴ cells/well) and treated with NEL at concentrations ranging from 0.125 to 4.0 µg/mL. **Results:** A significant reduction (9%, p < 0.01) in mitochondrial activity was observed at concentrations ≥8 µg/mL, leading to the use of lower doses in subsequent tests. In the scratch assay, treatment with NEL (0.5–4 µg/mL) resulted in a statistically significant increase in both cell proliferation (16% to 21%, p < 0.05) and cell migration (51% to 71%, p < 0.05) compared to the control. However, no significant differences were observed among the tested concentrations in either case. The BrdU assay confirmed the proliferative effect, showing a dose-dependent response at concentrations ranging from 0.125 µg/mL (51.72%) to 0.5 µg/mL (90.80%) (p < 0.001). Higher doses of 1.0 µg/mL (80.46%) and 2.0 µg/mL (35.63%) also showed statistically significant increases compared to the control (p < 0.001). **Conclusion:** Altogether, the data indicate that the *Lavandula dentata* nanoemulsion stimulates keratinocyte proliferation, migration, and regeneration. These findings suggest its potential as a therapeutic agent for skin repair and encourage further investigation into its mechanisms of action and pharmacological targets. **Financial support:** CNPq and Capes

04.010 Nitric Oxide Involvement in the Disability and Active Disease of Multiple Sclerosis: Systematic Review and Meta-Analysis. Kudsi SQ, Da Silva B, Viero FT, Rodrigues P, Trevisan G, UFSM, Graduate Program in Pharmacology, Brazil

Introduction: Multiple sclerosis (MS) is a chronic immune-mediated disease of unknown etiology that leads to progressive physical and cognitive disability. Evidence suggests that nitrosative stress plays a pivotal role in MS pathogenesis and disability. Although studies have assessed nitric oxide (NO) and its metabolites (nitrite and nitrate, collectively referred to as NOx) in MS patients compared to controls or other pathologies, the relationship between NOx levels, relapse/remission phases, and disability remains unclear. This study aimed to review systematically and meta-analyze NOx levels in MS patients, evaluating their association with disease phases and disability. **Methods:** This systematic review followed the PRISMA 2020 guidelines and was registered in PROSPERO (CRD42022327161). Electronic searches were performed in EMBASE, PubMed, and Scopus (February and August 2023). Inclusion criteria comprised studies assessing NOx levels in MS patients with a healthy control group. Exclusion criteria were reviews, pre-clinical studies, conference abstracts, case reports, studies without established MS diagnostic criteria, or non-English publications. Data extraction included sample size, MS subtype (relapsing-remitting MS [RRMS], primary progressive MS [PPMS]), clinical characteristics, NOx measurement methods, treatments, and disability assessment. Risk of bias in individual studies was evaluated using the Newcastle-Ottawa Scale, and publication bias was assessed using Egger's test, Begg's test, and funnel plots. Finally, we used the GRADE for non-randomized studies to evaluate the quality of evidence. **Results:** Twenty studies met the inclusion criteria, comprising 1,474 MS patients (1,010 RRMS, 221 PPMS) and 1,717 healthy controls. Meta-analysis revealed significantly elevated NOx levels in RRMS patients during relapse compared to remission and controls. Furthermore, higher NOx levels correlated with increased disability in MS patients. Study designs were predominantly observational, with heterogeneity in NOx detection methods and limited control for confounding factors such as treatment and lifestyle. **Conclusion:** NOx levels are elevated in RRMS during relapse and in MS patients with greater disability, supporting the role of nitrosative stress in MS pathophysiology. However, further studies are required to address confounding variables such as lifestyle, pain, and treatment effects on NOx levels. **Financial support:** FAPERGS, CNPq, and CAPES.

04.011 **Omega-3 Supplementation Attenuates Glucocorticoid Overproduction and Bone Mineralization Loss in a Type 1 Diabetes Mellitus Model.** Amanda Chaves, Lucas Carttman, Brunna Barbosa, Fabiana Pacheco, Livia Bergara, Patrícia Machado Martins e Silva, Marco Aurélio Martins, Vinicius Frias Carvalho IOC-Fiocruz, Lab of Inflammation, Center for Research, Innovation, and Surveillance in COVID-19 and Health Emergencies

Introduction: Diabetes has become a growing global concern problem, especially in low- and middle-income countries such as Brazil. Currently, Brazil ranks among the top ten countries with the highest prevalence of type 1 diabetes mellitus (T1DM). Over the past 30 years, there has been a 25% increase in T1DM cases among individuals over the age of 65, resulting in approximately 514 elderly individuals with T1DM per 100,000 inhabitants. The pathophysiology of T1DM is characterized by chronic hyperglycemia resulting from a relative or absolute deficiency in insulin production. Both Patients and experimental models with T1DM frequently exhibit elevated glucocorticoid levels. Additionally, hypercorticism observed in T1DM has been linked to the development of osteoporosis; however, the role of glucocorticoids in bone mineralization loss in diabetic conditions remains unclear. Long-chain fatty acids (LCFAs) have been investigated for their potential role in modulating immune responses and protecting bone microarchitecture. This study aimed to evaluate the effect of dietary LCFA supplementation on adrenal glucocorticoid overproduction and its impact on bone microarchitecture in an experimental T1DM model.

Methods: All procedures were approved by the Animal Ethics Committee (CEUA/IOC/FIOCRUZ – L-004/2024-A). Male Swiss-Webster mice (8 to 10 weeks old) were used. Type 1 diabetes was induced by a single intravenous injection of alloxan (65 mg/kg) seven days before treatment. Animals received daily oral supplementation with omega-3 (100 mg/kg, EPA: DHA ratio 1.5:1) for 14 consecutive days. Mice were divided into four experimental groups: non-diabetic + saline (0.9% NaCl); non-diabetic + omega-3; diabetic + saline (0.9% NaCl), and diabetic + omega-3 (0.9% NaCl). The endpoint was assessed on day 21 after the diabetes induction. The parameters evaluated included steroidogenic protein expression (western blotting), plasma corticosterone levels (ELISA), and bone microarchitecture (micro-CT). **Results:** In our T1DM model, mice exhibited increased plasma corticosterone levels, accompanied by elevated expression of the StAR enzyme and increased expression of ACTH receptor (MC2R) and MRAP2 in the adrenal glands. Diabetic mice also showed bone alterations consistent with osteoporosis, including reduced cortical bone mineral density (BMD) and trabecular thickness (Tb.Th), and increased total porosity (Ct.Po). Omega-3 supplementation significantly reduced corticosterone levels, as well as MC2R and StAR expression in the adrenal glands of diabetic animals, demonstrating a promising hormonal modulation effect. It also significantly improved cortical BMD parameters, cortex structure, and total porosity in the femurs. **Conclusion:** Our results indicate that the experimental model of T1DM presents significant hormonal dysregulation, evidenced by increased plasma corticosterone levels and elevated markers in the steroidogenesis cascade. Moreover, omega-3 supplementation showed promising effects in modulating glucocorticoid production as well as bone health in diabetic mice, as evidenced by the restoration of critical cortical bone quality parameters such as volume and porosity. These findings suggest that omega-3 exerts a protective role in the adrenal-bone axis, potentially mediated by the activation of anti-inflammatory pathways and improved metabolic regulation. **Financial support:** CNPq, CAPES, FAPERJ, NIM-INCT, JICA.

04.012 **Enhanced Anti-Inflammatory and Antioxidant Effects of H₂S-Releasing NSAID Derivatives on NLRP3-Activated Macrophages.** Coavoy-Sánchez SA¹, Teixeira SA¹, Wallace JL², Costa SKP¹, Muscará MN¹ ¹ICB-USP, Dept Pharmacology, São Paulo; ²Antibe Therapeutics Ltd., Toronto, Canada

Introduction: The NLRP3 inflammasome is a key component of the innate immune system that drives caspase-1 activation and the release of proinflammatory cytokines. Its dysregulation has been linked to the development of numerous chronic and inflammatory diseases. Hydrogen sulfide (H₂S), an endogenously produced gasotransmitter, plays a pivotal role in modulating inflammation and redox balance and has been shown to inhibit NLRP3 inflammasome activation. Hybrid compounds that conjugate H₂S-releasing moieties with nonsteroidal anti-inflammatory drugs (NSAIDs) have gained attention as potential therapeutic agents (Wallace et al., Br J Pharmacol, 2020). This study examines the effects of the NSAIDs indomethacin (Ind) and naproxen (Nx), their respective H₂S-releasing derivatives, ATB-344 and ATB-346, and the H₂S-releasing moiety 4-hydroxythiobenzamide (TBZ), on NLRP3 inflammasome activation and inflammatory responses in THP-1 macrophages. **Methods:** THP-1 cells were differentiated with 5 ng/mL PMA for 48 h and rested for 24 h. Cells were primed with lipopolysaccharide (LPS; 0.5 µg/mL, 3 h), washed, and then treated for 1 h with the test compounds at concentrations ranging from 100 to 1000 nM. Subsequently, nigericin (5 µM) was added for 90 min to trigger NLRP3 inflammasome activation. Cell viability (MTT assay) and lysis (LDH activity) were analysed, as well as the release of cytokines IL-1β, IL-18, TNF-α (quantified by ELISA). The activities of the antioxidant enzymes SOD, catalase, GR, and GST were also assessed. NLRP and ASC expression were evaluated by Western blot. **Results:** The presence of the H₂S-releasing moiety TBZ in the NSAID structures led to enhanced anti-inflammatory and antioxidant profiles, without increasing their toxicity. While Ind and Nx had limited or selective effects, Nx (1000 nM) reduced IL-18 and indomethacin (300–1000 nM) lowered TNF-α levels, their H₂S-releasing derivatives showed broader efficacies. ATB-344 (300 nM) and ATB-346 (300–1000 nM) significantly reduced LDH activity. Although no treatment reduced IL-1β levels, both ATB-344 and ATB-346 markedly decreased IL-18 release. TNF-α release was also more consistently reduced by the ATB compounds in comparison with the parent drugs. Both ATB-344 and ATB-346 (at 300 nM) reversed the reduction in SOD activity induced by inflammasome activation. TBZ alone also exhibited notable effects by reducing IL-18 (at 300–1000 nM) and TNF-α release (at 100–300 nM), restoring SOD and increasing GR activities (at 300 nM). **Conclusions:** H₂S-releasing NSAID derivatives exhibit enhanced anti-inflammatory and cytoprotective effects in comparison with their parent compounds, particularly by modulating IL-18 and TNF-α release and restoring antioxidant enzyme activities. These findings support the therapeutic potential of H₂S-donating NSAIDs by targeting NLRP3 inflammasome-related inflammation and oxidative stress. **Financial Support:** CNPq (150178/2024-7), CAPES and FAPESP (2023/13436-3).

04.013 **N2-like Neutrophil-Derived Extracellular Vesicles Enhance Malignancy of MDA-MB-231 Breast Tumor Cells.** Amorim CS¹, Amorim NS¹, Frony AC², Miranda BT³, De-Freitas-Junior JCM², RenovatoMartins M⁴, Barja-Fidalgo TC², Moraes JA¹ ¹UFRJ, Lab Biologia Redox; ²UERJ, Depto de Biologia Celular; ³IBCCF- UFRJ, RJ; ⁴UFF, Depto de Biologia Celular e Molecular

Introduction and Objective: Breast cancer is the second most common type of cancer in the world and the first most common among women. The relationship between cancer and inflammation is well established, and neutrophil infiltration has been described in tumors since 1863. Neutrophils are recruited by the tumor microenvironment and begin to favor tumor growth. Thus, they are called tumor-associated neutrophils (TAN) and can present two different phenotypes: N1 (anti-tumor) or N2 (pro-tumor). In previous work, our research group observed that neutrophils challenged with extracellular vesicles (EVs) derived from melanoma (MV3) or breast tumor cells (MDA-MB-231) were able to adopt an N2-like phenotype. As an *in vitro* polarization strategy for TAN N2, we used extracellular vesicles (EVs) derived from breast tumor cells (MDA-MB-231). We aimed to investigate the role of N2 TAN-derived EVs (N2-EVs) in MDA-MB-231 cells. **Methods:** Neutrophils were isolated using ficoll gradient from the peripheral blood of healthy donors (CAAE 65372522.4.0000.5257) and treated with MDA-EVs for three h at 37°C and 5% CO₂. After polarization, we observed some specific markers of neutrophil polarization to a pro-tumor or N2-like phenotype, such as VEGF, IL-8, arginase-1, total intracellular ROS production, CD184, and MMP-9 activity. N2-EVs were collected after two hours of neutrophil polarization and characterized for size (NanoSight) and L-selectin presence by flow cytometry. The effect of N2-EVs on AKT activation, mitochondrial ROS (using MitoSOX probe), invasion capacity (invasion assay with transwell), migratory capacity (wound healing), MMP-9 (gelatin zymography), and NF-κB activity (NF-κB responsive luciferase reporter) of MDA-MB-231 breast tumor cells was observed by treating the cells with the EVs by one h. **Results:** We observed that N2-EVs present L-selectin on their membranes and that fucoidan could block this selectin. Our findings demonstrated that N2-EVs interact with breast tumor cells through L-selectin, and these EVs could also increase mitochondrial ROS production in breast tumor cells via the PI3K/AKT axis. N2-EVs also regulated tumor cells' migratory and invasive capacity, probably through MMP-9 activity and NF-κB upregulation. **Conclusion:** Overall, our results show that N2-EVs can induce tumor cells *in vitro* to a greater migratory and invasive capacity of MDA-MB-231 cells in an L-selectin-dependent manner, increasing their malignancy *in vitro*. **Financial Support:** FAPERJ, Capes and CNPq.

04.014 DHA-Loaded Nanocapsules Enhance Microcirculation and Reduce Inflammation in Sepsis Experimental Model. Moraes BPT^{1,2}, Silva KP³, Moraes-de-Souza, I², Gomes, GLS², Saraiva, JSG¹, Costa, MF², Cunha, CMC², Almeida, MAP², Estado, V², Bozza PT², Castro-Faria-Neto HC², Ferrarini SR³, Sperandio M⁴, Silva AR², Gonçalves-de-Albuquerque CF^{1,2} ¹Unirio, Immunopharmacology Lab, Rio de Janeiro, Brazil ²IOC-Fiocruz, Immunopharmacology Lab, Rio de Janeiro, Brazil ³UFMT, HealthSciences Post-Graduation Program, Sinop, Brazil; ⁴Ludwig-Maximilians-University Munich, Institute of Cardiovascular Physiology and Pathophysiology, Biomedical Center, Planegg-Martinsried, Germany

Introduction: Sepsis is a life-threatening condition driven by an imbalanced host immune response to infection, often leading to microcirculatory dysfunction, organ failure, and death. Docosahexaenoic acid (DHA), an omega-3 fatty acid, exhibits well-documented anti-inflammatory effects. While effective on its own, DHA's therapeutic potential can be further enhanced through nanotechnology-based delivery systems, such as lipid-core nanocapsules (LNC_{DHA}), enhancing its bioavailability and efficacy. This study investigates the impact of LNC_{DHA} on neutrophil adhesion, microvascular function, and the host response in an experimental model of sepsis. **Methods:** LNC_{DHA} was developed and characterized using laser diffraction and Brownian motion analysis to determine particle size, distribution, and surface charge. Neutrophil-endothelial interactions were assessed both *in vitro* using flow chambers and *in vivo* through intravital microscopy of post-capillary venules in the cremaster muscle. Sepsis was induced in mice by cecal ligation and puncture (CLP), followed by evaluation of survival, bacterial burden, leukocyte infiltration, cytokine profiles in various tissues, brain intravital microscopy and perfusion. **Results:** LNC_{DHA} displayed a uniform size distribution (mean diameter: $0.223 \pm 0.00152 \mu\text{m}$) and a zeta potential of -12.83 mV . Treatment with LNC_{DHA} led to decreased adhesion and increased rolling velocity *in vitro*. *In vivo* analyses confirmed diminished leukocyte rolling and adhesion in TNF challenged venules. In septic animals, LNC_{DHA} administration resulted in reduced bacterial load, stabilization of body temperature, decreased leukocyte infiltration, and improved survival. Notably, it modulated cytokine responses by lowering TNF- α , IL-6, IL-1 β , CXCL1, and MCP1, while enhancing IL-10 and resolvins D1/D2 in plasma and brain tissue. LNC_{DHA} also modulated leukocyte-endothelial interactions in the brain and enhanced microvascular perfusion in septic mice. **Conclusion:** LNC_{DHA} ameliorated sepsis-induced microcirculatory dysfunction and systemic inflammation, translating into enhanced survival. These findings support the therapeutic potential of DHA-loaded nanocapsules as a novel intervention for sepsis and related inflammatory conditions. Funding: Rede Nano Saúde, FAPERJ, UNIRIO, CAPES, CNPq, IOC, Fiocruz.

04.015 **Lack of Effect of Dexamethasone in a Mouse Model of Influenza Infection with Bronchiectasis-Like Features as Monitored by Microcomputed Tomography.** Santana ACC¹, Ferreira TPT¹, Gomes HS¹, Santos HBS¹, Arantes ACS¹, Carvalho VF¹, Martins PMRS¹, Ball L², Martins MA¹ ¹IOC-FIOCRUZ, Lab of Inflammation, ²University of Genoa Dept Surgical Sciences and Integrated Diagnostics, Genoa, Italy

Introduction: Bronchiectasis is a disease associated with chronic airway inflammation, resulting from infections, genetic disorders, or autoimmune conditions. Repeated injury to the respiratory tract leads to structural airway changes, including irreversible bronchial dilation and mucus hypersecretion, which result in progressive pulmonary function impairment, and worsened quality of life. If not properly treated, or associated with other respiratory diseases, this condition can be fatal. Although computed tomography (CT) is one of the main diagnostic tools for bronchiectasis in humans, few studies have employed micro-computed tomography (micro-CT) or other imaging techniques to assess and quantify bronchiectasis in preclinical studies. This study aimed to investigate whether micro-CT scans can detect a phenotype of bronchiectasis in mice infected with H1N1 and assess its sensitivity to dexamethasone. **Methods:** The effects of H1N1 infection were examined in C57BL/6 mice using chest micro-CT scans. Male and female C57BL/6 mice were exposed to the H1N1 A/PR/8/34 strain (1000 PFU) or saline by oropharyngeal aspiration. We assessed body weight loss, leukocyte accumulation in bronchoalveolar lavage fluid and central airway volume (micro-CT). These analyses were performed before and 3, 5 and 7 days post infection. Results were compared to those of animals receiving dexamethasone on days 4–6 post-infection. All experimental procedures were approved by the Animal Ethics Committee of the Oswaldo Cruz Foundation (CEUA L002/20-A6). **Results:** The infection caused numerous pathological changes, including loss of body weight in parallel with lung neutrophilic inflammation, plasma leakage and increased airway resistance, which peaked at 7 days post-infection. The reconstructed 3D microtomographic images of the airways revealed an apparent bronchial enlargement on days 3, 5 and 7 post-infection, but showed no difference between males and females. The central airway volume values (mL) (mean $\pm$ SD) before and after infection were, respectively: $3,697 \pm 0,1767$ to $5,963 \pm 0,3209$ on day 3 ($n=12$, $p<0,0001$); $3,697 \pm 0,1767$ to $8,355 \pm 0,5967$ on day 5 ($n=6$, $p<0,0001$); and $3,697 \pm 0,1767$ to 14.78 ± 2.447 on day 7 ($n=6$, $p<0,0001$) with no effect of dexamethasone treatment. **Conclusion:** Our results suggest that C57BL/6 mice infected with the H1N1 virus presented marked pneumonia, associated with dexamethasone-resistant bronchiectasis-like features. These findings were confirmed and quantified through the evaluation of Micro-CT imaging. These discoveries may be a valuable addition to the currently available toolkit for preclinical drug discovery in bronchiectasis.

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04.016 **Effects of Colchicine on Lipopolysaccharide (LPS)-Induced Neuroinflammation in Astrocytes and PC12 Cells.** Araújo JR¹, Ananias CF¹, Bandeira SMA², Costa Júnior DC², Duarte ALS¹, Fernandes MO¹, Pinheiro KS¹, Rebouças MO³, Sales ISL³, Sampaio TL³, Silva MFS³, Sousa FCF³
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Introduction: It is well established that psychiatric disorders are associated with pathological mechanisms such as inflammation, oxidative stress, mitochondrial dysfunction, and cell death. Nuclear factor erythroid 2-related factor 2 (Nrf2) regulates antioxidant and cytoprotective genes, making it a promising therapeutic target. Although colchicine is traditionally used in inflammatory diseases, it has shown potential as a Nrf2 activator; however, its effects on central nervous system cells remain poorly understood. **Methods:** This study investigated the effects of colchicine on inflammatory parameters in astrocytes and PC12 cells stimulated with lipopolysaccharide (LPS) from *Escherichia coli*, a widely used model for inducing neuroinflammation *in vitro*. Initially, the cells were plated in 24-well plates and incubated for 24 hours. They were then exposed to LPS (50 µg/mL) for an additional 24 hours to induce the release of pro-inflammatory cytokines. Afterwards, the cells were treated with colchicine at concentrations of 0.312 µM (Col1) and 0.156 µM (Col2). After 24 hours, levels of the pro-inflammatory cytokines tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6), and interleukin-1 beta (IL-1β) were measured using *Enzyme-Linked Immunosorbent Assay* (ELISA) on the conditioned medium and cell homogenate. Statistical analysis was performed using ANOVA followed by Bonferroni's post hoc test, with $p < 0.05$ considered statistically significant ($n = 3$). **Results:** In astrocytes, the LPS exposure group showed an increase of TNF-α levels in the conditioned medium compared to the control, although not statistically significant. Treatment with colchicine at the 0.156 µM (Col2) showed a statistically significant reduction in TNF-α levels ($p = 0.0162$) compared to the LPS group. No statistically significant changes were observed in IL-6 and IL-1β levels in astrocytes. The values obtained were: TNF-α — Control: 1.716 ± 0.4635 ; LPS: 3.408 ± 0.3655 ; LPS+Col1: 2.340 ± 0.3655 ; LPS+Col2: 0.9707 ± 0.2598 . In PC12 cells, no statistically significant changes in cytokine levels were detected under any treatment condition, compared to the LPS group; therefore, the data are not shown. **Conclusion:** It is inferred that colchicine demonstrated anti-inflammatory effect, as evidenced by the significant reduction in TNF-α levels in astrocytes in the Col2 group (0.156 µM). This finding suggests that, at low concentrations, colchicine may modulate inflammatory responses in the central nervous system, possibly through mechanisms related to Nrf2 activation, although this remains to be investigated. The absence of detectable effects on IL-6, IL-1β and TNF-α in PC12 cells indicates that the substance's impact may be specific to certain mediators and cell types. Further studies, including *in vivo* models, are needed to confirm this neuroprotective potential and to clarify the role of Nrf2 in this context. **Financial support:** CAPES, CNPq (Grant number 402512/2023-5).

04.017 Interference of BDNF in Depressive Disorders: Effects of Cortisol-Modulating Substances.

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Introduction: The *Brain-Derived Neurotrophic Factor* (BDNF) has been found to play a critical role in modulating depressive disorders. Drugs that increase BDNF levels, such as cortisol-modulating substances have therapeutic potential in depression. Therefore, this review explores the current literature on BDNF regulation within the cortisol pathway and its implications for treating depressive disorders. **Methodology:** A literature review was performed using the PubMed and BVS databases and the descriptors “BDNF”, “Depression”, “Depressive Disorder”, “Major Depressive Disorder”, “Glucocorticoids”, “Cortisol”, and “Pharmacology”. We found 83 articles from PubMed and 91 articles from BVS. After excluding duplicates and applying filters for full and free-text availability, publication within the last five years, and languages (Portuguese, English, or Spanish), the total number of articles was reduced to 75. Articles in other languages or those unrelated to the theme, were excluded from the review. As a result, 29 studies were ultimately included. All phases of this review were manually carried out by the authors with the aid of Excel. **Results:** Depression is linked to hypothalamic-pituitary-adrenal (HPA) axis dysfunction, with hypercortisolemia in early stages and hypocortisolemia in severe phases. Crocin, genistein and 4-methylesculetin have demonstrated antidepressant effects associated with the inhibition of cortisol release, increased BDNF expression and regulation of inflammatory mediators. *Adansonia digitata* and Kaiyu Zhishen decoction showed antioxidant, anti-inflammatory properties and stimulation of the ERK/CREB pathway in the hippocampus. Ayahuasca and leonurine point to a more complex interaction between intermediate levels of cortisol and neurotrophic response. Among synthetic drugs, riparin IV, fluvoxamine, selanlyimidazopyridine (SIMP) and Ro41-5253 restored BDNF and improved behavioral parameters, while vortioxetine effectively regulates genes associated with stress. Mirtazapine demonstrated neuroprotective effects, partially restoring BDNF levels. Dexamethasone reduced inflammatory cytokines and preserved BDNF levels. **Discussion:** The persistent state of chronic stress induces significant biological changes in the brain, such as the loss of synapses in the hippocampus and prefrontal cortex. Inflammatory cytokines directly activate the HPA axis, increasing the expression of corticotropin-releasing hormone (CRH), glucocorticoid resistance, and serum cortisol levels. This hormonal imbalance leads to suppression of BDNF, which plays an essential role in neuroplasticity and neurogenesis. Synthetic substances stand out as endogenous neuromodulators, with anti-inflammatory, antinociceptive and antioxidant properties. Concerning natural compounds, despite their relevant modulation of the HPA axis and neuroplasticity in experimental data, there is still no clinical evidence supporting the effectiveness of these compounds in humans. **Conclusion:** These findings reinforce the importance of continued research into new therapeutic approaches for psychiatric disorders, exploring both natural and synthetic compounds to improve neuroprotection and restore neuronal homeostasis.

04.018 **Antioxidant Activity of New Synthetic Chalcones.** Marcarini VSM¹, Melo EDN¹, Falcão JS², Costa PRR², Caleffi GS², Muzitano MF¹, da Silva LL¹, Carmo PL¹ ¹UFRJ-Macaé Multidisciplinary Center, Bioactive Products Pharmacology Research Group, Brazil ²UFRJ, Health Sciences Center, Lab. of Bioorganic Chemistry, Brazil

Introduction: Pain, frequently intensified by oxidative stress, is a limiting factor that significantly impacts quality of life. This scenario has motivated the search for new therapeutic strategies, and bioactive compounds have emerged as promising alternatives, capable of both relieving pain and modulating oxidative mechanisms. Accordingly, the research group from the Laboratory of Bioactive Products (LPBio) aimed to evaluate the activity of five synthetic chalcones. Chalcones are intermediates in the biosynthesis of flavonoids—a large group of bioactive compounds widely recognized for their anti-inflammatory and antioxidant properties [1]. Therefore, as members of this class, chalcones have a high potential to exhibit such biological mechanisms. **Methods:** This study evaluated the antioxidant activity of chalcones LC5, LC24, LQB778, LQB779, and LQB781 through *in vitro* assays that mimic distinct antioxidant mechanisms observed in biological systems. Two tests were performed: 1) Evaluation of myeloperoxidase (MPO) cycle inhibition, using tetramethylbenzidine and H₂O₂ as substrates. Enzymatic activity was monitored by spectrophotometric readings at 450 nm. The five chalcones were tested at 100 µM and compared to the DMSO vehicle, quercetin, and chalcone LC4 [2]. Results were expressed relative to the control (%). 2) Assessment of superoxide anion scavenging capacity, based on the autoxidation of pyrogallol and monitored by spectrophotometric readings at 356 nm and 420 nm. The effect of chalcones at 100 µM was compared to DMSO and ascorbic acid. The potency of the most active chalcone was further determined. **Results:** 1) Regarding MPO inhibition, none of the chalcones evaluated in this study reproduced the inhibitory activity previously observed for LC4. 2) In contrast, regarding superoxide scavenging activity, LC5 and LC24 exhibited antioxidant effects compared to the control (0.65 ± 0.02), with values of 0.39 ± 0.06 and 0.13 ± 0.01 ($P < 0.05$), respectively. Since LC24 showed more than 50% antioxidant activity at 100 µM, its concentration–response relationship was assessed, resulting in an IC₅₀ of 67.67 µM. LQB781 initially appeared to display antioxidant activity; however, further analysis revealed a strong decrease in absorbance at 356 nm over the course of the assay, indicating possible instability of the compound under the test conditions. A shift in the detection wavelength to 420 nm suggested that no actual superoxide scavenging was occurring. Further chromatographic studies are needed to determine whether the observed reaction is spontaneous or involves interaction with the superoxide anion. **Conclusions:** LC24 demonstrated significant superoxide-scavenging capacity, which may contribute to its potential pharmacological effects. However, none of the chalcones tested appear to share the same MPO inhibition. **Acknowledgements:** This study was financed by the Carlos Chagas Filho Foundation for Research Support of the State of Rio de Janeiro (FAPERJ)

04.019 **Lipid Profile Evaluation in the Lung Response to Silica Particle Exposure in Mice.** Cabral, CL¹, Pezzella-Ferreira, GN¹, Pereira-Dutra, FS², Espinheira-Silva, H², Martins, MA¹; Bozza, PT², Silva, PMR¹ ¹IOC-Fiocruz, Lab of Inflammation, ²IOC-Fiocruz, Lab of Immunopharmacology, Rio de Janeiro, Brazil

Introduction: Silicosis is a chronic obstructive pulmonary disease of occupational origin, resulting from prolonged exposure to crystalline silica. This disease is characterized by pulmonary fibrosis and persistent inflammation. Lipids play a crucial role in the development and progression of fibrosis in various organs, including the lungs. They act as mediators in inflammatory responses, cell-cell communication, and even in the direct deposition of extracellular matrix. Furthermore, lipid droplets (LDs), organelles involved in lipid storage and metabolism, have been implicated in inflammatory responses. Although the role of these structures in silicosis remains underexplored, they may contribute significantly to the progression of pulmonary fibrosis. This study investigates the lipid profile in the lungs of mice exposed to silica particles, with a particular focus on lipid mediators that may be critical for understanding the inflammatory processes and the progression of fibrosis in silicosis. **Methodology:** Male Swiss-Webster mice (20–25 g) were exposed to silica via intranasal instillation (50 µL of a silica suspension, 10 mg/animal). Animals were euthanized at 7 and 28 days post-exposure, and their lungs were collected for analysis. Lipid mediators—including cysteinyl leukotrienes (CysLTs), resolvin D1 (RvD1), thromboxane B₂ (TBX₂), and prostaglandin E₂ (PGE₂)—were quantified using enzyme immunoassays (EIAs). The expression cyclo-oxygenase-2 (COX-2), 5-lipoxygenase (5-LO) was assessed by real-time PCR. The diacylglycerol O acyltransferase 1 (DGAT1), associated with lipid droplet formation was also evaluated. Lipid droplets (LD) were visualized using Oil Red O staining and quantified with Image J software. All experimental procedures were approved by the Ethics Committee for Animal Use of Oswaldo Cruz Institute/FIOCRUZ (L001/19-A6). **Results:** Silica-exposed mice exhibited elevated levels of CysLTs while TBX₂ was significantly reduced, as compared to controls, at 7 and 28 days post-silica. RvD1 levels as well as expression of COX-2 and 5-LO remained unchanged. Interestingly, DGAT1 expression was shown to be increased, indicating a potential upregulation of LD formation in response to silica exposure. Quantification of lipid droplets using Oil Red O staining supported this observation, revealing a higher accumulation in the lungs of silica-exposed mice at both 7 and 28 days post-exposure. **Conclusion:** Together, our findings suggest that silica exposure modulates lipid metabolism—particularly through the formation of lipid droplets (LDs)—which may contribute to the progression of pulmonary fibrosis. Additional experiments are needed to clarify the role of LP in silica-induced lung inflammation. **Financial support:** FIOCRUZ, FAPERJ, CNPq and CAPES (Brazil).

04.020 Effects of the β -Carboline Alkaloids Harmine and Harmaline on Neuroinflammation and Oxidative Stress *in vitro*. Morales-Lima G^{1,2}, Falconi-Souto A¹, Gray NE², Mendes FR¹ ¹UFABC, Center for Natural and Human Sciences, Brazil ²Oregon Health & Science University, Dept Neurology, USA

Introduction: Neuroinflammation and oxidative stress play a key role in the pathogenesis of neurodegenerative diseases, therefore substances that act intracellularly to attenuate these processes are promising prophylactic and therapeutic agents. Harmine (HAR) and harmaline (HML) are β -carboline alkaloids found in the *B. caapi* and *P. harmala* plant species, which have shown anti-inflammatory, antioxidant, and neuroprotective activities. The neuroinflammatory response can be induced by lipopolysaccharide (LPS), a Toll-like receptor 4 ligand capable of triggering an immune response that leads to microglial activation. Moreover, it is well known that the Nrf2-ARE antioxidant response pathway interacts with the inflammatory response, and the activation of this pathway has been reported as an important mechanism for antioxidant and anti-inflammatory agents. Considering the crosstalk between neuroinflammation and oxidative stress, this study aimed to assess the anti-inflammatory effects of harmine (CaymanChem) and harmaline (ThermoScientific) in LPS-stimulated BV-2 murine microglial cells. We also evaluated the β -carboline alkaloids' ability to act as Nrf2-ARE pathway inducers in HepG2-ARE cells, a cell line derived from hepatocellular carcinoma that stably expresses a firefly reporter under the control of the ARE transcription factor. **Methods:** We first assessed the β -carbolines' cytotoxicity in BV-2 and HepG2-ARE cells treated for 24h, as well as the LPS cytotoxicity in BV-2 cells using MTT (BV-2 cells) or MTS (HepG2 cells) assays. We also measured the release of interleukin-6 (IL-6) in the supernatant of LPS-stimulated BV-2 cells at concentrations of 0.5-5 μ g/ml over different treatment intervals (2-4-6-12-24h) using ELISA. Subsequently, we co-treated the BV-2 cells with HAR (1-5 μ g/ml) or HML (5-25 μ g/ml) and LPS (5 μ g/ml) for 6h and determined IL-6 and tumor necrosis factor (TNF) levels in the cell supernatant. The Nrf2-ARE pathway fold-induction was evaluated in the HepG2-ARE reporter cell line through the luminescence measurement in cells treated for 24h at the concentrations of 1-25 μ g/ml (HML) or 1-5 μ g/ml (HAR). **Results:** We have demonstrated that HML is not cytotoxic in BV2 cells across the concentration range of 1-50 μ g/ml, while HAR showed cytotoxicity at doses higher than 5 μ g/ml. Moreover, in HepG2-ARE cells, HML was cytotoxic in concentrations higher than 25 μ g/ml, while HAR was cytotoxic in concentrations higher than 5 μ g/ml. LPS treatment for 24h did not affect cell viability at the tested concentrations, but increased the supernatant IL-6 concentration in cells treated with LPS (5 μ g/ml) for 6h, an effect counteracted by the β -carbolines treatment ($p < 0.05$). Regarding the TNF levels, no statistically significant effects were observed. Additionally, we showed that the β -carbolines act as Nrf2-ARE inducers in HepG2-ARE cells in a concentration-dependent manner. **Conclusion:** Although our data are based on *in vitro* assays, the results support the anti-neuroinflammatory and antioxidant potential of β -carbolines in a cell line model, and these results can provide insights for future research using more complex models to assess their neuroprotective mechanisms. **Financial Support:** FAPESP (#2023/02031-2, #2024/18859-2, and #2022/12675-1)

04.021 Baricitinib Exhibits Antioxidant and Mitochondrial Protective Activity in Astrocytes and PC12 Cells Exposed to Lipopolysaccharide. Peixoto GPA¹, Bandeira SMA², Costa Junior DC³, Rebouças MO⁴, Lopes IS⁵, Silva DMA⁶, Sampaio TL⁷, Magalhães EP⁸, Teixeira IMM⁹, Sousa FCF¹⁰ ¹UFC, PPG Pharmacology, Brazil ²UFC, PPG Pharmacology, Brazil ³UFC, PPG Pharmacology, Brazil ⁴UFC, PPG Pharmacology, Brazil ⁵UFC, PPG Pharmacology, Brazil ⁶UFC, PPG Pharmacology, Brazil ⁷UFC, Dept Pharmacy, Brazil ⁸UFC, PPG Pharmaceutical Sciences, Brazil ⁹UFC, Pharmaceutical Sciences, Brazil ¹⁰UFC, PPG Pharmacology, Brazil

The treatment of depression still faces significant challenges. The neuroinflammation theory postulates that dysregulation of the inflammatory response is closely related to the pathophysiology of the disorder. In this context, baricitinib (BARI) emerges as a promising compound due to its anti-inflammatory and antioxidant properties. This study aimed to investigate the effect of BARI in an *Escherichia coli* lipopolysaccharide (LPS)-induced inflammation model. For this purpose, astrocyte and PC12 cell cultures were exposed to LPS at a concentration of 50 µg/mL for 24 hours. Subsequently, BARI was administered at three different concentrations (BARI 1 = 1.25 µM, BARI 2 = 0.63 µM, and BARI 3 = 0.31 µM) for an additional 24 hours. Following treatment, analyses were performed to assess the production of reactive oxygen species (ROS) in the cytoplasm and the mitochondrial membrane potential. The experiments were performed in independent triplicates. For statistical analysis, ANOVA with Tukey's post hoc test or Kruskal-Wallis with Dunn's test was used, and results were expressed as median [lower percentile-upper percentile] (statistical significance was considered when $p < 0,05$). The main findings indicate that LPS induced a significant increase in ROS production (LPS: 2.11 [1.98–2.16]; SAL: 1.06 [0.91–1.11] ($p < 0,01$ vs. SAL)), whereas BARI exhibited antioxidant activity at all concentrations (LPS + BARI 1: 1.06 [1.03–1.09] ($p < 0,05$ vs. LPS); LPS + BARI 2: 1.11 [1.04–1.32]; LPS + BARI 3: 0.67 [0.59–0.98] ($p < 0,0001$ vs. LPS)). Regarding mitochondrial membrane potential in astrocytes, fluorescence analysis revealed that the LPS group compromised membrane integrity (LPS: 0.54 [0.45–0.63]; SAL: 1.04 [0.75–1.17] ($p < 0,05$ vs. SAL)), while treatment with BARI 2 partially reversed the damage (LPS + BARI 2: 0.75 [0.69–0.76]). In PC12 cells, LPS induced a slight reduction in fluorescence (LPS: 0.68 [0.65–0.79]; SAL: 0.99 [0.99–1.00]), and BARI effectively reversed this effect (LPS + BARI 1: 1.16 [0.84–1.18] ($p < 0,01$ vs. LPS); LPS + BARI 2: 1.15 [0.90–1.19] ($p < 0,01$ vs. LPS); LPS + BARI 3: 1.16 [1.05–1.17] ($p < 0,001$ vs. LPS)). This set of results suggests that baricitinib exerts a protective effect on astrocytes and PC12 cells, exhibiting both antioxidant and mitochondrial protective activity under inflammatory conditions induced by lipopolysaccharide.

04.022 Anti-Inflammatory Property of Crude Geopropolis Extract from *Melipona quadrifasciata* In Human Macrophages Zapotoski LNK^{1,2}, Tambourgi DV², Pohl PC² ¹PPIB, University of São Paulo ²IBu, Immunochemistry Lab

Introduction: Geopropolis is a product of stingless bees (Meliponini), composed of plant resins, floral secretions, and with a distinctive addition of soil or clay. Predominantly found in tropical and subtropical regions, over 400 species are known globally—about 300 in Brazil— playing a key role in cross-pollination and ecosystem regeneration. Despite its ecological and economic significance, geopropolis has often been undervalued in meliponiculture due to its inorganic content. However, growing interest in its complex chemical and biological properties has revealed significant therapeutic potential. Considering that geopropolis contains bioactive compounds, its effects on immune cells are of interest. Macrophages are a key cell in immune response, they play a significant role in the local and systemic regulation of physiological processes, such as inflammation, tissue repair, metabolism, and cell proliferation. These cells can polarize according to the stimulus presenting a pro- or anti-inflammatory profile. Lipopolysaccharide (LPS), found in the outer membrane of Gram-negative bacteria, is one inducer of inflammation, its recognized by Toll-Like Receptor 4 which activates Factor Nuclear Kappa B (NF- κ B) via and in response triggers a cascade of inflammatory mediators, including cytokines such as Tumor Necrosis Factor Alpha (TNF- α), Interleukins- 1 β and 6 (IL-1 β , IL-6). Considering the potential of this unexplored product, the present study aims to evaluate the anti-inflammatory activity of the hydroalcoholic extract of geopropolis from the bee *Melipona quadrifasciata* using a human macrophage model. **Methods:** To analyze the cytotoxic effects, macrophages derived from THP-1 lineage were treated with concentrations ranging from 7.82 to 1000 μ g/mL of hydroalcoholic extract of geopropolis (HEG) for 24 and 48 hours. For immunomodulatory analysis, THP-1/ macrophages were stimulated with the presence or absence of LPS and two-sublethal concentrations of HEG for 24 and 48 hours. Then, the profile of cytokines (IL-1 β , IL-6, IL-8, IL-10 and TNF- α) secreted in the culture supernatants was analyzed using Cytometric Bead Array (CBA) and the relative expression of immunomodulatory factors (IL-6, IL-10, TGF- β , VEGFA and ARG 2) were analyzed by RT-qPCR. **Results:** The viability of THP-1 macrophages treated with the hydroalcoholic extract of geopropolis (HEG) remained unaffected at concentrations between 7.82 and 62.50 μ g/mL. However, concentrations of 125 μ g/mL or higher exhibited a reduction in viability exceeding 50%. Functional assays revealed that LPS-stimulated THP-1 macrophages treated with HEG exhibited a significant increase in IL-10 production and a concomitant decrease in IL-6 levels, indicating an anti-inflammatory effect in this *in vitro* model. In non-LPS-treated THP-1 macrophages, HEG treatment significantly elevated IL-8 levels. In a wound-healing context, this cytokine can be involved in angiogenesis and tissue remodeling. Gene expression analysis further supported these findings, showing upregulation of TGF- β and Vascular endothelial growth factor A (VEGFA), which are associated with cellular proliferation and tissue repair, as well as upregulation of arginase 2 (ARG2), a marker of a regulatory/repairative M2 phenotype. Additionally, IL-6 was downregulated, consistent with the CBA results. **Conclusion:** Collectively, these data highlight the anti-inflammatory and wound-healing potential of HEG, underscoring its relevance for future pharmaceutical applications

04.023 **Effect of Hypothalamic ETB Receptor Blockade on Serotonin and Dopamine Levels During Systemic Inflammation in Rats.** Alencar ST, Costa RA, Santos MO, Schneider VMM, Fernandes SMA, Borges AP, Zampronio AR UFPR Inflammation and Fever Lab, Dept Pharmacology

Introduction: Systemic infectious diseases are characterized by signs and symptoms such as fever, hyperalgesia, anorexia, and somnolence collectively referred to as sickness syndrome. Exacerbated systemic inflammatory responses or sepsis may lead to severe tissue damage or death. Previous studies have shown that increased hypothalamic serotonin (5-HT) levels during systemic inflammation activate the inflammatory reflex, thereby reducing the intensity of the inflammatory response (Mota et al., *Brain Behav Immun.* 66:372, 2017). Recent data showed that systemic inflammation induced by lipopolysaccharide (LPS) leads to decreased hypothalamic 5-HT levels in male rats but increased levels of 5-HT in females. Both the decrease and increase in 5-HT levels were reversed by the intracerebroventricular administration of the endothelin-1 (ET-1) receptor B (ETB) antagonist BQ788 (Costa et al., *J Neuroimmunol.* 394:578427, 2024). However, it remains unclear whether BQ788 exerts its effects directly within the hypothalamus or via the dorsal raphe nucleus, the primary source of serotonergic projections to the hypothalamus. This study aimed to investigate whether direct administration of BQ788 into the hypothalamus could restore hypothalamic 5-HT levels and reduce sickness syndrome (fever and hypolocomotion) in male rats. Additionally, we have also evaluated the hypothalamic levels of dopamine (DA) and its metabolites.

Methods: Male Wistar rats (90 days old) were implanted with remote temperature sensors in the peritoneal cavity. After a 7-day recovery period, animals were housed in a temperature-controlled room (28°C). BQ788 (6 pmol in 0.5 µL) or saline was microinjected into the hypothalamus, followed by intraperitoneal injection of LPS from *E. coli* (100 µg/kg) or vehicle. Body temperature was monitored for 5 hours. Subsequently, locomotor activity was assessed in an open field test, and the hypothalamus was collected for quantification of 5-HT and DA and the metabolites, 5-hydroxyindoleacetic acid (5-HIAA), homovanillic acid (HVA) and 3,4-dihydroxyphenylacetic acid (DOPAC) by HPLC. This study was approved by the Animal Ethics Committee of the Federal University of Paraná (CEUA #1601). **Results:** Intraperitoneal injection of LPS induced fever and hypolocomotion in male rats, and decreased hypothalamic levels of 5-HT and 5-HIAA by approximately 40%. LPS also reduced DA and its metabolites, HVA and DOPAC, by 60%, 67%, and 40%, respectively. Intrahypothalamic administration of the ETB receptor antagonist BQ788 significantly attenuated LPS-induced fever and hypolocomotion. BQ788 also reversed the LPS-induced reductions in hypothalamic 5-HT, 5-HIAA, DA, and its metabolites (HVA and DOPAC). **Conclusion:** These findings suggest that in male rats ET-1 acts directly within the hypothalamus to induce fever and hypolocomotion following LPS administration. The observed reductions in 5-HT and 5-HIAA levels indicate that ET-1 may activate ETB receptors in the hypothalamus, thereby suppressing local serotonergic signaling and dampening the descending anti-inflammatory pathway in males. Furthermore, LPS-induced reductions in DA and its metabolites suggest a dysfunction in dopaminergic neurotransmission during systemic inflammation. Financial Support: CNPq and UFPR.

04.024 **Muscarinic Antagonists Mitigate Structural Lung Damage in Experimental Silicosis.** Santos, BDF, Ferreira, TPT, Arantes, ACS, Santana, ACC, Martins, MA, Silva, PMR IOC-Fiocruz, Lab Inflammation, Brazil

Introduction: Silicosis is the leading cause of disability among occupational respiratory diseases in Brazil and worldwide. It is characterized by a chronic, progressive inflammatory response that culminates in granulomatous fibrosis. Despite the availability of several therapeutic approaches, no effective antifibrotic treatment currently exists. Acetylcholine, a neurotransmitter of the parasympathetic nervous system expressed in several cell types, has been implicated in the pathogenesis of inflammatory lung diseases. Recent studies have shown that blocking cholinergic signaling through muscarinic receptor antagonists, can be effective in treating chronic pulmonary inflammation. The objective of this study was to investigate the impact of treatment with the muscarinic receptor antagonist, ipratropium bromide on silica-induced chronic lung inflammation in mice. : Male Swiss-Webster mice were sedated with isoflurane and administered 10 mg of silica intranasally, dissolved in 50 μ L of sterile saline. Control animals received an equivalent volume of 0.9% sterile saline. From day 21 to 27 post-silica exposure, animals were treated daily via aerosolization with ipratropium bromide (IB) (0.15–5 μ g/mL). Analyses were performed 24 hours after the final treatment. The following parameters were evaluated: i) radiological changes by micro-CT system; ii) Lung function by barometric plethysmography; iii) Morphology and morphometry using tissue sections stained with hematoxylin-eosin (H&E) and Picrosirius; iv) Collagen content by Sircol technique. All experimental procedures were approved by the Committee on Use of Laboratory Animals of the Oswaldo Cruz Institute/FIOCRUZ (license L001/19A6). **Results:** Our data show by tomographic analysis an increase of lung weight and volume in silica-exposed animals, indicative of severe inflammation. Additionally, a marked reduction in normally aerated and increase in the non-aerated areas were detected. Pulmonary function assessed using the enhanced pause (Penh) parameter, in response to increasing methacholine doses, showed a significant increase in the silica-exposed animals compared to controls, indicating bronchial hyperreactivity. Also, an increase in the amount of total collagen was in the lungs of silica-challenge mice compared to controls. Therapeutic administration of IB led to a dose-dependent reduction of all parameters analysed, with the dose of 1.5 μ g/mL being most effective. **Conclusion:** Our findings demonstrate that pharmacological blockade of the muscarinic cholinergic pathway via ipratropium bromide administration effectively attenuates silica-induced pulmonary inflammation, extracellular matrix deposition, and granuloma formation. These results underscore the therapeutic potential of muscarinic antagonists in the treatment of silicosis and other chronic fibrotic lung diseases. **Financial support:** FIOCRUZ, CAPES, CNPq (Brazil).

04.025 **Hyperglycemia Induces Macrophages Senescence** *in vitro*. Dias DS, Pacheco FS, Mota RG, Chaves AS, Barbosa BL, Carvalho VF, Silva PR Martins MA Lab Inflammation, IOC-Fiocruz, Rio de Janeiro, Brazil

Introduction: Macrophages play a significant role in the development of both type 1 and type 2 diabetes, contributing to the inflammatory processes and insulin resistance associated with these conditions. These cells also are involved in promoting aging and age-related diseases, particularly through a process called inflammaging with promotes senescent cells accumulation. Senescence is a natural process where cells stop replication and change to a secretory profile, secreting inflammatory cytokines such as IL-6 and TGF- β . **Methods:** The peritoneal macrophage cell line, RAW 264-7 were cultivated with crescent concentrations of glucose 5.5mM, 7.5mM, 15mM, and 30mM for 24h and bone marrow-derived macrophages (BMM) in 25mM glucose and low glucose DMEM as control for 24h. Senescence associated beta galactosidase (SA- β -Gal) was evaluated by staining kit. Expression of cell cycle arrest proteins p21 and p16 was evaluated by western blotting assay. The experiment with BMM was performed in accordance with Committee on Use of Laboratory Animals of the Oswaldo Cruz Institute/FIOCRUZ (L-004/2024). **Results:** Our results show that a high glucose microenvironment induced macrophage senescence, attested by SA- β -Gal immunostained, in both RAW 264-7 cells ($2,2 \pm 0,6$; $20 \pm 8,1$; $20,9 \pm 8,7$; $31,6 \pm 12,1$ of in 5.5 mM, 7.5 mM, 15 mM, and 30 mM glucose, respectively; mean $\pm$ SEM; n=4; p<0.05) and BMM ($36,5 \pm 9,6$; $61 \pm 7,8$ of BMM in normoglycemic media and 25 mM glucose media, respectively; mean $\pm$ SEM; n=5; p<0.05) *in vitro*. Furthermore, hyperglycemic media increased the expression of cycle arrest proteins p16 and p21 in RAW 264-7 cell line. **Conclusion:** These results show partial conclusions that hyperglycemia may directly induce senescence of macrophages. **Financial support:** IOC, INCT-NIM, CAPES, FAPERJ and Ministério da Saúde.

04.026 Nano Emulsion with Omega 3 Fatty Acids Modulates Muscle Skeletal Tissue Degeneration and Regeneration Process in Venom of *Bothrops asper* Induced Lesion. Roschel GG¹, Sousa MBN¹, Escalante T², Purgatto E³, Moreira V¹ ¹Unifesp, Lab of Pharmacology of Inflammation, PPG Pharmacology, Brazil ²Clodomiro Picado Institute, Faculty of Microbiology, Costa Rica University, Costa Rica ³FORC-USP, Faculty of Pharmaceutical Sciences and Food Research Center, Brazil

Introduction: Omega-3 fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) exhibit anti-inflammatory effects via lipid mediators derived from omega-3 (LM3). LM3 are synthesized through enzymatic pathways, such as cyclooxygenase-2 (COX-2) and 12-lipoxygenase (12-LOX), modulated or not by acetylsalicylic acid (ASA). Skeletal muscle regeneration involves myogenic transcription factors, including paired box transcription factor 7 (PAX7), myoblast determination protein 1 (MyoD), and myogenic factor 5 (Myf5), as well as inflammatory mediators like the myokines interleukin (IL)-6, IL-15, leukemia inhibitory factor (LIF), osteonectin (OST), irisin (IRI), and myostatin. The regenerative potential of LM3, alone or combined with ASA, remains unclear and requires further investigation. **Methods:** Male Swiss mice (n=4/group) received intraperitoneal injection (i.p.) of fish oil nanoemulsion (FON) (6.0 µg/g DHA and 10.2 µg/g EPA in 500 µL) or 0.9% saline for 7 days. *Bothrops asper* venom (Bav) (2.5 mg/kg in 50 µL) was injected intramuscularly (i.m.) into the left gastrocnemius. A separate group received i.p. ASA (117 mg/kg in 500 µL) every 3 days. Muscles were collected after 24 h, 3, 7, and 28 days for analysis of myogenic factors PAX7, MyoD and Myf5, and enzymes COX-2, 12-LOX, myokines IL-6, -15, LIF, IRI, OST and miostatin, and creatine kinase activity. Fatigue resistance was assessed on day 28 using 15-minute tetanic isometric stimulation. **Results:** The first results observed were PAX-7 expression increased in FON (66%) and FON+ASA (65%) groups compared to saline after Bav (p<0.05) and remained elevated in FON+ASA (56%) at 3 days (p<0.05). The same was observed in MyoD and Myf5. These proteins were elevated in FON and FON+ASA 24h and 3 days post Bav i.m., 97% and 43% respectively, p<0,05, for MyoD and post 24h of Bav i.m. for Myf5, 47% for FON and 43% for FON+ASA. Data showed a reduction in IL-1β post-Bav injection at 24h, 3 and 7 days in the FON-treated groups by 23%, 46%, and 78%, respectively (p<0.05). IL-6 was reduced at 24h post-Bav injection in both FON (47%) and FON+ASA (48%) groups (p<0.05), and at 3d post-injection by 47% and 74%, respectively (p<0.05), compared to Bav alone. LIF and IRI were reduced by 32% and 68% at 24h post-Bav in both FON and FON+ASA groups, respectively, compared to Bav group (p<0.05). In contrast, high levels of OST in all treated groups post Bav i.m. (p<0,05) were observed when compared to saline group. CK levels decreased at 3 days post-Bav i.m. in both FON and FON+ASA (40%) compared to Bav alone (p<0.05). In the fatigue test, FON+ASA group showed reduced resistance at 5 min (34%) and 6 min (39%) compared to Bav i.m. (p<0.05), with values similar to the saline. **Conclusion:** Data showed that LM3 can modulate key pro resolutive mediators on muscle regeneration, but reduced resistance was observed when combined FON and ASA treatments, probably by alteration of muscle fibers, and need to be further investigated.

04.027 **A Dual FPR1/FPR2 Agonist Suppresses Pulmonary Inflammation and Fibrosis Development in a Murine Model of Silicosis.** Pezzella-Ferreira GN¹, Cabral CL¹, Arantes ACS¹, Martins MA¹, Perretti M², Silva PMR¹ ¹ Lab Inflammation, Oswaldo Cruz Institute, FIOCRUZ, Rio de Janeiro, Brazil. ²Queen Mary University, William Harvey's Research Institute, UK

Introduction: Silicosis is a chronic occupational lung disease caused by inhaling crystalline silica dust, and characterized by persistent inflammation followed by granuloma formation and progressive fibrosis. Evidence indicates that annexin A1 (AnxA1) mediates most of its cellular effects via interaction with formyl peptide receptors (FPR), G protein-coupled receptors involved in immune responses, chemotaxis, inflammation, and resolution pathways. Compound 43 (Cmp43) is a synthetic small molecule identified, in some studies, as a dual agonist for FPR1 and FPR2. Thus, this study was carried out to investigate the potential effect of Cmp43 on pulmonary inflammation caused by silica exposure in mice. **Methodology:** Anesthetized male Swiss-Webster mice were intranasally instilled with silica particles (10 mg/kg) or vehicle (0.9% saline). Cmp43 (10 mg/kg), was administered subcutaneously, daily from day 1 to day 7, starting 6 hours post-silica exposure. Analyses were conducted 1 day after the final treatment. Lung function was assessed by whole-body invasive plethysmography. Morphological and morphometric assessments were performed using H&E staining. Collagen deposition was measured using the Sircol colorimetric assay, and cytokine levels (TNF- α , MCP-1, TGF- β) were quantified by ELISA. All procedures were approved by the Committee on the Use of Laboratory Animals of the Oswaldo Cruz Institute/FIOCRUZ (L-001/19A6). **Results:** Silica instillation led to increased lung weight, total lung volume, and expansion of non-aerated compartments, indicating the initiation and progression of inflammation and fibrosis. Lung function analysis revealed elevated airway resistance and lung elastance, and also methacholine-induced airways hyperreactivity in the silicotic mice. These phenomena were accompanied by marked leucocyte infiltration (mainly neutrophils), collagen accumulation and granuloma formation, increased levels of cytokines (TNF- α and TGF- β) and chemokines (MCP-1). Treatment with Cmp43 showed reduction of neutrophil infiltration, levels of TNF- α and MCP-1 and TGF- β , along with collagen deposition points to reduce fibrosis and tissue remodeling. **Conclusion:** These findings suggest that therapeutic administration of Cmp43 effectively attenuates silica-induced lung inflammation and fibrosis in mice. The observed reduction in neutrophil infiltration, pro-inflammatory cytokines (TNF- α , MCP-1), and fibrotic markers (TGF- β , collagen deposition) supports a protective role of FPR1 and FPR2 signaling pathways in the pathogenesis of silicosis. Consequently, AnxA1 mimetics like Cmp43 hold significant potential as novel anti-inflammatory and anti-fibrotic therapeutic agents for treating silicosis and related pulmonary disorders. **Financial support:** FIOCRUZ, FAPERJ, CNPQ, CAPES.

04.028 Maternal Neutrophils Orchestrate Embryo Development and Reshape the Innate Immune System in the Offspring. Scharf P¹, Borges PP¹, Costa KAM¹, Fock RA¹, Urban S², Farsky SHP¹ ¹USP, PPG in Pharmacy (Physiopathology and Toxicology), São Paulo, Brazil ²UNIFESP, PPG in Functional and Structural Biology, São Paulo, Brazil

Neutrophils play emerging and multifaceted roles during pregnancy, contributing to uterine remodeling and embryo implantation. Their functional plasticity is crucial for creating a supportive environment for the developing embryo. Disruption of neutrophil dynamics may compromise maternal-fetal interactions and lead to long-lasting consequences across the early and late stages of life. However, the extent to which alterations in maternal neutrophil biology affect offspring development and immunity remains poorly understood. To address this, we investigated the role of neutrophils during early gestation and their long-term impact on offspring development and innate immune function. Allogeneic pregnancies were established by mating C57BL/6 females with BALB/c males (CEUA 663). Neutrophils were depleted 24 h post-mating (gestational day 1.5) using anti-Gr1 antibody or isotype control (100 µg, i.p.). Pregnancies progressed to gestational day 16.5 for embryo analysis or to term for postnatal evaluation (8–10 weeks), including immunophenotyping of bone marrow (BM) and peripheral blood leukocytes, and functional assays. Additionally, adult offspring were challenged with LPS to assess innate immune responses and resolution of inflammation upon an acute lung injury. At day 16.5, neutrophil depletion impaired embryonic development, resulting in reduced fetal and placental weights. Notably, 33% of the offspring exhibited congenital malformations, including congenital talipes equinovarus, umbilical hernia, and microcephaly. In adulthood, offspring from neutrophil-depleted dams showed reduced frequencies of common myeloid (Lin⁻Sca-1⁻IL7R⁻c-Kit⁺CD34⁺CD16^{low}) and granulocytic (Lin⁻Sca-1⁻IL7R⁻c-Kit⁺CD34⁺CD16^{high}) progenitors in the BM, without changes in erythroid progenitors (Lin⁻Sca-1⁻IL7R⁻c-Kit⁺CD34⁻CD16^{low}). Although total neutrophil counts were unaffected, their phenotype was altered, with increased CXCR2 and CXCR4 expression in the BM. Functionally, neutrophils showed increased migratory capacity and oxidative burst *in vitro*. Moreover, bone marrow-derived macrophages (BMDMs) from these offspring exhibited skewed M1/M2 polarization and impaired phagocytic and efferocytic abilities. Following an LPS challenge (10 mg/kg, i.p.), adult mice born to neutrophil-depleted mothers exhibited an exacerbated innate immune response, with elevated circulating neutrophils and increased bronchoalveolar lavage cellularity at 6 and 24 h. At 24 h, we detected higher CXCL1 levels, increased neutrophil apoptosis, and a predominance of M1-like alveolar macrophages, contrasting with the M2-like profile in controls. This was associated with reduced levels of the pro-resolving mediator annexin A1. Collectively, our findings demonstrate that maternal neutrophil depletion during early gestation disrupts embryonic development, increases the incidence of congenital anomalies, and programs long-term alterations in the immune system of the offspring. These results underscore a previously underappreciated role for maternal neutrophils in orchestrating both fetal development and the establishment of immune homeostasis. This study was supported by FAPESP 2022/11602-0

04.029 **Environmental enrichment alters FFA1 receptor-mediated responses in a mouse model of chronic pain.** Estrázulas M^{1,2}, Neculqueo GW^{1,2}, Farias ABS², Campos MM^{1,2} ¹PUCRS, Escola de Medicina, PPG Medicina e Ciências da Saúde, Brazil. ²PUCRS, Escola de Ciências da Saúde e da Vida, INTOX, Brazil

Introduction: Environmental enrichment (EE) increases brain-derived neurotrophic factor (BDNF) release, promoting resilience to pain (Falkowska et al., 2023), whereas FFA1 receptors modulate BDNF expression and impact neuroinflammatory pain (Xiao et al., 2021). We used the CFA model to induce inflammatory pain in mice to evaluate the effects of FFA1 receptor modulation under EE.

Methods: The local Animal Ethics Committee (CEUA-PUCRS11019) approved the protocols. C57BL/6J male mice were housed in standard (SE) or enriched (EE) conditions. SE consisted of a 19.5 cm×36.5 cm×16 cm cage. In EE, a 33.5 cm×36.5 cm×16 cm cage with the alternation of nesting cotton, PVC piping, nontoxic wooden toys, and cardboard paper was used. After 7 days of EE onset, animals were anesthetized and received CFA (50 µl; 1:1) in the right hind paw. Seven days later, they were treated with FFA1 receptor agonist GW9508 (8 and 2 mg/kg) or antagonist GW1100 (4 mg/kg) s.c. daily for another 7 days. Pain and paw edema were assessed on days 7, 14, and 21 after CFA, and samples were collected from the right hind paw and brain. **Results:** For mechanical sensitivity, GW9508 (8 mg/kg) displayed significant effects on day 21. The pain threshold was significantly lower when comparing the SE CFA group to the SE CFA+GW9508 group ($p=0.0028$) and the EE CFA group to the EE CFA+GW9508 group ($p=0.0205$). A significant effect for 2 mg/kg was observed on day 21 (SE CFA vs. SE CFA+GW9508 group; $p=0.0450$). The antagonist increased pain only in the SE group on day 14 ($p=0.0100$). Regarding thermal sensitivity, GW9508 (2 mg/kg) produced analgesic effects in the SE groups at 14 and 21 days ($p=0.0107$ and $p=0.0340$), as well as in the EE groups ($p=0.0069$ and $p<0.0001$), with a synergistic effect in the EE group on day 21 ($p=0.0048$). GW1100 affected only the EE group on day 21 ($p=0.0078$). No changes in edema formation were seen. CFA increased leukocyte counts in both the SE and EE groups ($p<0.0001$), an effect that was lessened in the SE group by GW9508 (8 mg/kg; $p=0.0450$) but enhanced by GW1100 ($p=0.0015$ vs. SE CFA). For BDNF cortical positive cells, there was a significant increase for GW9508 (8 mg/kg) vs. the SE control ($p=0.0006$) and the CFA ($p<0.0001$), and the EE control and EE CFA groups ($p<0.0001$). The 2 mg/kg dose also showed an increase in the SE group vs. CFA ($p=0.0437$) and the EE groups vs. control ($p=0.0003$) and CFA ($p=0.0011$). The antagonist showed no response in BDNF labeling. Cortical FFA1 positive cells significantly increased with GW9508 (8 mg/kg) compared to the SE control ($p=0.0058$) and the EE group (vs. control; $p<0.0001$, and CFA, $p=0.0006$). GW1100 elicited a decrease in the EE group (vs. control; $p=0.0261$, and vs. CFA, $p=0.0020$). Notably, the EE group alone showed a significant increase (SE control vs. EE control; $p=0.0311$); (SE CFA vs. EE CFA; $p=0.0351$); (SE GW9508 8 mg/kg vs. EE GW9508 8 mg/kg; $p<0.0001$). **Conclusions:** FFA1 receptor activation boosts BDNF-positive cells and synergizes with EE to enhance synaptic plasticity. EE may also modulate FFA1 receptor expression, supporting its positive effects on inflammatory pain. This research highlights the importance of EE in preclinical studies for analgesic drugs. **Keywords:** Experimental arthritis; FFA1 receptors; environmental enrichment; mice. **Financial support:** This study is supported by grants from CAPES (Financial Code 001) and CNPq (304205/2022-2).

04.030 Aging, Immunity and Metabolism: Divergent Effects of Rapamycin in Senescence-Prone and -Resistant Mice. Queiroz LAD¹, Barros RS¹, Assis JB², Pantoja KC¹, Bustia SX¹, Sá-Nunes A², Rodrigues SFP³, Turato WM⁴, Silva CCC⁵, Migliorini S¹, Martins JO¹ ¹FCF-USP, Dept. of Clinical and Toxicological Analyses, Lab. of Immunoendocrinology, Brazil; ²ICB-USP, Dept. of Immunology, Lab. of Experimental Immunology, Brazil; ³ICB-USP, Dept. of Pharmacology, Lab. of Vascular Nanopharmacology, Brazil; ⁴IQ-USP, Dept. of Biochemistry, Energy Metabolism Lab., Brazil; ⁵FCF-USP, Dept. of Clinical and Toxicological Analyses, Multi-user Preclinical Imaging Center, Brazil

Introduction: Aging is a multifactorial biological process characterized by progressive loss of physiological integrity and homeostasis. Among emerging pharmacological strategies, rapamycin (RAPA)—an mTOR inhibitor—has shown strong potential as an anti-aging agent. However, its impact on glucose metabolism and immune responses varies according to dosing and regimen. This study aimed to evaluate the effects of intermittent low-dose RAPA on metabolic and immunological parameters in Senescence-Accelerated Mouse Prone 8 (SAM-P8) and Senescence-Accelerated Mouse Resistant 1 (SAM-R1) strains. **Methods:** Twelve-week-old male SAM-P8 and SAM-R1 mice were treated orally with RAPA (0.78 µg/kg) or vehicle (water) every five days for six months, in accordance with animal ethics approval (CEUA no. 619). At the end of the treatment, glucose tolerance tests (GTT), insulin tolerance tests (ITT), and [¹⁸F] fluorodeoxyglucose (FDG) uptake were performed. After the intervention, liver, thymus, and spleen were collected, and we assessed the respiratory capacity of isolated liver mitochondria, the immunophenotype of spleen and thymus, as well as the lymphoproliferative and cytokine profiles of splenic cells. **Results:** In SAM-P8 mice, RAPA significantly reduced FDG uptake in the bladder and the frequency of splenic FoxP3⁺ regulatory T cells ($p<0.05$), while enhancing ATP production during state 3 respiration ($p<0.05$). In SAM-R1 mice, RAPA decreased thymic CD3⁺ T cell populations and splenic lymphoproliferative capacity ($p<0.05$), while increasing mitochondrial respiratory control ratio and ATP synthesis ($p<0.05$). Comparative analyses of vehicle-treated controls revealed that SAM-P8 mice exhibited greater glucose uptake in muscle, liver, heart, and bladder ($p<0.05$), lower T cell populations in spleen and thymus, altered CD4⁺/CD8⁺ ratios, elevated IL-2 and reduced IL-4 expression ($p<0.05$), and impaired insulin sensitivity, as indicated by a higher ITT area under the curve compared to SAM-R1 controls ($p<0.05$). **Conclusions:** Our findings confirm that intermittent low-dose RAPA retains its immunosuppressive effects and exerts strain-specific influences on metabolic and immune function. The drug enhances mitochondrial efficiency and energy metabolism, particularly in aged-prone SAM-P8 mice, while concurrently dampening immune activity. These results support the therapeutic potential of RAPA in age-related interventions, yet emphasize the critical need for individualized treatment strategies to optimize efficacy-safety balance in aging populations. Funding: FAPESP, CAPES, CNPq **Keywords:** SAM-P8, SAM-R1, RAPA, mTOR

04.031 **Evaluation of Different Estradiol Treatment Strategies in Lung Inflammation of Brain Death Donors After Lung Transplantation.** Wellichan G¹, Sousa PS, Vidal-dos-Santos M, Correia CJ¹, Breithaupt-Faloppa AC, Moreira LFP, Ricardo-da-Silva FY InCor-FM-USP Lab Cirurgia Cardiovascular e Fisiopatologia da Circulação, São Paulo, Brasil

Introduction: Transplantation is the last treatment option for patients with severe lung diseases. Most lung grafts are from brain dead donors, however brain death (BD) induced inflammation could determine transplant success. Female sex hormones are linked to sexual dimorphism in patients in BD, estradiol being considered a protection factor in the lung. Male patients have worse prognosis when lung grafts come from female donors. The aim of this study is to investigate the effects of different estradiol treatment strategies during BD, on transplanted lung grafts. **Methods:** Lung of brain dead (6h) female Wistar rats, were transplanted to male Wistar rats (4h) (ethic SDC 541321188), separated in 3 groups: F/M — lung of female donors transplanted to male recipients; FE2I/M — lungs of female donors were transplanted to male recipients, donors received E2 intermittent treatment (100µg bolus at 3, 4 and 5h after BD); and FE2C/M — lungs of female donors were transplanted to male recipients, donors received E2 continuous treatment (50µg/mL i.v. 2 mL/h starting 3 h after BD confirmation). Lung tissue was obtained for histopathology by score, and full blood count at 0, 3 and 6h after BD, and in the recipient at 0 and 4 h of reperfusion. Blood was sampled before BD (0h) and 6h after in the donors, and in the recipient at 0h and 4h of reperfusion, for blood gas analysis. **Results:** In histopathology of the donor's lung, FE2C/M ($p=0.0360$) and FE2I/M ($p=0.0853$) presented reduced leukocyte infiltrate compared to F/M. FE2C/M also had lower edema ($p=0.0169$) and haemorrhage ($p=0.0305$) compared to F/M group. In full blood count, F/M and FE2C/M increase total leukocytes after BD ($P_{ANOVA}=0.0417$) and after transplant ($P_{ANOVA}<0.0001$). All groups increase granulocytes count over time after BD ($P_{ANOVA}<0.0001$) and transplantation ($P_{ANOVA}=0.0001$). F/M ($p=0.0091$) and FE2C/M ($p=0.0088$) increased monocytes count over time of reperfusion. In blood gas analysis, FE2C/M group showed an increased pO_2 level compared to F/M group ($p=0.0409$) at the end of BD. It also had decreased levels of pCO_2 after transplantation ($p=0.0002$), while pCO_2 decreased over time in FE2I/M ($p=0.0059$) and FE2C/M group ($p=<0.0001$). Also, pCO_2 reduced in both the FE2I/M ($p=0.0254$), and the FE2C/M group ($p=0.0008$) compared to F/M group. **Conclusion:** E2 treatment improves gas exchange in the lungs, and reduces inflammation, by reducing leukocyte infiltrate, edema and haemorrhage. These results suggest E2 has potential positive effects in lung grafts and could lead to better organ quality. This study was financed by 2023/12247-2, 2021/13020-6, São Paulo Research Foundation (FAPESP).

04.032 **Methyl Gallate as a Modulator of Pro-Inflammatory Macrophage.** Rodrigues CS^{1,2}; Correa LB¹, Seito LN¹, Rosas EC¹ ¹FIOCRUZ, Lab Applied Pharmacology, Farmanguinhos, Rio de Janeiro, RJ, Brazil. ²IFRJ, Biological Sciences, Rio de Janeiro, RJ, Brazil

Introduction: Macrophages are highly plastic immune cells capable of adopting pro- (M1) or anti-inflammatory (M2) phenotypes in response to microenvironmental signals, a process known as polarization. While M1 macrophages produce iNOS, COX-2, and pro inflammatory cytokines (e.g., IL-6, IL-1 β), M2 macrophages express arginase and release IL-10 and IL-13. An imbalance favoring the M1 phenotype contributes to the progression of inflammatory diseases such as rheumatoid arthritis and lupus. Thus, modulating macrophage polarization has emerged as a promising therapeutic strategy. In this context, methyl gallate (MG), a plant-derived phenolic compound with antioxidant and reported anti-inflammatory properties, was investigated for its potential to influence macrophage polarization. Aim: This study aimed to evaluate the effect of MG on M1 polarization in bone marrow derived macrophages (BMDMs) from C57BL/6 mice. Methodology: Macrophages were polarized *in vitro* to the M1 phenotype using LPS and IFN- γ , or to the M2 phenotype using IL-4 and IL-13. MG was administered to M1 macrophages under both pre- and post-treatment conditions, with dexamethasone serving as a reference control. To assess the effect of MG on macrophage polarization, we evaluated the production of nitric oxide, TNF- α , IL-6, CCL-2, and CCL-5 using ELISA. Macrophage viability was also evaluated using the MTT assay. Additionally, MG effects upon enzymes and transcriptional factors were evaluated by western blotting. **Results:** Cell viability assays (MTT) performed on naïve macrophages (M0) confirmed that MG was non-cytotoxic at all tested concentrations. In M1-polarized macrophages, MG significantly reduced nitric oxide (NO) production and iNOS expression, along with decreased levels of IL-6, TNF- α , CCL-2, and CCL-5. COX-2 expression was also markedly downregulated following MG treatment. Notably, MG did not upregulate M2 associated markers such as arginase, STAT3, or STAT6, suggesting that its effects are primarily anti-inflammatory rather than indicative of macrophage repolarization. **Conclusion:** Collectively, these findings indicate that MG exerts inhibitory effects on M1 macrophages without promoting M2 polarization. Further studies are warranted to better understand its role in the phenotypic modulation of macrophages.

04.033 **Effectiveness of Nonsteroidal Anti-Inflammatory Drugs as First-Line Therapy in Pediatric Chronic Nonbacterial Osteomyelitis: A Systematic Review of Multicenter Cohorts.** Forte GV¹, Maraia ME^{2,3}, Lemes FS¹, Miguel CB^{1,3}, Rodrigues WF^{1,3} ¹Unifimes, Multidisciplinary Lab Scientific Evidence, Mineiros, GO, Brazil. ²UFTM, Biomedicine, Uberaba, MG, Brazil. ³UFTM, Postgraduate Course in Tropical Medicine and Infectious Diseases, Uberaba, MG, Brazil

Introduction: Chronic nonbacterial osteomyelitis (CNO), including its multifocal form (CRMO), is a rare autoinflammatory bone disorder that predominantly affects children and adolescents. Nonsteroidal anti-inflammatory drugs (NSAIDs) are widely adopted as first-line therapy, but treatment response is heterogeneous, and standardized guidelines remain lacking. **Objective:** To evaluate the effectiveness and response patterns to NSAIDs as initial therapy in pediatric CNO/CRMO across multiple international cohorts. **Methods:** This systematic review included cohort studies and case series without language or time restrictions. Comprehensive searches were conducted in six databases (PubMed, Embase, Cochrane, Scopus, SciELO, and Web of Science) and gray literature using MeSH terms. Eligible studies involved pediatric patients (≤ 18 years) treated initially with NSAIDs. Data were extracted to Excel and analyzed through narrative synthesis. **Results/Discussion:** Across eight studies encompassing more than 200 pediatric patients aged 3–17 years, NSAID monotherapy achieved complete or partial remission in approximately 50–75% of cases. The Indian cohort (Surendran et al., 2018) reported a 75% response, while the Belgian (Kaut et al., 2022) and multinational European-Indian cohort (Gamalero et al., 2020) showed success rates of 47% and 49.3%, respectively. However, in all cohorts, 30–50% of patients required escalation to second-line therapies. In the Turkish and Norwegian studies, persistent disease activity was observed despite NSAID use. Variation in NSAID type, dosage, and treatment duration may have influenced these outcomes and limits generalizability. **Conclusion:** NSAIDs are effective first-line agents for many pediatric CNO/CRMO cases, particularly in mild presentations. Nonetheless, a substantial proportion of patients require additional therapies, underscoring the importance of early identification of refractory cases and the need for clinical guidelines.

04.034 Effect of Gedunin on the Polarization of Differentiated Human Macrophages. Nascimento JAD^{1,3}, Guedes LS², Rosas EC³, Penido C^{3,4} Costa TEMM^{3,4} ¹UFRJ, Faculdade de Farmácia, Brazil, ²UERJ, Faculdade de Farmácia, Brazil, ³Farmanguinhos-Fiocruz, Lab Farmacologia Aplicada, Brazil, ⁴CDTS-Fiocruz, Brazil

Introduction: Macrophages are key cells of the innate immune system, playing a central role in both inflammation and its resolution. Due to their plasticity, macrophages can be activated into different phenotypes depending on environmental stimuli, including the pro-inflammatory (M1) and the pro-resolving (M2) types. M1 macrophages are activated by interferon-gamma (IFN- γ) and lipopolysaccharide (LPS) from the walls of Gram-negative bacteria and are characterized by the release of inflammatory mediators. In contrast, M2 macrophages, activated by interleukin (IL)-4 and IL-13, are involved in tissue repair and secrete anti-inflammatory, pro-resolving, and tissue-regenerative mediators such as IL-10, vascular endothelial growth factor (VEGF), and transforming growth factor (TGF)- β 1. Gedunin is a natural limonoid found in *Carapa guianensis* and *Azadirachta indica*, and has been described as an inhibitor of Heat Shock Protein 90 (HSP90), a chaperone involved in protein folding and stabilization. Previous studies from our group have shown that gedunin can modulate inflammation by reducing pro-inflammatory cytokines such as TNF- α and IL-6, increasing the expression of cytoprotective proteins such as heme oxygenase-1 (HO-1) and HSP70, and inducing IL-10 production by LPS-stimulated macrophages. **Aim:** In this study, we evaluated the effect of gedunin on the M2 polarization of THP-1-derived macrophages previously polarized toward the M1 phenotype. **Methods:** THP-1 cells were seeded in 24-well (5×10^5 cells/well) or 96-well plates (2×10^5 cells/well), differentiated with phorbol 12-myristate 13-acetate (PMA, 20 nM) for 24 h, and then stimulated with IFN- γ (20 ng/mL) and LPS (100 ng/mL) to induce M1 polarization. After 24 h, cells were treated with various concentrations of gedunin (5, 10, and 25 μ M) or dexamethasone (1 μ M) for an additional 24 hours. As a positive control for M2 polarization, THP-1 macrophages were stimulated with IL-4 (20 ng/mL) and IL-13 (20 ng/mL). Cell viability was assessed using the MTT assay. M2 phenotype-associated markers were evaluated by western blotting using specific antibodies against signal transducer and activator of transcription 6 (STAT6), phosphorylated STAT6 (pSTAT6), and arginase-1. Levels of IL-10, VEGF, and TGF- β 1 in the cell supernatants were evaluated by ELISA. **Results:** Gedunin treatment increased the production of IL-10, VEGF, and TGF- β 1 in the cell supernatants, particularly at concentrations of 10 and 25 μ M, although it did not affect M2-associated markers such as pSTAT6 or arginase-1. Notably, gedunin treatment did not induce cytotoxicity in either polarized or non-polarized THP-1 macrophages. **Conclusion:** Our findings confirm the immunomodulatory potential of gedunin, which acts by promoting a pro-resolving phenotype in macrophages previously polarized to the M1 state, through the induction of anti-inflammatory cytokine IL-10 and tissue repair mediators VEGF and TGF- β 1. **Financial support:** PIBITI/CNPq, CNPq, FAPERJ, Programa Inova Fiocruz, Farmanguinhos. **Ethics Committee Approval:** Not applicable. **Keywords:** gedunin; macrophage polarization; pro-resolving mediators, M2-macrophages.

04.035 **A Novel Sulfonylhydrazone Derivative of LASSBio-1632 as Potential Therapeutic for Inflammatory Pulmonary Diseases.** Ota RSN¹, Oliveira MS², Souza LM¹, Gomes HS¹, Ferreira TPT¹, Carvalho VF¹, Lima LM², Martins MA¹, Silva PMR¹, ¹Fiocruz-IOC, Lab of Inflammation, Rio de Janeiro, Brazil, ²UFRJ, LASSBio, Rio de Janeiro, Brazil.

Introduction: Phosphodiesterase (PDE) 4 has emerged as a promising therapeutic target for inflammatory lung diseases, particularly chronic obstructive pulmonary disease (COPD), a condition whose primary clinical manifestation is emphysema, characterized by the progressive destruction of alveoli due to a chronic inflammatory process. The sulfonylhydrazone derivative LASSBio-1632, reported for its PDE4 inhibition, served as the structural basis for the development of a novel and more potent analogue. The present study focuses on the pharmacological evaluation of this novel compound as a PDE4 inhibitor using both *in vitro* and *in vivo* approaches. **Methods:** Roflumilast was used as the reference compound to assess the therapeutic potential of this novel substance in both *in vitro* and *in vivo* models. The PDE4 inhibitory activity was evaluated using the IMAP® PDE FP (Fluorescence Polarization) assay. Furthermore, the analogue's ability to inhibit LPS-stimulated TNF- α production was assessed in alveolar macrophages (AMJ2C11), *in vitro*. For the *in vivo* study, acute lung injury (ALI) was induced in A/J mice by oropharyngeal instillation of LPS (10 μ g/40 μ L). Animals were pre-treated orally with either the analogue or roflumilast (10 μ mol/kg). The following parameters were analyzed: i) lung function and airway hyperresponsiveness via non-invasive whole-body plethysmography; ii) leukocyte infiltration in bronchoalveolar lavage fluid (BAL) and total protein concentration; iii) neutrophil infiltration by myeloperoxidase (MPO) of the lung tissue. All procedures were approved by the Animal Ethics Committee (CEUA L002/2020A6). **Results:** As the reference control, roflumilast validated the PDE4 inhibition assay, exhibiting IC₅₀ values consistent with literature reports (0.92 nM for PDE4A1A, 1.23 nM for PDE4B1, 5.42 nM for PDE4C1, and 0.26 nM for PDE4D3). The novel analogue displayed potent inhibitory activity, with selectivity toward PDE4A1A and PDE4D3 (IC₅₀ = 140 nM and 345 nM, respectively), while maintaining activity against PDE4B1 and PDE4C1 (IC₅₀ = 1.24 μ M and 2.0 μ M, respectively). Moreover, the compound was shown to attenuate LPS-induced activation of alveolar macrophages by demonstrating a significant inhibition of TNF- α production. In the *in vivo* ALI model, while the analogue did not markedly improve lung function parameters or reduce MPO activity, the treatment with the analogue significantly reduced total leukocyte counts and total protein levels in BAL, demonstrating anti-inflammatory effects and protection against LPS-induced alveolar damage. **Conclusion:** This new sulfonylhydrazone analogue demonstrated a promising pharmacological profile as a selective PDE4 inhibitor, exhibiting both anti-inflammatory and lung-protective effects, which suggests potential therapeutic applications for inflammatory pulmonary diseases. **Financial support:** FIOCRUZ, FAPERJ, CAPES e CNPq (Brazil). **Note:** Due to confidentiality agreements and intellectual property protection requirements, the chemical name and the structure of the novel analogue are withheld.

04.036 Melatonin Attenuates Vascular Hyporeactivity Induced by TLR4 and P2X7 Receptor

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Introduction: Sepsis is a life-threatening condition characterized by organ dysfunction due to a dysregulated immune response, often resulting in vascular hyporeactivity mediated by LPS/TLR4, and with limited treatment options (Singer *et al.*, 2016, JAMA, 315(8): 801; Gabarin *et al.*, 2021, J Innate Immun. 13: 323). The serum levels of ATP, an agonist of purinergic receptors, are elevated in septic patients (Leite *et al.*, Clinical and Experimental Medicine, 23: 3651). Emerging evidence also indicates that internalized LPS can interact with the P2X7 receptor (P2X7R), an ATP-gated ion channel expressed in endothelial cells (Gabarin *et al.*, 2021, J Innate Immun. (2021) 13: 323; Denlinger *et al.*, 2001, J Immunol. 167 (4): 1871). Melatonin is a hormone with pleiotropic effects and has been investigated in clinical trials due to its anti-inflammatory effects (Tamura *et al.*, 2009, J Pineal Res. 46:268; El-Gendy *et al.*, 2018, J Matern Fetal Neonatal Med. 31(17):2299). Thus, this study aimed to investigate the effects of TLR4 and P2X7R signaling on aortic contraction *in vitro* and to evaluate the potential protective role of melatonin (Mel). **Methods:** Male Wistar rats (2–3 months old, 250–350 g) were euthanized via intraperitoneal injection of ketamine/xylazine (CEUA 001/24). Thoracic aortas were excised and cut into 3 mm rings, which were mounted in organ baths for isometric tension recording. Vascular contractility was assessed by inducing contraction with 100 mM KCl, followed by 1 μ M phenylephrine (PHE). Endothelium-dependent relaxation was evaluated with 3 μ M acetylcholine (ACh) added at the contraction plateau. Rings that exhibited $\geq 70\%$ relaxation were classified as endothelium-intact (E+). Subsequent contractile responses to cumulative PHE (1 nM – 10 μ M) were measured in the absence or presence of the following treatments: melatonin (Mel; 10 μ M, 1 h), LPS (1 μ g/mL, 2 h), Benzoyl ATP (BzATP; 10 μ M, 30 min), LPS + BzATP, or LPS + BzATP + Mel. Maximal contractile response ($E_{\max}$, in mN) and the half-maximal effective concentration (EC_{50}) of PHE were determined using nonlinear regression analysis. Data were analyzed using Student's t-test, with significance set at $p < 0.05$. **Results:** In the E+ group, we did not observe alteration of EC_{50} values in the different groups. Also, neither Mel nor BzATP groups showed differences in $E_{\max}$ values of PHE compared to the control group. LPS reduced the $E_{\max}$ values from 9.2 ± 1.5 to 7.5 ± 0.7 mN. Moreover, the combination of LPS and BzATP reduced further the $E_{\max}$ (5.07 ± 0.7 mN; $n=8$) as compared to LPS. However, in the presence of melatonin the hyporeactivity induced by LPS+BzATP was not observed (9.9 ± 1.5 mN; $n=5$). We did not observe hyporeactivity in rings without endothelium (data not shown), suggesting TLR4/P2X7R signaling depends on intact endothelial cells and that melatonin mitigates the negative effects of TLR4 and P2X7R coactivation on vascular contractility. **Conclusion:** These findings suggest that co-activation of TLR4 and P2X7 receptors reduces vascular reactivity to phenylephrine, and this effect was attenuated by melatonin, suggesting a potential beneficial effect of melatonin in sepsis. **Acknowledgments:** CNPq, CAPES, FAPERJ.

04.037 **Sexual Dimorphism in a Model of Systemic Inflammation of Viral Origin.** Schneider VMM, Costa RA, Borges AP, Alencar ST, Santos MO, Zamprônio AR UFPR, Inflammation and Fever Lab, Dept Pharmacology

Introduction: Inflammatory diseases exhibit sex-related differences both in terms of prevalence and underlying pathophysiological mechanisms—a pattern also observed during the COVID-19 pandemic, in which men presented with more severe symptoms and higher mortality rates. Most of the studies evaluate sex differences related to inflammatory diseases using lipopolysaccharide (LPS), which is a model for bacterial infection. Previous studies (Costa et al., J. Neuroimmunol. 394:578427,2024) showed that male rats display a more intense LPS-induced febrile response compared to females, suggesting differences in central mechanisms, particularly within the hypothalamus. Males exhibit reduced hypothalamic serotonin (5-HT) levels, whereas females demonstrate an increase in this neurotransmitter after LPS injection, suggesting that they differentially regulate descending inhibitory pathways. Despite these differences, LPS induces similar systemic hyperalgesia and hypolocomotion in males and females. The present study aimed to investigate the systemic hyperalgesia, hypolocomotion, and alterations in hypothalamic 5-HT release in a model of systemic viral infection induced by polyinosinic-polycytidylic acid (Poly IC) at pyrogenic doses in male and female rats. **Methods:** For the experiments, 90-day-old male and female Wistar rats were used and received intraperitoneal (i.p.) injections of Poly I:C (300 or 600 µg/kg). Hyperalgesia was assessed using the electronic Von Frey test at 1, 3, and 5 hours after Poly I:C administration. At the end of this period, the animals were subjected to the open field test to evaluate locomotor activity. Subsequently, the hypothalamus was collected for quantification of serotonin (5-HT) and its metabolite, 5-hydroxyindoleacetic acid (5-HIAA), using high-performance liquid chromatography (HPLC). This project was approved by the Animal Research Ethics Committee of the Federal University of Paraná (Protocol No. 1658/2025). **Results:** Intraperitoneal injection of poly I:C at 300 or 600 µg/kg induced similar hyperalgesia in both sexes. Male rats exhibited a greater reduction in mechanical threshold at the 600 µg/kg dose at 1 h and 5 h compared to the 300 µg/kg dose. Female rats showed a reduction in mechanical threshold at the 300 µg/kg dose starting at 3 h and persisting until 5 h. Hypolocomotion (reduced crossing, speed and distance travelled in the open field test) was observed only in female rats. Hypothalamic levels of 5-HT was reduced and the 5-HIAA/5HT turnover was significantly increased in males. No changes were observed in hypothalamic 5-HT in females at the lower dose. **Conclusion:** These results showed that at pyrogenic doses, the injection of Poly I:C induces systemic hyperalgesia in males and females. However, hypolocomotion seems to occur only in females. Conversely, only males showed changes in 5-HT hypothalamic levels at these doses of Poly I:C. Altogether, these results suggest that there are sex differences in viral responses between males and females rats. **Financial support:** CNPq and UFPR.

04.038 **Bornyl Acetate Enhances Phagocytosis and M2-Associated Gene Expression in Macrophages.** Silva GRS, Barros ABB, Ferreira EGA, Fidelix MSP, Santana JS, SilvaJP, Reis MDS Barreto E UFAL, Lab Biologia Celular, UFAL, Brasil

Introduction: Macrophages are key regulators of tissue homeostasis and immune responses, particularly during inflammation and infection. Depending on their phenotype, macrophages can exacerbate inflammation (M1) or resolve it (M2), with the latter involved in tissue repair and regeneration. These phenotypes represent opposite ends of a dynamic activation spectrum influenced by the tissue microenvironment. Modulating macrophage polarization may be a promising strategy for treating immunoinflammatory conditions. In particular, M2 macrophages have shown therapeutic potential in regenerative medicine. Natural compounds, including plant-derived monoterpenes, have been reported to influence macrophage polarization. Bornyl acetate (BA), a bicyclic monoterpene found in medicinal plants, possesses anti-inflammatory and antioxidant properties, but its effects on macrophage function remain unclear. This study aimed to investigate the impact of BA on macrophage polarization and phagocytosis, as well as its effects on the expression of antioxidant and M2-associated genes. **Methods:** The murine macrophage cell line J774.1 was cultured in DMEM supplemented with 10% FBS and maintained at 37°C, 5% CO₂. BA (1–500 µM) cytotoxicity was assessed via MTT assay. Phagocytosis was evaluated after 24 h BA treatment using zymosan particles (1:10 ratio), followed by Giemsa staining. Phagocytic parameters—mean number of particles per cell (MNP), percentage of phagocytic cells (PP), and phagocytic index (PI = MNP × PP)—were quantified. Reactive oxygen species (ROS) were measured by the NBT reduction assay. Expression of SOD1, Nrf2 (antioxidant genes), and Arg-1, TGF-β1 (M2 markers) was analyzed by qPCR. Data were analyzed using one-way ANOVA and Tukey's test (P<0.05). **Results:** BA showed no cytotoxic effects at any tested concentration. BA treatment (100 µM) significantly increased all phagocytosis parameters (MNP, PP, and PI) by approximately 1.6-fold. Zymosan-induced ROS production increased at 27% compared to control, and BA at the highest tested concentration (100 µM) inhibited this effect by 75%. BA treatment also enhanced the expression of antioxidant genes: Nrf2 and SOD1 mRNA levels increased by 1.8-fold and 20-fold, respectively, while GPX expression decreased by 1.5-fold. Moreover, BA-treated macrophages exhibited significant upregulation of M2 phenotype markers: Arg-1 by 25-fold and TGF-β1 by 3-fold. **Conclusion:** Our findings demonstrate that BA modulate macrophage function by promoting polarization toward the M2 phenotype, enhancing antioxidant defense, and increasing the expression of M2-associated markers. These findings highlight BA's potential as a therapeutic agent in immunoinflammatory and regenerative medicine contexts. **Financial Support:** CNPq, CAPES, BioproFar-BA, FAPEAL.

04.039 **Neutrophils Polarization as a New Approach for Cancer Treatment.** Amorim NS¹, Amorim CS¹, Valença LS¹, Almeida PP², Moraes J¹ ¹UFRJ, Lab Biologia Redox, RJ; ²UERJ, Depto de Biologia Celular

Introduction AND OBJECTIVES: Cancer is a complex disease characterized by uncontrolled growth of abnormal cells invading surrounding tissues and spreading throughout the body. It remains one of the leading causes of death worldwide. In 2011, inflammation was recognized as a critical hallmark of cancer, as immune cell infiltration supports the tumor microenvironment. Neutrophils, first observed in tumors by Virchow in 1863, can be recruited by the tumor microenvironment and contribute to tumor progression. These tumor-associated neutrophils (TANs) exhibit functional plasticity and can polarize into two distinct phenotypes: N1 (anti-tumor) and N2 (pro-tumor). N1 neutrophils show enhanced cytotoxic activity against tumor cells, with elevated pro-inflammatory mediators such as CD95 (FAS), TNF- α , ICAM-1, and NOS2. They also show increased NOX activity, leading to the generation of cytotoxic reactive oxygen species (ROS), including peroxynitrite (ONOO⁻) and hydrogen peroxide (H₂O₂). In contrast, N2 neutrophils exhibit an anti-inflammatory and tumor-promoting phenotype that supports tumor growth, invasion, and metastasis. Our research group was the first to successfully polarize human neutrophils toward an N2-like phenotype and investigate their role in promoting tumor cell proliferation. Over the years, we identified pharmacological strategies capable of reducing N2-like neutrophils. Conversely, inducing polarization toward a N1-like phenotype may provide insights into how these cells can effectively kill tumor cells under controlled conditions. Therefore, the primary objective of this study was to polarize human neutrophils *in vitro* toward the N1-like phenotype and investigate their interaction with three tumor cell lines: MDA-MB-231, MV3, and U87-MG. **Methods:** Neutrophils were isolated from the peripheral blood of healthy donors using a Ficoll gradient and stimulated with LPS and IFN γ for 3 hours at 37 °C in 5% CO₂. Following phenotypic characterization and assessment of tumor cell viability after exposure to these neutrophils, we evaluated the redox state of N1-like neutrophils co-cultured with tumor cells. This included measurements of total ROS (DCF probe), nitric oxide (NO, DAF probe), and peroxynitrite (ONOO⁻, APF probe). Additionally, we analyzed lipid peroxidation in tumor cells treated with N1-like neutrophils using the TBARS assay and assessed the release of extracellular vesicles (EVs) by neutrophils using NanoSight analysis. **Results:** N1-like neutrophils significantly increased the production of total ROS, NO, and ONOO⁻ when co-cultured with all three tumor cell lines, compared to naïve neutrophils. Lipid peroxidation, as indicated by malondialdehyde levels, was also elevated in tumor cells after 24 hours of co-culture with N1-like neutrophils. Furthermore, these neutrophils released extracellular vesicles (N1-EVs) that contained higher protein content compared to vesicles from naïve neutrophils (N0-EVs). **Conclusion:** Our findings suggest that N1-like neutrophils represent a newly characterized polarization state with potent anti-tumor activity against MDA-MB-231, MV3, and U87-MG cell lines *in vitro*. This cytotoxicity appears to be mediated through lipid peroxidation involving diverse ROS. Moreover, the N1-EVs may serve as a promising therapeutic tool in cancer treatment strategies. **Financial Support:** FAPERJ, Capes, and CNPq.

04.040 **Estrogen Modulation of Febrile Response via GPER Receptors in Systemic Inflammation in rats.** Costa RA, Santos MO, Alencar ST, Schneider VMM, Borges AP, Zampronio AR. UFPR, Inflammation and Fever Lab, Departament of Pharmacology

Introduction: Systemic inflammatory diseases manifest symptoms such as fever, hyperalgesia, and loss of appetite, collectively known as sickness syndrome. The hypothalamus is an important area in the central nervous system for the control and integration of these responses through central mediators such as prostaglandins (PGE) and endothelin-1 (ET-1) and in the case of fever through EP3 and ETB receptor activation, respectively. ET-1 induces fever independently of PGE synthesis, but its action depends on female sex hormones, such as estrogen (E2). ET-1, through ETB receptors, increases the levels of serotonin (5-HT) in the hypothalamus of female rats after LPS injection. However, ovariectomized (OVX) female rats do not develop fever after ET-1 injection, differently from males and cycling female rats, suggesting that E2 influences the functionality of ETB receptors. Studies show that G protein-coupled estrogen receptors (GPER) may affect the functionality of endothelin receptors in other systems, such as the kidney. Thus, the objective of this study was to evaluate the role of GPER in the febrile response induced by LPS and its importance in hormonal regulation of fever in females with a functional estrous cycle and OVX. **Methods:** For this study, 90-day-old female Wistar rats were used. They underwent OVX, and experiments were conducted 3 weeks post-surgery to ensure complete hormone reduction. To assess the febrile response, remote temperature recorders were implanted in the animal's peritoneal cavity. For intracerebroventricular (i.c.v.) drug administration, a stereotaxic surgery was performed to implant a cannula. After 7 days, animals received lipopolysaccharide (LPS 50 and 100 µg/kg, i.p.), E2 (10 µg/kg, s.c., subchronic administration), and the GPER antagonist G36 (20 µg/kg, i.c.v.) according to the experimental design. Body temperature was recorded for 5 hours. At the end of this period, animals were placed in an open field to assess locomotion. Subsequently, the hypothalamus was collected for quantification of 5-HT and its metabolite 5-hydroxyindoleacetic acid (5-HIAA) by HPLC. This project was approved by the Animal Research Ethics Committee of the UFPR (Protocol No. 1444/22). **Results:** The i.p. injection of LPS at 100 µg/kg induced a similar and maximum febrile response (temperature change of 1.5°C) and hypolocomotion, reducing the number of crossings, velocity, and distance traveled in the open field test, in sham and OVX females. E2 replacement in OVX females induced a reduction in the febrile response (by 1°C). LPS at the same dose increased 5-HT levels and its metabolite 5-HIAA in cycling females, while OVX females showed a reduction of these neurotransmitters. The G-36 estrogen receptor antagonist did not alter the febrile response at 100 µg/kg of LPS. However, at 50 µg/kg, females that previously exhibited a submaximum febrile response (temperature change of 0.5°C) reached the maximum response (up to 1.5°C). **Conclusion:** These results indicate that E2 modulates the febrile response during systemic inflammation, which is linked to the release of 5-HT in the hypothalamus. At least in part, this action occurs via GPER receptors, as G-36 was able to increase the febrile response at the lower LPS dose, where females exhibited a submaximum response. **Financial support:** CNPq and UFPR.

04.041 Zika Virus (ZIKV) and Dengue Virus (DENV) NS1 Proteins Impair Blood-Epididymis Barrier Integrity in Mice. Camargo IA¹, Martini PV¹, Andrade AD¹, Kushima H¹, Amarilla AA², Modhiran N^{3,4}, Watterson D^{3,4}, Costa SM⁵, Alves AMB⁵, Silva, EJR¹ ¹UNESP, Institute of Biosciences of Botucatu, PPG Biomolecular and Pharmacological Sciences, Brazil; ²Commonwealth Scientific and Industrial Research Organization, Australian Centre for Disease Preparedness, Australia. ³University of Queensland, School of Chemistry and Molecular Biosciences, Australia; ⁴University of Queensland, Australian Infectious Diseases Research Centre, Australia; ⁵Fiocruz, PPG Cellular and Molecular Biology, Brazil

Introduction: Flaviviruses, such as ZIKV and DENV, cause severe human diseases, often accompanied by vascular, neurological, and reproductive outcomes. The nonstructural protein 1 (NS1) from ZIKV (NS1-ZIKV) and DENV2 (NS1-DENV2) plays a crucial role in these viral infections pathogenesis. We have recently demonstrated that NS1-ZIKV and NS1-DENV2 trigger acute inflammatory reactions in the epididymis, an organ crucial for sperm maturation and male fertility, in part via Toll-like receptor 4 activation. As an immune-privileged organ, the blood-epididymis barrier (BEB), formed by tight junctions between epididymal epithelial cells, plays a pivotal role in maintaining a specific luminal environment promoting sperm maturation and protection from autoimmunity. Here, we tested the hypothesis that NS1-induced epididymal inflammation impairs the integrity of the BEB. **Methods:** The epididymides from C57BL/6 mice (90-120 days; CEUA nº2132170322) were dissected, and initial segment (IS) and cauda (CD) regions collected. The IS and CD were then incubated in organotypic cultures (34°C, 5%/95% CO₂/air) in the absence (control) or presence of (1) ultrapure LPS from *E. coli* (50 ng/ml; positive control), or (2) NS1-ZIKV or NS1-DENV2 (20 µg/ml) for 3 hrs. The tissues were processed for RT-qPCR assays to evaluate transcript levels of BEB components (*Gja1*, *Cldn1*, *Cldn3*, *Cldn8*, and *Cdh1*). Culture medium was collected for Multiplex immunoassay to assess cytokine/chemokine levels (IL1B, IL6, IL10, IFNG, TNF, CCL2, CXCL2, CCL3, and CCL4). The results were analyzed using ANOVA, followed by the Bonferroni test ($p < 0.05$ was considered significant). **Results:** LPS decreased the expression of *Cldn1* and *Cdh1* (<2.0-fold) in IS and *Gja1*, *Cldn3*, and *Cldn8* (<2.5-, <1.9- and <1.8, respectively) in CD compared to the saline control. Notably, NS1-ZIKV and NS1-DENV decreased the transcript levels of all BEB components evaluated, both in the IS (*Gja1*: <3.9-fold; *Cldn1*: <5.7- and 5.4-fold; *Cldn3*: <3.0- and 3.4-fold; *Cldn8*: <3.5- and 2.8-fold; and *Cdh1*: <5.2- and <6.4-fold) and CD (*Gja1*: <4.1- and <3.9-fold; *Cldn1*: <3.1- and 2.9-fold; *Cldn3*: <3.1- and 1.2-fold; *Cldn8*: <6.1- and 3.0-fold; and *Cdh1*: <6.2- and <3.5-fold). These changes were associated with increased levels of CXCL3 and CCL3 above detection limits after incubation with LPS, NS1-ZIKV, and NS1-DENV2 both in the IS and CD. In addition, variable increases (at least >1.5-fold) were observed for other cytokines and chemokines evaluated. **Conclusion:** Our findings suggest that NS1-ZIKV and NS1-DENV may impair the molecular integrity of the BEB by affecting the expression of its components. This disruption could, at least in part, be secondary to the localized cytokine and chemokine responses. NS1-induced damage to the BEB emerges as a novel mechanism by which ZIKV and DENV infections may impair male fertility. **Financial support:** FAPESP, CAPES, CNPq.

04.042 **Modulatory Effects of Cholecalciferol on Inflammatory Cytokines and Autophagic Flux Proteins in RAW 264.7 Cells Stimulated with Lipopolysaccharide.** Freire ALP, Bella LM, Migliorini S, Martins JO FCF-USP, Dept. of Clinical and Toxicological Analyses, Lab. of Immunoendocrinology, Brazil

Introduction: Cholecalciferol, known as vitamin D, functions as a secosteroid hormone with immunomodulatory properties. Its biological activity depends on its conversion to the active form, calcitriol ($1,25(\text{OH})_2\text{D}_3$), mediated by the enzyme CYP27B1 (1α -hydroxylase). Vitamin D exerts regulatory effects on immune system cells, such as macrophages, as well as on cell lines like RAW 264.7, which is widely used in studies of inflammatory responses. Additionally, vitamin D influences autophagy, an essential intracellular degradation process that plays a key role in maintaining homeostasis and immunity. **Material and Methods:** RAW 264.7 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS). After reaching approximately 80% confluence, the cells were treated with lipopolysaccharide (LPS) (100 ng/mL) and cholecalciferol (1, 10, and 100 nM) for different time periods, and divided into four experimental groups: control, cholecalciferol, LPS, and LPS + cholecalciferol. Subsequently, supernatants were collected for cytokine quantification by ELISA and nitric oxide measurement using the Griess reaction. Additionally, total protein was extracted for analysis of CYP27B1, iNOS, beclin-1, LC3B II, p62 e mTOR by Western blot. **Results:** Treatment with cholecalciferol (100 nM) and stimulation with LPS (100 ng/mL) led to an increase in CYP27B1 expression ($p < 0.05$) in RAW 264.7 cells. While LPS induced the expression of inducible nitric oxide synthase (iNOS) ($p < 0.001$), the release of cytokines (TNF- α , IL-6, IL-1 β , IL-10) ($p < 0.001$), nitric oxide production ($p < 0.001$), and increased expression of the autophagy-related proteins beclin-1 ($p < 0.03$) and p62 ($p < 0.0013$), treatment with cholecalciferol significantly reduced iNOS expression ($p < 0.001$), cytokine release ($p < 0.001$), and nitric oxide production ($p < 0.001$), but did not alter the expression of beclin-1 or p62. **Conclusions:** Under the experimental conditions, cholecalciferol did not alter the expression of proteins related to autophagic flux in RAW 264.7 cells stimulated with LPS. However, immunomodulatory effects induced by cholecalciferol were observed. **Financing:** FAPESP; CAPES; CNPq. **Keywords:** Cholecalciferol; RAW 264.7 cells; Lipopolysaccharide; Immune system; Autophagy.

04.043 **Effects of *Tityus paraguayensis* and *Tityus confluens* venoms on the *in vitro* macrophages cell response.** Fernandes MML¹, Bonfá IS¹, Lencina JS¹, Lossavaro PKMB¹, Lencina DS¹, Toffoli-Kadri MC¹, Lucena MN², Silva-Filho SE¹ 1UFMS, Pharmaceutical Sciences, Food and Nutrition College, Brazil, ²UFMS, Biosciences Institute, Brazil

Introduction: The *Tityus* scorpions genus is widely distributed throughout Brazil due to its remarkable ecological plasticity and adaptability. These arachnids thrive even in environments altered by human activity, such as urban areas, where they can easily find shelter and food. In the Mato Grosso do Sul state, *Tityus paraguayensis* and *Tityus confluens* are the two main species involved in scorpion envenomation, causing both urban and rural accidents. Their venoms are complex mixtures containing enzymes such as metalloproteases, phospholipases, and hyaluronidases, which contribute significantly to the pathophysiology of envenomation. In recent years, research has shown that venoms and toxins from various scorpion species may act as modulators or activators of the immune response. This study aimed to investigate the effects of *T. paraguayensis* and *T. confluens* venoms on the cellular response of murine macrophages.

Methods: The experimental protocol was approved by the Ethics Committee in the Use of Animals of UFMS, under registration No. 1.308/2024. The peritoneal macrophages were obtained from C57BL/6 male mice, after intraperitoneal 3% thioglycolate solution injection, followed by cells harvesting 96 hours later. For this, the mice peritoneal cavity was washed and the exsudate was centrifuged, and the obtained cells suspended in RPMI-1640 medium at a concentration of 2×10^6 cells/mL. In all tests, the cells were stimulated with *Tityus paraguayensis* venom (PTP) and *Tityus confluens* venom (PTC), at concentrations of 0.1, 1 or 10 $\mu\text{g/mL}$. Nitric oxide (NO) production was assessed by nitrite (NO_2^-) quantification using Griess reagent after 48 h. Hydrogen peroxide (H_2O_2) was measured by phenol red oxidation after 1 h. Phagocytosis was evaluated using zymosan particles opsonized with mouse serum, followed by incubation with macrophages and exposure to PTP or PT, and macrophages that internalized at least one zymosan particle were counted under light microscopy. In all test, as a control, cells were maintained without stimulus. **Results:** Macrophages incubated with PTP, at concentrations of 0.1, 1, and 10 $\mu\text{g/mL}$ produced 6.6 ± 1.0 , 3.9 ± 0.3 , and 4.0 ± 0.47 μM of nitrite, respectively. PTC induced 3.1 ± 0.4 , 5.0 ± 0.24 , and 4.5 ± 0.3 μM of nitrite production. The unstimulated cells presented 1.0 ± 0.3 μM of nitrite production. PTP and PTC, at concentrations of 0.1, 1, and 10 $\mu\text{g/mL}$, induced H_2O_2 increase levels. PTP induced 47.2 ± 4.6 , 51.7 ± 7.1 and 41.7 ± 3.7 μM , and PTC 23.6 ± 2.3 , 36.8 ± 3.8 , and 25.7 ± 2.1 μM of H_2O_2 production. The unstimulated cells presented 2.7 ± 1.2 μM of H_2O_2 production. In the phagocytosis assay, the PTP treatment, at concentrations of 0.1, 1, and 10 $\mu\text{g/mL}$, stimulated the phagocytic activity in 49.8 ± 6.3 , 46.4 ± 5.5 , and 51.7 ± 5.9 , respectively, compared to control group (26.33 ± 3.3). The PTC treatment, at concentrations of 0.1, 1, and 10 $\mu\text{g/mL}$, induced the phagocity activity in 66.3 ± 3.5 , 60.8 ± 1.2 , and 56.1 ± 2.4 , respectively, compared to control group. **Conclusion:** Our data demonstrated that the PTP and PTC increase the macrophage activity, suggesting their potential for future applications in biotechnological and pharmacological research. **Financial support:** UFMS e CAPS

04.044 Evaluation of the Antimicrobial Effect of Anacardic Acid on Multidrug-Resistant *Staphylococcus epidermidis* and its Immunomodulatory Action on Macrophages. Lopes EC, Honório MS, Almeida JFS, Sforcin JM. UNESP, Institute of Biosciences, Brazil

Stingless bees produce geopropolis (GEO), which has several bioactive compounds, such as anacardic acid (AA), which has different biological activities, including antimicrobial and immunomodulatory activities. Immunosuppressed patients are at greater risk of nosocomial infections, which can be caused by commensal bacteria such as *Staphylococcus epidermidis*, which has become a public health problem due to its ability to form biofilms on medical devices and its resistance to antimicrobials, causing therapeutic failure. In this study, the antibacterial and immunomodulatory action of AA was evaluated, with the aim of investigating its action on macrophages treated or not with dexamethasone (DEX). The minimum inhibitory concentration (MIC) and bactericidal concentration (MBC) of AA and conventional antimicrobials (ceftaroline and gentamicin) were evaluated. The effect of the treatment was also evaluated in terms of the bacteria's ability to reduce biofilm formation. The immunomodulatory action of AA was analyzed in THP-1 cells differentiated into macrophages suppressed or not with DEX, assessing cell viability, production of eicosanoids (PGE2 and LTB4), cytokines (TNF- α , IL-1 β , IL-6 and IL-10) and bactericidal activity against *S. epidermidis* (ATCC 35983). Compared to conventional antimicrobials, AA had a higher minimum inhibitory and bactericidal concentration, with MIC₉₀ of 31.25 $\mu\text{g/mL}$ and MBC ranging from 62.5 to $>250 \mu\text{g/mL}$. AA was also effective in inhibiting bacterial biofilm, with a maximum inhibition of 45.41%. The treatment did not affect the cell viability of THP-1 macrophages. In the production of eicosanoids, there was no statistical difference in the production of PGE2, but it did inhibit the production of LTB4, with an average of 1.36 ng/mL. AA increased the bactericidal capacity of macrophages suppressed or not with dexamethasone and inhibited the production of IL-10. It can be concluded that AA has antibacterial and immunomodulatory action and may be an effective adjuvant in the treatment of *S. epidermidis* infections.

04.045 **Targeting PAR-1 to Attenuate Lung Fibrosis Induced by Silica Particles.** Souza LM¹, Ferreira TP¹, Ferreira GG¹, Pezzella-Ferreira GN¹, Villar LS¹, Cotias AC¹, Arantes AC¹, Lagente V², Martins MA¹, Silva PMR¹ ¹Lab Inflammation, Oswaldo Cruz Institute/FIOCRUZ, Rio de Janeiro, Brazil; ²University of Rennes I, Faculty of Pharmacy, Rennes, France

Introduction: Silicosis is an occupational lung disease caused by the inhalation of silica particles and is characterized by chronic inflammation, granuloma formation, and progressive fibrosis. Protease-activated receptor 1 (PAR-1) has been implicated in lung epithelial activation and the development of fibrosis in several organs, including the lungs. This study aimed to investigate the role of PAR-1 in silica-induced lung inflammation and fibrosis using two different biological systems.

Methods: Human lung epithelial cells (A549 lineage) were stimulated with silica particles (300 µg/mL) or with a PAR-1 agonist peptide (S1820, 30 µg/mL). Cells were pre-treated with the PAR-1 antagonist Vorapaxar (1 and 100 µM) 1h prior to stimulation, and supernatants were collected 24 h later for analysis. For the *in vivo* model, male Swiss-Webster mice were instilled with silica particles (10 mg, i.t.), while control animals received saline only. Vorapaxar was administered subcutaneously (1.5 and 5 mg/kg), once daily, from days 21 to 27 post-silica exposure. Analyses were conducted 24h after the final treatment. The following parameters were evaluated: i) Radiological changes using micro-computed tomography (microCT); ii) Morphology and morphometry on lung tissue sections stained with hematoxylin and eosin (H&E) and Picrosirius; iii) Collagen content assessed by the Sircol assay; iv) Cytokine and chemokine levels quantified by ELISA; v) PAR-1 receptor expression evaluated by Western blotting and RT-PCR. All experimental procedures were approved by the Animal Ethics Committee of the Oswaldo Cruz Institute/FIOCRUZ (CEUA L-001/19A6). **Results:** Stimulation of A549 cells with either the PAR-1 agonist peptide S1820 (3–30 µg/mL) or silica particles (30–300 µg/mL) induced the production of the inflammatory chemokine IL-8/CXCL8 and the fibrotic chemokine MCP-1/CCL2. These responses were markedly inhibited by Vorapaxar (100 µM). Additionally, PAR-1 mRNA expression was upregulated following silica exposure, suggesting its involvement in the cellular response to silica stimulation. *In vivo*, silica-exposed mice showed a significant increase in PAR-1 levels in lung tissue compared to control animals. Notably, several silica-induced lung alterations including reduction in normo-aerated lung areas and the increase in non-aerated regions, as assessed by microCT, were reversed by treatment with Vorapaxar. Furthermore, reduced granuloma formation, collagen deposition, and production of key inflammatory chemokines (IL-8/CXCL8, MCP-1/CCL2) and cytokines (IL-1β and TNF-α), in silicotic lung tissue, were also sensitive to Vorapaxar. **Conclusion:** These findings provide evidence that PAR-1 plays a central role in driving silica-induced lung inflammation and fibrosis. Notably, the blockade of PAR-1 with Vorapaxar reversed key pathological features of silicosis, including inflammation, granuloma formation, and collagen deposition. These results position PAR-1 as a highly promising therapeutic target and open new avenues for the development of effective treatments for silicosis and other fibrotic lung diseases currently lacking curative options. **Financial support:** FIOCRUZ, FAPERJ, CNPq, CAPES (Brazil).

04.046 **Effects of Intestinal Ischemia-Reperfusion (I/R) in Mice with Cholinergic Deficiency.** Queiroz VC¹, Silva CN¹, Lyoko CS², Silva AF¹, Freitas PLZ¹, Almeida FM¹, Tibério IFLC¹, Soriano FG¹, Barbeiro DF¹, Breithaupt-Faloppa AC¹, Prado CM² ¹FMUSP, School of Medicine, Brazil, ²Unifesp, Dept Bioscience, Brazil

Introduction: Intestinal ischemia and reperfusion (I/R) is a serious condition with risk of mortality, causing rupture of the intestinal barrier, bacterial translocation and multiple organ failure. In the lungs, it causes acute lung injury (ALI) and acute respiratory distress syndrome (ARDS), the main causes of death. The cholinergic pathway is crucial in controlling inflammation. **Objectives:** To evaluate the role of the cholinergic pathway in a model of acute I/R injury in mice. **Methods:** Male VACHT-KD^{HOM} mice (with reduced levels of the vesicular acetylcholine transporter) and wild-type controls (WT) were submitted to 45 min of intestinal ischemia through clamping of the superior mesenteric arteria followed by 2 h of reperfusion; SHAM controls were not submitted to I/R. Some animals received PNU-282987 ($\alpha 7$ nAChR nicotinic receptor agonist, 10 mg/kg, i.p). Parameters evaluated: mortality, lung integrity, inflammatory infiltrate, intestinal morphometry (villus height, crypt depth and villus/crypt ratio), cytokines and myeloperoxidase (MPO) activity. **Results:** I/R increased mortality in VACHT-KD I/R mice ($P=0.0018$) compared to WT, and this effect was attenuated by PNU. I/R-i induced lung inflammation in VACHT-KD^{HOM} mice, evidenced by elevated mononuclear cells infiltrate and hemorrhage, whereas PNU reduced polymorphonuclear cells in WT I/R mice and protected lung parenchyma from distension and collapse in both genotypes ($P < 0.0001$). In the intestine, VACHT deficiency reduced villus height ($P=0.0092$) and villus-to-crypt ratio ($P<0.05$), whereas I/R decreased crypt depth, regardless of genotype. PNU reduced IL-1 β , IL-6, and IL-10 in WT, but not in VACHT-KD^{HOM} mice. MPO activity was increased in VACHT-KD I/R vs. WT ($P<0.01$) and was differentially modulated by PNU, showing elevation in WT but no effect in VACHT-KD^{HOM}. **Conclusions:** Cholinergic deficiency modulates inflammation and I/R damage. PNU 282987 reduced inflammation in WT and protected VACHT-KD^{HOM} against mortality and lung injury, revealing distinct protective pathways. Cholinergic deficiency induces intestinal proliferation alterations in naïve mice, and this can be an important fact to explain distinct responses in mutant mice. **Financial support:** FAPESP (proc. 2020/13480-4, 2022-02510-5), Capes Code 001 and CNPq. **Animal Use Ethics Committee (CEUA):** (nº1967/2023).

04.047 **Differential Phenotype Modulation and Molecular Mechanisms of Lipoxin on Tumor Associated Macrophages.** Villanova Amaral MF, Cunha-da-Costa H, Duncan-Moretti J, De-Brito NM, Simões RL, Barja-Fidalgo TC. UERJ, Lab Farmacologia Celular e Molecular, Rio de Janeiro

Introduction: Macrophages are cells of the innate immune system and present as a heterogeneous population. Depending on the stimuli present in the inflammatory environment, they acquire two main profiles: M1, with pro-inflammatory characteristics, including high expression of markers such as CD86 and cytokines associated with the immune response, as well as the production of reactive oxygen species (ROS) and nitric oxide (NO), which are important for their cytotoxic capacity; and M2, with anti-inflammatory properties, characterized by an increase in markers such as CD206 and immunosuppressive mediators, playing a role in tissue remodeling. Once in the tumor microenvironment, tumor-associated macrophages (TAMs) exhibit an M2-like phenotype, with tumor-promoting characteristics. In this work, we investigated the signaling mechanisms involved in lipoxin, a pro-resolving lipid mediator, effects on the phenotypes of TAMs derived from different tumors, and the signaling pathway involved in these effects. **Methods:** For the experiments, human monocytes-derived macrophages were stimulated and differentiated to TAM by treatment with the conditioned medium (CM) from cultures of human melanoma (MV3), glioblastoma (U373), and prostate cancer cells (PC-3). After 72 hours, they were treated with lipoxin at 10nM for a further 72 hours. The phenotypic profile of TAMs was analyzed by flow cytometry, using specific antibodies (anti-CD86-APC and anti-CD206-PE). The analysis of ROS production was measured by labeled TAMs with luminol (100 μ M), while NO production was assessed with DAF-FM probe (5 μ M). The molecular mechanisms involved in the polarization of MV3 TAMs were demonstrated by western blotting, using specific antibodies for SHP-1, pVEGFR1 and VEGFR. **Results AND Conclusion:** Firstly, TAMs-derived from all types of tumor cells presented similar phenotype, expressing characteristic M2/TAM cells markers. The treatment with lipoxin was able to shift MV3-, U373-, and PC-3-derived TAMs from their M2 phenotype towards an M1-like profile. Additionally, we observed that lipoxin effects on TAMs depends on the presence of VEGF in the CM during the M2/TAM polarization. Further, we detected that lipoxin decreased VEGFR-1 phosphorylation and increases SHP-1 expression in TAMs. The results suggest that lipoxin, decreasing the activation of VEGFR-1 by VEGF in macrophages, during TAM-polarization, down-modulates the ability of these cells to assume the M2/TAM characteristic phenotype, driving the cell towards an M1-like profile. Together, the data support lipoxin as a potential modulator of TAMs in tumor microenvironment.

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04.048 Eicosapentaenoic Acid- Supplementation Decreases Adrenal Glucocorticoid Steroidogenesis in a Murine Model of Type 1 Diabetes. Barbosa BLSS, Chaves AS, Carttman L, Pacheco FS, Bergara L, Martins PMRS, Martins MA, Carvalho VF IOC-Fiocruz, Lab Inflammation, Center for Research, Innovation, and Surveillance in COVID-19 and Health Emergencies

Introduction: Type 1 diabetes mellitus (T1D) is a chronic metabolic disease characterized by the destruction of insulin-producing pancreatic β -cells, leading to severe metabolic disturbances. Emerging evidence suggests that omega-3 long-chain fatty acids (LCFAs), particularly eicosatetraenoic acid (EPA) and docosahexaenoic acid (DHA), play a beneficial role in modulating inflammatory and endocrine responses. The present study evaluates the potential of EPA in regulating steroidogenic pathways involved in glucocorticoid synthesis, providing insights into its therapeutic application in diabetes management. **Objectives:** This study aims to investigate the effects of EPA supplementation on glucocorticoid levels and steroidogenic pathway regulation in an experimental model of T1D. **Methodology:** All procedures were approved by the Animal Ethics Committee (CEUA/IOC/FIOCRUZ – L-004/2024-A). We used male Swiss-Webster mice. Type 1 diabetes was induced (alloxan, 65 mg/kg, i.v.), and the animals were treated with EPA (125 mg/Kg); or DHA (50 mg/Kg); and/ or booster (EPA 125 mg/Kg + DHA 50 MG/Kg) by gavage. Control animals received sterile saline (0.9% NaCl). Treatments began 7 days after diabetes induction and were administered daily for 14 consecutive days. **Results:** Our findings indicate that EPA administration significantly reduced circulating corticosterone levels in diabetic animals, while DHA did not alter the levels of corticosterone. Furthermore, the expression of steroidogenic cascade proteins, including MC2R and StAR, in the adrenals was markedly decreased in EPA-treated animals compared to diabetic non-treated animals. **Conclusion:** These results show that EPA exerts modulatory effects on adrenal steroidogenesis, contributing to lower glucocorticoid secretion in diabetes. The supplementation with EPA appears to be a promising candidate adjunct therapy in diabetic patients, as it effectively attenuates excessive glucocorticoid production and mitigates the associated metabolic disturbances. Future studies should explore the clinical implications of these findings, possibly improving patient outcomes when combined with dietary and pharmaceutical interventions.

04.049 Red LED Therapy Led to Tissue Remodulation and Decrease of Immune Cells in Skin of Murine Imiquimod-induced Psoriasis Experimental Model. Rodrigues-Silva N, Correia-Silva RD, Santos DD, Corrêa MP, Gil CD Unifesp-EPM, Brazil

Photodynamic therapy (PDT) has been used since ancient times and now represents an important treatment option for inflammatory skin diseases. Psoriasis (Pso) is an immune-mediated skin disease characterized by chronic inflammation and scaling due to hyperproliferation of keratinocytes of epidermis affected area. The disease can be triggered by pathogens, medications, and trauma. Due to the complexity of its pathogenesis, identifying biomarkers is essential for the development of personalized treatments. Among PDT approaches, LED phototherapy has gained attention, with red LED (LEDV) being the only one capable of penetrating the entire dermis. This study evaluated the effects of LEDV light on Pso progression in an imiquimod (IMQ)-induced murine model. C57BL/6 mice were divided into four groups: control (CTR), CTR+LEDV, Pso, and Pso+LEDV (n=5-7 animals/group). On day 0, animals were shaved; from days 1 to 8, Pso groups received topical IMQ (50 mg/kg), while CTR groups received Vaseline. LEDV treatment was applied on days 4, 6, and 8. Skin thickness (double skinfold), body weight, and PASI score were assessed on days 0, 2, 4, 6, and 8. Twenty-four hours after the last IMQ and LEDV application, animals were euthanized for skin and spleen collection (CEUA nº 5387071123). IMQ-induced Pso led to a significant increase in PASI score in the Pso+LEDV group on days 4 and 6 compared to CTR, whereas on day 8, this increase was observed only in the untreated Pso group. Skinfold thickness analysis showed a marked increase between days 4 and 6 in both Pso and Pso+LEDV groups. Histological analysis revealed significant epidermal thickening in LEDV and Pso+LEDV groups compared to CTR and Pso, respectively. In contrast, mast cell quantification in the dermis showed a significant increase only in the Pso group compared to the others. Densitometric analysis of picrosirius revealed some tissue remodeling, evidenced by a significant increase of collagen protein in Pso+LEDV compared to Pso group. Systemic response was indicated by an increase in relative spleen weight and size in both Pso and Pso+LEDV groups compared to its respective controls. In addition, we observed a significant increase in white pulp relative area in Pso and Pso+LEDV compared to CTR, while there was a significant decrease in red pulp relative area in Pso and Pso+LEDV compared to CTR. Skin immunohistochemistry showed a significant decrease of CD3-positive cells in Pso+LEDV epidermis when compared to CTR and Pso groups. Lastly, spleen immunohistochemistry revealed a decrease of CD45 in both Pso and Pso+LEDV when compared to CTR and CTR+LEDV, respectively; and the same profile was observed in CD68 reaction (One-way ANOVA). Although LEDV did not prevent Pso progression, it suggests a potential modulatory effect on the cutaneous inflammatory response, highlighting the need for further studies to elucidate its mechanisms.

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04.050 **Effects of Nor-JNJ-7777120, a Novel Potent Histamine H₄ Receptor Antagonist, on a DSS-Induced Colitis in Mice.** Silva PS, Fernandes JPS, Landgraf RG Unifesp-Diadema, Dept Pharmaceutical Sciences, Brazil

Introduction: Crohn's disease and ulcerative colitis are the most common forms of human inflammatory bowel disease (IBD), chronic pathologies that affect individuals throughout life. Current treatments for IBD may involve different drugs, but mesalazine remains one of the main anti-inflammatory agents effective in the acute phase of IBD. Histamine plays a fundamental role in the inflammatory response in several tissues (including the intestine), mainly through activation of the H₄ receptor (H₄R). Thus, the pharmacological blockade of this receptor is a therapeutic strategy for IBD. In previous work, our group tested the effects of the LINS01007 compound (pK_i 6.1, H₄R antagonist) in a murine DSS-induced model of colitis, showing to significantly reduce the disease activity, including a remarkable reduction in several inflammatory markers. Considering this, in the present work the compound nor-JNJ-7777120 (NorJNJ), a potent H₄R antagonist (pK_i 8.1), was assessed in a murine DSS-induced colitis model as a potential treatment for IBD. **Methods:** The compound NorJNJ was synthesized from commercial starting material with good yields. Two synthetic routes were employed: direct coupling of 2-(5-chloroindol)carboxylic acid with piperazine using coupling agents(EDC/HOBt), which produced byproducts and low yields; and a second strategy using Boc-protected piperazine, which enabled selective acylation and higher yields (60–94%). The final compound was characterized by ¹H and ¹³C NMR, showing purity >95%. The compound was converted into its maleate salt to increase solubility and administration to the animals. The compound was tested in male BALB/c mice, which were subjected to colitis induction using 3% dextran sulfate sodium (DSS). The animals were treated intraperitoneally with NorJNJ (5 mg/kg/day) and with mesalazine (10 mg/kg/day) for seven days. Clinical progression was monitored daily by the Disease Activity Index (DAI), considering weight loss, stool consistency, and presence of blood and compared with animals without any treatment (DSS group) and healthy animals. **Results:** While DSS animals exhibited progressive worsening of DAI, NorJNJ treatment significantly reduced disease severity, with clear improvement from day four onwards. The therapeutic effect was comparable to that of standard treatment with mesalazine. **Conclusion:** NorJNJ showed structural integrity, high purity, and relevant anti-inflammatory activity in the DSS-induced colitis model. These findings reinforce the pharmacological importance of H₄R antagonists and suggest that structural optimization of JNJ derivatives may yield promising candidates for the treatment of inflammatory bowel diseases. **Financial support:** FAPESP, grant 2024/15451-2 and 2024/02740-6; CNPq, grant 302348/2025-5 and 301135/2025-8. **Ethics statement:** This work was approved by the CEUA UNIFESP 5541101120

04.051 **Hydrogen Sulfide Pathways in a Male Murine Model of Experimental Cystitis.** Sombra VT^{1,2}, Santos LG¹, Costa de Souza AL¹, Teixeira AS¹, Muscara MN¹, Costa SKP¹.¹ICB-USP, Depto de Farmacologia, São Paulo, SP, ²Associação Crescer Sempre, São Paulo, SP, Brazil

Introduction: interstitial cystitis/bladder pain (IC/BPS) is a chronic inflammatory condition characterized by pelvic pain, urinary urgency, and frequency in the absence of active infection¹. Women are 4–9 times more likely to be diagnosed with IC/BPS, whereas men show a lower incidence, partly due to biological factors but largely as a result of underdiagnosis and misclassification as prostatitis². Previous studies from our group demonstrated that hydrogen sulfide (H₂S) donors, such as GYY4137, are effective in alleviating IC/BPS; however, the role of endogenous H₂S in this condition remains unclear.³ This study investigated the contribution of endogenous H₂S to inflammatory responses in experimental cystitis. **Methods:** Male C57BL/6 mice (8–10 weeks) were subjected to cyclophosphamide (CYP, 300 mg/kg, intraperitoneal)-induced cystitis. After 24 h, bladders were collected for assessment of inflammatory markers (myeloperoxidase [MPO], iNOS, F4/80, COX-2, and arginase) and endogenous H₂S production, as well as expression of its generating enzymes (cystathionine γ -lyase [CSE], cystathionine β -synthase [CBS], and 3-mercaptopyruvate sulfurtransferase [3-MST]) using colorimetric assays, Western blotting and immunoassay when appropriated. **Results:** Twenty-four hours after CYP-induced cystitis, MPO activity was increased in the bladder compared with control mice (17.65 ± 7.29 and 3.79 ± 1.94 , respectively $n = 4-5$), reflecting neutrophil infiltration. At this time-point, no significant changes were observed in F4/80, COX-2 protein expression or arginase activity. However, endogenous H₂S production was significantly reduced at earlier time points (4 h) following CYP-induced cystitis (3.73 ± 0.44 and $1.43 \pm 0.22^*$, respectively $n = 5$) and returned to baseline after 24 h (2.68 ± 0.36 , $n = 6$). This reduction was associated with downregulation of CSE (100 ± 2.0 and $82 \pm 3.1^*$, for control and CYP, respectively, $n = 5$) and 3-MST expression (109.73 ± 8.25 and $56.4 \pm 4.72^*$, for control and CYP, respectively, $n = 5$) particularly in the bladder mucosa. In contrast, CBS immunostaining was increased in the urothelium and detrusor muscle, suggesting a compensatory response. **Conclusion:** CYP-induced cystitis in male mice was characterized by increased MPO activity (neutrophil infiltration) and an early, transient reduction in endogenous H₂S production, accompanied by downregulation of CSE and 3-MST and compensatory induction of CBS. These findings demonstrate that bladder inflammation disrupts H₂S homeostasis and identify the H₂S pathway as a promising therapeutic target for IC/BPS. **Ethics approval:** This study was approved by the Ethics Committee on Animal Use (CEUA-ICB/USP, Protocol no. 2055050819). **References:** [1] Oliveira et al., Am J Physiol Renal Physiol. 2016 Jul 1;311(1):F85-93. [2] Lim Y, et al., StatPearls Publishing; 2025 Jan (2025). In: StatPearls [Internet]. Treasure Island (FL): -. Available from: : StatPearls [Internet]. Treasure Island (FL):. [3] Santos et al., WCP 2023, Glasgow, Scotland. **Acknowledgments:** Fapesp (2017/15175-1), CAPES (Finance Code 001) and CNPq (312514/2019-0).

04.052 **Reduction of Pro-Inflammatory Cytokines by Resveratrol and AGK-2 *in vivo* and *in vitro* Stimulated with *Klebsiella pneumoniae*.** Costa M^{1,3}, Thiele, E¹, Almeida MAP^{1,2}, Castro LVG¹, Bozza PT¹, Castro-Faria-Neto HC¹, Gonçalves-de-Albuquerque CF^{1,2,3}, Silva AR^{1,3} ¹IOC-Fiocruz, Immunopharmacology Lab and Postgraduate Program in Cellular and Molecular Biology, Rio de Janeiro, Brazil ²UFF, Post-Graduation Program in Neuroscience, ³UFRJ, Immunopharmacology Lab and Postgraduate Program in Molecular and Cellular Biology, Rio de Janeiro, Brazil

Introduction: Under physiological conditions, microglial cells contribute to maintaining brain homeostasis. In neurodegenerative diseases, they become activated, exhibiting morphological and functional changes, and release cytokines such as TNF- α and IL-6 (1). Sepsis induces glial activation (2) and is characterized by massive cytokine production, leading to hemodynamic and metabolic dysfunctions that may progress to septic shock, multiple organ failure, and death (3). Activated microglia and inflammation are also described in sepsis and sepsis-associated encephalopathy (4). Sepsis may result from infections by microorganisms, such as pneumonia caused by *Klebsiella pneumoniae* (KP), a Gram-negative opportunistic bacterium that primarily affects immunocompromised individuals. KP is of major concern to the World Health Organization due to its production of extended-spectrum beta-lactamases (ESBLs) and carbapenemases, enzymes that confer resistance to multiple classes of antibiotics, making treatment difficult (5). Sirtuins (SIRTs), part of the class III histone/lysine deacetylase family, are NAD⁺-dependent. SIRT1 plays neuroprotective roles, whereas SIRT2 is associated with neurodegeneration (6). Resveratrol is a pharmacological activator of SIRT1 (7), while AGK-2 is a selective SIRT2 inhibitor that counteracts neuroinflammation and neurodegeneration (8). **Methods:** Murine microglial cells (BV-2) were pre-treated with Resveratrol (100 μ M) or AGK-2 (20 μ M) and subsequently exposed to KP (MOI 2). After 24 hours, the supernatant was collected for cytokine analysis (IL-6 and TNF- α). Mice were subjected to intratracheal instillation with 5×10^8 CFU of KP and treated with AGK-2 (10 mg/kg) via intraperitoneal injection at 5 and 24 hours post-instillation. After 48 hours, animals were euthanized and brains were collected for cytokine quantification. NF- κ B (pP-65) activation was assessed in brain tissue 48 hours after instillation and in BV-2 cells one hour after KP stimulation. **Results:** Stimulation of BV-2 cells with KP significantly increased IL-6 and TNF- α production after 24 hours. Pre-treatment with Resveratrol or AGK-2 markedly reduced cytokine levels compared to KP alone. *In vivo*, KP instillation elevated IL-6 and TNF- α levels in brain tissue after 48 hours, while AGK-2 treatment significantly reduced this response in the cortex. NF- κ B (pP-65) phosphorylation was increased in BV-2 cells one hour post-KP stimulation, and both Resveratrol and AGK-2 effectively reduced its activation. Similarly, AGK-2 treatment in KP-instilled mice led to reduced NF- κ B activation in brain tissue after 48 hours. **Conclusion:** Resveratrol and AGK-2 demonstrated significant anti-inflammatory effects *in vitro* by reducing pro-inflammatory cytokine levels and NF- κ B activation in BV-2 microglial cells, while *in vivo*, AGK-2 reduced these inflammatory markers in the cortex of mice instilled with KP. These findings highlight the therapeutic potential of SIRT1 activation and SIRT2 inhibition in modulating neuroinflammation associated with bacterial infections. **Acknowledgments:** FAPERJ, CNPq, CAPES, IOC-FIOCRUZ/RJ, UNIRIO.

04.053 **Nanostructured DHA Attenuates Inflammatory Effects in BV-2 Microglial Cells Stimulated with *E. coli*.** Gomes-Reis GLS¹, Moraes BPT², Saraiva JSG², Ferrarini SR³, Santos FS⁴, Ferreira AM¹, Bozza PT¹, Castro-Faria-Neto HC¹, Silva AR¹, Gonçalves-de-Albuquerque CF^{1,2} ¹IOC-FIOCRUZ, Immunopharmacology Lab and Postgraduate Program in Cellular and Molecular Biology, Rio de Janeiro, Brazil, ²UFRJ, Immunopharmacology Lab and Postgraduate Program in Molecular and Cellular Biology, Rio de Janeiro, Brazil, ³UFMT, Institute of Health Sciences, Mato Grosso, Brazil, ⁴UFF, Post-Graduation Program in Neuroscience, Niterói, Brazil

Introduction: Microglia reside in the central nervous system (CNS) and acts in immune defense. When detecting changes in CNS homeostasis, microglia alter their morphology and physiology through the expression of pattern recognition receptors (PRRs), releasing cytokines such as tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) in neuroinflammatory processes, including sepsis, which is characterized by an exacerbated host immune response to a polymicrobial infection [1]. New therapeutic targets have been explored to reduce the consequences of inflammatory processes, such as omega-3. Docosahexaenoic acid (DHA) is an omega-3 fatty acid that exhibits neuroprotective action due to its anti-inflammatory activity in cells, such as microglia, through its pro-resolving mediators: maresins, protectins, and resolvins [2]. To enhance the effects of DHA, nanostructures have been produced due to their improved pharmacokinetic performance, ensuring increased potential and bioavailability of the active ingredient. DHA is a ligand of peroxisome proliferator-activated receptors (PPARs), which suppress the expression of cytokines involved in neuroinflammatory functions. When activated, PPAR- γ inhibits the nuclear factor kappa B (NF- κ B) signaling pathway, which reduces the translocation of RelA (p65) to the nucleus and suppresses the transcription of genes related to inflammation [3]. Therefore, we evaluated the effects of nanostructured DHA in BV-2 microglial cells stimulated with *Escherichia coli* (*E.coli*) in neuroinflammation. **Methods:** Murine BV-2 cells (5×10^4) were plated in RPMI medium, 10% fetal bovine serum (FBS), 1% penicillin/streptomycin (P/S) for 24h. Nanostructured DHA at 1 μ M and 10 μ M was used as treatment. After 1h, BV-2 were stimulated with *E. coli* at MOI (multiplicity of infection) 10. After 1h or 24h, the resazurin assay was performed. The supernatant was collected for the evaluation of TNF- α and IL-6 by ELISA, or the cells were fixed for the immunocytochemistry assay to evaluate p65. **Results:** Viability, as measured by resazurin, showed that all experimental groups remained highly viable, with a percentage greater than 80%. Stimulation with *E.coli* increased the production of pro-inflammatory cytokines TNF- α and IL-6, while treatment with 10 μ M nanostructured DHA reduced the level of TNF- α ($p < 0.05$). In an immunocytochemistry assay, treatment with 1 μ M and 10 μ M nanostructured DHA reduced the translocation of the p65 protein, a subunit of NF- κ B, to the nucleus. **Conclusion:** Nanostructured DHA attenuated the pro-inflammatory effects in BV-2 stimulated with *E. coli*, indicating a reduction in neuroinflammation through the activation of the NF- κ B transcription factor. References: 1.Vidal-Itriago, A. et al. Front, 2022. 2.Fourrier, C. et al. Jou of Neu, 2017. 3.Yang, Z. et al. Mol, 2019. Funding: CAPES, CNPq, FAPERJ, IOC, FIOCRUZ, REDE NANO SAÚDE, UFMT, UNIRIO.

04.054 PPAR- γ Profiling in Macrophage-Mediated Inflammatory Dysregulation Induced By *Klebsiella pneumoniae* Infection. Guedes LD, Carvalho MV, Castro LGV, Costa MF, Souza BB, Dutra FSP, Bozza PT, Farias Neto HCC, Albuquerque CG, Silva AR IOC-Fiocruz

Introduction: Peroxisome proliferator-activated receptor gamma (PPAR- γ) is a transcription factor expressed in innate immune cells that regulates cellular metabolism and inflammation. In pneumosepsis, PPAR- γ agonists reduce hyperinflammation, promote balanced immune responses, and improve bacterial clearance. During pulmonary infections, macrophages are key for host defense; however, multidrug-resistant *K. pneumoniae* can evade phagocytosis and exacerbate inflammation. Rosiglitazone, a PPAR- γ agonist, modulates the expression of inflammatory and metabolic genes, such as CD36, FABP4 and iNOS, potentially restoring cellular homeostasis and aiding infection resolution. **Methods:** RAW 264.7 murine macrophages were pretreated with rosiglitazone (20 μ M) for 1 hour and infected with *K. pneumoniae* at a multiplicity of infection (MOI) of 2.0 and 10.0. After 24 h, the culture supernatant was collected for quantification of pro-inflammatory cytokines tumor necrosis factor (TNF)- α , interleukin (IL)-6 and IL-1 β by enzyme-linked immunosorbent assay (ELISA) and nitric oxide (NO) production by Griess reagent. The adherent cells were lysed for RNA extraction and gene expression analysis via one-step quantitative RT-qPCR targeting *IL-1 β* , *IL-6*, *CD36*, *FABP4* and *iNOS*. Each experiment was performed in triplicate and repeated independently three times. Results are expressed as mean $\pm$ standard error of the mean (SEM) and analyzed by one-way ANOVA followed by Bonferroni's post hoc test. Statistical significance was set at $p < 0.05$. **Results:** *K. pneumoniae* induced the production of pro-inflammatory cytokines, NO and other inflammatory markers by RAW 264.7. Pretreatment with rosiglitazone reduced pro-inflammatory cytokine release, NO production and markedly downregulated IL-1 β , IL-6, iNOS, CD36, and FABP4 expression, 24 h after infection. **Conclusion:** These findings highlight that rosiglitazone serves as a promising therapeutic candidate for drug repositioning modulating excessive inflammation and promoting cellular homeostasis in conditions of antimicrobial resistance (AMR). **Financial support:** IOC, FIOCRUZ, FAPERJ, CNPq, CAPES, UNIRIO.

04.055 **Systemic Endotoxemia Differently Modulates the Expression of NLRP3 Inflammasome Complex in Mice Epididymis: A Pilot Study.** Bizzotto JQ, Martini PV, Torres RM, Andrade AD, Silva EJR IBB-Unesp-Botucatu, PPG Biomolecular and Pharmacological Sciences, Brazil

Introduction: The epididymis is a highly regionalized organ that is essential for post-testicular sperm maturation, protection, and storage until ejaculation. Epididymitis, inflammation of the epididymis, can lead to tissue damage, including destruction of epithelial and smooth muscle cells, mainly in its distal regions, compromising tissue integrity and ultimately resulting in subfertility and infertility. The severity of tissue lesions characteristic of epididymitis correlates with the intensity of the inflammatory response. The NLRP3 inflammasome, a multiprotein complex composed of the NLRP3 sensor, the accessory protein ASC, and the caspase 1 protease. The elements of the NLRP3 inflammasome are expressed at low levels in epithelial and immune cells and can be transcriptionally induced by an inflammatory stimulus. This is referred to as the “priming” step and is necessary to induce transcription of *Nlrp3* and the cytokine *Il1b* to become responsive to the “activation” step, which is triggered by different inflammatory- and stress-associated signals. The activation of the NLRP3 inflammasome is essential to the inflammatory response, since it causes the proteolytic cleavage of pro-inflammatory interleukins, such as *IL1B* and *IL18*, as well as the activation of the pyroptosis cell death pathway. Lipopolysaccharide (LPS) from Gram-negative bacteria, a Toll-like receptor 4 agonist, is a well-studied inflammasome activator. Here, we tested the hypothesis that the expression of the NLRP3 inflammasome complex components is increased in the epididymal regions during systemic endotoxemia as a priming step. **Methods:** Systemic inflammation was induced in adult male C57BL/6 mice (90 days, n=3-5/group, IBB/CEUA protocol 2132170322) by intraperitoneal injection of ultrapure LPS from *E. coli* (1.0 mg/kg). Sterile saline solution (vehicle) was used as a control. Mice were euthanized 6, 12, and 24 h post-injection. The initial segment and cauda epididymis were collected and processed for RT-qPCR to assess *Nlrp3*, *Asc*, and *Il1b* transcript levels with *Rps18* as the endogenous control. Results were expressed as mean $\pm$ SEM and analyzed by Student's t-test ($p < 0.05$ was considered significant). A naïve animal was used to collect different organs for conventional RT-PCR assays to detect *Nlrp3*, *Asc*, *Gsdmd*, and *Ppia* (endogenous control). **Results:** Constitutive expression of *Nlrp3* and *Asc*, as well as the pyroptosis marker *Gsdmd*, was observed in all epididymal regions. LPS upregulated *Nlrp3* in the initial segment (fold-change > 2.8) and cauda epididymis (> 2.5) after 12 h compared to the control group. Furthermore, LPS upregulated *Asc* and *Il1b* after 6 (> 2.5 and > 6.7), 12 (> 3.1 and > 5.4) 24 h (> 5.5 and > 2.4) in the initial segment. Concurrently, LPS upregulated *Asc* after 12 (> 2.7) and 24 h (> 1.3) and *Il1b* only at this later time point (> 21.3) in the cauda epididymis. **Conclusion:** Our findings suggest that systemic endotoxemia triggers the priming of the NLRP3 inflammation in a region- and time-specific manner in the mouse epididymis. Further studies are needed to elucidate whether systemic endotoxemia is sufficient to trigger the activation of the NLRP3 inflammasome in the epididymis and its contribution to epididymitis outcomes. **Support:** FAPESP, CAPES, CNPq.

04.056 **Anti-Inflammatory and Antiedematogenic Activity of the Isolated Trypsin Inhibitor from *Inga laurina* seeds (ILT_i)**. Lencina DS¹, Bonfá IS¹, Lossavaro PKMB¹, Venancio GS¹, Fernandes MML¹, Lencina JS¹, Silva SE¹, Jacobowski AC², Macedo MLR², Kadri MCT¹ ¹UFMS, Lab. of pharmacology and inflammation; ²UFMS, Lab Protein Purification and its Biological Functions

Introduction: Serineproteases are enzymes that modulate many functions such as immune response and coagulation. Currently their inhibitors have been important targets to drug discovery. Thus, trypsin inhibitors already demonstrated antiinflammatory activities. Therefore the objective of this work was the evaluation of the antiedematogenic and antiinflammatory activities of the isolated trypsin inhibitor from *Inga laurina* seeds (ILT_i). **Methods:** The assays were performed with male Swiss mice weighing 18–25 g pretreated with saline, dexamethasone, ILT_i 0.3, 3 or 30 mg/Kg (200 uL, subcutaneously). 30 minutes later, the mice were injected with carrageenan 1% (intraplantar, 40 uL) for edema and 48/80 compound 1 ug/mL (intraperitoneally, 500 uL) for the mast cell degranulation. The paw edema was measured at 30, 60, 120 and 240 minutes after the stimuli. For the evaluation of mast cell degranulation the animals were euthanized 30 minutes after the stimuli injection and mesentery was removed, washed and transferred to a slide were stained with HEMA3®. After, the cells were analyzed in light microscopy, counted and classified as degranulated or non-degranulated based on morphological standards and dispersion of granules. Data were shown as the mean ± S.E.M. Statistical differences were analyzed by one or two-way ANOVA followed by the Bonferroni test (p<0.05). The results were expressed as % of reduction in comparison with the control group. **Results:** ILT_i pretreatment inhibited paw edema at dose 3 mg/kg at all times and 30 mg/kg at 30 minutes (51%, 0.022 ± 0.005mL), 60 minutes (47%, 0.025 ± 0.006mL) and 120 minutes (57%, 0.020 ± 0.004mL). In mast cell degranulation at 17.8% (45.8 ± 3.17%) on the dose of 0.3 mg/Kg in comparison to the control group (19.3 ± 0.9%). **Conclusion:** These results showed a potential anti-inflammatory and antiedematogenic effect in ILT_i probably due to the inhibition of inflammatory proteases and non-activation of protease-activated receptors, which is a possible target to innovative antiinflammatory drugs. **Financial support:** UFMS, FUNDECT, CAPES and CNPq. **Ethics Committee on Animal Experimentation of UFMS approval protocol:** 1225/2022.

04.057 **Hydroethanolic Extract from *Schinus terebinthifolius* Raddi as Therapeutic Alternative for Joint Inflammation.** Nascimento, Sarah N.1;2; Correa, Luana B.1; Berenger, Ana L.R3.; Figueiredo, Maria R3; Rosas, Elaine C.1;2. 1Farmanguinhos-Fiocruz, Lab Applied Pharmacology, Rio de Janeiro, Brazil. 2. Rio de Janeiro State University IBRAG, Postgraduate Program in Biosciences. 3. Lab Natural Products (TecBio), Institute of Pharmaceutical Technology - Farmanguinhos, Oswaldo Cruz Foundation, Rio de Janeiro, RJ, Brazil

Introduction: *Schinus terebinthifolius* Raddi, commonly known as Aroeira, belongs to the Anacardiaceae family. This species is widely used for its antioxidant, antimicrobial, wound-healing, and anti-inflammatory properties. Previous studies by our research group demonstrated that the hydroethanolic extract from *S. terebinthifolius* leaves (ST-70) exhibited anti-inflammatory activity in zymosan-induced arthritis model, inhibiting cell migration and the production of inflammatory mediators such as TNF- α , IL-6 and IL-1 β . This study aimed to evaluate the anti-arthritic potential of ST-70 in antigen-induced arthritis model (AIA).

METHODOLOGY: Leaves of *Schinus terebinthifolius* Raddi were collected from the Mata Atlântica Campus of F in Jacarepaguá, Rio de Janeiro, Brazil. ST-70 was prepared by macerating *S. terebinthifolius* leaves in 70% ethanol. C57BL/6 mice were immunized subcutaneously (s.c.) with an emulsion containing methylated bovine serum albumin (mBSA) and complete Freund's adjuvant (CFA). Control animals (sham) received the emulsion without mBSA. On the twenty-first day post-immunization, animals were pretreated orally (p.o.) with ST-70 (25 - 200 mg/kg) and challenged with an injection of mBSA into the left femorotibial joint. Control animals receive only 1x PBS. Twenty-four hours (24 h) after induction, joint edema was measured using a caliper, synovial lavage fluid and inguinal lymph node were collected to assess leukocyte migration. Blood serum was also collected for immunoglobulin quantification. All procedures were approved by the Animal Use Ethics Committee (CEUA) of FIOCRUZ under protocol LW-15/2023. **Results:** ST-70 at a dose of 100 mg/kg significantly reduced joint edema 24 h after AIA induction. Furthermore, the 100 and 200 mg/kg doses of ST-70 significantly reduce total leukocyte (52.05%) and neutrophil (20.31%) infiltration into the joint. A reduction in lymph node leukocyte counts (64.84%) was also observed at all doses tested. Moreover, serum IgG levels decreased, indicating systemic immunomodulatory effects. **Conclusion:** ST-70 exhibits potent anti-inflammatory activity in the AIA model by attenuating both local joint inflammation and systemic immune responses. These findings support its potential as a phytotherapeutic agent for autoimmune inflammatory conditions such as rheumatoid arthritis. **Financial support:** FIOCRUZ, CNPq, FAPERJ and CAPES. **Key words:** *Schinus terebinthifolius* Raddi; anti-inflammatory; edema; antigen. **CEUA:** LW-15/2023

04.058 **Influenza A Virus Infection Exacerbates the Inflammatory Response and Bronchiectasis Induced by Cigarette Smoke Exposure in Mice Assessed by Microcomputed Tomography.** Nobrega MEGC^{1,2}, Cotias AC¹, Gomes HS¹, Lopes RR¹, Gaze LA¹, Arantes ACS¹, Ferreira TPT¹, Santos BDF¹, Carvalho VF¹, Silva PMR¹, Ball L³, and Martins, MA¹ ¹IOC-Fiocruz, Lab Inflammation, Rio de Janeiro, Brazil; ²UFRJ, PPG Farmacologia e Química Medicinal, Brazil; ³University of Genoa, Dept Surgical Sciences and Integrated Diagnostics, Genoa, Italy

Introduction: Influenza A (H1N1) virus infection is among the leading causes of exacerbations of Chronic Obstructive Pulmonary Disease (AECOPD), the development of which is strongly associated with prolonged exposure to cigarette smoke (CS). This combination leads to persistent and progressive airway obstruction and may favor the development of bronchiectasis, a condition of irreversible dilation of the bronchial lumen resulting from infection, inflammation and mucociliary damage. Failure to mitigate this condition generates a cycle of destruction and repair of the bronchial wall, which results in irreversible lesions and impairment of lung function, which may be intensified during exacerbations. Our study aimed to investigate the inflammatory response and bronchiectasis in a murine model of acute exacerbation of lung inflammation induced by the combination of H1N1 virus infection and exposure to cigarette smoke through analysis of the airway in images obtained by microcomputed tomography (Micro-CT). **Methods:** Female C57BL/6 mice were exposed to cigarette smoke for 1 h per day for 11 consecutive days in a system coupled to a commercially available ENDS smoke generating device (Data Sciences International, USA). Mice were exposed to 160 cigarette puffs, with a smoke volume of 70 mL. On day 7, the animals were infected with the Influenza A H1N1/A/PR/8/34 virus by oropharyngeal aspiration. Mice were treated with dexamethasone (1 mg/Kg, by gavage) on days 8, 9 and 10, 1 h before each CS exposure. Twenty-four hours after the last CS exposure, assessments of airway obstruction and total and differential leukocyte counts in the bronchoalveolar lavage (BAL) effluent were performed. We assessed the putative bronchiectasis using Micro-CT. All procedures involving the use of animals were approved by the Laboratory Animal Use Committee of the Oswaldo Cruz Institute (license number: L002/2020-A6). **Results:** We observed a gradual reduction in the mice's body weight on 3, 4, and 5 days post infection (DPI). Compared with animals exposed to cigarette smoke alone or those infected with H1N1, CSH1N1 animals presented clearly exacerbated Penh values. Concerning leukocyte infiltration, CSH1N1 mice also exhibited increased numbers of neutrophils and macrophages in the BAL effluent and lungs, as measured by lung myeloperoxidase (MPO) activity, compared to CS-exposed or H1N1-infected mice. A significant increase in plasma extravasation also accompanied these changes. Micro-CT analyses showed that H1N1 infection induced a substantial increase in bronchial volume (mm²) (0.4173 ± 0.07827 to 1.609 ± 0.2403 ; mean $\pm$ SEM, n=5; $p \leq 0.005$), suggestive of bronchiectasis, which was not observed in animals exposed to cigarette smoke alone (0.4173 ± 0.07827 to 0.6087 ± 0.06006 ; mean $\pm$ SEM, n=6; $p \leq 0.005$). The combination of H1N1 infection and cigarette smoke exposure further exacerbated this alteration. Treatment with Dexamethasone 1 mg/Kg (oral) was ineffective in all parameters, indicating that these changes are dexamethasone-resistant. **Conclusion:** Our findings demonstrate that exposure to cigarette smoke in conjunction with influenza A (H1N1) virus infection exacerbates key parameters involved in the pathogenesis of AECOPD, including lung function, inflammatory infiltrate, and alveolar damage. In agreement, we observed an increase in bronchial volume characteristics of bronchiectasis in mice infected with the H1N1 virus, confirmed by microcomputed tomography imaging analyses. **Financial support:** FIOCRUZ, CNPq, FAPERJ e CAPES (Brazil).

04.059 **Metformin Effect on *Klebsiella Pneumoniae* Infection *in vitro* and *in vivo*: Focus on Brain Cells.** Ferreira AM^{1,2}, Ferreira-Costa M^{1,2}, Castro LVG^{1,2}, Gomes-Reis GLS^{1,2}, Santos FS^{1,2,3}, Souza BB^{1,2}, Bozza PT¹, Castro-Faria-Neto HC¹, Gonçalves-De-Albuquerque CF^{1,2,3}, Silva AR^{1,2,3} ¹IOC-Fiocruz Immunopharmacology Lab and Postgraduate Program in Cellular and Molecular Biology, Rio de Janeiro, Brazil; ²UFRJ, Immunopharmacology Lab and Postgraduate Program in Molecular and Cellular Biology, Brazil; ³UFF, Postgraduate program in Neuroscience, Brazil

Introduction: Neuroinflammation affects the nervous system as a result of systemic inflammation. In hospital environments, sepsis is a common condition and is one of the biggest causes of death in ICUs in Brazil¹. A large proportion of admissions start with pneumonia and evolve into a septic condition, and *Klebsiella pneumoniae* plays an essential role in documented cases of pneumonia. It was declared by the WHO as one of the antimicrobial resistance (AMR) bacteria of concern due to its demonstrated potential for developing antimicrobial resistance to many classes of antibiotics. Considering drugs that already have known efficacy, metformin is a substance used to treat type 2 diabetes, but also has anti-inflammatory effect and can cross the blood-brain barrier³. Our work aimed to investigate the potential of drug repositioning by utilizing metformin to reduce the inflammatory markers associated with *K. pneumoniae* inflammation. **Methods:** Using three different concentrations of metformin (0.5 mM, 1 mM, and 2 mM) as pre-treatment and two MOI (multiplicity of infection) of *K. pneumoniae* (2 and 20) in BV-2 cells, we analyzed the levels of IL-1 β and IL-6 24h after infection. To verify the effects of metformin *in vivo*, we stimulated mice with 5×10^8 bacteria/animal by intratracheal instillation, treated them with metformin (200 mg/kg) 5 h and 24h after, and analyzed the parameters 48h after the infection (CEUA: L-005/2023; L-016/2025). **Results:** Our results showed an increase in IL-1 β and IL-6 in both *in vitro* and *in vivo* models, indicating that *K. pneumoniae* induces an inflammatory response in both. Metformin treatment reduced these inflammatory markers using MOI 2 and 20. We also observed whether *K. pneumoniae* could alter the microglia phenotype and found a decrease in microglial ramification in response to *K. pneumoniae* stimulation, and an increase with metformin pre-treatment, indicating that metformin can restore the phenotype. *In vivo*, we observed a decrease in TNF- α levels in the hippocampus and a reduction of IL-1 β levels in the cortex with metformin, demonstrating that different regions of the brain may respond differently, and the metformin effect may also be different in these regions. **Conclusion:** As an AMR bacteria, *K. pneumoniae* presents a risk to public health. Studies trying to reduce inflammation contribute to the possibility to increase the survival of infection and here we demonstrate these results with drug repositioning, raising the possibility of utilization of metformin. Our model of infection *in vitro* and *in vivo* develop an inflammatory response on BV-2 cells and mice, and metformin acted as an anti-inflammatory to aid in recovery from inflammation, reducing cellular inflammatory markers. **Financial support:** IOC, FIOCRUZ, FAPERJ, CNPq, CAPES, UNIRIO. **References:** ¹Zampieri FG, et al. Ann Intensive Care 2023; ²Muraya A, et al. Pathogens 2022; ³Koenig AM, et al. Alzheimer Dis Assoc Disord 2017

04.060 **Evaluation of Phenotypic changes Induced by Lipoxin in Macrophages Derived from Breast Cancer.** Duncan-Moretti J, Cunha-da-Costa H, Villanova Amaral MF, Simões RL, Barja-Fidalgo TC. UERJ, Lab Farmacologia Celular e Molecular, Rio de Janeiro

Introduction: Macrophages are very plastic cells able to modulate the immune-inflammatory response by assuming different phenotypes. While M1 phenotype shows pro-inflammatory profile, with increased expression of the CD86 marker, reactive oxygen species (ROS) and nitric oxide (NO), important for its anti-tumoral effects, M2 macrophages may present anti-inflammatory, repairing and pro-tumor properties. In breast cancer, macrophages (TAM) are key components of tumor microenvironment. By displaying M2 phenotype, TAMs contribute to tumor progression and are directly related to different stages of tumor development and clinical outcomes. We have early demonstrated that lipoxin, a pro-resolutive lipid mediator, triggered anti-tumoral effects in TAMs in melanoma, restoring their cytotoxic properties, such as the production of ROS and NO, leading to apoptosis of tumor cells. However, the work carried out so far has shown the effect of lipoxin only in TAMs polarized with conditioned medium from MV3, a human melanoma cell line. Herein, we investigate the effects of lipoxin on the profile of TAM derived from breast cancer. **Methods:** To obtain TAMs, macrophages derived from human monocytes were stimulated and differentiated to TAM with conditioned medium (CM) from two human breast cancer cells: (1) TAMs derived from MDAMB-231 (TAM/MDA); and (2) TAMs derived from MCF-7 (TAM/MCF-7). To investigate the lipoxin effects in TAM, cells were treated with ATL at 10nM. The phenotypic profile of TAMs was analyzed by flow cytometry, using specific antibodies (anti-CD86-APC and anti-CD206-PE). The analysis of ROS production was measured by labeled TAMs with luminol (100 μ M), while NO production was assessed with DAF-FM probe (5 μ M). Phagocytic activity was evaluated using FITC Dextran (1mg/ml). The content of TAM supernatant (SN) was assessed by ELISA assay. Next, the effects of polarized TAMs on tumor cells were analyzed by incubating them with the SN of TAMs treated or not with lipoxin. Migration was assessed using wound healing assay. Proliferation of tumor cells was performed with BrdU assay. **Results AND Conclusion:** TAMs polarized with CM from MDAMB-231 and MCF-7 have a similar phenotype, with high expression of CD206, characteristic of the M2-like profile. Notably, TAM/MDA showed low expression of M1 marker CD86. Lipoxin treatment in TAMs increased M1 marker expression, including CD86, production of ROS and increased the phagocytic activity of TAM/MDA and TAM/MCF-7. However, lipoxin treatment increased NO production, another M1 marker, only in TAM/MCF-7. Likewise, lipoxin treatment induces high secretion of IL-6 only in TAM/MCF7. The impact of TAMs on tumor cells showed that TAM/MDA-SN increased MDAMB-231 cell migration, with no effect from lipoxin treatment. Conversely, TAM/MCF-7-SN, when treated with lipoxin, decreased MCF-7 cell migration. Additionally, TAM/MCF-7-SN and TAM/MDA-SN did not alter proliferation of tumor cells, evidencing that cell-cell contact is necessary for ROS-induced tumor cell death. These findings indicate that CM-polarized TAMs from different breast cancer lines have similar functional profiles with M2-like characteristics. Lipoxin treatment induces a phenotypic shift from M2 to M1-like in TAMs, restoring their cytotoxic functions. However, lipoxin treatment appears to act differently on each TAM, impacting its action on each tumor cell lines.

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04.061 **Microglial Cannabinoid Receptor 2 Activation and Its Therapeutic Potential in Sepsis** Castro LVG¹, Guedes, LD¹, Souza BB^{1,2}, Pereira-Dutra F¹, Bozza PT¹, Castro-Faria-Neto HC¹, Gonçalves-de-Albuquerque CF^{1,2}, Silva AR¹ ¹IOC-Fiocruz, Immunopharmacology Lab and Postgraduate Program in Cellular and Molecular Biology, Rio de Janeiro, ²Unirio, Immunopharmacology Lab and Postgraduate Program in Molecular and Cellular Biology, Rio de Janeiro, Brazil

Introduction: Sepsis is a life-threatening condition characterized by organ dysfunction resulting from a dysregulated host response to infection. This condition leads to unresolved inflammation, tissue damage, and potential long-term sequelae, particularly in the central nervous system (CNS). *Klebsiella pneumoniae* is a pathogen common in immunocompromised ICU patients, being a leading cause of sepsis in newborns and adults and posing significant global health challenges due to its increasing antimicrobial resistance (AMR). Microglia are the resident immune cells and the primary line of defense of the CNS, being a prominent therapeutic target due to their multiple functions, maintaining homeostasis, and their central role in neuroinflammation. Increasing evidence suggests that cannabinoid receptor 2 (CB2R) serves as a promising anti-inflammatory target, primarily expressed in peripheral immune cells and microglia, which helps regulate cell activation and cytokine release. Peroxisome proliferator-activated receptor gamma (PPAR γ) has demonstrated efficacy in controlling the microglial phenotype, making it a key molecular target for mitigating neuroinflammation. This study aims to evaluate the role of the CB2 receptor and its relationship with PPAR γ activation in modulating microglia and its neuroprotective effects in sepsis, thereby slowing its progression. **Methods:** BV2 microglial cells were seeded in 24-well plates at a density of 5×10^4 per well in 500 μ L complete medium and incubated for 24 h. After this time, cells were treated with the CB2 agonist AM1241 (10 μ M) for 1 hour and then stimulated with *K. pneumoniae* for 24 h. Supernatant was collected for ELISA and total RNA for qRT-PCR. **Results:** *K. pneumoniae* triggered a pro-inflammatory response in microglia, leading to the production of pro-inflammatory mediators. AM1241 treatment resulted in significantly reduced levels of the pro-inflammatory cytokines IL-6 and TNF- α , also reducing IL-1 β mRNA expression. PPAR γ mRNA expression was upregulated in treated cells indicating a possible correlation between CB2 activation and the PPAR γ pathway. **Conclusion:** The *K. pneumoniae*-induced inflammation model demonstrated the successful stimulation of pro-inflammatory behavior in BV2 microglia cells. AM1241 showed anti-inflammatory effects by reducing the release and expression of pro-inflammatory cytokines. It also upregulated PPAR γ mRNA expression, indicating its possible involvement in the neuroprotective and anti-inflammatory properties usually associated with AM1241. These results suggest the need for further research into this correlation and its effects on microglia, which may become a promising therapeutic strategy for the development of new sepsis treatments and improved long-term cognitive outcomes. **Financial Support:** Supported by Capes, FAPERJ, CNPQ, UNIRIO, IOC, FIOCRUZ.

04.062 **Exploring the Therapeutic Potential of Luteolin and Quercetin Co-treatment in *E. coli*-induced Inflammation in BV-2 Cells.** Brito MADSM¹, Santos FSD², Cunha CMC³, Gomes-Reis GLS³, Ferreira AMS³, Costa MF³, Dutra FSP³, Bozza PT³, Farias-Neto HCC³, Silva AR³, Albuquerque CFG¹ Unirio¹, ²UFF, ³Fiocruz

Introduction: Sepsis is a life-threatening clinical syndrome marked by a dysregulated host immune response to an infection, leading to systemic inflammation and organ dysfunction (Singer *et al.*, 2016). The brain is often among the first organs affected, manifesting as sepsis-associated encephalopathy (SAE), a condition characterized by acute or chronic brain injury and a high risk of mortality (Gofton and Young, 2012). In the context of SAE, microglia are rapidly activated in response to systemic infections, adopting a pro-inflammatory phenotype that exacerbates neuroinflammation through the release of cytokines as TNF- α . Although this response is essential for pathogen clearance, excessive or sustained activation can lead to neuronal damage and impaired cognitive function (Liu *et al.*, 2022). Despite intensive care interventions, sepsis-related morbidity and mortality remain high, prompting ongoing research into novel therapies (Meyer and Prescott, 2024). Among potential alternatives, plant-derived compounds like the flavonoids luteolin (LUT) and quercetin (QUR) have shown antimicrobial, immunomodulatory, antioxidant, and neuroprotective properties. However, their therapeutic benefits may be limited by toxicity concerns (Bangar *et al.*, 2023; Georgiou *et al.*, 2023). In this context, drug combinations are suitable strategies to overcome this issue and are often multitargeted (Siqueira de Mello *et al.*, 2022), thus applicable to complex conditions as SAE. Our study aimed to evaluate the therapeutic potential of LUT-QUR co-treatment in BV-2 cells infected with *E. coli*. **Methods:** BV-2 microglial cells were cultured in RPMI1640 medium supplemented with 10% fetal bovine serum (FBS) and 1% penicilin/streptomycin. At 70-80% confluence, cells were seeded in 24-well plates and incubated for 24 hours. Subsequently, BV-2 cells were pre-treated for 3h with LUT and QUR, prepared as stock solutions in DMSO 0,1% and diluted in RPMI1640 medium supplemented with 2% FBS and 1% penicilin/streptomycin. Cells were then challenged with *E. coli* (ATCC 25922). Resazurin assay was performed to evaluate LUT:QUR cytotoxicity. Cytokine levels of TNF- α were quantified by ELISA. **Results:** The co-treatment of LUT:QUR (1:2) did not affect microglial survival. Furthermore, the individual concentrations of LUT (2 μ M) and QUR (4 μ M) were not effective in reducing TNF- α levels in BV-2. Notably, LUT:QUR (1:2) co-treatment significantly diminished TNF- α levels in BV-2 supernatants. **Conclusion:** Taken together, these findings suggest that LUT:QUR (1:2) treatment attenuates *E. coli*-induced microglial inflammation by modulating cytokine production without compromising cell viability. **Financial support:** CNPq, FAPERJ, CAPES, UNIRIO, FIOCRUZ, IOC, UFF.

04.063 **Therapeutic Potential of Citral in the Treatment of Periodontal Disease Induced by Systemic Microbial Challenge.** Solon IG¹, Passaglia P⁴, Trajano ⁴IP, Silva WS¹, Salvador SLS², Bulle D³P, Nascimento GC¹, Branco LGS¹ ¹FORP-USP, Dept Basic and Oral Biology, ²FCFRP-USP Dept Clinical Analyses, ³FORP-USP, Dept Oral and Maxillofacial Surgery and Periodontology, ⁴FMRP-USP, Dept Physiology

Introduction: Periodontitis is a chronic and noncommunicable inflammatory disease that affects the tooth-supporting tissues. Although conventional therapies are widely used, there is increasing interest in complementary approaches involving natural compounds with anti-inflammatory properties. Citral has well-documented anti-inflammatory and antibacterial activities; however, its role in periodontitis is not yet fully understood. Thus, we investigated the effects of citral in an experimental model of periodontitis induced by *Porphyromonas gingivalis* (Pg). **Methods:** Male BALB/c mice (6–8 weeks old, n = 8 per group) were used to induce periodontitis through six oral gavages on alternate days over a 12-day period. Two experimental protocols were established: (1) Citral (10 mg/kg) was administered concomitantly with *P. gingivalis* to evaluate its prophylactic effect; and (2) Citral was administered after periodontitis induction to evaluate its therapeutic effect. Forty-two days after the first gavage, the animals were euthanized, and blood, jaws, and gingival tissues were collected. The inflammatory response in the plasma and gingival tissue was assessed by ELISA: IL-6, IL-10, TNF- α , and IL-1 β levels. Alveolar bone loss was evaluated by histological, histometric, and micro-CT analyses. The activity of matrix metalloproteinases (MMPs) was assessed in the gingival tissue. Statistical analysis was performed using one- or two-way ANOVA, followed by Tukey's post hoc test ($p < 0.05$). **Results:** Citral treatment reduced IL-6, IL-10, TNF- α , and IL-1 β production compared with the Pg-treated group in both the prophylactic and therapeutic protocols. Alveolar bone loss was confirmed by histological, histometric and microCT analyses. A significant reduction in bone loss was observed in the prophylactic citral-treated group ($p < 0.05$), supporting citral's potential as a complementary therapeutic agent in the management of periodontitis. In contrast, citral treatment in the therapeutic group did not reduce bone loss. No interference in body weight gain was observed in either the prophylactic or therapeutic groups, nor in the Pg or citral-treated experimental groups. Prophylactic administration of citral reduced MMPs activity ($p < 0.05$), indicating decreased tissue degradation in citral-treated animals compared to the experimental group. However, in animals receiving curative citral treatment, no difference in tissue degradation was observed when compared to the experimental group. **Conclusion:** Administration of citral reduced both inflammation and bone loss, demonstrating a protective effect against the progression of systemically induced periodontal disease. **Financial support:** FAPESP grant number 2024/03285-0.

04.064 LPS- and LTA-Induced Epididymitis Modifies the Transcriptional Profile of Protein Disulfide Isomerase (PDI) Genes in Mice. Andrade AD^{1,2}, Silva EJR¹, Avellar MCW² ¹Unesp, Dept Biophysics & Pharmacology, Brazil. ²Unifesp-EPM Dept Pharmacology, Brazil

Introduction: Protein disulfide isomerases (PDIs) are a multifunctional family that is primarily involved in proper protein folding and quality control in the endoplasmic reticulum, and may also act as hormone-binding proteins, among other functions. Encoded by at least 21 genes, we previously characterized the transcriptional profile of *Pdi* genes in the developing rat epididymis. To investigate their specific role in epididymal biology, here we tested the hypothesis that epididymal *Pdi* gene expression is altered under inflammatory conditions induced by intravascular injections of lipopolysaccharide (LPS; TLR4 agonist) and lipoteichoic acid (LTA; TLR2/TLR6 agonist), via NF- κ B transcription factor, which are preclinical models of acute epididymitis. **Methods:** Epididymitis was induced in anesthetized adult male C57BL/6 mice (90 days) by retrograde intravascular injection of either ultrapure LPS from *E. coli* (50 μ g) or LTA purified from *S. aureus* (125 μ g) into the vas deferens towards the cauda epididymis. Control groups received sterile saline (vehicle). Mice were euthanized 2, 6, 24, and 72 h post-injection. Additional groups were pretreated with the NF- κ B inhibitor PDTC (100 mg/kg body weight, i.p.) 1 h prior to epididymitis induction, and mice were euthanized 6 and 24 h after PDTC treatment. The cauda epididymis was collected and processed for RT-qPCR to determine the transcript levels of selected *Pdi* genes (*P4hb*, *Pdia3*, *Pdia4*, *Pdia5*, *Pdia6* and *Erp44*), with the *Rpl19* gene serving as the endogenous control. Data were analyzed by ANOVA, followed by the Bonferroni test ($p < 0.05$ was considered significant). **Results:** Following LPS challenge, *P4hb*, *Pdia4*, and *Erp44* mRNAs exhibited a biphasic response: downregulation at 6 h (fold-change ~ 1.5 , ~ 1.8 and ~ 1.5 , respectively) and upregulation at 24 h (~ 1.4 , ~ 1.7 , and ~ 1.5 , respectively) compared to saline controls. *Pdia5* was transiently downregulated at 6 h (~ 1.6 and ~ 2.1 , respectively), and *Pdia6* at 6 and 72 h (~ 2.1 and ~ 1.6 , respectively), while *Pdia3* was only upregulated at 24 h (~ 1.6). In contrast, LTA treatment induced peak expression of *P4hb*, *Pdia3*, *Pdia4*, *Pdia5*, and *Erp44* mRNAs at 24 h (~ 1.9 , ~ 1.7 , ~ 1.5 , ~ 1.4 , and ~ 1.5 , respectively), while *Pdia6* was downregulated at 72 h (~ 2.3) relative to saline controls. Pre-treatment with PDTC prevented the LPS- and LTA-induced changes in *Pdi* transcript levels at 6 h and 24 h. **Conclusion:** Our findings demonstrate that activation of the TLR4 and TLR2/TLR6 signaling pathways, through NF- κ B activation, differentially regulates *Pdi* gene expression in the cauda epididymis during inflammation. This suggests that specific members of the *Pdi* gene family play distinct roles in mediating epididymal responses to acute inflammatory stimuli, which may influence epididymal function and consequently male reproductive health. **Ethics approval:** 6272050320-IBB/CEUA. **Support:** FAPESP/CAPES/CNPq.

04.065 **Oral JME-209, a Mexiletine Analogue, Prevents Glucocorticoid-Resistant Pulmonary Neutrophilic Inflammation and Atelectasis in a Mouse Model of Acute Lung Injury.** Gomes HS¹, Coutinho DS¹, Cotias AC¹, Gaze LA¹, Costa JCS², Carvalho VF¹, Silva PMR¹, Martins MA¹ ¹IOC-FIOCRUZ, Lab Inflammation, Brazil. ²Fiocruz, Production and Innovation in Health, Brazil

Introduction: Obstructive inflammatory lung diseases such as severe asthma and chronic obstructive pulmonary disease (COPD) remain important causes of global morbidity and mortality. A hallmark of these conditions is the persistent airway inflammation, airway obstruction and tissue remodelling. Although glucocorticoids are the mainstream treatment for these conditions, approximately 5 to 10% of asthmatics and the vast majority of those with COPD do not achieve symptom control, even when receiving high doses of systemic steroid-based anti-inflammatory medications, highlighting the urgent need for alternative therapeutic strategies. Prior investigations have demonstrated that local anaesthetics, such as lidocaine and mexiletine, can inhibit bronchospasm and inflammation in animal models and patients. However, adverse side effects, mainly related to local anaesthesia of the airways, have limited their efficacy and putative use in treating lung inflammatory diseases. We report here the effects of JME-209, 1-([1,1'-biphenyl]-4-aryloxy)-2-propanamine, a novel mexiletine derivative with optimised bronchodilator and anti-inflammatory properties, in a glucocorticoid-resistant model of lung inflammation induced by repeated LPS instillations. **Methods:** Male A/J mice were subjected to oropharyngeal aspiration of LPS for three consecutive days. Oral treatments with dexamethasone at 1 mg/Kg dose or JME-209 at doses of 0.3, 1, and 3 mg/kg were administered 1 hour before each LPS instillation. Twenty-four hours after the last LPS exposure, assessments were conducted, including MicroCT imaging, lung elastance measurement, airway leukocyte infiltration, catalase activity, lipid peroxidation levels, and NRF2 expression by Western blotting. FIOCRUZ's ethics committee, CEUA, previously approved all procedures involving the use of experimental animals – L002/2020-A6. The data were analysed using parametric One-Way or Two-Way ANOVA with a post-hoc Tukey test (significance set at $p < 0.05$). **Results:** While the glucocorticoid dexamethasone was ineffective across all evaluated parameters, oral treatment with JME-209 at 3 mg/kg significantly blocked the increased lung elastance responses by 95%, reduced neutrophil accumulation in the airways by 50%, and attenuated oxidative damage by 64,7 % through restoration of catalase activity via a mechanism involving NRF2 activation. Additionally, MicroCT imaging revealed that JME-209 at the same dose attenuated the extension of non-aerated lung regions (atelectatic areas) by 46%, following triple LPS challenge. **Conclusion:** These findings highlight the therapeutic potential of JME-209 as an effective strategy for controlling steroid-insensitive neutrophilic airway inflammation. MicroCT analysis further demonstrated the effectiveness of orally administered JME-209 in preventing the formation of atelectatic regions, reinforcing its relevance for treating chronic lung diseases such as severe asthma and COPD. **Financial support:** CAPES, CNPq and FAPERJ.

04.066 *Arthrospira platensis* Modulates Inflammatory Cytokines in the Intestine and Liver: A Preventive Approach Against Obesity Induced by a Hypercaloric Diet. Diniz AFA^{1,2}, Arruda RRA^{1,2}, Silva JMA^{1,2}, Ribeiro SB⁴, Lima CAS⁴, Sousa Filho JECS³, Melo MB³, Soares MFS^{1, 2}, Claudino BFO^{1,2}, Barros BC^{1,2}, Araújo AA⁴, Silva BA^{1,2,3} ¹UFPB, Prof. George Thomas Functional Pharmacology Lab, Institute of Research in Drugs and Medicines, Brazil ²UFPB, PPG Bioactive Natural and Synthetic Products, Brazil ³UFPB, Department of Pharmaceutical Sciences, Health Sciences Center, Brazil ⁴UFRN, Lab Experimental Model of Intestinal Mucositis, PPG Pharmaceutical Sciences, Brazil

Introduction: *Arthrospira platensis* (AP) is a cyanobacterium widely consumed as a dietary supplement, known for its antioxidant, immunomodulatory, and anti-inflammatory properties. However, studies evaluating its preventive effects on intestinal and hepatic cytokine modulation in experimental models of hypercaloric diet-induced obesity are still limited. Obesity is characterized by a chronic low-grade inflammatory state with increased levels of pro-inflammatory cytokines, such as interleukin-1 beta (IL-1 β), tumor necrosis factor-alpha (TNF- α), and interleukin-6 (IL-6), along with reduced interleukin-10 (IL-10), an anti-inflammatory cytokine. This imbalance significantly contributes to the pathogenesis of metabolic comorbidities including insulin resistance, dyslipidemia, and hepatic diseases (Aimaretti, E. *Nutrients*, v. 16, p. 552, 2024). This study aimed to investigate the preventive effects of AP supplementation on the expression of pro- and anti-inflammatory cytokines in the ileum and liver of male Wistar rats fed a hypercaloric diet.

Methods: Animals were randomly assigned to three experimental groups (n=5): standard diet (SD), hypercaloric diet (HCD), and hypercaloric diet supplemented with *A. platensis* 25 mg/kg (HCD+SP25), by gavage once daily for 8 weeks. At the end of the experimental period, ileum and liver samples were collected for quantitative analysis of IL-10, IL-6, IL-1 β , and TNF- α levels using enzyme-linked immunosorbent assay. **Results:** AP supplementation effectively prevented the excessive increase in pro-inflammatory cytokines in both tissues compared to the HCD group. Specifically, in the HCD+SP25 group, IL-6 levels were 87.8 $\pm$ 11.3 pg/mg in the ileum and 915 $\pm$ 22.4 pg/mg in the liver; IL-1 β levels were 113.1 $\pm$ 36.9 pg/mg in the ileum and 816.3 $\pm$ 195.8 pg/mg in the liver; and TNF- α levels were 45.2 $\pm$ 11.8 pg/mg in the ileum and 1053 $\pm$ 25.5 pg/mg in the liver. These values were significantly lower than those observed in the HCD group: IL-6 (87.8 $\pm$ 11.3; 1772 $\pm$ 220.3 pg/mg), IL-1 β (132.7 $\pm$ 6.9; 2158 $\pm$ 160.1 pg/mg), and TNF- α (337.5 $\pm$ 52.6; 1836 $\pm$ 55.4 pg/mg). Additionally, AP supplementation significantly increased IL-10 production in both the ileum (734.5 $\pm$ 71.5 pg/mg) and liver (2300 $\pm$ 200.1 pg/mg) compared to the HCD group. **Conclusion:** These findings suggest that *A. platensis* has preventive immunomodulatory effects, attenuating the chronic inflammatory response induced by hypercaloric diets and potentially contributing to the prevention of obesity-associated metabolic disorders. Further studies are necessary to elucidate the molecular mechanisms underlying these effects, particularly focusing on nuclear factor kappa B (NF- κ B) and nuclear factor erythroid 2-related factor 2 (Nrf2) pathways, which are critically involved in inflammation and oxidative stress regulation. **Ethical committee number:** 6914241024 (ID 002274). **Acknowledgements:** CNPq, CAPES, CFF.

04.067 **Micro-Computed Tomography (MicroCT) as a Non-Invasive Tool for Longitudinal Assessment of Lung Alterations in an Experimental Model of Elastase-Induced Pulmonary Emphysema.** Ferreira TPT, Arantes ACS, Silva KN, Cotias AC, Martins MA, Silva PMR IOC-Fiocruz, Lab Inflammation, Rio de Janeiro, Brazil

Introduction: Chronic Obstructive Pulmonary Disease (COPD) is a progressive and debilitating respiratory disorder, ranking among the leading global causes of morbidity and mortality. Emphysema, one of the pivotal manifestations of COPD, is characterized by irreversible alveolar wall destruction and pulmonary hyperinflation, resulting in impaired gas exchange. Reliable and sensitive animal models of COPD are essential for understanding emphysema pathophysiology and testing potential therapies. In this context, micro-computed tomography (MicroCT) has emerged as a powerful non-invasive imaging modality, enabling high-resolution, three-dimensional visualization of pulmonary changes in small animals, including accurate quantification of aerated versus non-aerated lung areas. **Methods:** Female C57BL/6 mice were subjected to oropharyngeal aspiration of either porcine pancreatic elastase (PPE), at doses of 0.25 U or 0.5 U/animal or sterile saline (control). Evaluations were conducted at 7, 14, 21, and 28 days post-PPE challenge. All procedures were approved by the Animal Ethics Committee of the IOC-FIOCRUZ (CEUA L-001/19A6). The following endpoints were assessed: i) Radiological changes via MicroCT: lung volume, tissue density, and aeration distribution; ii) Leukocyte infiltration in bronchoalveolar lavage fluid (BALF); iii) Histopathological alterations in lung tissue. **Results:** PPE instillation induced marked pulmonary damage in a time- and dose-dependent manner, with the most significant changes observed in the 0.5 U group. MicroCT analysis revealed progressive hyperinflation and parenchymal destruction, indicated by increased dark (low-density) areas consistent with hyper-aerated regions. Quantitative imaging showed a gradual increase in total lung volume and expansion of hyperinflated zones over the 28-day period. BALF analysis at day 28 confirmed sustained inflammation, with elevated total leukocyte counts, predominated by mononuclear cells, and a moderate increase in neutrophils numbers. Histological evaluation corroborated these findings, demonstrating alveolar wall destruction, enlarged airspaces, and loss of parenchymal integrity, particularly in the higher dose group, classic features of emphysema. **Conclusion:** The PPE-induced emphysema model in C57BL/6 mice is a robust and reproducible experimental platform that accurately reflects the progressive structural and inflammatory features of human COPD. MicroCT imaging proved to be an excellent non-invasive tool for longitudinal assessment of lung damage, especially in tracking the temporal and dose-dependent progression of hyperaeration. These findings support the utility of this model for future pathophysiological studies and the preclinical evaluation of novel therapeutic strategies targeting emphysema. **Financial support:** FIOCRUZ, FAPERJ, and CNPq (Brazil).

04.068 **Tyrosine Kinase Inhibitor Reduces Systemic and Cerebral Inflammation in Experimental Sepsis.** Cunha CMCD^{1,2,3}, Moraes BPT^{1,3,4}, Abreu VHP^{1,3}, Soares GVM^{1,3}, Moraes-de-Souza IM^{1,2,3}, Almeida MAP¹, Estado V¹, Sayão PGF^{1,3,4}, Souto HA^{1,3}, Bozza PT^{1,2}, Castro-Faria-Neto HC^{1,2}, Silva AR^{1,2}, Gonçalves-de-Albuquerque CF^{1,2,3,4} ¹IOC-Fiocruz, Immunopharmacology Lab, ²IOC-Fiocruz Postgraduate Program in Cellular and Molecular Biology, ³Unirio, Immunopharmacology Lab, Rio de Janeiro, Brazil. ⁴Unirio, Postgraduate Program in Molecular and Cellular Biology

Introduction: Sepsis is a leading cause of death worldwide (1). Its pathophysiology involves dysregulated inflammation, increased inflammatory mediators, increased leukocyte infiltration, vascular changes, and neuronal impairments (2). Sepsis-associated encephalopathy affects around 70% of septic patients and contributes to a worse prognosis (3). Src family kinases (SFKs) are a family of protein kinases involved in regulating chemotaxis, cell adhesion and migration, cytokine secretion, and endothelial permeability. Inhibition of SFK attenuates exacerbated leukocyte and endothelial responses in inflammatory reactions (4-7). Bosutinib is a multiple kinase inhibitor clinically used in the treatment of chronic myeloid leukemia with a potent effect on SFK (8). This study investigates the potential of bosutinib as a treatment for sepsis. **Methods:** Swiss mice were divided into 4 groups: Sham+Bosutinib; Sham+Vehicle; Cecal Ligation and Puncture (CLP)+Bosutinib, and CLP+Vehicle. CLP was used to induce a polymicrobial sepsis model. Treatments were administered orally 30 minutes before and 6 hours after surgery with bosutinib (3 mg/kg) or vehicle. Survival analysis was performed for 7 days, and clinical score assessment was performed 24 hours after surgery. Also 24 hours later, peritoneal lavage (PL), plasma, and brain were collected. Intravital microscopy was performed to assess leukocyte-endothelial adhesion, capillary density, and cerebral blood perfusion. Cytokines and chemokines were measured by enzyme-linked immunosorbent assay in PL, plasma, and brain tissue. PL were cultured to analyze the growth of colony-forming units, the total and differential leukocyte count was performed on these samples. All experimental procedures were approved by the Animal Use Ethics Committee of IOC (L015/2015; L026/2022) and UNIRIO (2019/03). Results and **Conclusion:** Bosutinib improved clinical scores and survival in sepsis, reduced sepsis-induced cytokine levels in PL, plasma, and brain tissue. It reduced leukocyte recruitment, mainly neutrophils, and bacterial growth in the PL of CLP animals. Bosutinib treatment also improved capillary density and blood perfusion, and reduced leukocyte recruitment and adhesion in the cerebral microcirculation of septic mice. These results demonstrate that bosutinib modulates leukocyte recruitment and attenuates systemic and cerebral dysregulated inflammation and neurovascular alterations in experimental sepsis. Funding: FAPERJ, UNIRIO, CNPq, CAPES (Grant 001), IOC, FIOCRUZ, BCM.

05. Pain and Nociception Pharmacology

05.001 **Unravelling the Anterior Cingulate Cortex Nitric Oxide Relevance in the Effects of Electroacupuncture on the Anxiety and Neuropathic Pain Interface in Rats.** Ribeiro Siqueira CM¹, Donizetti-Fonseca MA¹, de Souza MBRF¹, da Silva AL^{1,2}, da Silva ML^{1,2}, da Silva JRT^{1,2}, Falconi-Sobrinho LL^{1,2} ¹Unifal, Lab. of Neuroscience, Neuromodulation and Study of Pain, Brazil, ²Unifal, Postgraduate Program in Biosciences Applied to Health, Brazil

Introduction: It is known that chronic pain can trigger emotional disorders such as anxiety. Growing evidence shows that electroacupuncture (EA) may be a relevant adjuvant therapy in the management of anxiety related to neuropathic pain. In particular, recent studies in rodents have suggested that the anterior cingulate cortex (ACC) is implicated in the modulation of the pain-anxiety interface via nitrergic mechanisms. However, the influence of nitric oxide (NO) in the ACC in mediating the effects of EA on anxiety and allodynia related to peripheral neuropathy is unknown.

Aims: Our proposal was to investigate whether the decrease in nitrergic activity of the ACC through local microinjections of the NO inhibitor N(omega)-propyl-L-arginine (NPLA), potentiates the effects of a single application of EA on allodynia and anxiety related to peripheral neuropathy.

Methods: Male Wistar rats, initially weighing 120 g, had their mechanical nociceptive thresholds (baseline 1: BL1) measured through von Frey test. After 24h of BL1, the rodents were subjected to chronic constriction injury (CCI) of the ischiatic nerve. After 14 days of CCI, the animals had their mechanical nociceptive thresholds measured again (baseline 2: B2). The next day (15st), they underwent stereotaxic surgery to implant a guide cannula directed to area 24b (Cg1) of the ACC. On the 21st day of CCI, the rodents had their mechanical nociceptive thresholds measured by von Frey test (baseline 3: B3) and were treated with EA (single application, 2 Hz, acupoints ST36 and SP6, 20 min). After 24h of EA, the rats received microinjections of either physiological saline (vehicle) or NPLA (0.4 nmol/0.2 µL) in the ACC followed by Von Frey test at 5-min intervals for 30 min. After the von Frey test, the animals were subjected to the elevated plus maze (EPM) test, in which the time spent in the open and closed arms as well as the frequency of risk assessment were recorded.

Results: According to a two-way RM-ANOVA, there were significant effects of treatment ($F_{3,12} = 14.24$; $p < 0.001$), time ($F_{6,24} = 7.694$; $p < 0.001$) and in the interaction between them ($F_{18,72} = 23.6$; $p < 0.001$) on mechanical allodynia. CCI in the right paw induced neuropathic pain over 21 days ($p < 0.05$). A single application of EA in the right paw + Veh (ACC) was not able to reverse the mechanical allodynia induced by CCI ($p > 0.05$). In turn, single application of EA in the right paw + NPLA (ACC) increased mechanical nociceptive thresholds (antinociception) for 10 minutes ($p < 0.05$). According to Student's t-test, rats that received EA + NPLA (ACC) spent more time in the open arms of the EPM ($t = 3,130$, $df = 8$; $p < 0.05$) and exhibited less risk assessment behaviour in this same apparatus ($t = 3,776$, $df = 7$; $p < 0.01$), when compared with control animals that received EA in the right paw + vehicle (ACC). **Conclusion:** Our findings suggest that decreased nitrergic activity in the ACC potentiates the modulatory effects of EA on allodynia and anxiety-like behaviours triggered in animals with peripheral neuropathy. Ethical approved (CEUA-UNIFAL, 0041/2024). Financial support (FAPEMIG and PROBIC- UNIFAL).

05.002 **Antinociceptive Evaluation of the Ethanolic Extract from Bark and Leaves of *Abarema cochliacarpus* in an Animal Pain Model.** Guedes GN¹, Macario VSG¹, Oliveira BCCA¹, Rezende B¹, Bazilio YS¹, Maia EP¹, Martins RCC², Montes GC¹ ¹UERJ, Roberto Alcântara Gomes Institute of Biology (IBRAG), Lab of Pharmacology and Psychobiology, Brazil; ²UFRJ, Walter Mors Institute of Research on Natural Products (IPPN), Brazil

Introduction: *Abarema cochliacarpus* is a plant known for its analgesic and anti-inflammatory properties, attributed to the presence of flavonoids and saponins. This study aimed to evaluate its antinociceptive activity using an animal model of pain. **Methods:** This study was approved by the Ethics Committee on Animal Use (CEUA/IBRAG/UERJ – protocol nº 014/2022). Pain was induced in mice via intraplantar injection of 2.5% formalin in the hind paw, assessing both the neurogenic phase (0–5 min) and the inflammatory phase (15–45 min). Male and female mice (n = 8 per group) were treated intraperitoneally (i.p.) with vehicle (DMSO), acetylsalicylic acid (ASA, 150 mg/kg), morphine (10 mg/kg), or ethanolic extracts of *Abarema cochliacarpus* bark (EECA) and leaves (EEFA) at doses of 1, 10, and 30 mg/kg, 30 minutes before formalin injection. Pain response times were recorded for each phase. Data were analyzed statistically ($p < 0.05$) and expressed as mean $\pm$ standard error of the mean (SEM). To investigate the involvement of opioid and cholinergic pathways, naloxone and atropine (1 mg/kg) were administered 15 minutes before the 30 mg/kg extract dose. **Results:** EECA exhibited antinociceptive activity in the neurogenic phase. In males, response time decreased from 56 ± 4.9 s (vehicle) to 46 ± 9.2 s, 43 ± 5.3 s, and 29 ± 6.0 s at 1, 10, and 30 mg/kg, respectively. In females, times were reduced from 72 ± 8.5 s (vehicle) to 53 ± 6.6 s, 49 ± 11.7 s, and 28 ± 4.6 s. During the inflammatory phase, male response times dropped from 123 ± 13.1 s (vehicle) to 119 ± 22.1 s, 113 ± 18.2 s, and 28 ± 9.7 s. In females, times decreased from 182 ± 13.3 s to 107 ± 34.8 s, 152 ± 21.8 s, and 22 ± 22.0 s. For the 30 mg/kg dose, naloxone reversed the neurogenic effect in females (61 ± 11.9 s) and males (53 ± 6.4 s), while a partial reversal in the inflammatory phase was observed with naloxone (45 ± 24.9 s) and atropine (46 ± 16.0 s) in females, and 60 ± 16.2 s and 80 ± 29.7 s in males, suggesting the involvement of both pathways. EEFA also showed neurogenic antinociceptive activity. In males, response time decreased from 56 ± 4.9 s to 32 ± 8.0 s, 44 ± 4.1 s, and 57 ± 4.5 s. In females, times were 73 ± 7.4 s (vehicle) versus 81 ± 6.2 s, 52 ± 5.6 s, and 28 ± 5.3 s. In the inflammatory phase, male times dropped from 123 ± 13.1 s to 28 ± 14.7 s, 23 ± 8.7 s, and 7.5 ± 2.8 s; in females, from 164 ± 20.9 s to 109 ± 33.4 s, 1.5 ± 0.6 s, and 0.4 ± 0.4 s. Partial reversal of EEFA's neurogenic effect was observed in females with naloxone (65 ± 3.7 s) and atropine (59 ± 6.6 s), supporting the participation of these pathways. **Conclusion:** Ethanolic extracts from bark and leaves of *Abarema cochliacarpus* demonstrate significant antinociceptive activity, especially at 30 mg/kg, likely involving opioid and cholinergic mechanisms. Funding: FAPERJ, CAPES, CNPq, PIBIC/UERJ, IPPN/UFRJ.

05.003 Evaluation of the Antinociceptive and Anti-Inflammatory Profile of New P2X7 Receptor Antagonist Derivatives. Valério AMF¹, Gaspar LCSG¹, Faria RX², Salles JP¹, Miranda ALP¹ ¹FF-UFRJ, Lab Estudos em Farmacologia Experimental (LEFEx), Brasil. ²IOC-Fiocruz, Lab for Environmental Health Assessment and Promotion, Instituto Oswaldo Cruz, Brasil

Introduction: Inflammatory pain is characterized by the activation and sensitization of peripheral nerves by inflammatory cytokines, and it is common in conditions such as rheumatoid arthritis. Chronic pain, on the other hand, can persist even after the initial injury or inflammation has been resolved, becoming a debilitating condition, impacting significantly the quality of life. Among the therapeutic targets currently being studied for the treatment of inflammatory pain, there is the P2X7 receptor, an ion channel belonging to the P2X subfamily of purinergic receptors. This receptor is widely expressed in cells of the innate and adaptive immune system. Its activation is associated with the release of inflammatory cytokines and chemokines, as well as promoting events such as inflammation, proliferation, differentiation and cell death. **Objective:** To evaluate the antinociceptive and anti-inflammatory activity of new P2X7R receptor antagonist derivatives *in vivo* using the Formalin and Complete Freund's Adjuvant (CFA) induced inflammatory pain models in mice. **Methodology:** Eight-week-old adult Swiss Webster mice of both sexes were divided into experimental groups: vehicle group (gum arabic 5%), standard group (Dexamethasone 10 mg/kg) and treatment groups, PMM 80-17 and PMM 07-18, at doses of 1 and 10 mg/kg, orally administered (CEUA/UFRJ 054/22). Formalin was injected intraplantarly (20 µL; 2.5%) to animals' right hind paw. The nociceptive response is assessed by the time required to lick the paw in two phases: 0-5 minutes (neurogenic phase) and 15-30 minutes (inflammatory phase). In the CFA model, the animals received an intraplantar injection of CFA (20 µL) and pain and inflammation parameters were monitored for 15 days: mechanical allodynia/hyperalgesia (von Frey filaments), paw thickness (edema) and locomotor activity (open field test). At the end of the experiment, the animals were euthanized, and samples of plasma, plantar tissue and spinal cord were collected for biochemical and histological analysis. **Results:** In the formalin model, derivatives were able to be inhibited only the inflammatory phase. PMM 07-18 showed anti-hyperalgesic activity with 45% of inhibition at a dose of 10mg/kg, while PMM 80-17 inhibited by 20% at 1mg/kg, suggesting a anti-inflammatory potential. In the CFA model, repeated doses of 1 mg/kg of PMM 80-17 derivative promoted a significant reduction in pain responses to mechanical stimuli (*p<0.05), while PMM 07-18 did not show relevant effect even at doses of 10 mg/kg. **Conclusions:** These results show that the compounds have a promising action in the acute phase of inflammation and that at least one of them may have potential in context of chronic inflammation, although further studies are needed to confirm and optimize the activity. **Financial support:** CNPq, PIBIC/CNPq/UFRJ, FAPERJ. **References:** Savio LEB, Andrade Mello P, Silva CG, Coutinho-Silva R. The P2X7 Receptor in Inflammatory Diseases: Angel or Demon? *Front Pharmacol* 9:52, 2018. Treede R-D, Rief W, Barke A et al. Chronic pain as a symptom or a disease: the IASP Classification of Chronic Pain for the ICD-11. *Pain* 160:19–27, 2019. Raja SN, Carr DB, Cohen M et al. The revised IASP definition of pain: concepts, challenges, and compromises. *Pain* 161:1976-1982, 2020. Tjølsen A, Berge OG, Hunskaar S, Rosland JH, Hole K. The formalin test: an evaluation of the method. *Pain* 51:5-17, 1992.

05.004 TRPV4 Channel Participates in the Nociception and Spinal Neuroinflammation involved in a Model of Complex Regional Pain Syndrome Type I In Mice. Brito HA¹, Ruviano NA², Rodrigues P¹, Antoniazzi C¹, Viero FT¹, Peres DS¹, Frare JM², Trevisan G^{1,2} ¹UFMS, PPG Pharmacology, Brazil, ²UFMS, PPG Biological Sciences: Toxicological Biochemistry, Brazil

Complex Regional Pain Syndrome Type I (CRPS-I) is a debilitating pain condition that can arise after limb trauma, such as surgeries, fractures, and ischemia/reperfusion injury. Currently, there is no effective pharmacological treatment for the chronic pain associated with CRPS-I, highlighting the urgent need to identify new therapeutic targets. A potential target is the transient receptor potential vanilloid 4 (TRPV4), which is involved in the mechanisms that cause neuropathic and inflammatory pain. Therefore, the aim of the present study was to evaluate the role of the TRPV4 channel in nociception and neuroinflammation in chronic post-ischemia pain (CPIP). CPIP was the chosen animal model for inducing CRPS-I in mice. In this model, the blood flow to the animal's hind paw is blocked through the application of a tourniquet, promoting symptoms similar to those observed in humans. All experimental protocols were approved by the Animal Ethics Committee of the Federal University of Santa Maria (protocol #5749300620). Nociceptive tests were conducted to assess mechanical allodynia (vonFrey test), thermal hypersensitivity (hotplate test), body weight, and motor function, coordination, and balance impairments (rotarod test) using male C57BL/6 mice. These assessments were performed before (baseline) and after CPIP induction on days 1, 5, 10, and 15. Furthermore, after repeated treatment for 15 days, the antinociceptive and neuroprotective effects of the TRPV4 antagonist (HC-067047, 1 mg/kg, intraperitoneally) were evaluated. Open field testing was performed to evaluate the locomotor activity on days 1 and 15 post-CPIP induction, and nest-building behavior was assessed from day 15 to 16 to assess possible spontaneous nociceptive behavior. On day 16, the animals were euthanized and samples of spinal cord were harvested for hydrogen peroxide (H₂O₂) and nitric oxide (NO) levels analysis. In addition, gene expression of *Par2*, *Trpv4*, *Gfap*, and *Nrf2* by PCR was also evaluated in spinal cord samples. Following CPIP induction, animals exhibited mechanical allodynia and thermal hypersensitivity, without significant changes in body weight or locomotor performance. Repeated administration of HC-067047 showed antinociceptive effects from day 5 to day 15 post-CPIP induction (87,73 ± 4% for mechanical allodynia and 49 ± 7% for thermal hypersensitivity). The TRPV4 antagonist also increased the time spent in the central zone of the open field and helped preserve typical nest-building behavior, in contrast to the altered behavior observed in CPIP Vehicle (CPIP-Veh) animals. Elevated levels of putative endogenous TRPV4 ligands such as H₂O₂ and NO were observed in CPIP-Veh mice and were reduced after 15 days of treatment with HC-067047 (53% ± 7% and 66% ± 14%; respectively). CPIP induction also led to increased gene expression of *Par2*, *Trpv4*, *Gfap*, and *Nrf2*. Treatment with HC-067047 reduced the expression of these markers (32% ± 3% *Par2*, 67% ± 24% *Gfap* and 73% ± 69% *Nrf2*), except for *Trpv4*, which remained elevated. In conclusion, this study demonstrates that the TRPV4 channel may play a significant role in the nociceptive mechanisms induced in a mouse model of CRPS-I. **Financial support:** CNPq and CAPES.

05.005 **Analgesic Effectiveness According to Pain Intensities and Locations in Pediatric Cancer Patients.** Marotta FC¹, Leal EL², Oliveira IA¹, Gonçalves TG¹, Santos PCJL¹ ¹Unifesp-EPM, Dept Pharmacology, Brazil, ²Unifesp, Grupo de Apoio ao Adolescente e Criança com Câncer, Brazil

Background: Cancer in children and adolescents (ages 0 to 19) is frequently associated with pain, which is one of the most common and distressing symptoms reported by patients. Effective pain management remains a major concern for healthcare teams. Despite the existence of national and international pain management protocols since the mid-1980s, challenges persist in the assessment, treatment, and follow-up of pediatric patients. There is a lack of studies evaluating the most appropriate type and dosage of analgesics to achieve adequate pain control in pediatric oncology settings. **Objective:** To assess the effectiveness of selected analgesics based on pain intensity and anatomical location in pediatric cancer patients. **Methods:** This is a pharmacoepidemiologic study carried out in a pediatric oncology hospital, with inclusion of patients aged 0 to 17 years old with cancer and using analgesic drug. Information regarding cancer diagnosis, hospitalization diagnosis, analgesic scale and pain intensity before and after drug administration and pain site were collected from medical records. **Results:** A total of 1,465 episodes of pain from 335 patients were included, most of them diagnosed with leukemia (30.1%). We included 576 episodes of pain treated with dipyrone or morphine that occurred in the abdomen (n=283), head (n=155) and lower limbs (n=138). When pain was mild to moderate, dipyrone was the most used drug: 105 (65.2%) episodes of pain that occurred in the abdomen, 93 (86.9%) in the head and 50 (64.1%) in the lower limbs. However, when the pain was severe to unbearable, morphine was the most used drug: 79 (64.7%) in the abdomen and 36 (60.6%) in the lower limbs; except in the head (17 episodes of pain, 35.4%). The final pain scores indicated pharmacological effectiveness in all patient subgroups. Depending on pain intensity, mean initial pain scores ranged from 4 to 8, while mean final scores ranged from 0 to 1. Administration of dipyrone led to pain intensity reductions ranging from 84% to 98%, depending on pain intensity. When treated with morphine, pain intensity was reduced between 80% and 97%. **Conclusion:** The use of dipyrone and morphine, guided by pain intensities and locations, demonstrated effectiveness. These findings support the tailored use of analgesics according to pain characteristics to optimize symptom control in pediatric oncology patients. Funding: FAPESP (Fundação de amparo à pesquisa do Estado de São Paulo) 2024/07284-9 Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki, and approved by the Research Ethics Committee of the FEDERAL UNIVERSITY OF SÃO PAULO (64195522.8.0000.5505) and by the scientific committee of the GRAACC HOSPITAL (IOP-007-2020).

05.006 **Chemical Profile and Antinociceptive and Anti-inflammatory Activities of Ethyl Acetate and Butanolic Fractions of the Endophytic Fungus *Schizophyllum commune* Isolated from *Tocoyena bullata*.** Santos LL, Melo EDN, dos Santos MB, Guimarães DO, Muzitano MF, Carmo PL, Pinto SC UFRJ-Macaé, Lab of Bioactive Products, Federal University of Rio de Janeiro, Brazil, Multidisciplinary Center

Introduction: The search for safer, more effective analgesic and anti-inflammatory drugs continues. In this context, the endophytic fungus *Schizophyllum commune*, isolated from the plant *Tocoyena bullata*, has previously demonstrated anti-inflammatory activity *in vitro*. **Objective:** To evaluate the *in vivo* antinociceptive and anti-inflammatory effects of ethyl acetate (EtOAc) and butanol (BuOH) fractions obtained from the partition of the ethanol extract of the fungus *S. commune* cultivated in rice, and to perform their chemical profile using HPLC-DAD. **Methods:** Male Swiss mice (18–25 g) were treated intraperitoneally with dimethyl sulfoxide (DMSO, vehicle), morphine, indomethacin, or EtOAc and BuOH fractions (n= 6–8 animals/group). The following tests were performed: (1) Formalin test (20 μ L, intraplantar), measuring paw licking time during the neurogenic phase (0–5 min) and inflammatory phase (15–30 min); (2) Hot plate test (54.0 ± 0.5 °C), evaluating the latency of the nociceptive response at 30, 60, 90, and 120 min; (3) Carrageenan-induced paw edema (50 μ L), with paw volumetric evaluation up to 6 h after injection (CEUA/UFRJ, protocol 039/24). Data were analyzed in GraphPad Prism 5.0, the Shapiro-Wilk test was used, and differences between groups were analyzed by One-Way ANOVA, followed by Tukey's multiple comparisons. **Results:** In the neurogenic phase of the formalin test, the EtOAc fraction (10, 30 and 60 mg/kg) reduced paw licking time compared to the DMSO group by 39.3%, 81.3% and 74.8%, respectively, while the BuOH fraction (3, 10 and 30 mg/kg) produced reductions of 36.2%, 70.1% and 77.6%, morphine (10 mg/kg) reduced it by 60.8% and indomethacin (5 mg/kg) by 15% ($P < 0.05$). In the inflammatory phase, EtOAc led to reductions of 32.4%, 63.1%, and 38.6%, and BuOH of 34.3%, 63.6%, and 65.8%, respectively, morphine of 35.7%, and indomethacin of 63.5% ($P < 0.05$). In the hot plate test, no significant increase in response latency was observed, suggesting no effect on thermal nociceptive stimuli. In the paw edema model, the BuOH fraction (30 mg/kg) significantly reduced swelling 6 h after carrageenan administration, from $56.3 \pm 2.9\%$ (DMSO control) to $26.8 \pm 5.9\%$, indomethacin to $29.9 \pm 6.7\%$, $21.8 \pm 7.8\%$, and $20.0 \pm 8.5\%$ at 4, 5, and 6 h, respectively ($P < 0.05$). HPLC-DAD analysis of the EtOAc fraction revealed a main peak ($t_R = 33.28$ min; %A=73.1) with UV absorption maxima at 200, 259, and 308 nm. A second peak ($t_R = 31.48$ min; %A=24.4) exhibited absorption at 201, 287, and 291 nm. In the BuOH fraction, the main peak ($t_R = 32.66$ min; %A=74.5) presented bands at 201, 260, and 308 nm, while a secondary peak ($t_R = 31.46$ min; %A=22.7) exhibited absorption at 201, 287, and 292 nm. **Conclusion:** Both fractions showed significant antinociceptive activity, indicating central mechanisms. The BuOH fraction also had strong anti-inflammatory effects. Major compounds in both fractions shared chromophores, suggesting they belong to the same chemical class, despite being distinct.

05.007 **Learning and Memory Impairment in Preclinical Models of Orofacial Pain: A Systematic Review and Meta-Analysis.** Morais RIF¹, Viero FT¹, Rodrigues P¹, Kudsi SQ¹, Pereira LG¹, Trevisan G¹
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Introduction: Orofacial pain is a prevalent and debilitating form of chronic pain that compromises physical, emotional, and cognitive functions. It includes clinical entities such as trigeminal neuralgia, temporomandibular disorders, migraine, and dental pain, all associated with deficits in attention, learning, and memory. While clinical recognition of the link between pain and hippocampal dysfunction is growing, robust preclinical evidence focused on orofacial pain remains scarce. This review aimed to evaluate its impact on cognitive function in rodent models. **Methods:** This systematic review and meta-analysis followed PRISMA guidelines and was pre-registered in PROSPERO (CRD42023355502). Four databases (PubMed, Scopus, EMBASE, Web of Science) were searched for preclinical studies investigating cognitive performance in rodents subjected to orofacial pain. Methodological quality and risk of bias were assessed using SYRCLE and CAMARADES tools. Publication bias was analyzed using Begg's and Egger's tests and funnel plots. Meta-analyses were performed using standardized mean differences (SMD) and 95% confidence intervals (CI). **Results:** Twenty-one studies were included, totaling 604 animals (318 pain-induced and 286 controls). Orofacial pain models included trigeminal neuropathy (n = 12), migraine-like pain (n = 4), tooth nociception (n = 4), and acute models (n = 1). Cognitive assessments were primarily based on the Morris Water Maze (MWM), evaluating escape latency, target quadrant time, and platform crossings. Additional tasks included Y-maze (working memory), novel object recognition (recognition memory), passive avoidance (aversive memory), and reversal learning (cognitive flexibility). Meta-analysis revealed increased escape latency (SMD = 2.18; 95% CI = 1.28 to 3.08) and reduced target quadrant time (SMD = 1.74; 95% CI = 0.27 to 3.21), indicating deficits in spatial learning and memory. Orofacial pain also increased spontaneous nociceptive behaviors and facial mechanical allodynia (SMD = -5.63; 95% CI = -8.01 to -3.25). Heterogeneity was partially explained by differences in pain duration, test timing, and task parameters. Methodological quality was high (mean CAMARADES score: 9.85/10), but most studies lacked baseline cognitive data, used only male rodents (90%), and some employed individual housing, a known confounder. **Conclusion:** Rodent models of orofacial pain consistently demonstrate cognitive impairments, particularly in spatial learning and memory. These findings reinforce the relevance of including cognitive endpoints in pain research. Nonetheless, improved study design, including both sexes, baseline evaluation, visual screening, and environmental control, is essential to enhance translational value. This is the first meta-analysis to systematically demonstrate such deficits across orofacial pain models. **Financial support:** CAPES, CNPq, FAPERGS.

05.008 TRPM3 as a Therapeutic Target for Osteoarthritis Pain: Effects of Local and Systemic Antagonism. Pereira S², Almeida BL², Nunes MC², Andersson D¹, Bevan, SJ¹, Costa R^{1,2} ¹Wolfson SPaRC, King's College London, London, UK; ²UFRJ, School of Pharmacy, Rio de Janeiro, Brazil

Osteoarthritis (OA) is a progressive joint condition characterized by persistent chronic pain, associated with sensitization of the peripheral and central nervous systems (Goldring, SR. *Nat. Rev. Rheumatol.*, 2016). The Transient Receptor Potential Melastatin 3 (TRPM3) ion channel, expressed in sensory neurons, is involved in the detection of noxious heat and inflammatory thermal hyperalgesia (Behrendt, M. *Biol. Chem.*, 2019). Preliminary data from our group demonstrated that global or sensory neuron-specific deletion of the *Trpm3* gene prevents the development of pain in OA models induced by monosodium iodoacetate (MIA) and partial meniscectomy (MSC), without affecting the progression of joint pathology. Furthermore, the specific TRPM3 antagonist ononetin (ONO) was able to reverse established pain in these models. These findings suggest that TRPM3 in sensory neurons is a critical mediator of pain in OA. Based on these data, our current objective was to investigate, through pharmacological approaches, the effects of TRPM3 antagonists – ononetin and isosakuranetin (ISOS) – on OA-associated pain in MIA- and MSC-induced models in mice. Female C57BL/6J mice (2 to 3 months old) were subjected to OA induction by MIA or MSC. Animals were treated with ONO or ISOS via intraperitoneal (i.p.) injection to assess systemic effects, and via intra-articular (i.a.) injection into the ipsilateral knee to evaluate local effects, 42 days after induction of the MIA model and 68 days for the MSC model (n10 for each model). As a control for potential systemic spillover from the intra-articular administration, we also performed i.a. injection in the contralateral knee. Mechanical allodynia was assessed using the von Frey test at multiple time points (0.5h to 24h) after treatment. Following i.p. administration, both compounds exhibited antiallodynic effects more prominently during the early hours post-treatment. In the MIA model, ONO reduced mechanical allodynia between 0.5 and 2 hours, while ISOS showed significant effects up to 1 hour, with a shorter response. In the MSC model, isosakuranetin had a more prolonged effect (0.5 to 4 hours), whereas ONO was effective only up to 1 hour. Intra-articular injection into the ipsilateral knee also alleviated pain, with ONO showing sustained effects up to 4 hours in the MIA model and an early response (0.5 to 2 hours) in the MSC model. ISOS, in turn, had a modest effect in the MIA model (mainly at 2 and 4 hours) and limited action at 0.5 and 1 hour in the MSC model. These results confirm the local efficacy of the treatments and highlight temporal and model-specific differences in responsiveness. Injection into the contralateral knee did not result in behavioral changes, suggesting no systemic effect due to intra-articular spillover. These findings reinforce the role of TRPM3 in mediating joint pain in this condition and suggest that its pharmacological inhibition represents a promising therapeutic strategy for managing OA-related pain.

05.009 **Pharmacopuncture with Apitoxin and Melittin: A Novel Approach to Chronic Pain Control and Prevention.** Boaventura de Oliveira, AM¹, Leonor, MGR¹, Silva, DF¹, Sant'anna, MB¹, Almeida, TC¹, Silva GSA¹, Marques-Porto R², Picolo G¹ ¹Lab of Pain and Signaling, Butantan Institute, São Paulo, Brazil; ²Lab of Development and Innovation, Butantan Institute, São Paulo, Brazil

Background & Aims: Chronic pain remains one of the main public concerns since some types of pain are still resistant to the current available pharmacotherapy, causing suffering to patients due to uncontrolled pain or side effects of medicines. Furthermore, the search for new compounds or therapies remains necessary. Bee venom (apitoxin) injection has been used since ancient times to relieve pain and to treat chronic inflammatory diseases. However, controlled preclinical studies are scarce or inexistent, and unstandardized. Then, it was our aim to evaluate the analgesic potential of apitoxin in a chronic pain model and to investigate whether melittin, the main bee venom component, may reproduce its effect, avoiding the toxicity induced by other components of the venom. **Methods:** Neuropathy was induced in rats by chronic constriction of the sciatic nerve (CCI) (CEUA 7835170522 and 7218300924). Apitoxin (1mg/Kg) or melittin (0,6 mg/Kg) was injected, 15 days after surgery, in the stomach 36 acupoint (ST36), a described target for inflammatory disorders. Von Frey Filaments, the Acetone test, and the Hargreaves test were used to evaluate nociceptive threshold. The involvement of endocannabinoid and serotonergic pathways in the pharmacopuncture effect was investigated using the respective antagonists. Further, we investigated the potential of these compounds to prevent the establishment of pain. **Results:** CCI decreased mechanical and thermal nociceptive thresholds (hypernociception), an effect which was reverted by apitoxin and melittin for 96 h or 120 h, respectively. Endocannabinoid and serotonergic antagonists blocked the analgesic effect, indicating the involvement of these systems in pharmacopuncture analgesia. Moreover, we tested whether apitoxin and melittin would prevent neuropathic pain. For that, the treatment started on day 1 after surgery. Surprisingly, four applications of apitoxin and melittin delayed pain onset in 44 days and 50 days, respectively, and booster sessions of toxins delayed pain onset for 101 days. Additionally, preventive treatment promotes better body distribution and weight gain. **Conclusion:** Our results demonstrated apitoxin and melittin as attractive therapies for chronic pain control due to their efficacy and their prolonged duration. Moreover, the delay observed in the onset of pain suggests that they may be potential agents capable of postponing or even preventing the establishment of chronic pain. **Keywords:** Analgesia, Apitoxin, Melittin. **Supported by:** Fapesp/CeTICS 2013/07467-1, Fapesp 2024/04901-7, CAPES, Butantan Foundation

05.010 **Sex Differences in Behavioral Responses Across Rat Models of Migraine.** Sex Differences in Behavioral Responses Across Rat Models of Migraine. Baggio DF, Da Luz FMR, Lejeune VBP, Chichorro JG UFPR – Lab of Craniofacial Pain, PPG Pharmacology, Brazil

Introduction. Migraine is a neurological syndrome characterized by unilateral headache attacks that are aggravated by physical activity and associated with visual and auditory hypersensitivity, as well as nausea and vomiting. Migraine is classified based on attack frequency: episodic migraine (EM) is defined by at least five lifetime attacks, whereas chronic migraine (CM) is diagnosed when individuals experience at least 15 headache days per month over a three-month period. Migraine affects approximately 15% of the global population, predominantly during the most productive period of life, and is 2 to 3 times more prevalent in women than in men. Despite these well-established sex differences in the clinic, most preclinical migraine studies do not consider sex as a biological variable, which may limit translational relevance. Therefore, this study aims to characterize sex differences in rat models of migraine. **Methods.** Three different models of migraine were tested in adult male and female Wistar rats (250–300 g). In the EM model, calcitonin gene-related peptide (CGRP) or pituitary adenylate cyclase-activating polypeptide (PACAP) were administered into the trigeminal ganglia of rats and periorbital mechanical allodynia was evaluated by Von Frey filaments at 1-h intervals for up to 6 hours. Twenty-four hours after injection, rats were exposed to a bright light for 1 h followed by the assessment of periorbital cutaneous allodynia for 4 h after photosensitivity induction. For CM model, nitroglycerin (NTG) was administered every other day for 9 days. Periorbital cutaneous allodynia was assessed 2 h after the injection and on days 11, 15 and 17. At day twenty, rats were exposed to a bright light for 1 h followed by the assessment of periorbital cutaneous allodynia at 1-h intervals up to 5 h after photosensitivity induction. Data were analyzed using Two-way ANOVA with repeated measures followed by Bonferroni's post hoc test ($n = 10$). **Results.** Intraganglionic administration of CGRP induced periorbital mechanical allodynia and photosensitivity in both sexes; however, female rats exhibited more intense and longer-lasting responses in both parameters compared to males. In contrast, sex-dependent responses were observed in the intraganglionic PACAP model and in the NTG-induced CM model, in which only female rats developed periorbital mechanical allodynia and photosensitivity. **Conclusion.** Our results contribute to the characterization of sex differences in rat models of migraine. These results are consistent with clinical observations showing a higher prevalence and severity of migraine in women, reinforcing the importance of including both sexes in preclinical migraine research to enhance the translational relevance of experimental models. **Financial support:** This work was supported by the Coordination for the Improvement of Higher Education Personnel (CAPES, Brazil, finance code 001). F.M.R.L. is a recipient of CNPq undergraduate scholarships. D.F.B. and V.B.P.L. are recipients of CAPES PhD scholarships. J.G.C. is the recipient of a CNPq Research Productivity scholarship (#303781/2020-3).

05.011 Anterior Cingulate Cortex Mediates Cannabigerol-Induced Antinociception in a Mouse Model of Chemotherapy-Induced Peripheral Neuropathy. Ferreira MV¹, Wade QW², Morris NM², Cunha JM¹, Graziane NM² ¹UFPR, Dept Pharmacology, Curitiba, PR, Brazil. ²Dept Anesthesiology and Perioperative Medicine, Pennsylvania State University, Hershey, PA, USA

Introduction: Chemotherapy-induced peripheral neuropathy (CIPN) is a prevalent and debilitating adverse effect of cancer treatment, characterized by mechanical allodynia, hyperalgesia, and paresthesia. Current therapeutic options are limited and often ineffective, underscoring the need for novel interventions. CIPN is associated with both peripheral nerve damage and maladaptive plasticity in central pain-processing circuits, particularly the anterior cingulate cortex (ACC), a key region involved in the affective dimension of pain. Phytocannabinoids, including cannabigerol (CBG), exhibit promising antinociceptive properties; however, the contribution of the ACC to CBG-induced analgesia in CIPN remains poorly understood. This study aimed to evaluate the effects of systemic and intra-ACC CBG administration on neuropathic pain behaviors and neuronal excitability in a murine model of CIPN. **Methods:** CIPN was induced in adult male C57BL/6 mice (20–30 g) by once-a-week cisplatin (CIS; 5 mg/kg, i.p.) injection for four weeks. Mechanical thresholds were assessed using electronic von Frey (VF) testing at baseline, post-CIPN induction, and following treatment with CBG (20 mg/kg, i.p.) or indomethacin (IND; 10 mg/kg, i.p.). After a one-week washout, mice were given an acute CBG dose before euthanasia for whole-cell patch clamp recordings of pyramidal neurons on ACC to measure membrane excitability (IME) and rheobase. To determine the functional role of the ACC, DREADD-based chemogenetic inhibition was achieved via systemic administration of clozapine-N-oxide (CNO; 3 mg/kg, i.p.). Additionally, bilateral microinjections of CBG (20 nM and 20 μ M) into the ACC were performed to assess region-specific effects on mechanical allodynia. All procedures were approved by the Institutional Animal Care and Use Committee at the Pennsylvania State University (Protocol #01327). **Results:** CIPN induction significantly reduced mechanical thresholds compared to vehicle controls (54%, 7 days after the last injection). Systemic CBG effectively reversed mechanical allodynia in CIPN mice (98%, $p < 0.01$), whereas no effect was observed in non-neuropathic animals. IND partially attenuated allodynia (76%, $p < 0.05$). Electrophysiological analysis revealed no significant differences in IME or rheobase between CIPN and control groups; however, a non-significant trend toward reduced rheobase was observed in CIS+CBG animals. Chemogenetic inhibition of ACC neurons via CNO significantly increased mechanical thresholds in CIPN mice (100%, $p < 0.01$), with peak effects at 60 minutes post-injection and a return to baseline within 72 hours. Furthermore, local microinjection of CBG into the ACC at both concentrations (20 nM and 20 μ M) significantly reversed mechanical allodynia (94% and 133%, respectively, $p < 0.01$). **Conclusion:** These results demonstrate that CBG exerts robust antinociceptive effects in a mouse model of CIPN and highlights the ACC as a critical site mediating this response. The efficacy of local CBG administration suggests that CBG modulates pain via ACC-dependent mechanisms. Despite the absence of alterations in IME of ACC pyramidal neurons following CIS treatment, the behavioral effect of chemogenetic inhibition further reinforces that the ACC remains a key player in CIPN-associated pain modulation. The absence of postsynaptic excitability changes suggests that CBG's antinociceptive effects and possibly CIPN-related adaptations may rely on presynaptic modulation within the ACC, underscoring the need for further studies on synaptic mechanisms. **Support:** CAPES (Finance Code 001).

05.012 Characterization of a Peripheral Neuropathy Model Induced by the Chemotherapeutic Agent Sorafenib in Mice. Characterization of a Peripheral Neuropathy Model Induced by the Chemotherapeutic Agent Sorafenib in Mice Rasga GL, Almeida BL, Pereira S, Costa R, Passos GF UFRJ, Lab Neurofarmacologia e Comportamento, Programa de Pós-Graduação em Ciências Farmacêuticas, Universidade Federal do Rio de Janeiro, Brazil

Sorafenib is a multikinase inhibitor chemotherapeutic agent used for treating advanced hepatocellular carcinoma, renal cell carcinoma, and metastatic thyroid carcinoma. By modulating cellular pathways related to tumor growth and angiogenesis, sorafenib has shown promising results in slowing cancer progression and extending patient survival. However, there are several adverse

effects associated with sorafenib treatment, such as fatigue, hypertension, and palmar-plantar erythrodysesthesia (PPE). PPE is the main adverse effect related to sorafenib treatment, affecting between 21% and 51% of patients undergoing treatment. It manifests as a sensory hypersensitivity syndrome, causing symptoms like burning sensation, pain, and hyperkeratosis in peripheral areas, thereby reducing patients' quality of life. The mechanisms underlying PPE manifestations remain poorly understood, often leading to dose reduction or treatment interruption once it reaches more severe stages, as all current therapeutic options are ineffective in managing symptoms. This study (CEUA-CCS-UFRJ nº A29-23-051-20) aims to characterize sorafenib-induced neurotoxicity and investigate the mechanisms related to PPE using a mouse model. Swiss mice (6–8 weeks old; n = 10/group) received vehicle (0.5% carboxymethylcellulose) or sorafenib (320 mg/kg, p.o., 5 times per week for 30 days). Behavioral assessments were conducted to evaluate the impact of sorafenib treatment on peripheral sensitivity to mechanical and thermal stimuli to cold and heat, through von Frey filaments, the acetone drop test, and the hot plate test, respectively. At the end of the treatment, animals were culled, and the spinal cord (SC) and dorsal root ganglia (DRG) were collected for molecular analysis by RT-qPCR and immunohistochemistry (IHC). Sorafenib-treated mice showed a significant increase in mechanical sensitivity in the von Frey test after three weeks of treatment. Similar effects were observed in the acetone drop test and hot plate test, indicating that sorafenib treatment induced behavioral changes in response to those stimuli. To determine whether neuronal damage was induced by sorafenib, ATF-3 gene expression was evaluated in the DRG and SC by RT-qPCR. Its expression was significantly increased in the DRG but not in the SC of treated mice. Neuroinflammation was also explored as a potential cause of sorafenib-related hypersensitivity by assessing TNF- α and TLR4 gene expression, which showed an increase of both markers in the DRG but not in the SC of the treated group. EGFR gene expression was also measured due to its role in pain processing, and a significant increase was observed in the SC after sorafenib treatment. Additionally, sorafenib induced astrocyte activation in the SC, indicated by increased GFAP immunostaining. These findings suggest that neuropathic and neuroinflammatory components may be contributing to the neurotoxic effects associated with sorafenib treatment. This research is funded by FAPERJ, INCT-INOVAMED/CNPq, CAPES, PIBIC-UFRJ.

05.013 Stigmasterol Attenuates Nociceptive Behaviours in a Fibromyalgia Experimental Model.

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Introduction: Fibromyalgia (FM) is a disabling chronic disease that affects 2–3% of the world's population. The main symptom reported by FM patients is widespread pain, characterised by mechanical and thermal allodynia, muscle pain, and spontaneous nociception. In addition to pain, fibromyalgia patients have comorbidities such as nonrestorative sleep, chronic fatigue, depression, anxiety, and migraine. Gabapentinoids (pregabalin) and serotonin and noradrenaline reuptake inhibitors (SNRIs) (duloxetine and milnacipran) are approved by the Food and Drug Administration for FM treatment, but other therapies may also be employed, including amitriptyline, nortriptyline, gabapentin, tramadol, and low-dose naltrexone. However, these medications present limited efficacy and cause adverse effects that limit their use. Thus, to improve pain management, it is necessary to investigate new analgesic agents that are consistently effective and with fewer adverse effects. Earlier studies demonstrated that stigmasterol possesses significant antinociceptive properties in mouse models of both acute and chronic pain. Here, we explore the potential of stigmasterol in reducing nociceptive behaviour in a fibromyalgia experimental model. **Methods:** To induce the fibromyalgia experimental model, reserpine (1 mg/kg) was injected subcutaneously (s.c.) once daily into the back of C57/BL6 mice for three consecutive days. Mechanical allodynia (von Frey test), thermal hyperalgesia (Hargreaves apparatus), and muscle strength (Grip test) were evaluated before reserpine administration (baseline) and at day 4 h after the first reserpine administration (baseline 2). After baseline 2, animals were orally treated with either vehicle (10 mL/kg), stigmasterol (0.3, 1, and 3 mg/kg), or pregabalin (30 mg/kg). Nociceptive behaviours were assessed 0.5 hours up to 6 hours after the treatments. Mice treated with reserpine developed mechanical allodynia, thermal hyperalgesia, and loss of muscle strength. Stigmasterol (3 mg/kg, p.o.) alleviated the mechanical allodynia, thermal hyperalgesia, and loss of muscle strength with inhibitions of $80 \pm 9\%$, $81 \pm 12\%$, and $27 \pm 5\%$, respectively, similar to pregabalin. Parametric data were expressed as mean + SEM for 6 animals per group. Two-way ANOVA followed by Bonferroni post hoc test. Non-parametric data were expressed as median with interquartile range and analyzed by the Kruskal–Wallis test followed by Dunn's test. **Conclusions:** Stigmasterol reduced the nociceptive behaviours in an experimental fibromyalgia model in mice. Thus, stigmasterol represents a natural product with potential for the development of new medications for the treatment of fibromyalgia-related symptoms. **Financial Support:** Coordenação de Aperfeiçoamento de Pessoal de Nível Superior — Brasil (CAPES); Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul-FAPERGS; Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). We thank CNPq, PIBITI/CNPq, CAPES/PROEX, and PROBIC/FAPERGS for their fellowship support. **Ethical Permissions:** All procedures performed in studies involving animals were in accordance with the ethical standards of the institution at which the studies were conducted and ethical approval was obtained from Institutional Animal Care and Use Committee of the Federal University of Santa Maria (Process #9104130923).

05.014 **Sex Differences in Pain and Long-Lasting Analgesia Induced by Apitoxin and Melittin Treatment.** Leonor MGR¹, Boaventura de Oliveira AM¹, Silva DF¹, Sant'anna MB¹, Paulo, MEFV², Pícolo G¹ ¹Lab Pain and Signaling, Butantan Institute, São Paulo, Brazil; ²Pharmacology Lab, Butantan Institute, São Paulo, Brazil

Introduction: Sex-related differences in pain sensibility and analgesic efficacy are reported. However, most basic searches, pre-clinical assays, and clinical trials are still conducted in male rats and men. This accounts for the poor understanding of the painful mechanism, hormonal influence, and the effectiveness of new compounds in females. Chronic pain is a public health concern, which has become worse in women because they represent 60% of neuropathic pain cases. Considering that the treatment of chronic pain is still a challenge and the search for new analgesic compounds is necessary, toxins have been highlighted as sources for new compounds for millennia, and among them, apitoxin is outstanding because of the prolonged antinociception effects. Then, this project aimed to compare the analgesic effect of apitoxin and its main compound, melittin, in female and male rats, investigating the interference of the estrous cycle with the painful sensibility and analgesia. **Methods:** Neuropathy was induced in Wistar female (CEUA 9080280324) and male (CEUA 7835170522) rats (3-6 weeks, 180– 200g), by the chronic constriction of the sciatic nerve (CCI). Initially, 15 days after CCI induction, treatment was performed to determine the analgesic effect of apitoxin, manual acupuncture, and melittin applied as pharmacopuncture in and out of the Suzanli (ST36) acupoint. Pain nociceptive behavior was executed by using the stimulus-evoked test (Von Frey filaments test). The estrous cycle was analyzed by vaginal lavage in the same period of the behavioral assessments. **Results:** Evaluating the basal sensibility, males exhibit a higher mechanical basal threshold than females, which was not influenced by the estrous cycle. Apitoxin can develop analgesia in both sexes with the same dose (1mg/kg). Based on that, melittin was used in a corresponding dose (60%, 0.6mg/kg), since it represents this percentage of apitoxin content. We observed that although the same dose promotes analgesic effect in both sexes, this is longer-lasting in males than in females in both treatments. Thereby, while in males apitoxin promotes analgesia for four days, in females this lasts only two days; in the same way, while melittin promotes analgesia for five days in males, it lasts only three days in females. All the treatments realized out of acupoints were not able to promote analgesia. **Conclusion:** Although sex-dependent differences in basal nociceptive threshold were observed between male and female rats, reflecting differences in apitoxin-induced long-lasting analgesia were observed after apitoxin and melittin treatment in chronic neuropathic pain in both genders. **Keywords:** Sex difference, Bee Venom, Analgesia. **Financial support:** FAPESP PROCESS NUMBER 2024/04664-5, 2024/04901-7 and 2013/07467-1 (CETICS PROGRAM), CAPES, Butantan Foundation and CNPq.

05.015 **Evaluation of the Antinociceptive Effect of Creams Containing *Cannabis sativa* Extracts in an Animal Model of Pain.** Bazilio YS¹, Rezende B¹, Macario VS¹, Oliveira BC¹, Guedes GN¹, Andrade MEB¹, Calumbi KS¹, Maia EP¹, Oliveira L², Maluf D², Antunes F³, Montes GC¹, ¹UERJ, Roberto Alcântara Gomes Institute of Biology (IBRAG), Brazil, ²UFPR, Dept Pharmacy, Brazil, ³UENF, Animal Clinic and Surgery Lab, Brazil

Introduction: The absence of topical cannabis-based products is due to the limited national preclinical evidence proving their efficacy in pain control. Methodology: Experiments approved by CEUA/IBRAG/UERJ (No. 030/2023) evaluated creams containing cannabidiol (CBD) or cannabigerol (CBG) (0.3%, 1%, 3%) in Wistar rats, using negative (cream without active ingredient) and positive controls (3% methyl salicylate or 1% hydrocortisone). In the formalin test (n=6-9/group), the creams were applied to the right hind paw. The time of interaction with the paw was recorded after injection of 60 µL of 2.5% formalin in the neurogenic (0-5 min) and inflammatory (15-45 min) phases. In the tail immersion test (n=6/group), the latency to tail withdrawal in 55°C water was evaluated at predefined intervals (15-180 min) after applying the cream to the animal's tail tip. In the hyperalgesia test (n=6-9/group), a 1% carrageenan solution (100 µL) was injected into the right hind paw. After two hours, hyperalgesia was confirmed in the animals, which were then treated with CBD and CBG creams (1%). Mechanical hyperalgesia was evaluated using the Randall Selitto analgesimeter, and thermal hyperalgesia was evaluated by immersing the paw in water heated to 46°C. Neuropathic pain was induced in animals by spinal nerve ligation (SNL) and treated with 1% CBG cream for 14 days, with thermal and mechanical hyperalgesia assessed throughout treatment.

Results: In the formalin test, during the neurogenic phase (0-5 min), the control group showed a reactivity time of 60.4 ± 7.9 s. CBD treatment reduced this time to 27.0 ± 3.0 s (0.3%) and 29.4 ± 4.4 s (1%), while CBG reduced it to 22.9 ± 4.5 s (0.3%) and 28.5 ± 7.1 s (3%). In the inflammatory phase (15-45 min), CBD reduced reactivity time from 187.4 ± 20.5 s (control) to 46.3 ± 6.1 s (0.3%), 87.4 ± 13.4 s (1%), and 88.9 ± 10.8 s (3%), while CBG reduced it to 51.2 ± 7.1 s (0.3%), 106.1 ± 13.7 s (1%), and 111.4 ± 7.9 s (3%). In the tail immersion test, 1% CBD increased baseline latency from 3.5 ± 0.4 s to 4.0 ± 0.4 s at 45 min, while 3% CBD showed a progressive increase from 3.7 ± 0.3 s to 4.2 ± 0.5 s at 90 min. 1% CBG increased latency from 3.7 ± 0.8 s to 4.0 ± 0.4 s between 60-90 min, and 3% CBG maintained elevated latencies from baseline (5.3 ± 1.7 s) up to 90 min (5.0 ± 1.7 s). In thermal hyperalgesia assessment, 1% CBD and CBG increased thermal latency from 2.3 ± 0.3 s (control group) to 4.3 ± 0.9 s and 4.2 ± 0.4 s, respectively, at 135 min, remaining elevated until 240 min. In mechanical hyperalgesia assessment, groups treated with 1% CBD and CBG showed improved response thresholds, increasing from 66.0 ± 13.9 g (control group) to 89.7 ± 12.2 g and 98.8 ± 17.9 g, respectively, at 135 min, with sustained effects up to 240 min. In chronic pain-associated hyperalgesia, animals treated with 1% CBG showed an increased response threshold from 96.6 ± 4.3 g (control group) to 127.8 ± 4.1 g and 121.5 ± 7.8 g on days 14 and 17 of treatment, respectively. **Conclusion:** Creams containing CBD and CBG demonstrated antinociceptive effects in an animal model of pain. Funding: Faperj, Capes, CNPq, PIBIC/UERJ, APEPI

05.016 **Decreased Anterior Cingulate Cortex Activity Optimizes the Effects of Electroacupuncture on Anxiety Related to Neuropathic Pain.** Souza MBRF¹, Ribeiro-Siqueira CM¹, Donizetti-Fonseca MA¹, da Silva AL^{1,2}, da Silva ML^{1,2}, da Silva JRT^{1,2}, Falconi LLS^{1,2} ¹UNIFAL, Lab. of Neuroscience, Neuromodulation and Study of Pain, Brazil, ²UNIFAL, PPG Biosciences Applied to Health, Brazil

Introduction: Chronic pain has been considered one of the main triggers of emotional changes, as evidenced in cases of anxiety that occur in individuals with allodynia due to neuropathic diseases. In this scenario, electroacupuncture (EA), a therapeutic approach that combines acupuncture with electrical current stimulation has been shown to be effective in relieving neuropathic pain and pain-related anxiety through medullary opioidergic and forebrain excitatory mechanisms. However, the role of cortical structures in mediating the modulatory effects of EA on pain and anxiety is not fully understood. The anterior cingulate cortex (ACC), has been proposed as a pain and anxiety interface because it is a major source of modulatory projections to pain- and emotion-related areas. **Aims:** Our proposal was to investigate whether decreasing ACC activity through intra-ACC microinjections of lidocaine, a voltage-dependent sodium (Na⁺) channel blocker, alters the effects of a single EA application on anxiety related to neuropathic pain. **Methods:** Male Wistar rats, initially weighing 120 g, had their mechanical nociceptive thresholds (baseline; BL1) measured through von Frey test. After 24h of BL1, the rodents were subjected to chronic constriction injury (CCI) of the ischiatic nerve of the right hind paw. After 14 days of CCI, the animals had their mechanical nociceptive thresholds measured again (baseline 2; BL2). The next day (15st), they underwent stereotaxic surgery to implant a guide cannula directed to area Cg1 of the ACC. On the 21st day of CCI, the rodents had their mechanical nociceptive thresholds measured by von Frey test (baseline; BL3) and were treated with EA (single session, 2 Hz, acupoints ST36 and SP6, 20 min) on the right hind paw. After 24h of EA, the rodents received microinjections of either physiological saline (Vehicle) or lidocaine (2%/0.2µL) in the ACC followed by von Frey test at 5-min intervals for 30 min. After the von Frey test, the animals were subjected to the elevated plus maze (EPM) test, in which the time spent in the open and closed arms were recorded. **Results:** Two-way RM-ANOVA showed effects of treatment ($F_{3,24} = 444.3$; $p < 0.05$), time ($F_{6,48} = 44.05$; $p < 0.05$) as well as the interaction between them ($F_{18,102} = 23.87$; $p < 0.05$) on mechanical allodynia. CCI induced neuropathic pain over 21 days ($p < 0.05$). Single EA + Vehicle (ACC) was not able to reverse the mechanical allodynia induced by CCI ($p > 0.05$). In turn, single EA followed by Na⁺ channel blockade in the ACC by intra-Cg1 microinjections of lidocaine increased mechanical nociceptive thresholds (antinociception) for 30 minutes ($p < 0.05$). According to Student's t-test, rats that received EA + lidocaine (ACC) spent more time in the open arms of the EPM (t_{2382} , $df = 13$; $p < 0.05$), when compared to the EA + vehicle (ACC)-treated control group. **Conclusion:** Our findings suggest that reducing ACC activity by blocking Na⁺ channels, which in turn affect fibres of passage, optimise the modulatory effects of EA on both mechanical allodynia and anxiety-like behaviours in rats with peripheral neuropathy. Ethical approved (CEUA-UNIFAL, 0041/2024). Financial support (FAPEMIG, 19/2024).

05.017 **Resveratrol Modulates Neurochemical Parameters and Produces Dose-dependent Analgesia in Rats Subjected to a Rat Model of Chronic Orofacial Inflammatory Pain** Roland S^{1,2,3,4}, Morais ITS^{1,2,3,4}, Farias VEF¹, Stieven A^{1,2,4}, Melo AS¹, Stein DJ^{1,2,4}, Torres ILS^{1,2,3,4} ¹Lab Farmacologia da Dor e Neuromodulação: Investigações Pré-Clínicas, Centro de Pesquisa Experimental, ²HCPA, Núcleo de Farmacologia da Dor e Neuromodulação, Porto Alegre, RS, Brasil ³UFRGS Programa de Pós-Graduação em Ciências Biológicas: Farmacologia e Terapêutica, ⁴UFRGS, Programa de Pós-Graduação em Medicina: Ciências Médicas, Porto Alegre, RS, Brasil

Introduction: Chronic orofacial pain is a common yet debilitating condition that adversely impacts the quality of life of affected individuals. There has been a notable increase in interest concerning the therapeutic potential of natural compounds, such as Resveratrol (RES). This polyphenol is recognized for its antioxidant and anti-inflammatory properties and is being investigated for its possible role in modulating chronic inflammatory pain, particularly orofacial pain. **Aim:** To determine the most effective dose of RES for the management of chronic orofacial inflammatory pain in rats. **Methods:** Fifty adult male Wistar rats (3 months) were initially allocated to two distinct groups: control (CT) and pain (Pain). The pain model was established by administering 50 µL of Complete Freund's Adjuvant (CFA) into the right temporomandibular joint (TMJ), while the CT group received 50 µL of saline. After three days, the animals in the Pain group were assigned to four subgroups: Pain (no treatment), Pain+RES20 (20 mg/kg, oral), Pain+RES40 (40 mg/kg), and Pain+RES80 (80 mg/kg), with treatment administered for 14 consecutive days. Mechanical allodynia was assessed using the facial electronic von Frey test at baseline, and repeated on days 3 (D3), 10 (D10), and 17 (D17) thereafter. Following euthanasia, the brainstem (BST) and spinal cord (SC) were harvested for the analysis of TNF- α , IL-10, and IL-4 levels. The protocol was approved by CEUA/HCPA under #2019.0763. **Results:** Treatment with RES resulted in a dose-dependent analgesic effect, with intermediate (40 mg/kg) and high (80 mg/kg) doses promoting partial reversal of allodynia during the early phase of inflammation, in line with previous evidence on its pain-modulating properties. The absence of significant differences in late phases suggests spontaneous remission of the pain condition, limiting the assessment of the compound's therapeutic window. Furthermore, RES decreased levels of TNF- α and increased IL-4 in the SC, demonstrating the compound's anti-inflammatory properties. No changes were observed in the levels of these cytokines in CT animals, nor IL-10 in the BST and SC ($P>0.05$ for all analyses). **Conclusion:** The findings of this study indicate that RES exerts a dose-dependent analgesic effect, partially reversing mechanical allodynia in rats subjected to a model of chronic orofacial inflammatory pain, likely by modulating the levels of TNF- α and IL-4 in the SC. Collectively, these results suggest that the analgesic effect of resveratrol may be linked to the modulation of neuroinflammation. **Financial Support:** FIPE HCPA #2019.0763; BIC/PRAE UFRGS; CAPES; CNPq; FAPERGS. **Ethical approval:** Approval granted by the Ethics Committee on the Use of Animals (CEUA-HCPA), protocol #2019.0763.

05.018 Antinociceptive Effect of Repeated Administration of the TRPA1 Antagonist in a Model of Relapsing-Remitting Multiple Sclerosis in Mice. Luiz ACM¹, Peres DS¹, Antoniazzi C¹, Trevisan G^{1,2},

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Introduction: Relapsing-remitting multiple sclerosis (RRMS) is a chronic, autoimmune neurodegenerative disease that affects the central nervous system (CNS). Among the clinical signs observed in the disease, we can highlight neuropathic pain, which leads to a decrease in patients' quality of life. Pain treatment in RRMS is still limited, which reinforces the need for new therapeutic approaches. It is known that pain and neuroinflammation in RRMS is related to oxidative stress and the infiltration of inflammatory cells in the CNS. The involvement of the transient receptor potential ankyrin 1 (TRPA1) in the process of neuroinflammation and nociception in RRMS models. This receptor is an ion channel permeable to calcium and activated/sensitized by different oxidizing substances. Thus, the present study aims to evaluate the TRPA1 channel repeated pharmacological blockage with a selective antagonist to treat nociception in the RRMS model in mice. This strategy supports clinical management by focusing on symptomatic patients, aiming to generate robust evidence for the development of therapies targeting pain and neuroinflammation in MS. **Methods:** Female C57BL/6 mice (20–25 g, CEUA nº 2381301120/2020) were used, and relapsing-remitting experimental autoimmune encephalomyelitis (RR-EAE) was induced through immunization with MOG_{35–55} and Quil A adjuvant (subcutaneous), followed by pertussis toxin administration on days 0 and 2 (post-induction). Mice received repeated treatment with A-967079 (selective TRPA1 antagonist, 30 mg/kg, intragastric) for 20 days, from day 16 to 36 post-induction. After model induction, behavioral tests were performed to assess disease progression and nociception. Clinical scores were recorded on days 21, 28, and 35. Motor function and balance were evaluated using the rotating cylinder test. Locomotor and exploratory activities were analyzed by the open field test on days 21, and 34, and nest-building behavior was assessed on day 35. Mechanical allodynia was measured using von Frey filaments on days 21, 28, and 35. **Results:** EAE-RR-induced mice showed increases in clinical scores throughout the study, which were significantly reduced by treatment with the TRPA1 antagonist A-967079 starting at day 21 post induction. No significant changes in body weight or general locomotor activity were observed among groups. In the open field test, while total crossings remained similar, EAE-RR-Vehicle animals spent less time in the center of the apparatus compared to controls on days 21 and 34; this behavior was reversed by A-967079 treatment. Mechanical allodynia was significantly attenuated by the antagonist, with maximal inhibition observed at day 35. Additionally, nest-building behavior, used as a measure of spontaneous nociception and well-being, was reduced in EAE-RR-Vehicle mice but restored by A-967079. **Conclusion:** Thus, our findings demonstrate that repeated administration of the TRPA1 antagonist produces a significant antinociceptive effect in a RR-EAE model, without inducing motor impairments. These results reinforce the involvement of TRPA1 in the neuropathic pain and support its therapeutic potential as a novel targeted strategy of pain in RRMS. **Financial support:** FAPERGS, CNPq and Capes.

05.019 **Molecular Docking Study of Cannabinol with Human TRPV1 Channel: Insights to Treatment of Fibromyalgia.** Costa AEA^{1,2}, Santos PVS¹, Santos BS¹, Veras JF¹, Ferreira VGF¹ Freire NAEP¹, Silva JLV¹ FMO, ²Unicamp

Introduction: The transient receptor potential vanilloid type 1 (TRPV1), also known as the capsaicin receptor, is a polymodal, nonselective cation channel expressed by all major classes of nociceptive neurons and is important for the detection of noxious stimuli. Central sensitization and upregulation of the TRPV1 signaling pathway has been observed in a murine model of fibromyalgia, indicating the direct involvement of this receptor in the pathogenesis of the disease. *Cannabis sativa* oils can be a great ally for the composition of the traditional treatment of fibromyalgia. Besides phytocannabinoids activates and desensitizes TRPV1 at *in vitro* and *in silico*, there have not been docking studies reported that present cannabinol (CBN) binding sites on TRPV1. **Aim:** To analyze the interactions of CBN in the hTRPV1 and compare to cannabidiol by docking molecular. **Methods:** A quantitative and experimental research with an *in silico* approach. The compounds used were CBN (CID 2543) obtained from PubChem. For docking studies, the structure of human TRPV1 was retrieved from Protein Data Bank (ID 8GFA). The output conformers from DockThor®, a receptor-ligand docking program, were ranked in order of increasing affinity with the protein (Aff). The virtual screening was 5,000.000 evaluations using blind docking and 884,736 grid points. The generated positions were grouped by the DTStatistics tool. Data were analyzed using the Chimera1.14 and PyMOL programs. **Results and Discussion:**

CBN presented Aff of - 8.9 and CBD of - 6.7 kcal/mol in the hTRPA1. Those energy scores were less than - 5 kcal/mol, indicating both a specific binding activity. Spatial analysis showed CBN was sited on alpha-helices, nearly, pore domain channel and realized bonds with Ile446, Ala443, Ile439, Met383, Cys412, Leu390, Ile387, Tyr386, Ile438, Tyr409, Phe391 and Phe416 residues. Thus, either CBD (data not shown) and CBN may modulate hTRPV1, suggesting *Cannabis sativa* oil with both phytocannabinoids (*full spectrum*) present synergism. **Conclusion:** The data corroborate cannabinol effect on fibromyalgia that, probably, it acts inhibiting hTRPV1. These findings support the modulation of Transient Receptors Potential *in vitro* studies by cannabinol and reinforce the action of phytocannabinoids in targets distinct from cannabinoid receptors.

05.020 Antinociceptive Effects of Different Full Spectrum Oils of Phytocannabinoids in Mice Acute Models of Pain. Junger MG^{1,4}, Matheus ME², Oliveira DR^{3,4}, Miranda ALP^{1,4} ¹LEFEx-FF-UFRJ, Lab Studies in Experimental Pharmacology, Brazil. ²FF-UFRJ, Brazil. ³FF-UFRJ, Lab Bioprospecting and Applied Ethnopharmacology, Brazil. ⁴FF-UFRJ, Postgraduate Program in Pharmaceutical Sciences, Brazil

Introduction: Pain is a condition that affects millions of people globally, negatively impacting quality of life and generating significant costs for healthcare systems. In this context, phytocannabinoids have gained prominence due to their therapeutic potential, especially in modulating pain through interactions with the endocannabinoid system (ECS) (Ulugol, Balkan Medical J. 31, 115-120, 2014). The ECS, identified in the 1990s, comprises receptors (CB1 and CB2), endogenous ligands such as anandamide (AEA) and 2-AG, and enzymes responsible for their synthesis and degradation. It plays a crucial role in regulating physiological processes, including pain perception, memory, and inflammation, acting through retrograde signaling that modulates neurotransmitter release (Finn et al., Pain 162, 5-25, 2021). **Objective:** This study aimed to evaluate the antinociceptive profile of five full spectrum oils containing different compositions of phytocannabinoids: Extract 1 (CBG 30 mg/ml, <0,2% de THC/ml), Extract 2 (THC 12 mg/ml), Extract 3 (CBD 10 mg/ml, THC 6 mg/mL), Extract 4 (CBD 33 mg/ml, <0,2% de THC) e Extract 5 (THCV 33,3 mg/ml, THC 35,5 mg/m), using Swiss mice *in vivo* preclinical models such as the hot plate, capsaicin, formalin and open field tests. **Methodology:** Eight-week-old adult mice of both sexes were divided into experimental groups and all tests conducted as single-day experiments. Extracts were orally administered at a doses of 10 mg/kg or 20 mg/kg (formalin test) one hour prior the plantar injection (capsaicin 10µl; 1.6 µg/paw or formalin 20µl; 2.5%). The hot plate test was conducted for a period of 120 min. **Results:** The results highlighted extract 4, containing mainly CBD, as the most effective, inhibiting by 54% (*p<0.05) the licking time of capsaicin test and increasing significantly by 40% (**p<0.01) the latency time in the hot plate test, similar to morphine at the same dose (43%). These findings corroborate previous studies showing modulation of the TRPV1 receptor by CBD. Extracts 1, 2, 3 and 5 did not show significant antinociceptive effects at the dose used, although higher doses may yield different outcomes. The open field test confirmed that the observed effects were not associated with sedation or impaired locomotion, reinforcing that the action was specifically antinociceptive. In contrast, none of the extracts at 20 mg/kg significantly reduced pain behavior in the formalin test compared to the vehicle (coconut oil), indicating the need for higher doses to observe inflammatory pain effect. **Conclusions:** Results confirm the antinociceptive effect for CBD. Further studies with higher doses and in the CFA (Complete Freund´s Adjuvant) induced chronic inflammation will be carried out to better understanding the analgesic potential of these oils and their effects on inflammatory and persistent pain contexts. It will allow for a more comprehensive evaluation of the underlying mechanisms and contribute to the development of new phytocannabinoid-based strategies for chronic pain management. **Financial support:** PIBIC/UFRJ, FAPERJ.

05.021 Search for Therapeutic Targets against Multiple Sclerosis Through Global Gene Expression Analysis: Use of the Crotoxin:SBA-15 Complex and Genetically Susceptible Mice in the Experimental Autoimmune Encephalomyelitis Model. Moraes JG¹, Corcino LFC¹, Sant'Anna MB¹, Lopes FR¹, Boaventura de Oliveira AM¹, Borrego A², De Franco, M³, Sant'Anna OAE⁴, Picolo G¹ ¹IB, Lab Pain and Signaling; ²IB, Development and Innovation Lab; ³IB, Diagnostic Section, Pasteur Institute; ⁴IB, Immunochemistry Lab

Introduction: Multiple sclerosis (MS) is an autoimmune and neuroinflammatory disease, characterized by demyelination of the central nervous system (CNS) and neurodegeneration. It affects approximately 2.8 million individuals worldwide, and it remains without cure, since medications are only effective in delaying the disease progression. Experimental autoimmune encephalomyelitis (EAE) is the most widely used animal model for studying MS, since it allows researchers to understand the molecular mechanisms involved in the immune response associated with MS. Previous data from our group demonstrated that crotoxin, a neurotoxin isolated from the venom of *Crotalus durissus terrificus* well-known for its immunomodulatory property, when complexed with silica SBA-15 (CTX:SBA-15), an inert nanostructured particle, was effective in modulating hyperalgesia and clinical signs of EAE, inhibiting disease development and reducing neuroinflammation. In this study, we aimed to identify, through global gene expression analysis, genes and pathways altered by the disease and up- or downregulated by CTX:SBA-15 and to compare these genes with those altered in genetically selected mouse strains that are susceptible or resistant to EAE. The strains currently used include HIII and LIII, which were genetically selected for their high and low abilities to produce antibodies, respectively. **Methods:** EAE was induced by MOG35-55 injection, and clinical signs were evaluated daily using scores from 0 to 5 (CEUAIB 3687050922 and 3086040919). C57BL/6 mice were treated with the CTX:SBA-15 complex on the 5th day after immunization. CNS tissues (brain and spinal cord) were collected at the peak of the disease (16 days after immunization). Total RNA was extracted using the MagMax™ CORE kit, followed by cDNA synthesis using the Clarion™S kit. Gene expression profiling was performed using the GeneChip® Mouse Array (Affymetrix, encompassing around 27k transcripts). The resulting data were analyzed using the Transcriptome Analysis Console (TAC, version 4.0.1 - ThermoFisher) and the Gene Ontology database. Gene expression changes induced by the disease and modulated by CTX:SBA-15 were compared with gene profiles from HIII and LIII mice. **Results:** A total of 1,313 differentially expressed genes were identified when comparing the EAE (disease), EAE treated with CTX:SBA-15, and CFA (control) groups, with 359 genes related to the disease and modulated after treatment. From then, 73 were upregulated and 286 were downregulated compared to the EAE group. The acetylcholine pathway was highlighted as a key pathway involved in the disease. Genes associated with this pathway were also altered in HIII susceptible mice. **Conclusion:** Based on these data, we infer that acetylcholine pathway genes play a relevant role in the development of EAE and could be further investigated as potential therapeutic targets for the control of multiple sclerosis. Keywords: Crotoxin, SBA-15, Experimental Autoimmune Encephalomyelitis, Gene Expression, Transcriptome, Mouse Model

05.022 **Targeting Neuronal Nitric Oxide Synthase to Reduce Migraine-Like Nociception Demyelination, and Anxiety-Like Behavior in a Mouse Model of Multiple Sclerosis.** Fim YV¹, Da Silva B¹, Viero FT², Antoniazzi CTD¹, Kudsi SQ¹, Peres DS¹, Morais RIF¹, Pereira LG¹, Trevisan G¹ ¹UFSM, Graduate Program in Pharmacology, Federal University of Santa Maria, Santa Maria, RS, Brazil. ²IQ-USP, Neuroscience Lab, Dept Biochemistry, São Paulo, SP, Brazil

Introduction: Multiple sclerosis (MS) is a complex neuroinflammatory disease often associated with migraine and anxiety, both of which impair quality of life. MS involves inflammation and oxidative stress, including increased nitric oxide (NO) production. However, the role of NO in MS-related migraines remains unclear. This study investigated whether repeated administration of 7-nitroindazole (7-NI), a selective neuronal nitric oxide synthase (nNOS) inhibitor, could alleviate migraine-like pain, anxiety-like behavior, and neuroinflammatory biomarkers in a mouse model of relapsing-remitting experimental autoimmune encephalomyelitis (RR-EAE). **Methods:** RR-EAE was induced in female C57BL/6 mice (20–30 g) using myelin oligodendrocyte glycoprotein (MOG35-55) and Quillaja saponin (Quil A) in PBS. The emulsion was injected subcutaneously into both flanks of the animal, and pertussis toxin was also administered. Control animals received Quil A and pertussis toxin only. Mice were treated with 7-NI (120 mg/kg, intragastric, in peanut oil) from day 20 to 35 post-induction. Four groups (n=15) were used: Control + vehicle, Control + 7-NI, RR-EAE + vehicle, RR-EAE + 7-NI. Disease progression (clinical score), mechanical and spontaneous allodynia (Von Frey and grimace), and anxiety-like behavior (elevated plus maze) were assessed. At the end, animals were euthanized for analysis of NOx (modified Griess method), H₂O₂ (horseradish peroxidase with phenol red method), CGRP (commercial kit), IL-4, and IL-10 (CBA BD Mouse Th1/Th2/Th17 kit) in plasma, brainstem, and trigeminal ganglion. All procedures were approved by CEUA/UFSM (protocol no. 219260423). **Results:** Treatment with 7-NI not only reduced pain-related behaviors, including periorbital and mechanical allodynia and spontaneous nociception, but also demonstrated anxiolytic-like effects. Additionally, 7-NI prevented the elevation of critical oxidative and nitrosative markers (H₂O₂ and NOx) in the brainstem, trigeminal ganglion, and plasma, reflecting its role in attenuating neuroinflammatory responses. Furthermore, our findings show that 7-NI prevents the elevation of calcitonin gene-related peptide (CGRP) levels, a marker associated with migraine. We also demonstrated that cumulative administration of 7-NI promotes an increase in the anti-inflammatory cytokines IL-4 and IL-10, suggesting a beneficial effect in the control of neuroinflammation. These effects were observed in RR-EAE animals treated with 7-NI when compared to RR-EAE animals receiving vehicle. **Conclusion:** Repeated treatment with 7-NI modulates nociception and neuroinflammation in RR-MS. Combined with improvements in clinical scores, pain, and anxiety behavior, these results support the therapeutic potential of nNOS inhibition in MS. Collectively, these results explore the potential of nNOS inhibition for managing the complex neuroinflammatory and nociceptive dimensions of RR-MS. Further studies are needed to confirm its efficacy and safety in broader contexts.

05.023 **Flavonoid Diosmetin Reduces Paclitaxel-Induced Nociceptive Symptoms in Acute and Chronic Pain Models in Rodents.** Dias R¹, Atuati SF^{1,2}, Becker G^{1,2}, Oliveira SM^{1,2} ¹UFSM, Pain Neurobiology Lab, ²UFSM, Graduate Program in Biological Sciences: Toxicological Biochemistry, Brazil

Introduction: Cancer patients experience painful symptoms for various reasons, including cancer progression, surgery, or as an adverse effect of antineoplastic therapy (Glare et al., 2014). Painful symptoms resulting from antineoplastic drugs can lead to treatment discontinuation and compromise patients' health (Glare et al., 2014). The paclitaxel chemotherapy causes peripheral neuropathic pain, characterised by mechanical and thermal hypersensitivity, manifesting hours or days after infusion, which can last for years (Loprinzi et al., 2020). Clinically, duloxetine is used for the relief of chemotherapy-induced peripheral neuropathy (Gupta et al., 2020). Despite this, the benefits of duloxetine remain limited; therefore, it is essential to explore new therapies, and natural plant products represent a valuable source of therapeutic agents (Santos et al., 2022). Diosmetin is a flavonoid found in natural plant products and is also chemically synthesised. Since systemic diosmetin attenuates neuropathic pain in experimental models, we investigated the effects of diosmetin in paclitaxel-induced neuropathy models in mice (Marquezin et al., 2024). **Methods:** To assess mechanical and cold allodynia, and nociceptive-induced affective-motivational behaviour (AMB), male C57BL/6 mice (CEUA #4400060223) were used. We assessed the diosmetin effects (0.015, 0.15, and 1.5 mg/kg, oral) in mice submitted to paclitaxel-induced acute (1 mg/kg, intraperitoneal) and chronic (4 x 1 mg/kg, intraperitoneal) pain syndromes. Duloxetine (30 mg/kg, oral) was used as a reference drug. Parametric data are expressed as mean + SEM for 6 animals per group. Two-way ANOVA followed by Bonferroni post hoc test. Non-parametric data are expressed as median with interquartile range and analyzed by the Kruskal–Wallis test followed by Dunn's test. **Results:** diosmetin reduced paclitaxel-induced acute responses, with inhibitions of $56 \pm 19\%$ at 2 hours and $91 \pm 14\%$ at 1 hour for 0.15 and 1.5 mg/kg, respectively, in mechanical allodynia. It achieved 100% inhibition within 0.5 hours for both doses in AMB, and 100% inhibition at 1 hour for both doses in cold allodynia. For chronic pain syndrome, it inhibited $90 \pm 12\%$ at 2 hours with 0.15 mg/kg in mechanical allodynia, reached maximum inhibition of 100% at 1 hour with 0.15 mg/kg in AMB, and achieved 100% inhibition within 2 hours for both doses in cold allodynia, and duloxetine reduced mechanical allodynia in an acute model and all nociceptive parameters in the chronic model, similar to diosmetin. **Conclusion:** In this sense, diosmetin presents a potential alternative to treatments currently used to relieve pain induced by antineoplastic drugs (DOI 10.1007/s12035-025-04939-w). Financial support: FAPERGS (21/2551–0001966-2), CAPES, CAPES/PROEX (1333/2023; 88887.568915/2020-00; 88887.875443/2023-00), CNPq (309404/2023-1; 152071/2024-5). CNPq/PIBIC-UFSM.

05.024 Antinociceptive Effect of Cannabigerol in Experimental Diabetic Neuropathic Pain Depends on CB1 and CB2 Receptors. Rampim LR¹, Ferreira MV¹, Miranda JM¹, Rauchbach L¹, Wolaniuk L¹, Demeu TA¹, Crippa JA², Zanolini JM¹, Cunha JM¹ ¹UFPR, Dept Pharmacology, Curitiba, PR, Brazil. ²FMRP-USP, Ribeirão Preto, SP, Brazil

Introduction: Neuropathic pain is a prevalent and debilitating complication of diabetes, characterized by increased sensitivity to mechanical and thermal stimuli. Current treatments offer limited efficacy and are often associated with significant side effects, underscoring the need for novel therapeutic strategies. Cannabigerol (CBG), a non-psychotropic phytocannabinoid, has shown promising anti-inflammatory and analgesic properties and may represent a potential alternative for managing diabetic neuropathic pain (DNP). This study aimed to investigate the antinociceptive effects of CBG and elucidate the involvement of cannabinoid receptors (CB1 and CB2) in a model of experimental DNP. **Methods:** Experimental diabetes was induced by streptozotocin (STZ; 60 mg/kg, i.p.) in male and female Wistar rats (180-240 g). Treatment with CBG (1, 3, 10, or 30 mg/kg; i.p.) was initiated 14 days after STZ administration and continued for 14 days. CB1 antagonist (AM251, 1 mg/kg, i.p.), CB2 antagonist (AM630, 1 mg/kg, i.p.), or both, were administered 30 minutes before acute injection of CBG. Mechanical allodynia was evaluated using Electronic Von Frey apparatus and the total locomotor activity was assessed in the open field test (OFT). All data were analyzed using two-way ANOVA followed by Bonferroni post hoc test and Student's t-test for comparison between NGL+VEH vs DBT+VEH groups. All procedures were approved by CEUA-BIO- UFPR (#1584). **Results:** Compared to normoglycemic rats (NGL), diabetic (DBT) rats showed: 1) a significant reduction in mechanical threshold starting on day 7 (female rats) or 14 (male rats) after STZ administration, persisting until the 4th week and 2) a decrease in the number of crossings on the OFT (49% in males and 21% in females). Compared to the DBT control group, the CBG-treated DBT rats exhibited: 1) No changes in the locomotor activity neither on the weight gain or hyperglycemia; 2) an increase on the mechanical thresholds, peaking at 120 (females) or 60 (males) minutes after the acute treatment; 3) consistently increased mechanical thresholds after sub-chronic treatment. In male and female diabetic rats, AM251 and AM630 partially blocked the antinociceptive effect of CBG (males: 13.61% and 29.8%; females: 17.80% and 17.15%, respectively). When AM251 and AM630 were combined, a slight reduction (4.14%) was observed in male rats. **Conclusion:** Our findings demonstrate that CBG exhibits antinociceptive properties, supporting its potential as a therapeutic candidate for DNP. Notably, sex-related differences were observed in this antinociceptive response. Both CB1 and CB2 receptors appear to contribute to the antinociceptive mechanism of CBG through the same pathway, as the combination of antagonists was unable to completely block its antinociceptive effect in male rats. Taken together, other receptors may contribute to the antinociceptive effect of CBG on mechanical allodynia associated with DNP, such as α 2-adrenergic receptors and TRPV1 channels, which is currently under investigation. **Financial support:** CNPq, CAPES (Finance Code 001).

05.025 **Evaluation of the Antinociceptive Potential of Neril Acetate in Neuropathic Pain Model-Investigation of Its Molecular Targets.** Gomes LS¹, Martins RYC¹, Dos Reis ACF¹, Castro JLS¹, Barbosa BS¹, Santos ALS¹, Ferreira MKA², Menezes JES², Santos HS², Almeida FRC¹. ¹UFPI, Graduate Program in Pharmacology, Teresina-PI, Brazil; ²UECE, Graduate Program in Natural Sciences, Fortaleza-CE, Brazil

Introduction: Neuropathic pain is a chronic condition difficult to treat and is often caused by lesions in the nervous system. Paclitaxel, a chemotherapeutic agent, can induce painful neuropathy, complicating cancer treatment. New compounds with antinociceptive action and low toxicity profiles are needed. Neril acetate (NA), a monoterpene found in essential oils, has a pharmacological potential that remains scarcely explored for neuropathic pain. This study evaluated the toxicity and antinociceptive effect of NA in zebrafish and mice, as well as investigated its possible molecular mechanisms through docking studies. **Methodology:** The NA acute toxicity was assessed in adult zebrafish (*Danio rerio*, CEUA/UECE No. 04983945/2021) at doses of 4, 20, and 40 mg/kg via intraperitoneal injection, monitoring lethality for 96 hours to determine the median lethal concentration (LC₅₀). The antinociceptive effect was evaluated using the formalin test on the fish tail, recording locomotor activity and investigating the involvement of the TRPA1 receptor using the antagonist camphor. In parallel, female Swiss mice (CEUA/UFPI No. 740/2022) received paclitaxel (2 mg/kg for 4 days, i.p.) to induce neuropathy. After confirming pain with the von Frey test, animals were treated with NA (100, 200, and 400 mg/kg, p.o.). The acute effect was evaluated up to 6 hours after a single dose, and the subacute effect was assessed with daily treatments from day 7 to day 19, with evaluations on alternate days. Morphine (10 mg/kg, s.c.) and pregabalin (10 mg/kg, p.o.) were used as positive controls for the acute and subacute protocols, respectively. Molecular docking studies were performed with TRPA1, CaV2.2 channels, μ -opioid receptor (MOR), and NOS enzymes using AutoDock Vina. An in silico ADMET analysis was also conducted to predict NA's pharmacokinetic and toxicological properties. **Results:** NA did not exhibit significant toxicity in zebrafish, with an LC₅₀ greater than 1.0 mg/mL and no morphological alterations. In the formalin test, NA (20 mg/kg) significantly reduced nociceptive behavior in the neurogenic phase, similar to morphine, and this effect was reversed by the camphor, suggesting modulation of TRPA1. In mice, NA (400 mg/kg) showed a significant acute antinociceptive effect, increasing the mechanical threshold during the first hours post-administration. NA (100 mg/kg) presented a delayed and sustained effect, while 200 mg/kg was ineffective. In the subacute protocol, 100 and 400 mg/kg increased the nociceptive threshold from day 9 onward, with pregabalin showing effects from day 15, and 200 mg/kg remaining ineffective, suggesting a biphasic dose-dependent effect. Docking studies showed NA favorable affinity with TRPA1, CaV2.2 channels, and MOR receptor. ADMET analysis predicted good oral absorption, low toxicity, and absence of hepatotoxicity. **Conclusion:** In summary, neril acetate demonstrated safety and efficacy in controlling neuropathic pain, possibly mediated by modulation of the TRPA1 channel, corroborated by behavioral data and molecular docking. These findings highlight its potential as a promising candidate for new therapies against neuropathic pain.

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05.026 Diosmetin Reduces Nociception Caused by Antineoplastic Anastrozole in a Preclinical Study. Favarin A, Atuati SF, Becker G, Oliveira SM UFSM, Graduate Program in Biological Sciences: Toxicological Biochemistry, Centre of Natural and Exact Sciences, Brazil

Pain is the most prevalent symptom in cancer diagnosis and also one of the most common adverse effects of oncological treatments. With advances in early detection and available therapies, patient survival rates have increased. However, this increase in survival has also increased the number of patients who live with pain after treatment. Anticancer treatments include antineoplastic therapy, such as anastrozole, used in the treatment of hormone receptor-positive breast cancer. Although its efficacy is undeniable, its use is associated with adverse effects, such as musculoskeletal pain, which reduce patients' quality of life and often leads to therapy discontinuation. Currently, therapies used to control these pain symptoms have limited efficacy and cause adverse effects. In view of this, the search for new treatments is essential to treat painful symptoms, and products of natural origin are promising molecules. Diosmetin, a flavonoid found in several plants, has shown promise as it has demonstrated activities in neuropathic, nociplastic and inflammatory pain. Thus, we investigated the effects of diosmetin on anastrozole-induced nociceptive parameters. Firstly, C57BL/6 mice (#4400060223) were treated with a single oral (p.o.) administration of anastrozole (0.2 mg/kg, p.o.) or vehicle (10 ml/kg) to induce a musculoskeletal pain model. Then, paw withdrawal threshold, affective-motivational pain behavior and muscle strength were evaluated. Furthermore, C57BL/6J mice subjected to anastrozole-induced musculoskeletal pain model were treated orally with diosmetin (0.15 mg/kg, p.o.) or duloxetine (30 mg/kg, p.o.), and paw withdrawal thresholds, pain affective-motivational behavior, and muscle strength were evaluated. Anastrozole induced mechanical allodynia, improved pain affective-motivational behavior, and caused loss of muscle strength in the mice. Furthermore, both diosmetin and duloxetine reduced mechanical allodynia, pain affective-motivational behavior, and loss of muscle strength caused by anastrozole (Atuati et al., 2025 – Molecular Neurobiology 10.1007/s12035-025-04939-w). Thus, these results suggest that diosmetin may be an effective therapeutic alternative for the treatment of painful symptoms caused by antineoplastic drugs, such as anastrozole. Acknowledgments: CAPES/PROEX, FAPERGS, and CNPq. **Financial support:** Coordenação de Aperfeiçoamento de Pessoal de Nível Superior — Brasil (CAPES/PROEX); Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul- FAPERGS; Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq). We thank CAPES and CNPq for their fellowship support. **Ethical Permissions:** All procedures performed in studies involving animals were following the ethical standards of the institution at which the studies were conducted, and ethical approval was obtained from the Institutional Animal Care and Use Committee of the Federal University of Santa Maria (Process #4400060223).

05.027 Aldehyde Dehydrogenase-2 Plays a Key Role in the Development of Alcoholic Neuropathy.

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Chronic alcohol consumption leads to alcoholic neuropathy (AN). The mechanisms underlying the etiology of this neuropathy are not yet fully understood. Previous studies demonstrate a strong correlation between AN and a mutation that leads to loss of function of the enzyme aldehyde dehydrogenase-2 (ALDH2), known as ALDH2*2, found in approximately 40% of the East Asian population. ALDH2 is the primary enzyme responsible for clearing acetaldehyde, which accumulates after ethanol ingestion. ALDH2 also metabolizes painful aldehydes produced by oxidative stress, such as 4-hydroxy-2-nonenal (4-HNE). Our research has shown that both aldehydes are produced in response to inflammation, and ALDH2 activation induces antinociception by reducing the levels of these aldehydes. Despite this evidence, the role of ALDH2 in the etiology of AN pain remains unknown. Thus, we aimed to investigate the role of ALDH2 in the development and maintenance of AN. We employed wild-type (WT) and transgenic animals with reduced ALDH2 function (ALDH2*2). To induce AN, ethanol was administered daily (1g/kg, orally) for six weeks. AD6626, an ALDH2 activator, was administered before ethanol (40 mg/kg/day, orally). The mechanical and thermal nociceptive thresholds were evaluated using von Frey filaments and the tail immersion test, respectively. Motor coordination was assessed using the rotarod test. ALDH2 expression and activity were evaluated in the spinal cord and liver, respectively. The 4-HNE levels were assessed in the spinal cord by Western blot. Chronic ethanol administration induced mechanical and thermal hypernociception in both strains, suggestive of neuropathy. However, animals with reduced ALDH2 activity (ALDH2*2) exhibited mechanical hypernociception earlier than WT animals (second vs third week, respectively). Notably, this hypernociception was more pronounced in the ALDH2*2 group compared to WT animals (44.73% vs. 8.72% in the second week; 90.39% vs. 79.05% in the fourth week; and 90.71% vs. 75.21% in the sixth week). Similar trends were observed for thermal hypernociception. The administration of AD6626 effectively reduced hypernociception in WT and ALDH2*2 animals throughout the observation period. As expected, ALDH2 expression is decreased in the spinal cord (71.02%) in ALDH2*2 when compared to WT mice. Ethanol reduced ALDH2 activity in WT and ALDH2*2 (44.34% and 14.46%, respectively). AD6626 treatment restored the ALDH2 activity. Additionally, ethanol led to increased 4-HNE load in the spinal cord of WT and ALDH2*2 mice (44,8% and 49,9%, in the ventral horn, and of 184,2% and 196,7%, in the dorsal horn). AD6626 treatment partially prevents 4-HNE increase in WT mice (6,3% in the ventral horn and 10,7% in the dorsal horn), and ALDH2*2 (7,27% ventral horn and 8,7% in the dorsal horn). Our data indicate that decreased ALDH2 activity aggravates neuropathic hypernociception. The present work reveals a pivotal role of ALDH2 in modulating the onset of AN, and identifies a new therapeutic class for its treatment. Supported by: PIBIC (Butantan Institute), CNPq 131439/2023-5, FAPESP 2022/15640-4, FAPESP 2021/14831.

05.028 **Influence of Calcitonin Gene-Related Peptide on Cutaneous Healing and Nociception: Insights from CGRP-Knockout and Wild-Type Mice.** Jamba MF¹, Tavares YPST¹, Teixeira SA¹, Brain SD², Muscará MN¹, Costa SKP¹ ¹ICB-USP Depto de Farmacologia; ²Kings College, School of Cardiovascular Medicine & Sciences, Waterloo Campus, London

Introduction: The skin, the body's largest sensory organ and protective barrier, displays high regenerative capacity. Injury to this barrier initiates a highly regulated wound-healing process involving immune, vascular, stroma and epithelial cells. Calcitonin gene-related peptide (CGRP), a sensory neuropeptide abundant in sensory nerves regulates vasodilation, inflammation and early wound healing¹. Similarly, hydrogen sulfide (H₂S) has been implicated in protective mechanisms against pain and inflammatory disorders², partly through stimulation of capsaicin-sensitive sensory neurons³. Whether endogenous CGRP modulates nociceptive behavior and wound healing plasticity remains unclear. Using α -CGRP knockout (KO) mice, we investigated the role of CGRP in nociception and skin wound healing. **Methods:** A 5-mm excisional wound was induced in the dorsal skin of male and female C57Bl/6 wild-type (WT) and α -CGRP KO mice (8 weeks old)⁴. Nociception was assessed over time by primary and secondary mechanical withdrawal thresholds (MWT) using von Frey filaments and spontaneous pain behavior using the facial grimace scale⁵. Wound closure was monitored longitudinally through digital imaging and caliper measurements. Data are expressed as mean $\pm$ SEM or median $\pm$ interquartile range for n= mice; significance was set at p<0.05. **Results:** Wound healing progressed significantly faster in WT than in α -CGRP KO mice, independent of sex. WT animals showing higher rates of complete wound closure (AUC0-15 days; 183.1 $\pm$ 14.8 and 207.4 $\pm$ 25.2*, respectively, n=5/6), enhanced epithelialization and hair follicle regeneration, whereas α -CGRP KO mice exhibited delayed closure (AUC0-15 days; 287.5 $\pm$ 3.2 and 341.8 $\pm$ 30.6*, respectively, n=5/6) and reduced regenerative outcomes. Longitudinal analysis (0–15 days) confirmed a greater proportion of fully healed wounds in WT animals compared to α -CGRP KO mice, suggesting lower epithelialization and hair follicle regeneration in α -CGRP KO mice (image not shown). Nociceptive testing revealed that α -CGRP KO mice displayed higher mechanical withdrawal thresholds in the primary wound region (0.5 cm score 0,6 g; AUC0-15 days; 4.27 $\pm$ 1.08 n=6) and reduced facial grimace scores (15.67 $\pm$ 3.37, n=6), compared to WT mice (6.02 $\pm$ 0.68 and 23.92 $\pm$ 5.40; n=5), respectively. No major differences were observed in secondary hyperalgesia between groups. **Conclusion:** CGRP exerts a dual role in cutaneous repair. It supports wound closure and tissue regeneration, while its absence attenuates nociceptive sensitivity. Thus, CGRP emerges as a key neuroimmune mediator linking tissue repair to pain. Animal Ethics Committee (CEUA number): Protocol No. 2055050819. **References:** 1. Russell et al (2014). Physiological reviews, 94(4), 1099-1142. 2. Costa, et al (2020). Antioxidants & redox signalling, 33(14), 1003-1009. 3. Hajna et al., (2016). Journal of Molecular Neuroscience, 60(2), 157-170. 4. Dunn et al., (2013). Journal of visualized experiments: JoVE, (75), 50265. 5. Langford, et al (2010). Nature methods, 7(6), 447-449. **Financial Support:** PDCT (Projecto de Desenvolvimento de Ciência e Tecnologia, Angola), CAPES/PROEX (001), CNPq. Fapesp.

05.029 **Investigation of the Antinociceptive Potential of Geranyl Acetate in an Animal Model of Non-Inflammatory Diffuse Pain.** Pereira SAP¹, Martins RYC¹, Castro JLS¹, Ferraz SLNS¹, Martins DFA¹, Acha BT¹, Sousa DP², Oliveira, FA¹, MLS¹, Almeida, FRC¹ ¹UFPI, Lab Pain Pharmacology, Medicinal Plants Research Center ²UFPB, Dept Pharmaceutical Sciences, Health Sciences Center

Introduction. The current landscape underscores the need for further research aimed at developing new pharmacological alternatives for the treatment of chronic pain conditions, such as fibromyalgia. Geranyl acetate (GA), an acyclic monoterpene, exhibits several bioactive effects depending on the plant source. In *Helichrysum microphyllum*, it shows antimicrobial activity; in *Cymbopogon martinii*, antibacterial and antifungal effects; in *Ferula szowitsiana*, antioxidant properties; and in *Citrus limon*, possible sedative and anxiolytic actions. Despite these findings, studies on its analgesic potential remain scarce. The aim of this study is to investigate the antinociceptive potential of geranyl acetate in animal models of non-inflammatory diffuse pain.

Methods. GA was kindly provided by Prof. Dr. Damião Pergentino de Sousa (UFPB). GA was prepared in an aqueous solution with 2% Tween 80 and 0.9% NaCl for oral administration. Female Swiss albino mice (25–35 g) were used, housed under controlled conditions. Fibromyalgia was induced by subcutaneous reserpine injections (0.25 mg/kg) once daily for three days. Control groups received the vehicle solution without reserpine. Mechanical and cold nociception were assessed using the Von Frey and acetone tests to confirm model development and evaluate the efficacy of GA. In the acute treatment, mice (n=6 per group) received GA at various doses (6.25 to 50 mg/kg, orally) or morphine (10 mg/kg, subcutaneous). Behavioral assessments were conducted from 0 to 8 hours of post-treatment. In the subacute protocol, animals were treated daily with GA, vehicle, or duloxetine (30 mg/kg, oral) until day 15, with evaluations every two days. Investigating GA's mechanism of action, the involvement of the opioid system, the nitric oxide pathway, and the cytokine levels of IL-1 β were evaluated. All experimental protocols were approved by the Animal Ethics Committee (CEUA-UFPI No. n^o 836/2024). **Results.** In acute treatment, GA at doses of 6.25 to 50 mg/kg significantly increased mechanical nociceptive thresholds from 60 minutes, with the 25 and 50 mg/kg doses lasting up to 480 minutes. Morphine (10 mg/kg) increased thresholds up to 240 minutes. GA and morphine reduced thermal nociception scores in the first hour, but only GA doses of 12.5 mg/kg and above maintained this effect longer. No changes were seen in the Sham group. In chronic treatment. In subacute treatment, GA increased mechanical nociceptive thresholds at doses of 6.25 to 50 mg/kg throughout treatment, like duloxetine, except the lowest dose lost effect on the last day. In the subacute cold sensitivity test, GA at all doses significantly reduced nociceptive scores on various days, with stronger and sustained effects at 25 and 50 mg/kg. These results indicate GA's dose-dependent antinociceptive potential in both mechanical and thermal pain models. Naloxone blocked the antinociceptive effect of GA (25 mg/kg) and morphine in reserpine-induced pain. L-arginine reduced GA's effect, while L-NOARG increased the nociceptive threshold, indicating nitric oxide's role. This increase was reversed by L-arginine. The doses of 6.25 and 25 mg/kg of geranyl acetate (GA) reduced IL-1 β levels, indicating a potential anti-inflammatory effect associated with the treatment. **Conclusion.** Based on these results, it can be suggested that the antinociceptive effect of geranyl acetate (GA) is related to the opioid and nitric oxide pathways. Additionally, GA reduced cytokine levels such as IL-1 β , indicating a potential anti-inflammatory effect. **Keywords:** Geranyl acetate. Behavioral assessment. Fibromyalgia. Nociception. **Financial support:** Emergency Postgraduate Development Program (PDPG) for Strategic Consolidation of Postgraduate Programs (PPGs) *stricto sensu* 3/4 of the aid / Process number: 1895/2022/88881.707502/2022-01

05.030 **Standardization of a Bilateral 6-OHDA Mouse Model for Nociceptive Assessment in Parkinson's Disease and Evaluation of Pregabalin as a Therapeutic Strategy.** Pereira LG¹ Brito HA¹ Moraes RIF¹ Luiz. ACM¹ Peres DS¹ Kudsi SQ¹ Viero FT² Bressan GN¹ Antoniazzi CTD¹ Fachinetto R¹ Trevisan G¹ ¹UFSM, PPG Pharmacology, Brazil. ²IQ-USP, Brazil

Introduction: Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by the neurodegeneration of dopaminergic neurons. Beyond its well-known motor symptoms, PD is also associated with non-motor symptoms such as chronic pain, which significantly impacts patients' quality of life. However, many conventional animal models of PD exhibit substantial motor impairments that hinder accurate nociceptive assessment. **Objectives:** This study aimed to standardize a bilateral 6-hydroxydopamine (6-OHDA) injection model in C57BL/6 mice that allows reliable nociceptive assessment without major motor deficits. Additionally, we evaluated the efficacy of oral pregabalin treatment in alleviating PD-induced nociceptive alterations. **Methods:** Male and female C57BL/6 were divided into Sham, 6-OHDA, and pregabalin-treated groups. PD was induced by bilateral intrastriatal injection of 1 μ L of 6-OHDA (4 μ g/ μ L) per site. Pregabalin (60 mg/kg, i.g.) was administered acutely. Behavioral analysis was performed at baseline and on days 7, 14, and 21 post-induction. Motor function and balance deficits were assessed using the rotarod test and cylinder test. Mechanical allodynia was measured using the von Frey up-down method, cold allodynia with the acetone test on the hind paw, and heat allodynia was assessed using the hot plate test at a constant temperature of 38 °C. Anxiety-like behavior was assessed on day 21 using the open field and elevated plus maze (EPM) tests. Euthanasia and sample collection occurred on day 21 post-induction. Western blotting for tyrosine hydroxylase was performed to confirm dopaminergic degeneration (CEUA N° 3164260423). **Results:** The PD model did not impair general locomotor activity, as confirmed by the rotarod and cylinder test and total distance traveled. In the open field test, PD animals of both sexes spent significantly more time in the peripheral zone (239.6 $\pm$ 4.5 for female; 261.6 $\pm$ 11.4 for male, p <0.05) compared to sham mice (167.8 $\pm$ 12.3 for female; 155.1 $\pm$ 9.0 for male, p <0.05), indicating increased spontaneous nociception and anxiety-like behavior, which was not observed in pregabalin-treated animals. In the EPM, PD animals spent more time in the closed arms (271.0 $\pm$ 4.9 for females; 261.6 $\pm$ 2.8 for male, p <0.05), again reversed in pregabalin-treated animals (245.8 $\pm$ 7.5 for female; 244.1 $\pm$ 8.8 for male, p <0.05). PD animals exhibited significant mechanical allodynia at all time points, peaking on day 21 post-induction (0.93 $\pm$ 0.2 for female; 0.54 $\pm$ 0.1 for male, p <0.05). Heat allodynia peaked on day 14 after induction (49.5 $\pm$ 11.4 for female; 86.4 $\pm$ 13.1 for male, p <0.05) compared to Sham animals (156.0 $\pm$ 15.9 for female; 164.7 $\pm$ 7.6 for male, p <0.05), while cold allodynia was not consistently altered. Pregabalin administration significantly reduced mechanical allodynia at 1 h post-treatment, with maximum inhibition (I_{max}) of 62.4 $\pm$ 3% in female and 44.2 $\pm$ 5% in males. For heat allodynia, pregabalin produced an I_{max} of 51.4 $\pm$ 10% in females and 40 $\pm$ 6% in males. The reduced tyrosine hydroxylase immunocontent (1.8 $\pm$ 0.1 to sham group; 0.4 $\pm$ 0.06 for 6-OHDA group, p <0.05) and the cylinder test confirmed the successful lesion to induce the PD model (47.5 $\pm$ 2.7 before the surgery; 28.8 $\pm$ 1.4 after the surgery, p <0.05). **Conclusion:** The bilateral intrastriatal 6-OHDA model in C57BL/6 mice successfully induced PD without impairing motor performance, enabling the investigation of pain-related behaviors. Pregabalin showed efficacy in reducing mechanical and thermal allodynia, supporting its therapeutic potential for PD-associated pain. This model provides a valuable setting for future studies targeting non-motor symptoms of PD. Financial: CNPq and Capes.

05.031 **Joint Pain Modulation by *Hibiscus sabdariffa*: Functional Evaluation in a Monoiodoacetate-Induced Osteoarthritis Model.** Cruz ACSN, Mesquita MJTAM, Reis ATF, Correia LCP, Carvalho TGPM, Frota GM, Lima RF, Sousa LR, Garcia JBS, Carvalho RC, Andrade MS, Cartagenes MSS UFMA, Experimental Lab for Pain Studies, Brazil

Introduction: *Hibiscus sabdariffa* (HS), known by the vernacular name "vinagreira" in Maranhão, is an edible plant species that contains a concentration of polyphenolic compounds, which justifies its antioxidant capacity and anti-inflammatory activity. Knee

osteoarthritis (OA) is a chronic and degenerative disease that affects the cartilage and the surrounding structures of the joint. The available pharmacological therapies are palliative, aimed at symptomatic relief of inflammation and pain, but cause adverse effects with chronic use. The search for new therapeutic compounds has stood out in the field of scientific investigation, and plant-derived products emerge as an option. Objective: To evaluate the effect of HS leaf extract in OA. Methodology: Study approved by CEUA under (CIAP: 02.0341.2019). This study was financially supported by CAPES and CNPq. OA was induced by intra-articular injection of 2 mg of monoiodoacetate (MIA) in the knee of 25 *Rattus norvegicus*. The animals were divided into 5 groups: healthy control, without MIA intervention, and 4 other groups induced to OA with MIA, distributed into: meloxicam (MLX) (2 mg/kg), saline group, treated with HS1 (45 mg/kg/day), HS2 (90 mg/kg/day), and ethyl acetate fraction (EAF) (1 mg/kg/day). The animals were evaluated by behavioral tests: weight bearing (WB) and grimace scale (GS) before induction (D0), and on days (D) 7, 14, 21, and 28 after induction, aiming to analyze nociception. **Results:** When evaluating the weight distribution on the paws, the WB test showed that HS1 (45 mg/kg) decreased the imbalance between the paws on D7 ($p = 0.006$) and on D21 ($p = 0.02$). Treatment with EAF (1 mg/kg) equalized the weight distribution on the paws during D7 ($p = 0.0002$) and D14 ($p = 0.0296$), with a moderate and sustained effect over the period. In the GS test results, facial expressions were observed, indicating that treatments with HS1 (45 mg/kg), HS2 (90 mg/kg), and EAF (1 mg/kg), on D7, showed a significant effect in reducing pain, with $p = 0.0019$, 0.0019 and $p = 0.0027$, respectively. On D21, the 45 mg dose showed significance ($p = 0.0209$), maintaining analgesic effect. On D28, EAF reappeared as effective in controlling late spontaneous pain, a biphasic action profile (D7 and D28).

Conclusion: HS extract at a dose of 45 mg/kg and EAF (1 mg/kg) exhibit antinociceptive effect in an experimental osteoarthritis model in rats. These findings reinforce the potential of HS in chronic pain management, possibly due to the presence of bioactive compounds promising for the development of new analgesic approaches. Keywords: osteoarthritis, *Hibiscus sabdariffa*, weight bearing, joint pain, grimace scale.

05.032 **Targeting Drp1, a Key Regulator of Mitochondrial Fission, to Mitigate Paclitaxel-Induced Neurotoxicity.** Schiess MC¹, Troitino VC¹, Souza MM² deOcesano-Pereira C² Bufalo MC² ChudzinskiTavassi AM² Zambelli VO^{1,2} ¹IB, Lab Pain and Intracellular Signalization, Brazil, ²IB, Centre of Excellence in New Target Discovery, Brazil

Introduction: Paclitaxel (PTX) is a chemotherapy commonly used to treat solid cancer and, although effective, it induces chemotherapy-induced peripheral neuropathy (CIPN) in up to 97% of patients, resulting in sensory abnormalities, such as numbness, tingling and neuropathic pain. Mechanistically, PTX binds to and stabilize microtubules and, consequently, interrupts axonal transport, leading to an insufficient supply of ATP and increased oxidative stress. Recently data from our group showed that PTX modifies the mitochondrial dynamics of sensory neuron-like cells, inducing mitochondrial fragmentation and dysfunction. The dynamin related protein (Drp1) is the main GTPase involved in pathological contexts of mitochondrial fragmentation. P110 is a seven-amino acid peptide that restores mitochondrial dynamics by acting as an inhibitor of mitochondrial fission. Therefore, we aimed to investigate whether preventing excessive mitochondrial fission would mitigate paclitaxel-induced neurodysfunction in sensory neuron-like cells in culture.

Methods: P110 (Ac-YGRKKRRQRRRGDILLPRGS-NH₂) was stored at a concentration of 1 mM. Sensory neurons-like cell were obtained from the culture and differentiation of SH-SY5Y cells in the presence of all trans-retinoic acid (10 mM) and brain derived neuron factor (50 ng/mL). Cells were seeded in 75 cm³ flasks until 80% of confluence and 1x10⁴ neurons were transferred to 96 well plates in collagen matrix (100 mg/mL) for differentiation. The neuroprotective effect was evaluated in mature neurons treated with paclitaxel (0.5, 5.0 and 10.0 mM; 48 h) in the presence or absence of P110 (1 mM) or control groups (vehicle, DMSO 1%; positive control, DMSO 20%). Cell viability was assessed by inhibition of 3-(4, 5-dimethylthiazol-2-yl)2,5-diphenyl-tetrazolium bromide (MTT) reduction. Cells were stained with β III-tubulin for neurite outgrowth evaluation in the High-Content Screening and mitochondrial fragmentation was evaluated by combining MitoTracker staining with confocal microscopy. **Results:** Cells incubated with (0.5, 5.0 and 10.0 μ M) displayed decreased viability (86.1 $\pm$ 0.3%*, 81.8 $\pm$ 2.5%*, 77.6 $\pm$ 1.5%* vs control 100%, respectively). However, the pre-incubation with P110 significantly prevented PTX-induced neurotoxicity, when compared to control groups (*p<0.05, n=4, One-way ANOVA, Post-Test Tukey). Moreover, P110 restored PTX-induced impairment in neurite outgrowth (7.6 $\pm$ 0.1 μ m, 8.7 $\pm$ 0.2 μ m vs 9.3 $\pm$ 0.2 μ m, respectively) and the mean process length (2.9 $\pm$ 0.02 μ m, 3.3 $\pm$ 0.05 μ m vs 3.7 $\pm$ 0.05 μ m (*p<0.05, n=1 in triplicate, One-way ANOVA, Post-Test Tukey). Finally, P110 protected from PTX- induced mitochondrial fragmentation, detected by the rescue of mitochondrial mean area (0.19 $\pm$ 0.004 μ m² vs 0.22 $\pm$ 0.003 μ m² vs control 0.24 $\pm$ 0.007 μ m²) and mean perimeter (2.2 $\pm$ 0.05 μ m vs 2.6 $\pm$ 0.01 μ m vs control 2.4 $\pm$ 0.05 μ m) of sensory-neurons when compared to groups. **Conclusion:** Our findings indicate that P110 display neuroprotective effects in sensory neurons-like cells, restoring the axonal degeneration and inhibits PTX-induced mitochondrial fragmentation. Thus, our study suggests that the use of pharmacological inhibitors, such as P110, may offer a relevant strategy for preventing PTX-induced neuropathy. **Financial support:** CAPES (001).

05.033 Investigation of Alpha-Terpinene in Chemotherapy-Induced Nociception: Insights from *in vivo* Model and *in silico* ADMET Analysis. Gouvea MC^{1,2,3}, Romeiro NC³, Carmo PL^{1,2}, Barth T¹, Raimundo JM², Bonavita AGC^{1,2} ¹UFRJ, Lab Produtos Bioativos, Centro Multidisciplinar UFRJ-Macaé. ²UFRJ, Grupo de Pesquisa em Farmacologia de Produtos Bioativos, Centro Multidisciplinar UFRJ-Macaé. ³UFRJ, Laboratório Integrado de Computação Científica, Centro Multidisciplinar UFRJ-Macaé

Neuropathic pain is a type of chronic pain resulting from damage to the central and peripheral nervous system, caused by a series of pathologies such as diabetes, viral infections, and others. Conventional analgesic treatments are often ineffective for this condition, requiring the use of antidepressant and anticonvulsant drugs. Therefore, the search for new treatments for this type of pain is necessary. The objective of this work was to evaluate the antinociceptive potential of 1-isopropyl-4-methyl-1,3-cyclohexadiene (α -terpinene), a monoterpene present in several essential oils, in a model of chemotherapy-induced neuropathic pain. Additionally, using computational resources, we aimed to predict pharmacokinetic properties and possible molecular targets. To assess nociception, we used female Swiss mice (30 to 35g) maintained on 12-hour light/dark cycles with food and water *ad libitum*. Neuropathic pain was induced by intraperitoneal (ip) administration of the chemotherapeutic agent vincristine (0.1 mg/kg) for 14 consecutive days. On the 15th day, nociception was evaluated using the hot plate test. The antinociceptive potential was evaluated using α -terpinene (100 to 200 mg/kg, ip) administered 30 minutes before measuring nociception. All experiments were approved by CEUA/UFRJ nº 055/23. The *in silico* analyses, was performed using PubChem database (<https://pubchem.ncbi.nlm.nih.gov>), and predictions were made using free web tools such as PkCsm, SwissADME, and SwissTargetPrediction. As an initial result, it was identified that vincristine induced neuropathic pain, with a reduction in latency time from 7.6 ± 0.4 s (control) to 4 ± 0.8 s to thermal stimulus (thermal hyperalgesia). Treatment with α -terpinene increased the latency time at the evaluated doses, indicating its analgesic potential. Regarding the *in silico* evaluation, it was possible to predict good permeability to biological barriers, such as the blood-brain barrier, using LogP descriptor values. This value is similar to that identified for amitriptyline and morphine, indicating that terpenes can reach the CNS. Furthermore, this substance has zero violations of Lipinski's Rule of 5. The prediction did not indicate interaction with hepatic metabolism enzymes (CYP) or possible hepatotoxic effects, using pKCSM. Finally, using SwissTargetPrediction, it was possible to predict that nuclear receptors and G-protein coupled receptors would be among possible molecular targets for pharmacological action. Thus, we can conclude that α -terpinene has therapeutic potential in the treatment of neuropathic pain, with *in vivo* effects and favorable pharmacokinetic parameters.

05.034 **Oleic Acid Alleviates Mechanical Hypersensitivity in an Experimental Inflammatory Pain Model.** Dambros LV¹, Serafini PT^{1,2}, Brum ES^{1,2}, Favarin A^{1,2}, Becker G^{1,2}, Brusco I^{1,2}, Oliveira SM^{1,2}
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Introduction: Sunburn represents a relevant public health issue, particularly in countries with high sun exposure, such as Brazil. Ultraviolet B radiation from sunlight can induce pain, inflammation, oedema, erythema, and generate reactive oxygen species in the affected tissues. While current treatments can be effective, their long-term use is often limited by undesirable side effects. Despite the effectiveness of current sunburn treatments, their adverse effects restrict prolonged use. Consequently, the development of new pharmacological strategies is essential, with natural products representing a promising source of bioactive compounds. Among them, oleic acid (OA), a naturally occurring molecule present in a variety of foods and cell membranes, has gained attention for its well-established anti-inflammatory effects. **Objective:** Evaluate the antinociceptive, anti-inflammatory, and antioxidant effects of topical OA application in a UVB-induced sunburn model. **Material and methods:** We used male Swiss mice (25–30 g; Ethical approval 140270722) to achieve this objective, exposing the right hind paw to UVB radiation (0.75 J/cm² for 20 min - single exposure). After exposure, 15 mg/paw of topical treatment gel (vehicle), gel containing OA (3%) and gel containing dexamethasone 0.1% (reference drug) were applied. Mechanical sensitivity, polymorphonuclear cell count, and oxidative stress were analyzed. **Results and Conclusion:** Oleic acid reduced mechanical sensitivity, cellular infiltration, and serum levels of reactive oxidative species. Similar results were obtained with topical treatment with dexamethasone. Therefore, OA appears to be a promising choice for treating skin conditions related to sunburn. **Keywords:** Sunburn; inflammation; inflammatory cells; nociception; pain.

05.035 **Anterior Cingulate Cortex NMDA Receptors Regulate the Effects of Electroacupuncture on Allodynia in Rats with Peripheral Neuropathy.** DonizettiFonseca MA¹, de Souza MBRF¹, Ribeiro-Siqueira CM¹, da Silva AL^{1,2}, da Silva ML^{1,2}, da Silva JRT^{1,2}, Falconi-Sobrinho LL^{1,2} ¹Unifal, Lab. of Neuroscience, Neuromodulation and Study of Pain, Brazil, ²Unifal, Postgraduate Program in Biosciences Applied to Health, Brazil

Introduction: Recent studies suggest that the medial prefrontal cortex (MPFC) is implicated in mediating the analgesic effects of electroacupuncture (EA) in chronic pain related to peripheral nerve injury. In this scenario, the anterior cingulate cortex (ACC), a structure that composes part of the MPFC, has been shown to play a relevant role in modulating neuropathic pain induced by sciatic nerve constriction in laboratory animals. However, little is known about the neural mechanisms of the ACC that are involved in mediating the analgesic effects of EA in chronic neuropathic pain. **Aims:** The purpose of this work was to investigate the effects of pharmacological manipulation of ACC, through local microinjections of NMDA, an agonist of NMDA glutamate receptors, on the effects of a single application of EA on mechanical allodynia induced to peripheral nerve injury. **Methods:** Male Wistar rats, initially weighing 120 g, were subjected to chronic constriction injury (CCI) of the sciatic nerve. Prior to this, each rodent was subjected to the von Frey test to assess the basal mechanical sensory threshold (baseline 1). After 14 days of CCI, the animals had their mechanical nociceptive thresholds measured again (baseline 2). The next day (15st), they were subjected to stereotaxic surgery to implant a guide cannula directed to area 24b (Cg1) of the ACC. On the 21st day of CCI, the rodents had their mechanical nociceptive thresholds measured by von Frey test (baseline 3). Then, they were treated with EA (single application, 2 Hz, acupoints ST36 and SP6, 20 min). After 24h of EA, the rodents received microinjections of either physiological saline (vehicle) or NMDA (1 nmol/0.2 µL) in the ACC followed by Von Frey test at 5-min intervals for 30 min. After the end of the von Frey test, rats were perfused and their brains processed for histological study of vehicle or drug microinjection sites. **Results:** According to a two-way RM-ANOVA, there were significant effects of treatment ($F_{5,20} = 37,42$; $p < 0,001$), time ($F_{6,24} = 3,010$; $p < 0,001$), as well as treatment-by-time interaction ($F_{30,120} = 7,048$; $p < 0,001$) on mechanical nociceptive threshold of rats. CCI in the right paw induced neuropathic pain over 21 days ($p < 0.05$). EA in the right paw followed by 24h of vehicle microinjection in the ACC reduced the CCI-induced mechanical allodynia at 10 min, when compared to the group that received EA Sham in the right paw followed by 24h of vehicle microinjection in the same cortical area ($p < 0.05$). In contrast, NMDA microinjection in this same cortical area, 24h after a single application of EA, decreased the mechanical nociceptive thresholds (pronociception) at 10 and 20 min, when compared to the control group that received intra-ACC microinjections of vehicle also 24h after EA. **Conclusion:** Our data provide new insights into the involvement of glutamatergic excitatory mechanisms of the ACC in mediating the effects of EA on mechanical allodynia, suggesting that ACC NMDA receptors activation impairs the antinociceptive effects of EA in rats with on neuropathic pain. Ethical approved (CEUA-UNIFAL, 0041/2024). Financial support (FAPEMIG and PROBIC-UNIFAL).

05.036 **Neryl Acetate, derived from the Esterification of Nerol, shows a Possible Neuroprotective Effect in Glioblastoma(U-87) Tumor Cells Exposed to Paclitaxel Induced Chemotherapy.** Martins RYC, Gomes LS, Cavalcante MLS, Dittz Junior D, Cavalcante AACM, Ferreira PMP, Almeida FRC UFPI Teresina, PPG Farmacologia, Brasil

Introduction: Neuropathic pain is a chronic condition caused by injuries, diseases, or induced by drugs, such as paclitaxel. The treatment induced by chemotherapy is limited by the low efficacy of the drugs used and the adverse effects. Therefore, research for new therapeutic agents is necessary. Neryl acetate (AcN), derived from the esterification of nerol, has antinociceptive activity in animal models of acute glutamate-induced pain. This study's goal was to evaluate the effect of AcN on the neurotoxic activity of paclitaxel (PACLI). **Methodology:** First, triple-negative human breast adenocarcinoma cells (MDA-MB-231, 5x10³ cells/well), cultured in RPMI-1640 + FBS 10% medium, were incubated with AcN [2000 – 7.81 µg/mL] for 72 h and evaluated for their viability by the metabolization capacity of alamar blue [10 µg/mL], and the determination of the fluorescence intensity (excitation: 520 nm; emission: 580 – 640 nm). Subsequently, the neuroprotective activity of AcN, against the cytotoxic effect of paclitaxel, was analyzed in U-87 glioblastoma cells (5x10³ cells/well), cultured in DMEM High glucose medium [4.5 g/L] + FBS 10%. These were treated with PACLI [10 – 0.0781 µg/mL] and AcN [1000 – 7.81 µg/mL], alone and in association [PACLI 1: AcN 100], for 3 days, followed by the determination of the affected fraction and the combination index (ci) with the CalcuSyn software (v.2.1), according to the Chou-Talalay method. In view of the relationship between PACLI neurotoxicity and oxidative stress, the antioxidant activity of AcN was evaluated in $fa=1-(\% \text{ of viability} / 100)$ *Saccharomyces cerevisiae* strains, by the central disc test, proficient and mutated in antioxidant enzymes (EG103 (SODWT); EG118 (Sod1Δ); EG110 (Sod2Δ); EG133 (Sod1ΔSod2Δ); EG223 (Cat1Δ); EG (Sod1ΔCat1Δ)). The lines were cultured in YEL medium (0.5% yeast extract, 2% Bacto-peptone, 2% glucose) at 28 °C. Suspension cells were seeded from the center to the margin of a petri dish in a continuous cycle. The antioxidant evaluation was performed considering the oxidative damage induced by hydrogen peroxide (10 mM). The results were evaluated by Analysis of Variance (ANOVA) and by the Newman-Keuls test as a post hoc test using the GraphPad Prism program, version 6.00 **Results:** The nerol derivative showed no cytotoxic effect on human breast adenocarcinoma cells at the concentrations tested ($p>0.05$). In the evaluation of possible neuroprotection, the combinations of PACLI + AcN [2.5:250; 1.25:125; 0.625:62.5; 0.3125:31.25] attenuated the inhibitory effect of paclitaxel on the viability of glioblastoma cells ($p<0.05$), and presented a mostly antagonistic interaction profile (>1.2). The antioxidant evaluation, using *S. cerevisiae*, suggests that AcN did not oxidize the genetic material of the tested strains, showing antioxidant activity in the modulation of oxidative damage induced by hydrogen peroxide, for the SODWT strains, Sod1Δ, Sod2Δ, Sod1/Sod2Δ, Cat1Δ, Cat1/Sod1Δ. **Conclusion:** The results suggests that AcN showed neuroprotective activity *in vitro*, framing it as a potential drug for paclitaxel-induced neuropathy. However, knowing the complexity of the pathology investigated, it is necessary to investigate the pharmacological action of this natural product *in vivo*.

05.037 Long-Lasting Thermal Sensitization Following SARS-CoV-2 Spike Protein Injection in Mice.

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COVID-19, a disease caused by the SARS-CoV-2 virus, has become the world's greatest public health emergency. It was initially treated as pneumonia, as it affected the lungs of infected individuals. However, as knowledge about the disease has advanced, other pathophysiological manifestations in different organs have been noticed, characterizing post-COVID syndrome. The symptoms include myalgia, arthralgia, back pain, joint pain, and headaches, which is still affecting patients' quality of life. Given the increase in cases of post-COVID pain and the current therapeutic limitations to treat chronic pain, the aim of this study was to evaluate the pathophysiological mechanisms of musculoskeletal pain associated with post-COVID syndrome using the experimental model of intraplantar injection (i.pl.) of the SARS-CoV-2 Spike protein (20 µg/paw) in Swiss mice. During 35 days, thermal (hot and cold) and mechanical sensitivity assessments were performed using the Hargreaves, Dry Ice and von Frey methods, respectively. In addition, automated mechanical sensitivity assessments were performed using the Blackbox One equipment. The results showed that the injection of the Spike protein into the animals' hindpaw plantar surface caused thermal hypersensitivity to heat stimulus, and mechanical hypersensitivity, for 20 days after the injection in both ipsi and contralateral hindpaws. Interestingly, automated luminance analyses (FTR frame) of the paws demonstrated a significant difference between the paw luminescence of ipsi and contralateral hindpaws when evaluated 30 days after i.pl. injection. These results suggest that, the structure of the virus not only is responsible for pneumonia, but also for pain sensitization, also referred by some patients after the virus infection. Further analyses will be performed to investigate the biological and molecular mechanisms behind this symptom.

05.038 **Physiopharmacological Evaluation of Anthelmintics against Inflammatory, Neuropathic and Oncological Pain.** Rodrigues DCN¹, Sousa RWR¹, Santos IL¹, Conceição MLP², Freitas MM¹, Acha BT³, Porto JCS¹, Feitoza LLS¹, Almeida ML¹, Santos IKS¹, Silva JN¹, Barbosa SLM¹, Dittz, D⁴, Almeida FRC³, Ferreira PMP¹ ¹UFPI, Lab Experimental Cancerology (LabCancer), Dept Biophysics and Physiology, Brazil; ²USP, Lab Inflammation and Pain (Lid), Ribeirão Preto Medical School, Brazil; ³UFPI, Lab Pain Pharmacology, Brazil; ⁴UFPI, Antineoplastic pharmacology Lab (Lafan), Brasil.

Introduction Cancer pain is one of the most common and debilitating clinical manifestations associated with cancer. Translational research addressing this paraneoplastic symptom has gained prominence due to its clinical relevance and potential therapeutic impact. Drug repurposing has emerged as an alternative strategy, and among the promising candidates for this approach are anthelmintic agents. **Objective** This study aimed to evaluate the effects of different classes of anthelmintics on inflammatory, neurogenic, and oncological nociception. **Methods** Different classes of anthelmintics were tested in nociception protocols using *Mus musculus* (Swiss) mice. Inflammatory nociception was assessed through the abdominal writhing test and the formalin-induced paw-licking test. While supraspinal thermal nociception was evaluated using the hot plate test. Oncological nociception was analyzed in a cancer pain protocol, in which S-180 tumor cells (2×10^6 cells/mL) were inoculated intraplantarly, and nociceptive thresholds were measured 14 days post-inoculation using the Von Frey test. Behavioral alterations were monitored and cold allodynia was assessed using the acetone drop test. **Results** In inflammatory nociception, all anthelmintics tested, except ivermectin, reduced abdominal writhes. Paw-licking times varied according to phase: in the neurogenic phase, Niclosamide (51.5 ± 7.3 s), Mebendazole (69.3 ± 4.2 s), Praziquantel (53.5 ± 3.3 s) and Piperazine (62.5 ± 5.6 s) significantly reduced licking time compared with the negative control (119 ± 14.9 s; $p < 0.05$). In the inflammatory phase, Ivermectin (23 ± 8.9 s), Mebendazole (15 ± 4.4 s), and Albendazole (26.1 ± 6.9 s) showed significant reductions compared with the control (182 ± 12.6 s; $p < 0.05$). During the evaluation of central analgesia, Mebendazole increased hot plate latency ($p < 0.05$), suggesting supraspinal effects. In specific cancer pain tests, mebendazole treatment significantly increased the mechanical nociceptive threshold ($p < 0.05$) in animals with paw tumors. This tumor-associated antinociceptive effect may be related to the reduction in neuropathic pain, as the treatment also reduced the perception time in cold allodynia ($p < 0.05$). **Conclusion** Among the anthelmintics tested, Mebendazole showed the best antinociceptive effect in all types of nociception investigated. These findings demonstrate its translational potential as an adjuvant for the management of cancer pain. **Financial Support** This was partially supported by CNPq. **Approval** Ethics Committee on Animal Use (nº 710/2022 CEUA/UFPI).

05.039 **FLT3L Dependent Neuro-Immune Interaction in Peripheral Neuropathic Pain.** Ajas D^{1,2}, Aníbal E¹, Lucena-Silva GV¹, Pigatto G¹, Rivat C², Cunha TM¹ ¹FMRP-USP, Dept Pharmacology, Ribeirão Preto, Brazil. ²University of Montpellier, Institut for Neurosciences of Montpellier (INM) INSERM, Montpellier, France

Introduction: Chronic pain is a major health issue due to its high prevalence and socioeconomic impact. Neuropathic pain (NP), caused by damage or dysfunction of the somatosensory system, involves complex changes in neuronal excitability. However, neuronal alterations alone do not fully account for the persistence of pain. Recent studies highlight the crucial role of neuro-immune interactions in pain chronification. In this context, we previously identified the cytokine Fms-like tyrosine kinase 3 ligand (FLT3L) together with its receptor Fms-like tyrosine kinase 3 (FLT3) as key contributors to NP. However, the source and timing of FLT3L production in the Spared Nerve Injury (SNI) model remain unclear. **Methods:** NP was induced using the SNI model in adult male C57BL/6J mice, in this surgical model, two branches of the sciatic nerve, the peroneal and tibial nerves, are transected, while the sural nerve is spared. This selective lesion leads to the development of NP. To investigate the cellular sources of FLT3L, we performed single-cell RNA sequencing analysis of immune cells accumulating in the meningeal tissues surrounding the dorsal root ganglia (DRG) following SNI. Complementary to transcriptomic data, protein levels of FLT3L were quantified using ELISA assays in tissues collected at different time points post-SNI (3 and 7 days). **Results:** Single-cell data analysis revealed that FLT3L expression is found predominantly in immune cell populations. Strong expression was detected in ILC2s (average expression ~1.2, ~80% of cells), NK cells (average expression ~1.4, ~85% of cells), and T cells (average expression ~1.5, ~90% of cells). Complementary, ELISA assays revealed that FLT3L levels were increased in DRG and cerebrospinal fluid (CSF), but reduced in the meninges compared to naïve mice, Corroborating the hypothesis that meningeal FLT3L may have a role in NP, we also found that intrathecal injection of FLT3L promoted pain hypersensitivity in naïve mice. Our preliminary data lead us to hypothesize that meningeal immune cells transiently release FLT3L after SNI and it may reach the DRG and promotes DRG neurons hyperexcitability. **Conclusion:** Overall, these findings indicate that FLT3L may act as an early and short-lived immune-derived signal contributing to the initiation of NP. Building on these observations, our next step is to generate knock-in mice of FLT3L in specific immune cell, allowing us to directly test the contribution of distinct immune cell subsets to NP mechanisms. Understanding how FLT3L/FLT3 signaling shapes neuropathic pain will provide a foundation for designing new targeted treatment approaches. This project is support by FAPESP, CAPES, FAEPa, University of Montpellier and INSERM.

05.040 **Mechanistic Insights into the Anti-Inflammatory Action of IMT504.** Miguel B¹, Anibal Silva, CE², Brumovsky, PR¹, Cunha TM² ¹Universidad Austral Instituto de Investigaciones en Medicina Traslacional (CONICET-Universidad Austral), Argentina. ²FMRP-USP, Dept Pharmacology, Brazil

Introduction: Neuropathic pain is a type of chronic pain caused by a lesion or disease of the somatosensory nervous system. Its prevalence is around 10% of the general population and only in Brazil over 31 million people suffer from it. The immune system is increasingly recognized as a relevant player in developing chronic neuropathic pain. Macrophages have been recently shown to play a crucial role in initiating and maintaining neuropathic pain. Despite our growing understanding of the mechanisms of neuropathic pain, its management remains difficult due to a lack of both effective and safe treatments. IMT504, a non-CpG oligodeoxynucleotide, has been proven to reduce pain-like behavior in neuropathic pain models and it does so by shifting the inflammatory response towards an anti-inflammatory profile. However, the precise molecular mechanism by which IMT504 exerts these effects remains unclear. The aim of this study is to determine the cytokine profile of bone marrow derived macrophages (BMDM) when stimulated with IMT504 and, through this mechanistical approach, uncover the specific signaling pathways IMT504 influences. **Methods:** BMDMs were cultured from C57BL/6 mice (n=12). To determine if IMT504 is internalized by BMDMs, these were incubated with a conjugated form of IMT504 and AlexaFluor 488 (IMT504-AF488) at 10 µg/ml for 15, 30, 60, and 180' to be analyzed by flow cytometry. A subset of samples was treated with 0.1% Trypan Blue immediately before acquisition. As for cytokine quantification, BMDMs were plated following this conditions: negative control, IMT504-treated control (at 10,30 and 100 ug/ml for either 6 or 24h), LPS-stimulated control (at 100 ng/ml for either 6, 12 or 24h) and IMT504 treated LPS positive cells (cells were pre-treated with IMT504 for 6 or 24h followed by a stimulation with LPS for 6,12 and 24h. Cytokine concentrations of TNFα, IL-6, IFN-β, IL-10, TGF-β, CXCL1, CCL2 in supernatant was measured. Cell viability was assessed. **Results:** In regards internalization, more than 96% of cells were positive for AF488 across all timepoints. Mean fluorescence intensity (MFI) significantly increased in a time-dependent manner. Trypan Blue addition did not alter these results. On cytokine quantification, BMDMs pretreated with IMT504 prior to LPS stimulation showed a significant increase in the release of IL-10 when compared to the negative controls, observed across all tested conditions. This increase was not seen in BMDMs only treated with IMT504. Cells viability remained comparable to negative control throughout all experiments. **Conclusion:** We confirmed that IMT504 is rapidly internalized by BMDMs, in a time-dependent manner. IMT504 pretreatment increases IL-10 release in response to LPS. The fact that IMT504 alone did not induce IL-10 release suggests that IMT504 acts as a primer rather than a direct activator. This may point to a key molecular mechanism for IMT504's anti-allodynic properties, as IL-10 is known to alleviate neuropathic pain. Future *in vivo* studies using IL-10 KO mice with neuropathic pain will be necessary to confirm if this induction is sufficient to produce a significant analgesic effect. This study was supported by Fondo Nacional de Ciencia y Tecnología de Argentina (PICT-2021-00401), CONICET & Universidad Austral (80/23;04/25).

05.041 **Advanced Oxidation Protein Products (AOPPs) Activate TRPA1 in a Neuropathic Multiple Sclerosis Model.** Rodrigues P¹, Frare JM², Ruviano NA², Peres DS¹, Moraes RIF¹, Antoniazzi C¹, Machado AC¹, Araújo DSM⁴, Marini M⁴, Landini L⁴, De Logu F⁴, Geppetti P⁴, Nassini R⁴, Bochi GV¹, Trevisan G. ¹UFSM, Graduate Program in Pharmacology, Brazil. ²UFSM Graduate Program in Biological Sciences, Brazil. ³UFSM, Graduate Program in Pharmaceutical Sciences, Brazil. ⁴University of Florence, Dept Health Sciences, Italy

Introduction: Relapsing-remitting multiple sclerosis (RRMS) is associated with oxidative stress and neuroinflammation, often accompanied by neuropathic pain and anxiety. The advanced oxidation protein products (AOPPs), generated via nicotinamide adenine dinucleotide phosphate oxidase (Nox)/myeloperoxidase (MPO) pathways, are elevated in MS and may act as endogenous agonists of TRPA1, a redox-sensitive ion channel involved in pain signaling. This study aimed to investigate whether AOPPs activate TRPA1 and contribute to relapsing-remitting experimental autoimmune encephalomyelitis (RR-EAE)-associated nociception and anxiety-like behavior. **Methods:** We assessed AOPP-induced calcium influx in HEK293 cells expressing TRPA1 and in DRG neurons from *Trpa1*^{+/+} and *Trpa1*^{-/-} mice. *In vivo*, AOPPs (0.1–30 nmol/site) were administered intrathecally (i.t.) in female C57BL/6J *Trpa1*^{+/+} and *Trpa1*^{-/-} protocols (#9746010620; #7956291123; #6792240423; #4575031022; #4053081020, protocol#1194/2015-PR) mice with or without TRPA1 antagonists (HC030031, A967079) or the RAGE antagonist (FPS-ZM1, 10 nmol/site, i.t.). RR-EAE mice were treated with apocynin (APO, 100 mg/kg, i.g., 15 days), or with a single i.t. dose of anti-AOPP antibody. Behavioral tests included nociception (von Frey and acetone), nest building, spontaneous behavior, anxiety-like behavior (open field, elevated plus maze), and motor performance. Biochemical assays evaluated AOPP levels, Nox/MPO activity, neuroinflammatory/demyelination markers by PCR and Immunofluorescence, and demyelination by Luxol fast blue. Data were expressed as mean ± SEM and analyzed using ANOVA or Kruskal–Wallis, > 0.05 were considered significant. Principal component analysis (PCA) was used to explore multivariate associations. **Results:** AOPPs selectively induced calcium influx in TRPA1-expressing cells and triggered nociceptive behavior and spinal p-Erk activation in *Trpa1*^{+/+} mice, but not in *Trpa1*^{-/-}. TRPA1 antagonists, but not the RAGE antagonist, blocked AOPP-induced effects. In RR-EAE mice, APO reduced clinical score (90% of the animals), nociception (von Frey 67.3 ± 6.8), anxiety-like behavior, AOPP levels (spinal cord 96.3 ± 5.4), MPO/Nox activity, neuroinflammation, and demyelination in spinal cord and brain. Acute anti-AOPP injections exerted antinociceptive and anxiolytic-like effects in RR-EAE- and AOPP-induced nociception by lowering spinal cord and brain AOPP levels. **Conclusion:** Our findings demonstrate that AOPPs are endogenous TRPA1 agonists that mediate nociception, anxiety-like behavior, and neuroinflammation in the RR-EAE model. Pharmacological blockade of AOPPs or inhibition of their formation through Nox inhibition alleviates these symptoms. These results support targeting the AOPP/TRPA1 axis as a promising therapeutic strategy in MS-related neuropathic pain and emotional comorbidities. **Financial support:** FAPERGS, CNPq and Capes.

05.042 **TRPV1 Channel Sensitization Mediates Orofacial Thermal Allodynia in Mice Mucositis Model.** Benso B¹, Bustos D², Madrid D³ ¹Pontificia Universidad Catolica de Chile, School of Dentistry, Faculty of Medicine, Santiago, Chile ²Universidad Católica del Maule, Laboratorio de Bioinformática y Química Computacional, Depto de Medicina Traslacional, Facultad de Medicina, Talca, Chile ³Universidad de Santiago de Chile, Facultad de Química y Biología, Santiago, Chile

Introduction: Oral mucositis is a frequent and debilitating adverse effect of chemotherapeutic agents. Its clinical course begins with erythema and progresses to pain, xerostomia, hemorrhage, and secondary infections, significantly impairing patients' quality of life. Transient receptor potential (TRP) channels are central to thermo-nociception and inflammation. TRPV1 has been proposed as a nociceptive sensor that amplifies inflammation in oral mucositis. The objective of the study was to investigate the role of TRPV1 in chemotherapy-induced oral mucositis and assess potential compensatory mechanisms involving TRPA1. **Methodology:** Oral mucositis was induced in Trpv1-null (KO) and wild-type (WT) mice with 5- fluorouracil (5-FU) for 14 days. Macroscopic evaluation of the oral epithelial barrier was performed under direct visualization. Histological changes in the tongue were assessed by H&E staining and neutrophil quantification. Immunohistochemistry was used to evaluate TRPV1 and TRPA1 expression in the trigeminal ganglion. Nociceptive mechanisms were evaluated through orofacial pain assessment (lick/face ratio) and thermal place preference tests for thermal sensitivity. **Results:** 5-FU treatment induced marked oral mucosal damage in WT mice, characterized by epithelial disruption and ~30% higher neutrophil infiltration compared to controls ($p<0.01$), while KO animals exhibited attenuated lesions with only ~9-15% increase in neutrophils ($p<0.05$). Quantified IHC showed upregulation of TRPV1 in trigeminal ganglia of WT-5FU mice ($p<0.01$); as expected, TRPV1 was absent in KO animals, which instead displayed a ~36% increase in TRPA1 expression ($p<0.05$), suggesting compensatory signaling. KO animals retained partial nociceptive responses. **Conclusions:** Our findings demonstrate that TRPV1 and TRPA1 contribute to the sensitization of the orofacial region during chemotherapy-induced oral mucositis by integrating inflammatory and thermal nociceptive signals. TRPA1 compensation in the absence of TRPV1 suggests overlapping roles of TRP channels in pain modulation, offering potential targets for therapeutic intervention. Ethical Approval: CEC-USACH #473/2024 **Support Funding/Grant Number:** ANID/FONDECYT de Iniciación #11230490 and DICYT USACH 022443MM

06. Cardiovascular and Renal Pharmacology

06.001 **Treatment with Grape Pomace (*Vitis vinifera*) Improves Cardiac Morphological Parameters in Spontaneously Hypertensive Rats.** Magalhães VHL¹, Costa KCM², Pertuzatti PB¹, Freitas RA¹, Giachini FRC^{1,2} ¹UFMT, Institute of Biological Science and Health, Lab. Vascular Biology and Histopathology, Brazil, ²UFG, Institute of Biological Sciences, Brazil

Introduction: Bioactive compounds are found in winery waste, including pomace, representing a valuable source to prevent and treat various comorbidities, mainly due to their antihypertensive, antioxidant and anti-inflammatory effects. The aim of this study was to evaluate the potential effects of grape pomace (*Vitis vinifera*, Syrah variety) treatment in the cardiac morphology from hypertensive rats. **Methods:** Normotensive and spontaneously hypertensive male rats (SHR), aged 8 to 10 weeks (250-300g), were used. Hypertensive rats (SHR UVA, n=5) received a special diet enriched with grape pomace (300 mg/kg/day, 14 days), whether control groups received a standard diet (Wistar and SHR, n=5). Histological analyses were carried out in the heart, using eosin-hematoxylin or picrosirius staining. **Results:** The number of cardiomyocytes, intercellular spaces, cardiomyocyte diameter and interstitial collagen deposition were quantified. Collagen subtypes I and III were estimated by polarization. All procedures were approved by the Ethics Committee (CEUA/UFMT/23108.010699/2023-18). There were no changes in the number of cardiomyocytes and their intercellular spaces, in the groups evaluated. The cardiomyocytes diameter was increased in SHR rats (11.0 ± 0.02) compared to Wistar (9.78 ± 0.02) and the treatment with grape pomace was able to reverse this parameter in SHR rats (9.44 ± 0.18). Interstitial collagen deposition (%) was increased in the SHR group (33.62 ± 1.77) compared to the normotensive control group (23.94 ± 2.29), while after treatment with grape pomace, a reduction in deposited collagen was observed in the hypertensive rats (30.19 ± 0.50). Greater collagen type I deposition was observed in SHR, compared to Wistar and treatment with grape pomace reduced it in the hypertensive rats, without normalizing this parameter. **Conclusion:** Therefore, these results indicate that grape pomace supplementation may offer beneficial properties to improve cardiac remodeling observed during hypertension.

06.002 **Serotonin Amides: A Renal Safety Pharmacology Analysis.** Brasil JPP, Pereira T, Esteves GAB, Santos MARFD, Morcillo LSL UFRJ

Introduction: Serotonin amides, or β N-alkanoyl-5-hydroxytryptamides (Cn-5HT), are bioactive molecules found in coffee wax. Composed of a serotonin moiety bound to fatty acid chains of varying lengths, they exhibit anti-inflammatory and antinociceptive properties. Considering that such pharmacological effects often pose risks to the cardiorenal system, we investigated whether β N-octadecanoyl-5-hydroxytryptamide (C18-5HT) affects renal function. **Methods:** Thirty-one Male Wistar rats (100–120 g; CEUA 009/22) were divided into three groups: Control (PBS, 0.1 mL/Kg), C18-5HT 10 mg/Kg, and C18-5HT 30 mg/Kg, administered via oral gavage. The rats were placed in metabolic cages for 24 h for urine collection and water intake measurement. Afterward, blood and kidneys were collected. Renal function was assessed via plasma and urinary creatinine, blood urea nitrogen (BUN), proteinuria, and glomerular filtration rate (GFR) estimated from creatinine clearance. Sodium handling was evaluated by filtered load (FL_{Na}) and fractional excretion of sodium (FE_{Na}). Ouabain sensitive ($Na^+ + K^+$)-ATPase and ouabain resistant, furosemide sensitive Na^+ -ATPase activities were also measured. All results are represented by Mean $\pm$ SEM, One-way ANOVA followed by Tukey's post-test (n= 10). **Results:** At 10 mg/Kg, C₁₈-5HT reduced urinary volume by 28%, with no other significant changes. The 30 mg/Kg dose decreased plasma and urinary creatinine (57% and 36%), BUN (19%), and Na^+ -ATPase activity (51%), while increasing GFR (51%) and FE_{Na} (20%). **Conclusion:** C18-5HT at 10 mg/Kg, previously shown to have anti-inflammatory and antinociceptive effects, appears safe for renal function. However, the 30 mg/Kg dose induces alterations suggestive of nephrotoxicity. Thus, serotonin amides at 10 mg/Kg present a favorable therapeutic profile with preserved renal safety. **Financial support:** Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro. Award number: E-26/210.561/2024, Emendas Parlamentares do Governo Federal.

06.003 The Influence of a Synthetic Broad-Spectrum Metalloprotease Inhibitor on the Coagulotoxic Properties of *Bothrops alternatus* (Viperidae: Crotalinae) Venom. Azevedo SNS¹, Dias SR¹, Ghirotti HA¹, Souza-Gomes GC¹, Torres-Bonilla KA², Hyslop S², Sant'Anna SS³, Sousa EP⁴, Morais-Zani K⁴, Floriano RS¹ ¹Unoeste, Lab. of Toxinology and Cardiovascular Research, Graduate Program in Health Sciences, Brazil, ²Unicamp, Dept Pharmacology, Faculty of Medical Sciences, Brazil, ³IB, Lab. of Herpetology, Brazil, ⁴IBu, Lab. of Pathophysiology, Graduate Program in Toxinology, Brazil

The genus *Bothrops* comprises the most extensive group of venomous snakes in Latin America. In Brazil, twenty-eight species of *Bothrops* are currently documented and distributed across all regions of the country, being implicated in more than 80% of the snakebites annually registered by the Brazilian Ministry of Health. Envenomation by *B. alternatus* is characterized by intense local symptoms, including pain, oedema, hemorrhage, and necrosis, accompanied by significant systemic alterations, which include blood pressure disorders, coagulopathy, and renal failure. Marimastat (MMT), a broad-spectrum metalloprotease inhibitor, has demonstrated a satisfactory interaction with snake venom metalloproteases, making it a promising pharmacological agent to replace antivenom when it is unavailable or to enhance its neutralization. In this work, we assessed the metalloprotease inhibitory action of MMT as a stand-alone pharmacological tool or combined with anti-*Bothrops* antivenom (AV) on the coagulant effects of *B. alternatus* venom (BAv). Enzymatic activity was assayed using azocasein as substrate, and changes in absorbance were determined by a multiwell plate reader. Factor II (prothrombin) and X activation and thrombin-like activity were assessed using chromogenic substrates (S-2238 and S-2765); minimum coagulant dose (MCD) and fibrinogenolytic activity were assayed in human citrated plasma (CAAE protocol no. 43560620.0.0000.5493). MMT (0.3 mM) was determined as the minimum inhibitory concentration to abolish the caseinolytic activity of BAv. The MCD of BAv was determined in 6.4 mg of venom/ml. MMT (0.3 mM) abolished the procoagulant effect of BAv. AV (antivenom:venom ratio of 1:5 v/w) did not prevent the clotting formation in 240 s of monitoring, although it partially delayed the clotting formation at a higher antivenom:venom ratio (1:1, v/w) ($p < 0.05$, $n = 3$). The thrombin-like activity of BAv was slightly reduced by MMT (0.3 mM) and AV (1:5) in ~15% and ~36%, respectively ($p < 0.05$, $n = 3$), and the combination of both agents prevented the factor X activation by this venom ($p < 0.05$, $n = 3$). MMT (0.3 mM) and AV (1:5), including their combination, successfully prevented the prothrombin (factor II) activation by BAv ($p < 0.05$, $n = 3$). BAv exhibited a- and b-chain fibrinogenolysis. MMT (0.3 mM) reduced by $53 \pm 4\%$ and $57 \pm 5\%$ ($p < 0.05$, $n = 3$) the venom-induced a-chain fibrinogenolysis only at 30 and 90 min of incubation, respectively, with AV (1:5) being effective in reducing $70 \pm 10\%$ at 5 min incubation. MMT (0.03 mM) failed to prevent b-chain fibrinogenolysis, whereas AV (1:5) produced b-fibrinogenolysis inhibition at 5 ($65 \pm 9\%$) and 30 ($54 \pm 3\%$) min of incubation. In conclusion, BAv exhibits hemostatic activity *in vitro*. MMT by itself or combined with AV seems to be a plausible pharmacological strategy to prevent hemostatic disorders caused by BAv. However, the drug does not prevent BAv-induced factor X activation and b-fibrinogenolysis.

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06.004 Dual-Action Phenylhydrazide Derivatives as Novel MPO Inhibitors and Vasodilators: Therapeutic Potential for Cardiovascular Disease. Teixeira AP¹, Navarro TR², Aguiar TBS², Pimentel PP², Lima EC², Silva LL¹, Raimundo JM^{1,3} ¹UFRJ-Macaé, FarPBio, Brazil, ²UFRJ-Macaé, LACB, Brazil, ³UFRJ-Macaé, PPG-ProdBio, Brazil

Myeloperoxidase (MPO) is an important enzyme in neutrophil-mediated innate immunity that catalyzes the formation of hypochlorous acid (HOCl) from hydrogen peroxide and chloride ions. In cardiovascular system, neutrophil recruitment and activation with MPO release contributes significantly to cardiovascular pathophysiology by promoting oxidative stress, endothelial dysfunction, and atherosclerosis through multiple mechanisms including LDL oxidation, HDL dysfunction, and reduction of nitric oxide bioavailability. This study aimed to evaluate a series of eleven phenylhydrazide derivatives (LACB15-25) for their ability to inhibit chlorinating activity of MPO, scavenge DPPH radical, and evaluate the most promising derivatives on vascular activity. MPO activity was measured by 5,5' dithiobis(2-nitrobenzoic acid oxidation in a system containing physiological substrates tyrosine, urate, and thiocyanate, with H₂O₂ and Cl⁻. Absorbance at 412 nm was monitored over 10 minutes. The antioxidant activity was assessed using the 2,2-Diphenyl-1-picrylhydrazyl (DPPH) assay. Samples were incubated with methanolic DPPH for 30 min in the dark and absorbance was measured at 517 nm. To assess vascular activity, aortic rings from male Wistar rats (200–250 g) were isolated and mounted in organ baths containing oxygenated Krebs-Henseleit solution (37°C, pH 7.4). After equilibration (1.5 h, 1 g tension), rings were pre-contracted with phenylephrine (10 µM). Endothelial integrity was confirmed by acetylcholine-induced relaxation (≥85%, intact; ≤10%, denuded). Cumulative concentration-response curves (0.1–30 µM) for derivatives were generated. The protocols were approved by the CCS/UFRJ Animal Use Ethics Committee (132/24). Seven compounds demonstrated inhibition above 50% of HOCl production at 5 µM concentration: LACB15 (61.7±4.7%, IC₅₀ = 7.3±1.2 µM), LACB17 (82.1±4.1%, 2±0.7 µM), LACB18 (63.1±5.3%, 1.8±0.2 µM), LACB19 (58.1±6.9%, 2.5±0.6 µM), and LACB21 (54.3±8.1%, 1.9±1 µM), LACB22 (53.7±8 %, 1.1±0.5 µM), LACB23 (102.6±0.9%, 0.8±0.2 µM). The antioxidant evaluation revealed LACB23 as the most potent compound (EC₅₀ 29.3±2.9 µM), outperforming ascorbic acid (EC₅₀ 39.6±14.7 µM). While LACB15 (42.5±13.3 µM) showed comparable activity to ascorbic acid, other derivatives exhibited weaker effects: LACB17 (52±14 µM), LACB20 (52.1±15.5 µM), LACB16 (65.3±13.4 µM), LACB19 (64.5±13.6 µM), LACB22 (76.1±13.4 µM), LACB21 (80.2±12.8 µM), and LACB18 (88.9±12.3 µM). No antioxidant or MPO inhibitory activity was observed for the LACB24 and LACB25 derivatives. LACB23 showed the most favorable pharmacological profile, we further investigated its vascular effects using isolated rat aortic rings. LACB23 showed potent vasodilator effect in both endothelium-intact (EC₅₀ = 3.4±1.2 µM, R_{max} = 65.9±6.3%) and endothelium-denuded rings (EC₅₀ = 10.2±1.7 µM, R_{max} = 63.4±7.2%, p=0.0052), indicating endothelium-dependent and -independent mechanisms. LACB23 stands out as a promising multifunctional candidate, combining MPO inhibition with vasodilation. This dual action - reducing oxidative damage while improving vascular function - may provide enhanced therapeutic benefits. **Financial support:** FAPERJ; Scientific initiation grant PIBIC/CNPq.

06.005 **Taurine Reduces High Glucose Levels in *Caenorhabditis Elegans*.** Sant'Ana BH¹, Machado JC¹, Viçozzi GP², Vidal BA¹, Laurentino AOM¹, Pereira FSO³, Garcia S², Leal MB¹, Gomez R¹ ¹UFRGS, Programa de Pós-Graduação em Farmacologia e Terapêutica, Brasil ²UFRGS, Lab Toxicologia (LATOX), Programa de Pós-Graduação em Ciências Farmacêuticas, Brasil ³Unijuí, Brasil

Introduction: Diabetes is a highly prevalent disease associated with elevated glucose levels, morbidity, and mortality. Taurine, a semi-essential amino acid, has shown potential anti-diabetic effects in rodent models. However, its anti-diabetic effects on *Caenorhabditis elegans* (*C. elegans*) are poorly explored. This study aimed to evaluate taurine effect on the glucose concentration in a *C. elegans* diabetic-like model. **Methodology:** To carry out the experiments, we used *C. elegans* N2 Bristol strain (wild type) grown in agar Petri dishes (100 mm) containing nematode growth medium (NGM) and fed with *Escherichia coli* OP50 bacteria. To ensure that all worms were at the same larval stage (L1) at the time of exposure to the different treatments, gravid hermaphrodite worms were synchronized using a lysis solution. After synchronization, 3,000 worms were exposed to a 500 mM concentration of fructose as a standard to induce high glucose conditions in the worms. Petri dishes without fructose were used as a negative control. Metformin, at a concentration of 50 μ M, was used as a positive control for the glucose-reducing effect on the worms. After exposure to the high fructose diet, the plates were subjected to different concentrations of taurine (10, 50, and 100 μ M) for 48 hours. The surviving worms were recorded and evaluated for length and body area, and then subjected to freezing and thawing, steps necessary to facilitate the breaking of the cuticle by sonication. The sample was centrifuged, and the supernatant was used to determine glucose with the commercial kit by an enzymatic method. **Results:** Fructose produced high glucose concentration in *C. elegans* ($p = 0.006$), and metformin reduced glucose levels by 35.5% ($p = 0.0009$). The highest taurine concentration reduced elevated glucose levels by 28% ($p = 0.0130$). This diabetic-like condition decreased worm survival ($p = 0.0338$), and the lowest dose of taurine increased this parameter (0.0213). **Conclusion:** Taurine reduces glucose in *C. elegans* in a concentration-dependent manner, showing a possible antidiabetic effect. Further studies will be conducted to explore the mechanisms underlying this effect. **Keywords:** High glucose levels, *Caenorhabditis elegans*, Taurine **Funding:** CAPES, CNPq

06.006 Phosphodiesterase 5 Inhibition Attenuates Cardiovascular Dysfunction in Sepsis Surviving

Rats. Delfrate G, Neves PG, Albino LB, Moreira DH, Nardi G, Pinheiro LC, Assreuy J, Fernandes D UFSC, PPG Pharmacology, Brazil.

Introduction: Sepsis is a life-threatening and prevalent condition. Sepsis survivors face an increased risk of mortality due to cardiovascular events, as myocardial infarction. However, mechanisms underlying the post-sepsis syndrome (PSS) are not well understood. We investigated the hypothesis that nitric oxide (NO) signaling is impaired in PSS. Our aim was to evaluate the effect of the increase of cGMP levels by the phosphodiesterase 5 (PDE5) inhibitor, tadalafil, on the cardiovascular dysfunction of sepsis-surviving rats. **Methods:** Male Wistar rats (90-days old, 300–400 g; CEUA/UFSC 9785211221) were subjected to sepsis by cecal slurry (CS) model (1 g/kg, i.p.). Control animals received PBS-5% glycerol i.p. Sepsis survivors received tadalafil (Tad, 5 mg/kg/day, p.o.) or vehicle (0.5% CMC) treatment, starting 4 days post-sepsis until 15 or 30 days, when analyses were performed. **Results:** Approximately 50% survival was observed following the CS-induced sepsis. Animals showed increased blood lactate levels 24 hours after CS compared to control (1.8 ± 0.8 vs 4.2 ± 0.5 nmol/dL; $p < 0.05$), confirming that the animals were septic. Cardiovascular parameters were not altered in animals 15 days after CS. However, after 30 days, surviving rats showed a reduced hypotensive response to acetylcholine 30 nmol/kg i.v. (CS = -824 ± 299 vs Control = -1259 ± 193 mmHg/s; $p < 0.05$), reduced acetylcholine potency in isolated aorta rings (Log EC₅₀: CS = 7.1 ± 0.2 vs Control = 7.4 ± 0.3 ; $p < 0.05$), and increased contraction to noradrenaline (AUC: CS = 358 ± 81 vs Control = 265 ± 79 ; $p < 0.05$), indicating endothelial dysfunction. The treatment with Tad prevents these changes as well as increased aortic cGMP levels (3 ± 1 vs 1 ± 0.3 pmol/mL; $p < 0.05$) and NO stained by a fluorescent probe (24 ± 7 vs 17 ± 4 AU; $p < 0.05$), compared to control. In the isolated heart (Langendorff preparation), a reduction in left ventricular developed pressure (LVDP) and the ventricular relaxation index ($-dP/dT$) during isoproterenol challenge (0.01 nM) was seen in the CS group compared to controls, while Tad treatment restored these parameters to control values (Control: 78 ± 4 ; CS: 60 ± 7 ; CS + Tad: 79 ± 23 mmHg; Control: -1.6 ± 0.2 ; CS: -1.13 ± 0.1 ; CS + Tad: -1.6 ± 0.6 mmHg·s⁻¹; $p < 0.05$). Collagen deposition was increased in the left ventricle of the CS group compared to control (5.7 ± 1.6 vs $3.8 \pm 0.6\%$; $p < 0.05$), along with prolongation of QT interval (67.6 ± 13.4 vs 52.7 ± 18.4 ms; $p < 0.05$) during isoproterenol infusion (0.3 nmol/kg), indicating structural and electrical conduction alterations in the heart. These changes were not observed in the Tad-treated group, which also increased reduced glutathione (GSH) levels in cardiac tissue compared to the CS group (0.9 ± 0.21 vs 0.6 ± 0.1 μmol/mg; $p < 0.05$), suggesting an increased antioxidant capacity. **Conclusion:** Sepsis-surviving rats exhibited signs of late endothelial and cardiac dysfunction. Tad treatment had a positive impact, improving cardiac function and reducing tissue remodeling. These findings highlight the therapeutic potential of PDE5 inhibition in mitigating long-term cardiovascular alterations following sepsis. **Keywords:** Tadalafil. Post-sepsis. Cardiac function. Endothelial dysfunction. **Financial support:** CNPq, CAPES, FAPESC, Cristália.

06.007 Cardiovascular Protective Effects of Syrah Grape (*Vitis vinifera*) Pomace Supplementation in Spontaneously Hypertensive Rats. Costa KCM¹, Magalhães VHL², Soares KS², Freitas RF², Pertuzatti PB², Giachini FR^{1,2}. ¹UFG, Institute of Biological Science, Brazil. ²UFMT, Institute of Biological Science and Health, Brazil

Introduction: Wine by-products, particularly those rich in polyphenols, have garnered attention for their potential in supporting cardiovascular health. Their antioxidant properties play a crucial role in mitigating oxidative stress, a key contributor to hypertension and related cardiovascular disorders. As interest grows in alternative and adjunctive therapies, natural compounds with bioactive potential are increasingly being explored as candidates for novel antihypertensive treatments. This emerging area of research underscores the importance of comprehensive scientific evaluation to establish the therapeutic efficacy and safety profiles of these natural interventions. This study aimed to identify and quantify the phenolic compounds present in Syrah (*Vitis vinifera*) grape pomace, and to evaluate the effects of its dietary supplementation on blood pressure, vascular reactivity, and cardiac remodeling. **Methods:** Grape pomace was subjected to identification and quantification using HPLC-DAD-ESI-MSn. Ethical procedures were approved by CEMAE-UFMT/CUA (23108.010699/2023-18). Normotensive male rats (Wistar) and spontaneously hypertensive rats (SHR) received grape pomace-enriched (300 mg/kg/day, 14 days) or standard chow. Arterial pressure was assessed indirectly by tail plethysmography. Endothelium-dependent and -independent relaxation, as well as vascular contractile response were evaluated in mesenteric resistance arteries. Histological procedures were done in the heart to evaluate the number of cardiomyocytes, intercellular spaces, cardiomyocyte diameter, collagen deposition. **Results:** Pomace samples exhibited higher total phenolic compound concentrations and five derivatives of hydroxycinnamic acids and twelve flavonols were identified. Quercetin-3-glucoside and ethyl caffeate were the most abundant phenolic compounds. Grape pomace-enriched chow promoted hypotensive effect [diastolic blood pressure (mmHg) 144±1.2 vs. 165±0.8 non-treated SHR]. Supplementation improved the endothelium-dependent (E_{max} 93.7±4.1% vs. 73.3±3.1% non-treated SHR), but not independent, relaxation, as well as reduced phenylephrine induced contraction (E_{max} 23.8±1.8 vs. 30.2±1.8 non-treated SHR) in resistance arteries from SHR. There were no changes in the number of cardiomyocytes or in the number of intercellular spaces between the groups evaluated. However, SHR showed augmented cardiomyocyte diameter (μm), compared to the normotensive group (11.0±0.02 vs 9.8±0.02 respectively), and grape pomace supplementation reverted it in the hypertensive treated rats (9.4±0.18). Greater collagen deposition was observed in the SHR group compared to the normotensive control group (33.6±1.8% vs. 23.9±2.3% respectively), and grape pomace-treatment evoked the reduction of deposited collagen (30.2±0.5%). SHR rats showed greater deposition of type I collagen compared to the Wistar group, and grape pomace treatment reduced type I collagen in the hypertensive rats. **Conclusion:** The high amounts of flavonols and hydroxycinnamic acids found in the grape pomace contributed both to the hypotensive and cardioprotective effects observed in hypertensive rats submitted to chow-enrichment.

06.008 **Bufalin-Induced Epithelial-Mesenchymal Transition in LLC-PK1 Cells *in vitro***. Barros GMO, Bagri KM, Mermelstein CS, Quintas LEM ICB-UFRJ Rio de Janeiro, Brazil

Introduction: Aging at cellular level is a widespread process that leads to gradual loss of function as a result of accumulated damage over time. This deterioration hampers the ability of tissues to regenerate and heightens the risk of developing various diseases. The kidneys, in particular, exhibit significant age-related changes, even in the absence of underlying pathological conditions. In this study, we evaluated the morphological and molecular alterations triggered by the endogenous cardiotonic steroid (CTS) bufalin in renal epithelial LLC-PK1 cells subjected to *in vitro* aging. To model cellular senescence, we compared high ($P > 80$) and low passages ($P < 40$) cells, representing aged and young populations, respectively. **Methods:** LLC-PK1 cells at high passages ($P > 80$) were exposed to 20 nM bufalin for 48 hours. After treatment, the aged cells were analyzed for the expression of cell adhesion molecules and epithelial-mesenchymal transition (EMT) markers using immunofluorescence. Statistical analysis was performed using Student's t-test ($p < 0.05$). Data are presented here as percentages relative to the control group. **Results:** Bufalin treatment induced significant changes in the cellular phenotype. A marked reduction in pan-cadherin (16.5%, $p = 0.0014$) and E-cadherin (70.7%, $p = 0.0001$) fluorescence intensity was observed, indicating disruption of adherens junctions. While no significant changes were detected in β -catenin and α -tubulin, the loss of E-cadherin already points to an early epithelial disorganization. Furthermore, tight junction proteins such as occludin, claudin-1, and ZO-2 showed 26, 24.7, and 39% decreased fluorescence, respectively ($p < 0.005$), whereas ZO-1 remained unaffected. Interestingly, the transcription factor ZEB1, known to regulate EMT, also exhibited reduced expression, retaining only 45% of the control level ($p = 0.0001$). **Conclusion:** These findings suggest that aged LLC-PK1 cells are more susceptible to bufalin, displaying a molecular profile suggestive of an EMT-like shift. This supports the idea that cellular aging increases sensitivity to endogenous CTS and may be implicated in mechanisms underlying renal injury.

06.009 **Treatment with Wedelolactone Reverses AKI Caused by *Bothrops jararacussu* Envenomation.** Santos MARF, Souza PDN, Accioli DSG, Esteves GAB, Pinto HMC, Brito ES, Albernaz L, Pereira T, Oliveira SSC, Amorim CS, Santos AL, Moraes JA, Gonzalez SR, Melo PA, Morcillo LSL UFRJ

Introduction: Snakebite-induced acute kidney injury (sAKI) is a severe complication of *Bothrops* envenomation that can progress to renal failure and chronic kidney disease. The World Health Organization classifies snakebite as a Neglected Tropical Disease and aims to reduce related mortality and disability by 50% by 2030. Although antivenom is the only specific therapy available, it has limitations such as restricted accessibility, risk of adverse reactions, and limited efficacy against local tissue damage. Thus, complementary therapeutic strategies are needed. This study investigated the nephroprotective effects of wedelolactone (WEL), a coumestan isolated from *Eclipta prostrata* with known anti-inflammatory and antioxidant properties, in a preclinical model of sAKI. **Methods:** Wistar rats (100-125 g) were intramuscularly administered 3.5 mg/kg *Bothrops jararacussu* (Bj) venom to induce sAKI, followed by treatment with WEL (2, 5, or 10 mg/Kg) 2 h post-envenomation (CEUA/UFRJ 009/22). Controls received saline. The rats were placed in metabolic cages for 24 h to assess water intake and urinary output. Blood and kidneys were collected for biochemical, histological, and morphometric analyses. Data were analyzed using one-way ANOVA followed by Sidak's test ($p < 0.05$). **Results:** WEL did not alter renal function or kidney cytoarchitecture in healthy rats. None of the doses prevented venom-induced muscle damage. WEL (5 mg/Kg) mitigated kidney dysfunction, preserving glomerular filtration rate (Bj: 2285 ± 425 vs. Bj+WEL: 1312 ± 320 mL/min) and reducing proteinuria (Bj: 43 ± 6.6 vs. Bj+WEL: 22 ± 3.0 mg/dL). Histological analysis revealed preserved kidney cytoarchitecture and reduced collagen deposition. Mechanistically, WEL inhibited the Bj-induced 20 % increase in matrix metalloproteinase activity and the 25 % increase in nitrite levels in the cortex of Bj rats, although myeloperoxidase activity that remained elevated. Neither envenomation nor treatment activated endoplasmic reticulum stress via the unfolded protein response pathway. Interestingly, WEL (2 mg/Kg) was the only dose that prevented Na^+ handling dysfunction, whereas the 10 mg/Kg dose showed broader nephroprotective effects. **Conclusion:** WEL (5 mg/Kg) demonstrated nephroprotective effects against Bj-induced nephrotoxicity via antioxidant and anti-fibrotic mechanisms, without impairing renal function in healthy rats. These findings support the potential of WEL as an adjunctive therapy for sAKI.

06.010 **Bergamot (*Citrus bergamia*) By-Product as a Preventive Strategy on Heart and Kidney Doxorubicin-Induced Oxidative Stress: A Preclinical Model New Perspectives.** Berretta F¹, Agostino ME¹, Palacio TLN², Siqueira JS², Corrêa CR³ ¹Unesp, Institute of Biosciences, Undergraduate Program, Brazil, ²Unesp, School of Medicine, Postgraduate Program in Pathology, Brazil ³Unipex-Unesp, Experimental Research Unit () – Laboratório Redox, Brazil

Introduction: Doxorubicin (Dox) is a widely used antineoplastic agent; however, it can lead to several organs toxicity primarily mediated by oxidative stress. Therefore, it is essential to identify preventive strategies capable of alleviate the Reactive oxygen species (ROS) production, increasing the antioxidant capacity in affected organs as heart and kidneys. Bergamot by-product (B) is recognized for its antioxidant activity and may represent a preventive strategy against Dox-induced oxidative damage. **Objective:** Determine the impact of the bergamot by-product on reducing oxidative stress markers in the heart and kidneys of rats before an acute Dox administration.

Methods: Male Wistar rats were distributed into two experimental groups with 16 animals each. Control + 250 mg/kg of bergamot by product (C+B) and control + potable water (C+W) administered by gavage for 7 days. After this period, the rats were redistributed into four new groups with 8 animals each. Groups C+W and C+B received an intraperitoneal (IP) injection of saline solution, while Dox+W and Dox+B animals received an IP dose of 4 mg/kg of Dox. After 48 hours, the animals were euthanized, and their hearts and kidneys were collected for analysis of malondialdehyde (MDA, nmol/mg protein), protein carbonylation (CBO, nmol/mg protein), and advanced oxidation protein products (AOPP, $\mu\text{mol/L/Uchloramine/mg protein}$). Data were evaluated by two-way ANOVA or Kruskal-Wallis, with Tukey's or Dunn's post-hoc tests ($p < 0.05$). **Results:** In cardiac tissue, the Dox+W group compared to C+W showed a significant increase in CBO levels (0.55 [0.74–0.34] vs. 0.22 [0.24–0.19]), MDA (594.65 $\pm$ 133.20 vs. 477.69 $\pm$ 64.47), and AOPP (19.55 [21.57–16.21] vs. 10.62 [12.92–8.17]) ($p < 0.001$), demonstrating that Dox increased oxidative stress products. In contrast, the Dox+B group showed reduced levels of these products: CBO (0.31 [0.39–0.24]), MDA (472.44 $\pm$ 81.59), and AOPP (13.52 [14.89–12.95]) ($p < 0.001$). In the kidneys, the Dox+W group compared to C+W also showed increased CBO (8.10 $\pm$ 1.67 vs. 5.95 $\pm$ 0.99), MDA (226.72 [253.61–182.34] vs. 151.29 [163.14–133.11]), and AOPP (13.49 [14.65–11.64] vs. 12.62 [14.48–11.01]) ($p < 0.001$). The B administration was capable of attenuating these renal products: CBO (6.79 $\pm$ 1.32), MDA (164.92 [185.53–147.56]), and AOPP (14.28 [16.90–13.23]) ($p < 0.001$). **Conclusion:** The bergamot by-product modulated cardiac and renal oxidative stress in rats subjected to an acute dose of Dox. **Ethics Committee Approval:** (1429/2023). **Financial support:** FAPESP (2023/09457-5).

06.011 Semaglutide Treatment Reduced Inflammatory Adipokines on PVAT from Hypertensive Ovariectomized Female Rats with Overweight. Queiroz MCT, Ramos IT, Sousa JH, Eichler RA, Akamine EH, Costa TJ, ICB-USP, Dept Pharmacology, Brazil

Cardiovascular disease remains the leading cause of death worldwide, and each year new insights emerge on how to prevent vascular dysfunction. Semaglutide, a glucagon-like peptide-1 receptor agonist (GLP-1R), has shown promising results in the treatment of diabetes and, more recently, in reducing obesity and cardiovascular risk, a new alternative for cardiovascular diseases. During the postmenopausal, women present several metabolic alterations, including increased body weight, metabolic syndrome and elevated blood pressure. Estrogen therapy, while beneficial in some contexts, is typically contraindicated in individuals with established vascular disease or more than 5 years on menopause. Notably, GLP-1 has been shown to act on visceral adipose tissue by inhibiting lipogenesis, promoting lipolysis, and reducing inflammation—mechanisms that collectively support weight loss and metabolic improvement. Recently, our group demonstrated that ovariectomized (OVX) female rats exhibit a proinflammatory profile in perivascular adipose tissue (PVAT), which impairs its anti-contractile effect on resistance arteries. We hypothesized that GLP1R activation by semaglutide exerts anti-inflammatory effects on PVAT in OVX-induced overweight and inflammation, which would explain its cardiovascular protection. The aim is to identify the cytokines involved in the anti-inflammatory profile induced by semaglutide treatment. Twelve-week-old female spontaneously hypertensive rats (SHR) were randomly assigned to three groups: sham-operated, ovariectomized (OVX), and OVX treated with semaglutide (GLP) (dose: 25 nmol/kg, sc, 30 days). PVAT from the mesenteric circulation was collected for semi-quantitative proteomic profiling using the Rat Adipokine Array (#ARY016; R&D Systems), according to the manufacturer's instructions. We used 200 ug of protein to analyze cytokines levels. Data are presented as means $\pm$ standard error (SE). Statistical comparisons among groups were made using one-way ANOVA followed by Bonferroni's post hoc test, with significance set at $p < 0.05$. All statistical analyses were conducted using Prism 10 (GraphPad Software, San Diego, CA). All experimental procedures were approved by the Animal Research Ethics Committee at ICB-USP (protocol #9725100724). We observed that OVX increased body weight (Sham: 196 $\pm$ 3g; OVX: 255 $\pm$ 7g*; GLP: 231 $\pm$ 2g*; * $p < 0,05$ vs sham) compared to the sham group, while semaglutide treatment for 30 days reduced the body weight around 5%, compared with the OVX group treated with the placebo. Blood pressure did not differ significantly among the groups (Sham: 165 $\pm$ 1mmHg; OVX: 158 $\pm$ 3mmHg; GLP: 159 $\pm$ 3mmHg). Qualitative array analysis revealed six pro-inflammatory adipokines (IGFBP-3, IGFBP-5, NGAL, VEGF, IGFBP-6, IGF-II) increased by OVX. Notably, the treatment with semaglutide reduced three pro-inflammatory cytokines on PVAT (ANGPT-L3, MCP-1, PREF-1) from OVX females, suggesting the impact of GLP-1 activation on inflammatory response. These findings underscore the therapeutic potential cytokines of semaglutide in modulating the PVAT inflammatory response associated with OVX-induced weight gain. **Financial support:** FAPESP 2024/20653-3.

06.012 Increase in Oxidative Stress and Organ Injury in Mice Subjected to Hemorrhagic Shock.

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Hemorrhagic shock (HS) is a critical condition caused by acute blood loss, resulting in systemic hypoperfusion and tissue hypoxia. The body attempts to compensate through hemodynamic mechanisms, but when blood loss is excessive, hypotension and organ failure occur. In mouse, HS is investigated using fixed-volume, fixed-pressure, or uncontrolled hemorrhage models, which have low reproducibility, high variability, and high mortality rates. This study aimed to standardize a fixed-pressure model of hemorrhagic shock in mice (target MAP of 40 mmHg) and to characterize the associated hemodynamic alterations, oxidative stress responses, and biochemical markers of organ injury. **Methods:** Male and female C57BL/6 mice were anesthetized and catheterized for continuous monitoring of mean arterial pressure (MAP) and heart rate. Hemorrhagic shock (HS) was induced by controlled blood withdrawal to maintain MAP at 40 ± 2 mmHg. During decompensation, reinfusion of blood was used to sustain pressure. After 90 minutes, resuscitation was performed with remaining blood plus Ringer's Lactate. Two hours later, animals were euthanized for blood and tissue collection. Oxidative stress markers and plasma injury biomarkers were analyzed. Sham animals underwent identical procedures without hemorrhage. Protocol feasibility was assessed by survival analysis. **Results:** MAP significantly decreased during the ischemic phase in HS animals (Sham: 77.90 ± 6.40 mmHg; HS: 51.53 ± 5.64 mmHg) compared to baseline values (Sham: 75.91 ± 2.29 mmHg; HS: 74.68 ± 7.50 mmHg). Ten minutes after reperfusion, MAP values were restored (Sham: 73.79 ± 5.45 mmHg; HS: 69.95 ± 5.88 mmHg). However, two hours later, MAP remained lower in HS animals (Sham: 75.31 ± 4.77 mmHg; HS: 62.74 ± 3.52 mmHg). A marked depletion of reduced glutathione (GSH) levels was observed in the kidneys of HS animals (8.95 ± 0.60 nmol/mg) compared to the control group (65.15 ± 41.96 nmol/mg), suggesting impaired antioxidant defense and increased oxidative stress in renal tissue. Additionally, levels of malondialdehyde (MDA), a marker of lipid peroxidation, were elevated in HS animals (0.86 ± 0.14 nmol/mg) relative to controls (0.62 ± 0.07 nmol/mg), indicating enhanced oxidative damage in these tissues. Conversely, no significant differences were observed between groups in the liver for either GSH (Sham: 113.6 ± 32.9 ; HS: 102.8 ± 32.9 nmol/mg) or MDA levels (Sham: 0.77 ± 0.16 ; HS: 0.84 ± 0.20 nmol/mg). Nevertheless, a significant increase in the hepatic enzymes alanine aminotransferase (ALT) (Sham: 13 ± 6.63 ; HS: 28 ± 8.40 nmol/mg) and aspartate aminotransferase (AST) (Sham: 45.33 ± 9.60 ; HS: 112 ± 63.55 nmol/mg) was observed in HS animals compared to controls, suggesting the presence of liver injury. Additionally, blood urea nitrogen (BUN) levels were significantly elevated in HS animals (83.25 ± 6.23) compared to controls (59.67 ± 11.84), indicating possible renal dysfunction. **Conclusion:** The fixed-pressure model targeting a MAP of 40 mmHg demonstrated feasibility for inducing oxidative stress and liver injury in HS model. Future studies will focus on therapeutic strategies to reduce organ injury and dysfunction associated with this condition. **Financial support:** Capes; CNPq.

06.013 Resveratrol Administration During Adolescence in Hypertensive Female Rats Does Not Reduce Vascular Matrix Metalloproteinase (MMP)-2 Activity and Hypertension in Adulthood

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Introduction: Systemic arterial hypertension (SAH), defined by a persistent elevation of blood pressure, induces vascular alterations that affect systemic circulation and target organs, such as vasculature, heart and kidney. In the vasculature, these changes lead to hypertrophic remodeling, characterized by thickening of the tunica media, hypertrophy and hyperplasia of vascular smooth muscle cells (VSMC), as well as increased deposition of extracellular matrix (ECM) components such as collagen, fibronectin and elastin. Matrix metalloproteinases (MMPs), especially MMP-2, a zinc-dependent endoprotease secreted by VSMC, participate in this process by degrading ECM, thus contributing to maladaptive vascular remodeling and dysfunction. Oxidative stress, resulting from excessive reactive oxygen species (ROS), is also involved in the pathophysiology of SAH and the activation of MMP-2. Therefore, the use of antioxidants as a therapeutic strategy has been investigated. Resveratrol is a phenolic compound with antioxidant properties and potential estrogen-mimicking effects. Prevent the occurrence of hypertension (HT) is an ideal alternative to control malefic effects in the cardiovascular system. As the neonatal and adolescent periods have significant plasticity thus being generally modulated by the exogenous environment, the use of resveratrol in these phases may be key for preventing or reducing HT later in life. From this perspective, the present study aims to evaluate the effects of resveratrol, administered during the adolescence of female rats, on vascular MMP-2 activity and whether it attenuates vascular alterations of HT in adulthood. **Methods:** Female Wistar Kyoto and Spontaneously Hypertensive Rats (SHR) were treated with resveratrol at 4 g/kg diet, from weeks 4 to 6 (1081/2022). Systolic blood pressure (SBP) was measured by tail-cuff plethysmography at 8 and 12 weeks. Gelatinolytic activity of 64 and 72 kDa MMP-2 was analyzed by in-gel zymography, using aorta samples, and the gel was quantified by ImageJ software (NIH). Statistical analysis was performed using two-way ANOVA followed by Tukey correction ($p < 0.05$), and data presented like Mean $\pm$ Std. Error of Mean. **Results:** SBP was significantly higher in the SHR rats compared to controls at weeks 8 ($167,5 \pm 2,945$ vs $143,1 \pm 2,987$; $p < 0.05$; $n = 10$) and 12 ($159,4 \pm 3,551$ vs $142,8 \pm 3,272$; $p < 0.05$; $n = 10$). In in-gel zymography, the 64 kDa gelatinase MMP-2 showed increased activity in the aortas from SHR ($0,5849 \pm 0,1176$ and $0,6594 \pm 0,09608$ vs $0,2137 \pm 0,02880$; $p < 0.05$ vs. Control; $n=5-6$), while no difference was observed for the 72 kDa form. Treatment with resveratrol did not decrease SBP and the 64 kDa MMP-2 activity in the female SHR. However, it is known that this phenolic compound binds to the zinc ion in the catalytic site of MMPs, and during in-gel zymography electrophoresis, resveratrol may dissociate from the protease, thereby losing its inhibitory effect. **Conclusion:** Resveratrol during adolescence was not able to attenuate SBP or the increased MMP-2 activity in the adult female SHR, although further experiments with larger sample sizes are necessary for more conclusive results. **Financial support:** FAPESP, CNPQ, CAPES.

06.014 Estrogen Attenuates Ovariectomy-Induced PVAT Dysfunction in SHR: Role of NOX4, H₂O₂, and Inflammatory Adipokines. Ramos IT, Queiroz MCT, Sousa JH, Eichler RA, Akamine EH, Costa TJ, ICB-USP, Dept Pharmacology, Brazil

This project was approved by the Animal Research Ethics Committee (#9725100724). Postmenopausal women experience increased cardiovascular disease, however, the vascular protective effects of estrogen treatment remain unclear. Studies suggest that estrogen contributes to vascular homeostasis by promoting anti-inflammatory responses and redox balance. Notably, endothelial cells from postmenopausal present a more pronounced proinflammatory profile than premenopausal women, indicating a potential hormone-mediated regulation of cytokine levels. Recent attention highlights estrogen's role in modulating adipose tissue, particularly perivascular adipose tissue (PVAT), which secretes vasoactive mediators with anti-contractile effects. We hypothesize that PVAT from ovariectomized spontaneously hypertensive rats (SHR) exhibits an exacerbated proinflammatory and oxidative stress profile, impairing anti-contractile function and that estrogen prevents this. This study aims to investigate estrogen's impact on this PVAT anti-contractile function underlying inflammatory and oxidative mechanisms. 12-week-old female SHR were divided into sham, ovariectomized (OVX), and OVX treated with estrogen (0.1mg/pellet, sc, 15 days). Vascular reactivity was assessed in isolated mesenteric resistance arteries (with(+)/without (-) PVAT) using phenylephrine concentration–response curves and nonspecific NOX1/4 inhibitor. PVAT samples were collected for NOX4 protein expression (western blot), H₂O₂ levels, and semi-quantitative proteomic profiling (Rat Adipokine Array). Protein quantification used BCA assay; densitometric analyses used ImageJ. Data are presented as means ± SE. Statistical comparisons (one-way ANOVA, Bonferroni post hoc test, *p < 0.05) were performed using Prism. Final percent body weight gain differed between groups: SHAM (7.4%), OVX (23.5%), and OVX+estrogen (16%), indicating that estrogen treatment partially attenuated ovariectomy-induced weight gain. The anti-contractile effect of PVAT was lost in ovariectomized (OVX) arteries, evidenced by comparable LogEC50 and Rmax values (LogEC50 for PVAT(+) was 5.4 [5.3 to 5.5] vs PVAT(-) 5.4 [5.3 to 5.6], and Rmax values were 188.5 ± 5.4 vs 186.4 ± 3.7) compared to the Sham (LogEC50 PVAT(+): 5.3 [5.4 to 5.5] vs PVAT(-): 5.1 [4.8 to 5.2]; Rmax: 178±3.7 vs 205.9±3.4*). Estrogen treatment successfully restored this function, indicated by a rightward shift in LogEC50 for PVAT(+) (5.3 [5.2 to 5.4] vs PVAT(-) 4.8 [4.5 to 5.0]) and attenuated Rmax (165.7±3.7 vs PVAT(-) 180±1.7*). Inhibition of NOX4 similarly recovered PVAT's anti-contractile properties in OVX animals, suggesting estrogen's impact on these parameters. H₂O₂ levels were significantly reduced in OVX PVAT but restored by estrogen. NOX4 protein expression also decreased in OVX SHR PVAT and was restored by estrogen. Furthermore, inflammatory cytokine analysis showed OVX PVAT exhibited a more proinflammatory profile (elevated IL-11 and IL-1β), which was reversed by estrogen treatment. Together, these results suggest that estrogen positively modulates the anti-contractile effect of PVAT in mesenteric arteries by NOX-4-, H₂O₂ and inflammatory cytokines dependent mechanisms. Support: FAPESP 2024/14968-1.

06.015 **Bariatric Surgery Prevents Nitrate Treatment-Induced Decreases in Circulating Matrix Metalloproteinases Concentrations in Overweight Women.** Cássia-Barros A¹, Sanches-Lopes JM², Conde-Tella SO^{1,2}, Coelho EB³, Kemp R⁴, Salgado Júnior W⁴, Moreno Junior H⁵, Tanus-Santos JE^{1,2}
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Introduction: Nitrate is bioactivated to nitrite and nitric oxide (NO) and has been used to treat cardiovascular and metabolic diseases. Patients with obesity or overweight are exposed to increased cardiovascular risk, which is in part attributable to increased matrix metalloproteinase (MMP) activity and cardiovascular remodeling. Here, we examined the effects of NO supplementation with oral nitrate treatment on the circulating concentrations of MMP-2, MMP-9, and their endogenous inhibitors, the tissue inhibitors of MMPs (TIMP-1,-2,-3 and -4) in overweight women who had previously undergone Roux-en-Y gastric bypass (RYGB) surgery (n=15), and those with no history of bariatric procedures (control, n=13). **Methods:** This study was approved by the Research Ethics Committee of the Hospital das Clínicas, at the Ribeirao Preto Medical School, University of São Paulo (CAAE:88796918.2.000.5440). Patients received 5g of beetroot extract enriched with sodium nitrate to daily intake of 0.1 mmol/kg body weight of nitrate for 14 days. Ambulatory blood pressure monitoring, carotid-femoral pulse wave velocity (PWV), and the Augmentation Index (Aix) were assessed at baseline and after nitrate treatment. MMP-2, MMP-9, and TIMP-1, -2, -3 and -4 were assessed using two Human Luminex® Discovery Assays. Nitrite and nitrate concentrations were assessed using ozone-based reductive chemiluminescence methods. **Results:** Nitrate treatment decreased net MMP-2/TIMP-2 by 31.42% ($p < 0.05$) and MMP-9/TIMP-1 ratios by 45.22% ($p < 0.05$) (which reflect net MMP activity) in control women, but not in those with previous bariatric surgery ($p > 0.05$). Nitrite and nitrate concentrations were negatively associated with net MMP-2 (NO₂⁻: $r = -0.028$, $p > 0.05$; NO₃⁻: $r = -0.275$, $p < 0.05$) and MMP-9 activity (NO₂⁻: $r = -0.324$, $p < 0.05$; NO₃⁻: $r = -0.293$, $p < 0.05$). While VOP showed no significant differences ($p > 0.05$), MMP-2 concentrations or MMP 2/TIMP-2 ratio associated positively with Aix (MMP-2: $r = 0.465$, $p < 0.05$; MMP 2/TIMP-2 ratio: $r = 0.395$, $p < 0.05$). **Conclusion:** Our results provide compelling evidence that nitrate treatment reduces net MMP-2 and MMP-9 activity in overweight women, highlighting its therapeutic potential to prevent cardiovascular remodeling. However, this effect appears to be attenuated in women with prior RYGB surgery. **Funding:** This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq Grant number 406442/2022-3) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) – Finance Code 001.

06.016 **Vasorelaxant Effect of Myrtenyl Pelargonate Derived from Biotransformation by *Cladosporium cladosporioides* in Aorta of SHR Rats: *in silico*, *in vitro* and *in vivo* Approach.** Barros LSR^{1,2}, Rego AF^{1,2}, Sena EP^{1,2}, Viana FSC^{2,3}, Barbosa BS^{2,4}, Acha BT^{5,6}, Kutyla M⁹, Trytek M⁹, Sabino JPJ^{7,8}, Oliveira AP^{1,2,7} ¹UFPI, Lab Cardiovascular Pharmacology, NPPM, Brazil ²UFPI, Graduate Program in Pharmacology, Brazil ³UEMA, Brazil ⁴UFPI, Smooth Muscle Lab, NPPM, Brazil ⁵UFPI, Lab Functional and Molecular Studies in Physiopharmacology, Brazil ⁶RENORBIO-UFPI, Graduate Program in Biotechnology, Brazil ⁷UFPI, Dept Biophysics and Physiology, CCS, Brazil ⁸UFPI, Lab Reflex Control of Pulmonary Ventilation, Brazil ⁹Maria Curie-Skłodowska University (UMCS), Poland

Introduction: Myrtenyl pelargonate was obtained by biotransformation of the monoterpene alcohol (-)-myrtenol by the filamentous fungus *Cladosporium cladosporioides*. Previous studies demonstrated that (-)-myrtenol exhibits hypotensive, bradycardic, and vasorelaxant effects in hypertensive rats (Mendes Neto, 2020). This study aimed to investigate the pharmacokinetic and pharmacodynamic properties of Myrtenyl pelargonate through *in silico* analysis, and to evaluate its vasorelaxant, antioxidant, and coagulation effects in spontaneously hypertensive rats (SHR).

Methods: All protocols were approved by the Ethics Committee (CEUA No. 563/2019). *In silico* analysis was performed using ADMETlab to predict biopharmaceutical and toxicological properties, and AutoDock (v1.5.7) for evaluating molecular docking with target proteins and enzymes. Pharmacokinetic analysis revealed high intestinal absorption, broad tissue distribution, stable hepatic metabolism, and moderate clearance. Docking analysis showed binding affinity to active site residues of L-type calcium channels (CavL) and endothelial nitric oxide synthase (eNOS). *In vitro* vascular reactivity assays were performed using isolated thoracic aorta rings from SHR rats precontracted with phenylephrine (10⁻⁵ M), followed by cumulative addition of Myrtenyl pelargonate (10⁻¹² to 3×10⁻⁴ mol/L).

Results: Myrtenyl pelargonate induced concentration-dependent vasorelaxation in both endothelium-intact (EC₅₀ = 8.02 × 10⁻⁸ M) and endothelium-denuded rings (EC₅₀ = 7.23 × 10⁻⁸ M), with no statistical difference (p > 0.05). Additional assays indicated direct action by CavL inhibition. In rings precontracted with KCl (80 mmol/L), vasorelaxation was concentration-dependent (p<0.05), with an EC₅₀ of 7.24 × 10⁻⁸ M. In the presence of BAY K 8644, the effect remained concentration-dependent (p<0.05), with an EC₅₀ of 7.94 × 10⁻⁸ M. *In vivo* treatment (12.5 mg/kg orally for 7 days) reduced malondialdehyde (MDA) levels in cardiac tissue, similarly to captopril (20 mg/kg), with no changes in reduced glutathione (GSH) levels. A reduction in nitrite levels was also observed, possibly related to eNOS inhibition, as predicted *in silico*. Coagulation parameters (PT, aPTT, INR) showed no significant changes.

Conclusion: Myrtenyl pelargonate promotes vasorelaxation via CavL inhibition, exhibits antioxidant activity, and does not significantly alter coagulation parameters, supporting its potential as a vascular agent for hypertension management. **Financial support:** UFPI, FAPEPI, CAPES, and CNPq.

06.017 **Salt-Inducible Kinase Regulates (Na⁺+K⁺)-ATPase Activity in Pig Kidney Outer Cortex.** Ribeiro DDS^{1,2}, Hilário-Souza E¹, Sarmiento GC³, Lowe J⁴, Gomes DS^{1,2}, Bastos GA^{1,2}, Dick CF⁴, Lara LS^{1,2}. ¹ICB-UFRJ, ²IBCCF-UFRJ, Centro de Pesquisa em Medicina de Precisão, ³ IBCCF-UFRJ, ⁴UFRJ, Instituto de Bioquímica Médica, Brazil

Introduction: It is well established that a high salt diet exacerbates hypertension and inappropriately increases Na⁺ reabsorption, but the underlying mechanisms remain unclear. Salt-inducible kinase (SIK) is a serine/threonine kinase belonging to the AMP-activated protein kinase (AMPK) family. We aimed to test the hypothesis that SIK activation increases renal primary Na⁺ transporters activities: (Na⁺+K⁺)-ATPase and Na⁺-ATPase. **Methods:** Pig kidneys were dissected into three distinct regions: (i) medulla, (ii) inner cortex, and (iii) outer cortex. Samples were centrifuged to obtain homogenates, which were incubated with increasing NaCl concentrations (1–320 mM), in the presence or absence of the SIK inhibitor YKL-05-099 (1 μM). CEUA/UFRJ: 136/24. ATPase activity was measured according to the colorimetric method described by Fiske and Subbarow (Fiske and Subbarow, 1925). **Results:** The homogenate fractions contained (Na⁺+K⁺)-ATPase, SIK1, SIK2, and AQP1. Regional markers were used to validate fraction identity: (i) medulla contained Tamm-Horsfall protein (THP, marker of the thick ascending limb of the loop of Henle) and aquaporin-2 (AQP-2, collecting duct marker); (ii) inner cortex contained THP; (iii) outer cortex showed low THP and no AQP-2 protein content. (Na⁺+K⁺)-ATPase activity was undetectable in the inner cortex. In the outer cortex, the activity was stimulated by Na⁺ in a dose-dependent manner, reaching a peak at 200 mM (218.8 ± 17.7 nmol Pi·mg⁻¹·min⁻¹), followed by a 41% decrease at 320 mM. In the medulla, activity was already elevated at 10 mM NaCl. SIK inhibition significantly reduced (Na⁺+K⁺)-ATPase activity only in the outer cortex at 200 mM NaCl (53.3 ± 11.8 mmol Pi·mg⁻¹·min⁻¹; p = 0.0031). Conversely, Na⁺-ATPase activity in the outer cortex remained unchanged by SIK inhibition at 200 mM NaCl (112.9 ± 50.3 vs. 122.8 ± 69.1 mmol Pi·mg⁻¹·min⁻¹; p = 0,9103). **Conclusion:** The Na⁺-dependent activation profile of (Na⁺+K⁺)-ATPase differs between the outer cortex and medulla, possibly reflecting regional variations in interstitial Na⁺ concentration. These findings suggest that (Na⁺+K⁺)-ATPase is a functional target of SIK in the outer cortex under high-salt conditions. **Financial support:** Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro. Award number: E-26/210.561/2024, Emendas Parlamentares do Governo Federal. CAPES Master's Scholarship, grant number: 88887.902995/2023-00. CEUA/UFRJ: Comissão de Ética no Uso de Animais em Pesquisas da Universidade Federal do Rio de Janeiro

06.018 **CB₂ Receptors as a Therapeutic Target for Cardiovascular Dysfunction in Pneumonia-Induced Sepsis.** Borges LB, Simas A, Albino LB, Mariot, LN, Da Silva-Santos, JE Sordi R UFSC, PPG in Pharmacology, Dept Pharmacology

Sepsis is defined as life-threatening organic dysfunction caused by a dysregulated host response to infection. It is one of the biggest causes of death worldwide and is the most common cause of admissions to intensive care units. The endocannabinoid system has emerged as a potential therapeutic target due to its modulatory effects on immune and cardiovascular functions. Although cannabinoid receptors have been implicated in sepsis pathophysiology, the specific role of CB₂ receptors in sepsis-induced cardiovascular alterations remains incompletely understood. Therefore, this study investigated the effects of an agonist of CB₂ receptor on cardiovascular dysfunction in a pneumonia-induced sepsis model. **Methods:** Animals were divided in 3 groups: naïve (control; without any intervention or treatment), sepsis+vehicle and sepsis+AM1241. Sepsis was induced in male mice by intratracheal inoculation of *Klebsiella pneumoniae*. CB₂ agonist (AM1241, 3 mg/kg) or vehicle (Dimethylsulfoxide, 0.3 mL/kg) was administered (i.p.) 6 and 12 hours after *K. pneumoniae* inoculation. Mean (MAP), systolic (SAP), and diastolic (DAP) arterial pressures, as well as heart rate (HR) were measured via a carotid artery catheter connected to a pressure transducer. Cardiac function was assessed using the Langendorff isolated heart preparation, while cardiac electrical activity was recorded by electrocardiography (ECG). Endothelial dysfunction was evaluated through immunofluorescence staining of aortic tissue using the dihydroethidium (DHE) probe to detect reactive oxygen species (ROS). Additionally, vascular reactivity was examined in isolated aortic rings mounted in an organ bath system to assess contractile and relaxation responses. All parameters were evaluated 24 hours after sepsis induction. Data are presented as mean ± SD (10 animals per group). *p < 0.05 compared with naïve group and #p < 0.05 compared with sepsis+vehicle group. **Results:** Septic animals treated with vehicle exhibited reduced HR when compared with naïve group, while treatment with AM1241 restored these values (naïve: 599.8 ± 47.57; sepsis+vehicle: 488.2 ± 64.00*; sepsis+AM1241: 559.5 ± 56.45# beats per minute; bpm). Arterial pressure was significantly reduced in septic animals treated with vehicle compared to naïve mice. AM1241 improved all these parameters [MAP (naïve: 88.16 ± 7.06; sepsis+vehicle: 68.39 ± 17.63*; sepsis+AM1241: 84.06 ± 16.71# millimeters of mercury; mmHg), SAP (naïve: 94.01 ± 9.70; sepsis+vehicle: 77.38 ± 16.50*; sepsis+AM1241: 94.62 ± 18.89# mmHg) and DAP (naïve: 78.24 ± 8.39; sepsis+vehicle: 59.54 ± 18.91*; sepsis+AM1241: 75.07 ± 15.43# mmHg)]. ECG analysis revealed an increase in P wave duration (naïve: 0.0129 ± 0.0044; sepsis+vehicle: 0.0187 ± 0.0043* s) and RR interval (naïve: 0.1004 ± 0.003 s; sepsis+vehicle: 0.1236 ± 0.01* s) in septic animals. Treatment of septic mice with AM1241 restored RR interval values (sepsis+AM1241: 0.1104 ± 0.0095# s) but not P wave duration. No significant differences in cardiac function were observed among groups using the Langendorff technique. ROS levels were increased in septic mice and treatment with the CB₂ agonist was able to reduce these levels (naïve: 12.59 ± 1.39; sepsis+vehicle: 15.37 ± 1.24*; sepsis+AM1241: 12.94 ± 0.61# arbitrary units; AU). **Conclusion:** Treatment with the CB₂ agonist AM1241 improved heart rate and blood pressure parameters in septic animals. Additionally, the treatment with AM1241 attenuated ROS levels in aortic tissue, suggesting a reduction in oxidative stress. Therefore, the activation of CB₂ receptors exhibited a protective role in vascular and cardiac changes associated with sepsis. Further studies are required to elucidate the mechanisms behind the protective effect of CB₂ receptors activation in cardiovascular complications of sepsis. All procedures used in this report were approved by the Institutional Animal Use Ethics Committee (CEUA-UFSC n. 3137210323).

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06.019 Impact of Esomeprazole on Nitric Oxide Bioavailability in a Rat Model of Gestational Hypertension. Da Silva MLS, Da Silva AAF, Toghi CJ, Dragoneti BJ, Da Costa LC, Dias-Junior CA IB-UNESP-Botucatu, Dept Biophysics and Pharmacology

Proton pump inhibitors (PPIs) are commonly used during pregnancy to relieve gastric symptoms. However, studies suggest that their use may reduce nitric oxide (NO) bioavailability, potentially impacting cardiovascular health. We hypothesized that reduced NO bioavailability represents a cardiovascular risk during pregnancy. Therefore, we evaluated the effects of esomeprazole, alone and in combination with sodium nitrite, in a rat model of preeclampsia induced by reduced uteroplacental perfusion pressure (RUPP). Pregnant rats were randomly assigned to six groups (n = 10 per group): Preg+Saline (normotensive control), Preg+NO (normotensive treated with sodium nitrite, 15 mg/kg/day), RUPP (subjected to the RUPP model), RUPP+NO (RUPP treated with sodium nitrite), Preg+ESO (normotensive treated with esomeprazole, 3.5 mg/kg/day), and RUPP+ESO (RUPP treated with esomeprazole). We evaluated blood pressure, fetal and placental weights, gastric pH, vascular reactivity, and plasma NO metabolites. Nitrite and nitrate levels were measured using the Griess method. Systolic blood pressure was increased in RUPP compared to Preg+Saline (125 ± 3 vs. 105 ± 3 mmHg), as well as in RUPP+ESO (130 ± 3 mmHg) and Preg+ESO (125 ± 4 mmHg), and was reduced in RUPP+NO (100 ± 3 mmHg). Fetal weight decreased in RUPP vs. Preg+Saline (3.17 ± 0.06 vs. 3.74 ± 0.07 g) and increased in RUPP+NO (3.72 ± 0.08 g) and RUPP+ESO (3.56 ± 0.09 g). Placental weight was lower in RUPP compared to Preg+Saline (0.29 ± 0.003 vs. 0.34 ± 0.004 g), but increased in RUPP+NO (0.31 ± 0.001 g) and RUPP+ESO (0.40 ± 0.008 g). Gastric pH was higher (indicating lower acidity) in Preg+ESO, RUPP+NO, and RUPP+ESO compared to RUPP and Preg+Saline (4.58 ± 0.1 , 4.53 ± 0.1 , and 4.38 ± 0.1 vs. 3.44 ± 0.1 and 4.01 ± 0.1 , respectively – $\log[H^+]$). Phenylephrine-induced vasoconstriction was enhanced in endothelium-intact aortic rings in RUPP vs. Preg+Saline (1.11 ± 0.27 vs. 0.67 ± 0.17 g), and attenuated in RUPP+NO (0.70 ± 0.19 g), with no significant change in RUPP+ESO (0.89 ± 0.20 g). In endothelium-denuded rings, hyperreactivity was observed in RUPP vs. Preg+Saline (1.82 ± 0.36 vs. 1.03 ± 0.25 g), with reductions in RUPP+NO (0.99 ± 0.25 g) and RUPP+ESO (0.94 ± 0.22 g). Acetylcholine-induced relaxation was impaired in RUPP vs. Preg+Saline ($54 \pm 7\%$ vs. $84 \pm 7\%$), but improved in RUPP+NO ($65 \pm 3\%$) and RUPP+ESO ($71 \pm 4\%$). No differences were observed in endothelium-denuded relaxation curves. Plasma NO metabolites were reduced in RUPP vs. Preg+Saline (82 ± 2 vs. 131 ± 8 mmol/L), increased in Preg+NO and RUPP+NO (181 ± 8 and 172 ± 7 mmol/L), and decreased in Preg+ESO (90 ± 3 mmol/L), with the lowest levels in RUPP+ESO (74 ± 3 mmol/L). Esomeprazole and sodium nitrite exhibited distinct effects in the RUPP model. While sodium nitrite increased NO bioavailability, improved endothelial function, and reduced hypertension, esomeprazole did not replicate these benefits and further increased blood pressure in normotensive pregnant rats. Ethical Approval (CEUA): No. 6707090320/2020 – IBB/UNESP
Keywords: Preeclampsia, Nitric Oxide, Endothelial Dysfunction, Esomeprazole, RUPP **Financial support:** FAPESP 2020/03135-8

06.020 Early Hypercaloric Diet: A Trigger for the Development of Contractile Dysfunction in Mice.

Mariot LN, Neves PG, Simas A, Borges LB, Delfrate G, Andrade CA, Dal Secco D, Da Silva-Santos JE, Fernandes D, Victorio JA, Sordi R UFSC, PPG in Pharmacology

Obesity is a chronic metabolic disease characterized by excessive accumulation of adipose tissue. In this context, an increase in ectopic lipid deposition around blood vessels – referred to as perivascular adipose tissue (PVAT), is commonly observed. PVAT classically modulates vascular function and exhibits regional phenotypic differences that influence its anticontractile properties. Obesity induced by a high-fat diet leads to vascular dysfunction, with studies reporting either enhanced or reduced vascular contraction. Moreover, high-calorie (HC) diets have been shown to impair PVAT function in males more severely than high-fat diets. Therefore, this study aimed to evaluate the impact of an HC diet initiated after weaning on the contractile function of mouse aortas in the presence of PVAT. **Methods:** (CEUA no. 9378250324) Female and male C57BL/6 Jackson mice (4 weeks old) were fed either a high-calorie diet (obese – OB; 45% of calories from fat and 30% sucrose, 4,82 kcal/g, Prag Soluções – São Paulo/BR) or a standard diet (control – CT) immediately after weaning, for a period of 12 weeks. After 12 weeks, body mass was assessed and the animals were anesthetized for the collection of thoracic aorta cross-sections (2 mm) in the presence (+) or absence (–) of perivascular adipose tissue (PVAT). In some arterial rings, the endothelium was left intact (E+). The rings were mounted in a wire myograph system (model 620M, Danish Myo Technology A/S) under 5 mN of tension to assess the vascular response to phenylephrine, and norepinephrine. Data are presented as mean $\pm$ SD of the area under the curve (AUC; *p < 0.05). **Results:** Animals that consumed the HC diet showed increased body mass gain compared to animals fed the standard diet (OB = $281.2 \pm 26.5\%$ vs. CT = $129.08 \pm 43.7\%$, relative to birth weight; OB = 8.6 ± 1.5 g vs. CT = 11.5 ± 2 g). OB animals exhibited a reduced contractile response to phenylephrine compared to the CT group (AUC: PVAT+E+ $\rightarrow$ CT = 17.3 ± 4.9 vs. OB = 5.8 ± 3.6 AU). Endothelium removal from PVAT+ rings increased contraction only in the CT group (AUC: CT = 23.1 ± 8.4 vs. OB = $4.4 \pm 1.3^*$ AU). The contractile response to norepinephrine was similar to that of phenylephrine, resulting in hypocontractility in PVAT+/E– rings (AUC: CT = 45.2 ± 9.1 vs. OB = $9.6 \pm 2.5^*$ AU). **Conclusion:** Our findings show that an HC diet initiated after weaning leads to thoracic aorta hypocontractility in the presence of PVAT, suggesting a relevant role in the development of vascular dysfunction. **Keywords:** perivascular adipose tissue; endothelium; thoracic aorta; obesity. **Financial Support:** Capes; CNPq.

06.021 Nebivolol Modulates Nitric Oxide Independently of eNOS in Endothelial Cells Exposed to Preeclamptic Placental Factors. Bueno-Pereira TO, Santos KN, Matheus MB, Zampieri GM, Passeti LFP, Nunes PR, Sandrim VC IBB-Unesp-Botucatu, Dept Biophysics and Pharmacology – Brazil

Introduction: Preeclampsia (PE) is the most common hypertensive disorder of pregnancy and remains a leading cause of maternal mortality in Brazil. Its pathophysiology is best described by the two-stage model: Stage 1 involves abnormal placentation, resulting in placental hypoxia and oxidative stress; Stage 2 is marked by systemic maternal endothelial dysfunction. A hallmark of this dysfunction is the imbalance between nitric oxide (NO) and reactive oxygen species (ROS), leading to reduce NO bioavailability. In this context, nebivolol a β_1 -adrenergic antagonist, stands out among antihypertensive agents due to its vasodilatory and antioxidant properties mediated by NO-dependent pathways. To investigate its effects on NO and ROS levels, we tested an *in vitro* two-stage model of PE in which Human Umbilical Vein Endothelial Cells (HUVECs) are exposed to supernatants from placental explants (SPE) of women with PE or healthy pregnant women (HP). **Methods:** HUVECs were incubated with 10% SPE from PE (n=3) or HP (n=2), in the presence or absence of 5 μ M nebivolol and/or 100 μ M NG-nitro-L-arginine methyl ester (L-NAME, a NOS inhibitor), for 4 hours. NO and ROS levels were assessed using the fluorescent probes DAF-FM and DCFH-DA, respectively. **Results:** NO levels were significantly higher in HUVECs treated with HP SPE compared to both the untreated control and PE SPE groups (fold change $\sim$ 1.5 and $\sim$ 1.2, respectively). Nebivolol did not increase NO production in the HP group, even in the presence of L-NAME. Interestingly, in the PE group, nebivolol treatment led to a reduction in NO levels (fold change $\sim$ 1.4), and this effect was not reversed by L-NAME, suggesting a NOS-independent mechanism. ROS levels remained unchanged across all experimental groups. **Conclusion:** Nebivolol reduced NO levels in endothelial cells exposed to supernatants from preeclamptic placental explants via an eNOS-independent mechanism, suggesting activation of a non-canonical pathway. ROS levels remained unchanged, suggesting a lack of detectable antioxidant effect in this model. These findings suggest that, under PE-induced endothelial dysfunction, nebivolol may act through an alternative mechanism of action beyond its canonical pathways. These are preliminary results, and the sample size will be increased to strengthen the analysis and further elucidate nebivolol's potential pleiotropic roles in endothelial dysfunction associated with PE. **Ethics approval:** Committee of Botucatu School of Medicine (n^o 6.747.398) **Financial support:** FAPESP (2021/01945-5; 2024/01935-8; 2024/10080-6).

06.022 Testosterone-driven Cardiac Remodeling in a Gender-Affirming Hormone Therapy Experimental Model (Testo-GAHT) Relies on CD4⁺ T Cell Effector Profile Activated by the Glycolytic Pathway. Oliveira-Neto JT^{1,4}, Oliveira CV², Machado MR¹, Cebinelli GCM², Rodrigues D¹, Bayer AL⁴, Robbe ZL⁴, Silva CAA³, Silva JS², Salgado HC³, Cunha FQ^{1,2}, Alves-Filho JCF^{1,2}, Santos JD¹, Alcaide P⁴, Tostes RC¹. ¹FMRP-USP, Graduate Programs in Pharmacology, ²FMRP-USP, Graduate Programs in Immunology and ³FMRP-USP, Graduate Programs in Physiology, Brazil. ⁴Tufts University, School of Medicine, Dept Immunology, USA

Testosterone in the context of gender-affirming hormone therapy (GAHT) promotes cardiac hypertrophy, glycolysis in cardiomyocytes and a Th17 cell effector profile. CD4⁺ T cells contribute to adverse cardiac remodeling, and pyruvate kinase M2 (PKM2)-regulated glycolysis modulate CD4⁺ T cells activation and function. We hypothesized that testosterone in a GAHT mouse model disrupts cardiac function by enhancing the glycolytic metabolism in CD4⁺ T cells, leading to a pro-inflammatory Th17 phenotype. Female C57BL/6 wild-type (WT), CD4^{Cre}Pkm2^{fl/fl}, and IL-17 receptor A knockout (IL-17Ra^{-/-}) mice were treated with testosterone cypionate (48 mg.kg⁻¹.wk⁻¹) or vehicle (peanut oil), for 8 weeks (CEUA-FMRP/USP-1026/2021 and IACUC-Tufts University-B2023-25). Echocardiogram analysis showed that testo-GAHT in WT females promoted cardiac morphological changes compatible with concentric hypertrophy - characterized by increased intraventricular septum thickness (IVSd), and decreased left ventricular internal diameter (LVIDd); and functional changes - characterized by increased E/A wave ratio (an indicator of diastolic dysfunction) and fractional shortening (FS%, a contractility index), preserved ejection fraction (EF%), and decreased left ventricular stroke volume (LV Vols). Flow cytometry analysis of *ex vivo* hearts showed increased endothelial cell activation (CD31⁺ICAM⁺), reduced myeloid cell infiltration (CD45⁺CD11b⁺), and no differences in the frequency of CD4⁺ or CD8⁺ in testo-GAHT WT mice. While T cell frequency remained stable in mediastinal lymph nodes (mLN) and decreased in the spleen, both sites showed an increased frequency of effector CD4⁺CD44^{hi}CD62L^{lo}. To assess the role of glycolysis in this phenotype, *ex vivo* extracellular flux assays (extracellular acidification rate – ECAR using a Seahorse XF96 analyzer) were performed. Testo increased glycolysis, glycolytic capacity, and non-glycolytic acidification, as well as GLUT-1 and PKM2 total protein content and gene expression of solute carrier family 2 (GLUT1), lactate dehydrogenase A (Ldha) and Pkm2, reinforcing an increased metabolism in CD4⁺ T cells. Testo did not change morphological or functional parameters in CD4^{Cre}Pkm2^{fl/fl} female mice, as it did in littermate controls. CD4⁺IL17a⁺ cells were increased in mLN, but not in the spleen [normalized by Mean Fluorescence Intensity (MFI)] of testo-GAHT WT mice. Importantly, IL-17Ra^{-/-} mice did not exhibit testo-GAHT-induced cardiac changes, suggesting a crucial involvement of Th17 cell-related mechanisms. Beyond to adverse effects on cardiac function, testosterone reprogramed CD4⁺ T cell metabolism, enhancing glycolysis and promoting pro-inflammatory differentiation. This immunometabolic axis places testosterone as a key upstream modulator of effector T cell responses, linking immune activation to adverse cardiac remodeling in GAHT, and suggesting metabolic and immunophenotypic signatures as potential biomarkers of cardiovascular risk. Funding: FAPESP, CAPES, CNPq and NIH.

06.023 **Metformin-Mediated Cardiac and Antitumor Effects in Doxorubicin-Treated Mice with Triple-Negative Breast Cancer.** Cavalcante KDM^{1,2}, Cavalcante FAS¹, Ferreira LPF^{1,2}, Lago JPAD³, Nagai MA⁴, Mangone FR⁴, Pavanelli AC⁴, Silva MTB⁵, Leal FLT⁶, Sousa JMC³, Silva VC⁷, Ferreira PMP², Amaral DT⁸, Dittz D^{1,2} ¹UFPI, Antineoplastic Pharmacology Lab, Brazil; ²UFPI, PPG Pharmacology, Brazil; ³UFPI, PPG Pharmaceutical Sciences, Brazil; ⁴FMUSP-ICESP, Molecular Genetics Lab, Brazil; ⁵University of Porto, Institute of Biomedical Sciences Abel Salazar, Portugal; ⁶UFPI, Dept Biophysics and Physiology, Brazil; ⁷Fiocruz, Campus Piauí, Brazil; ⁸UFABC, Center for Natural and Human Sciences, Brazil

Introduction: Doxorubicin-induced cardiomyopathy (DIC) is a cumulative, dose-dependent adverse effect of breast cancer (BC) chemotherapy that significantly impacts patient prognosis and quality of life. It is primarily driven by reactive oxygen species (ROS), leading to oxidative stress and mitochondrial dysfunction, thereby limiting the efficacy of cancer treatment. Metformin (MET), an anti-hyperglycemic drug, has shown cardioprotective and antitumor activity in experimental BC. However, the molecular mechanisms of co-treatment with DOX in DIC remain poorly understood. Thus, *in silico* approaches coupled with experimental validation provide valuable insights for developing therapeutic strategies for complex diseases, such as cancer. Here, we aimed to investigate the combined effects of DOX and MET using *in silico* and murine models of BC and DIC.

Methods: Targets associated with the antitumor and cardiotoxic effects of DOX were identified through a literature review utilizing curated data from metabolic maps and protein interaction networks (KEGG Pathway, DrugBank, PubChem, and STRING). Docking studies were performed using AutoDock Vina and PyMol. To assess the cytotoxicity, 4T1 cells were exposed to MET (25–0.049 µg/mL) or DOX (10–0.020 µg/mL) for 72 hours, and cellular metabolism was quantified via MTT at 560 nm. *In vivo*, BC-bearing mice with DIC were co-treated with DOX (37.5 mg/kg) and MET (150, 250, or 500 mg/kg) for 12 days. Electrocardiographic parameters, PHLDA1 expression, hepatic GSH levels, tumor mass, survival rates, body weight, and organ weights were assessed.

Results: Fourteen proteins linked to the antitumor and cardiotoxic effects of DOX were identified. Among them, MET and DOX showed the lowest binding energies with NADPH/P450 (-4.345 and -7.048 kcal/mol, respectively). In 4T1 cells, DOX's IC₅₀ was 0.2975 µg/mL, while MET reduced cell viability by ~45% between 0.195 and 0.391 µg/mL. *In vivo*, DIC induced QT, QTc, and QRS prolongation, along with ST-segment elevation, which were not reversed by MET. All treatments reduced body and organ weights (except for MET at 250 mg/kg). At 150 mg/kg, MET prevented the GSH depletion caused by BC, while MET at 250 mg/kg reduced both PHLDA1 expression and tumor mass without impairing DOX's antineoplastic activity. Survival remained at 100%, except for animals treated with DOX and MET at the lowest dose. **Conclusion:** While DOX remains a potent chemotherapeutic agent, its efficacy is undermined by dose-dependent cardiotoxicity mediated by mitochondrial oxidative stress. Molecular docking and *in vivo* analyses underscore the involvement of mitochondrial enzymes and redox cycling in DIC pathogenesis. MET exhibits cardioprotective potential by modulating oxidative stress pathways and attenuating cardiomyocyte apoptosis, with no systemic damage as well.

06.024 **Hypertension Impairs Tissue Accumulation of Nitric Oxide Metabolites after Oral Nitrite Administration.** Lima-Silva AK¹, Vitorino TR¹, Rebelo MA¹, Conde-Tella SO², Tanus-Santos JE^{2,1}FCM-Unicamp, PPG Pharmacology, Brazil. ²FMRP-USP, Lab. Cardiovascular Pharmacology, Brazil

Introduction: Hypertension is a main risk factor for the development of cardiovascular diseases (CVDs). Nitric oxide (NO) plays a central role in the regulation of vascular tone, particularly in vasodilation and the maintenance of endothelial homeostasis. In addition to the classical enzymatic pathway of NO production, a non-enzymatic route known as the entero-salivary cycle has emerged, in which metabolites such as nitrate, nitrite, and nitrosylated species (RXNO) are generated from dietary sources and systemically recycled, promoting NO release. Recently, sodium nitrite has been investigated as a promising therapeutic agent for modulating NO levels, especially in conditions involving cardiovascular impairment. In this study, we examined whether oral sodium nitrite supplementation increases tissue levels of NO metabolites in various organs of normotensive and spontaneously hypertensive rats (SHR). **Methods:** We used male Wistar and SHR rats (CEUA:1272/2023), which received either water (vehicle) or sodium nitrite (15 mg/kg) by oral gavage daily for 6 weeks. Systolic blood pressure (SBP) was measured weekly in mmHg using the non-invasive tail cuff plethysmography method. At the end of the treatment period, animals were euthanized one hour after the final dose administration. Venous blood, heart, liver, brain, and muscle samples were collected for further analysis. NO metabolites concentrations were determined by ozone-based reductive chemiluminescence using the Sievers NO Analyzer Model 280 (Boulder, CO). The results were analyzed using two-way ANOVA. **Results:** The results demonstrated that nitrite significantly decreased SBP by 16% in SHR (<0.05), but not in Wistar rats (p>0.05). After nitrite treatment, the mean cardiac nitrite concentrations were 53.8 pmol/mg in the control group vs. 16.8 pmol/mg in the SHR group (p=0.008), while RXNO concentrations were 17.7 pmol/mg in the control group vs. 4.2 pmol/mg in the SHR group (p=0.005). In the liver, the mean nitrite concentrations were 11 pmol/mg in the control group vs. 5.3 pmol/mg in the SHR group; nitrate concentrations were 133.2 pmol/mg in the control group vs. 124.2 pmol/mg in the SHR group; and RXNO concentrations were 4.2 pmol/mg in the control group vs. 2.2 pmol/mg in the SHR group (p<0.001). In brain tissue, nitrite concentrations were 32.5 pmol/mg in controls vs. 21.4 pmol/mg in SHR rats, while RXNO concentrations were 5.53 pmol/mg in controls and 3.8 pmol/mg in SHR animals (p<0.001). In soleus muscle, nitrite concentrations were 39.4 pmol/mg in the control group vs. 23.6 pmol/mg in the SHR group; nitrate levels were 343.4 pmol/mg vs. 279.1 pmol/mg, and RXNO concentrations were 17.1 pmol/mg vs. 8.5 pmol/mg, respectively (p<0.001). **Conclusions:** The results show that nitrite treatment significantly increased the concentrations of nitrite, nitrate, and RXNO in the tissues from normotensive rats, which were severely attenuated in SHR, thus indicating that hypertension impairs tissue accumulation of NO metabolites. **Financial support:** This study was funded by National Council for Scientific and Technological Development (CNPq) (N° 130908/2024-0).

06.025 **Supplementation with *Arthrospira platensis* Prevents Arterial Blood Pressure Increase in Asthmatic Rats.** Oliveira GNM¹, Vieira MM¹, Oliveira TA², Cavalcanti CO³, Cruz JC^{2,3}, Vasconcelos LHC^{1,4} ¹UFPB, Programa de Pós-graduação em Produtos Naturais e Sintéticos Bioativos, Brazil; ²UFPB, Programa Multicêntrico de Pós-graduação em Ciências Fisiológicas, Brazil; ³UFPB, Depto de Biotecnologia, Brazil; ⁴UFPB, Depto de Ciências Biomédicas, Brazil

Introduction: Asthma can cause damage to the cardiovascular system beyond the respiratory tract (Guo, J. *Nat. Card. Res.* v. 1, p. 417-430, 2022). Although current treatments are effective, their side effects can impair disease management (GINA, p. 23, 2024). The cyanobacterium *Arthrospira platensis* (AP), an approved dietary supplement, emerges as a promising alternative due to its antihypertensive, anti-inflammatory, and antispasmodic effects. **Objectives:** Thus, the present study investigated the impact of supplementing asthmatic rats with AP on cardiorespiratory parameters. **Methods:** For this, male Wistar rats were randomly divided into control group (CG), asthmatic group (AG) and asthmatic group supplemented with AP 100 mg/kg/day (AGAP100). The asthma induction protocol with ovalbumin (OVA) occurred over 21 days. One day after the last nebulization, the femoral vein and artery were catheterized, and baseline mean arterial pressure (MAP) and heart rate (HR) were evaluated. Additionally, baroreflex sensitivity (BRS) was measured by L-(-)-phenylephrine (8 µg/kg, i.v.) and sodium nitroprusside (25 µg/kg, i.v.) administration, as well as peripheral chemoreflex by potassium cyanide (40 µg/kg, i.v.) injection. **Results:** MAP of GA was higher (129.71 ± 3.3 mmHg, n = 3) than on CG (113.4 ± 2.5 mmHg, n = 3), indicating that asthma induces arterial blood pressure increase, while in AGAP100, this increase was prevented (MAP = 112.0 ± 4.6 mmHg, n = 3). HR was not different among the groups (358.5 ± 14.0; 410.6 ± 25.5 and 366.5 ± 18.8 bpm, respectively, n = 3). Regarding BRS, there was no difference among the groups (-4.2 ± 0.7, n = 7; -3.8 ± 0.9, n = 4, and -4.8 ± 0.5 bpm.mmHg⁻¹, n = 4, respectively). After stimulation of peripheral chemoreceptors with KCN, there was an increase in MAP in CG (ΔMAP = 51.0 ± 14.1 mmHg, n = 4), AG (ΔMAP = 54.4 ± 9.8 mmHg, n = 3), and AGAP100 (ΔMAP = 42.7 ± 14.7 mmHg, n=3) without difference among the groups. In summary, we observed 1) an increase in MAP in asthmatic animals, 2) that AP prevents the asthma-induced increase in MAP in rats, and 3) the baro- and peripheral chemoreflexes are not influenced by asthma. **Conclusion:** Taken together, these data indicate a possible preventive effect of *A. platensis* on asthma-induced hypertension in rats. **Financial support:** FAPESQ-PB. **Animal Research Ethical Committee Number:** (CEUA/UFPB: 3255100522).

Introduction: Systemic arterial hypertension is a multifactorial clinical condition with significant public health implications. Excessive Na⁺ intake is linked to the development and progression of hypertension, with potential harmful effects independent of high blood pressure. Cardiotonic steroids, such as ouabain (OUA), are classic ligands of Na⁺/K⁺-ATPase (NKA) and studies show that volume expanded hypertensive patients exhibit elevated plasma levels of endogenous OUA. The role of endogenous OUA is not well understood, but it is speculated to affect long-term salt homeostasis and blood pressure. Elevated plasma Na⁺ levels may correlate with increased endogenous OUA and potential kidney damage. However, the combined effects of OUA and Na⁺ on kidneys are obscure. This study aims to evaluate how OUA interacts with a hypernatremic medium affecting LLC-PK1 renal tubular cells *in vitro*. **Methods:** High passage LLC-PK1 cells (P>80) were submitted to different concentrations of OUA, NaCl, and mannitol (osmolality control) for 48 h and assessed for morphology and cell viability (MTT assay). Western blot analyses were conducted to examine NKA α 1 and MAPK expression in these experimental groups. The comparison between groups was performed by the ANOVA followed by Tukey posttest (p<0.05 was considered as statistically significant). **Results and Conclusion:** Cellular distress, observed via phase contrast microscopy, was pronounced in groups exposed to high OUA (100 nM), Na⁺ ($\geq$ 232 mM), and corresponding mannitol levels. The MTT assay confirmed the reduced cell viability in these groups compared to controls, with significant 10% enhanced viability in cells treated with 1 nM and 10 nM OUA alone, as well as those exposed to 261 mM or 290 mM Na⁺ medium + 1 nM OUA (22% and 20%, respectively), but the same was not observed for mannitol. These results suggest that high concentrations of Na⁺ and OUA decrease cell viability, whereas low OUA concentrations partially block the deleterious effect of Na⁺ overload. Preliminary protein expression analysis indicated a decline of NKA α 1 and p-ERK1/2 (100 nM OUA). In contrast, low passage LLC-PK1 cells (P<40) exhibited resistance to NaCl, mannitol, and OUA. Decreased cell viability and distress were observed with higher concentrations (>350 mM Na⁺; 1000 nM OUA) Ongoing investigations are examining proteins related to cellular stress, viability, and transport mechanisms.

06.027 Renal Impact of Subacute Exposure to Endocrine Disruptors in Female Rats: Evidence of Preserved Function. Esteves GAB^{1,2}, Buhler GV, Accioli DSG^{1,2}, Alves LM¹, Morcillo LSL^{1,2} ¹ICB-UFRJ, ²IBCCF-UFRJ, Centro de Pesquisa em Medicina de Precisão

Introduction: Endocrine-disrupting chemicals (EDCs) are exogenous substance or mixture that alters function(s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub)populations," according to the World Health Organization (WHO). This study aimed to investigate whether subacute exposure to doses lower than those permitted by regulatory agencies of the endocrine disruptors Bisphenol S (BPS), Tributyltin (TBT), or their combined exposure, would affect renal function. **Methods:** Female Wistar rats (12 weeks old) were randomly assigned to four experimental groups and treated via oral gavage for 30 days (CEUA/UFRJ: 141/24): CTRL – vehicle control (0.4% ethanol); BPS – bisphenol S (50 ng·kg⁻¹·day⁻¹); TBT – tributyltin (100 ng·kg⁻¹·day⁻¹); and MIX – combination of BPS and TBT. After the treatment period, rats were housed in metabolic cages for 24 h to measure food and water intake and collect urine. Subsequently, rats were euthanized for blood and kidney collection. Renal function parameters were assessed. Data were analyzed using GraphPad Prism 8.0.2 Statistical significance was determined by one-way ANOVA followed by Tukey's post hoc test, considering p < 0.05. Results are expressed as mean ± standard error of the mean. **Results:** Exposure to BPS, TBT, or their combination did not affect: (i) food and water intake, or urinary volume. The mean values were similar to the CTRL group: 17.71±1.017 mg, 46.63±6.745 mL and 31.54±21.39 mL, respectively; (ii) kidney-to-body weight ratio (CTRL: 7.238±0.122); (iii) plasma and urinary creatinine (CTRL: 0.397±0.065 and 1.820±0.307, respectively), blood urea nitrogen (CTRL: 39.19±4.288), proteinuria (CTRL: 9,310±2,141), or albuminuria (CTRL: 0.0009513±0.0001685); and (iv) renal sodium balance (CTRL: 0.4002±0.076). **Conclusion:** These findings suggest that subacute exposure to low doses of BPS and TBT does not impair renal function in female rats, despite known effects on triode regulation. Further studies are warranted to evaluate whether exposure to these compounds induces subtle or progressive kidney injury through latent pathological mechanisms.

06.028 **Chronic Lead and Fluoride Exposure During Early Life Reduces Circulating and Cardiac Nitric Oxide Metabolites in Rats.** Conde-Tella SO^{1,2}, Figueiredo JT³, Ribeiro NM³, Rebelo MA¹, Gerlach RF³, Tanus-Santos JE^{1,2} ¹Unicamp, Faculty of Medical Sciences, Dept Translational Medicine, PPG Pharmacology, Brazil ²FMRP-USP, Dept Pharmacology, PPG Pharmacology, Brazil ³FORP-USP, Dept Basic and Oral Biology, Brazil

Introduction: Environmental contamination remains one of the most pressing challenges of our time. Studies have shown that contaminants such as lead (Pb) and fluoride (F), originating from many sources, can accumulate in soil and contaminate groundwater and atmospheric air, leading to prolonged population exposure. This persistent exposure may result in numerous public health issues. Evidence suggests that chronic exposure to Pb and F can impair cognitive function in children, and that long-term Pb exposure may alter circulating nitric oxide (NO) levels, potentially contributing to cardiovascular disorders. Based on these findings, our hypothesis is that chronic exposure to Pb and F leads to reduced plasma and cardiac concentrations of nitrite (NO_2^-), nitrate (NO_3^-), and nitrosylated species (RxNO). The objective of this study was to evaluate the effects of chronic Pb and F exposure on the plasma and cardiac levels of NO metabolites in juvenile rats exposed from gestation through early life. **Methods:** This study was approved by the CEUA at the School of Dentistry of Ribeirão Preto, University of São Paulo (0035/2022R2). Male and female Wistar rats, 10 weeks of age, were used for breeding. On gestational day 1, randomly assigned to one of four groups, and received the following treatments in their drinking water: vehicle; lead acetate (30 mg/L); hydrofluorosilicic acid (100 mg/L); or lead acetate (30 mg/L) combined with hydrofluorosilicic acid (100 mg/L). Treatment continued throughout the lactation period, after weaning, the offspring were separated from their mothers and continued to receive the same treatment for an additional 30 days. At the end of the protocol, animals were euthanized, and plasma and heart samples were collected for analysis. Concentrations of NO_2^- , NO_3^- , and RxNO were measured using ozone-based reductive chemiluminescence. **Results:** Our data show that, in plasma, there was a significant decrease in NO_2^- and NO_3^- concentrations with Pb treatment ($p = 0.001$ NO_2^- and $p = 0.002$ NO_3^-) and F treatment ($p = 0.001$ NO_2^- and $p = 0.02$ NO_3^-), as well as an interaction between treatments ($p = 0.006$ NO_2^- and $p = 0.008$ NO_3^-). In contrast, RxNO concentration was decreased only by F treatment ($p = 0.001$). In the heart, we observed that NO_2^- and RxNO concentrations showed a significant decrease only in the Pb group ($p = 0.02$ and $p = 0.04$, respectively), whereas NO_3^- concentration decreased significantly only in the F group ($p = 0.05$). **Conclusion:** Based on these data, we can conclude that Pb treatment significantly reduced plasma concentrations of NO_2^- and NO_3^- , and in the heart, it caused a decrease in NO_2^- and RxNO concentrations. F treatment significantly reduced plasma concentrations of NO_2^- , NO_3^- , and RxNO; however, in the heart, this treatment led to a decrease only in NO_3^- concentration. Finally, we conclude that there was an interaction between Pb and F treatments, which produced a significant reduction in plasma concentrations of NO_2^- and NO_3^- . **Funding:** This work was supported by the FAPESP and the CAPES.

06.029 Resistance versus Conduit Artery Endothelial Cells: Divergent Roles in T Cell Activation.

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Introduction: Endothelial cells (EC) work as antigen-presenting cells (APC) and can activate T cells, contributing to the activation of immune response. APCs specifically express MHC class II and co-stimulatory molecules, such as CD80. There are different types of vessels in the body, each with distinct functions, including resistance arteries, conduit vessels, and veins. However, it remains unclear whether the location of ECs can affect their immunological function. Aim: We aimed to investigate whether ECs from resistance arteries are more efficient at activating the immune response than ECs from conduit arteries. This project was approved by Animal Care and Use Committee at the University of South Carolina (IACUC# 2595-101693-041122). We isolated endothelial cells from mesenteric resistance arteries (MRA) and aortas from Wistar rats. CD4 cells were isolated from the spleen using beads. We stimulated the ECs with IFN- γ (10ng/mL) for 24 hours before co-culturing them with CD4 T cells for three days. The cells were analyzed using flow cytometry, and the supernatant was used to measure cytokines and chemokines. All experiments were conducted at least in triplicate. **Results:** The results indicated that under basal conditions, endothelial cells (EC) from the aorta exhibited high levels of MHC II and IL-6 expression, while showing lower levels of ICAM, MCP-1, VEGF, IL-1 β , and IL-2 compared to ECs from MRA. After co-culturing the ECs with CD4+ T cells, ECs from both vessels displayed elevated ki-67 expression (EC aorta: 97.1%; EC MRA: 96.3%), suggesting proliferation when in contact with T cells. Notably, only the ECs from the MRA demonstrated a significant increase in MHC II in the presence of CD4+ T cells (28.6% to 89.3%). Regarding cytokine release, co-culturing CD4+ T cells with aortic ECs resulted in increased production of IL-6 and TNF- α . In contrast, the co-culture with MRA ECs raised the concentrations of IL-1 β , TNF- α , CCL5, VEGF, IL-2, and IL-10 in the supernatant. The CD4+ T cell proliferation was observed in co-culturing with both types of ECs. Interestingly, co-culturing with aortic ECs (82.5%) led to a 30% greater increase in proliferation compared to MRA ECs (54.9%). Furthermore, only the aortic ECs showed a rise in double-positive cells (MHC II + CD80) (41.5% in aorta versus 22.12% in MRA). **Conclusion:** In conclusion, our findings suggest that ECs from resistance arteries have a basal pro-inflammatory profile that intensifies in the presence of CD4+ T cells. Aortic ECs appear to be more effective APC than those from MRA. This suggests that aortic ECs modulate the CD4+ T cell response more effectively, while MRA ECs are more influenced by IFN and CD4+ T cells. This discovery is significant for enhancing our understanding of vascular diseases in different arteries and developing targeted treatments.

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06.030 Aging Differentially Affects Male and Female Thoracic Aorta Perivascular Adipose Tissue: A Study Using a Murine Model of Accelerated Aging. Marques BVD, Akamine EH USP - Dept Pharmacology, São Paulo, Brazil

Aging is a major risk factor for several diseases, including cardiovascular conditions. Biological sex plays a key role in the development of aging-related physiological changes. Perivascular adipose tissue (PVAT) surrounds most blood vessels and is essential for regulating vascular tone and other responses. Aging induces PVAT dysfunction, contributing to vascular impairment. Cellular senescence is an underlying process of aging. Senescent cells accumulate over time, losing their original function while remaining metabolically active and exerting paracrine effects via secreted factors. We hypothesized that PVAT aging and associated molecular changes contribute differently to vascular responses in males and females. The objective was to evaluate PVAT's influence on vascular reactivity and the expression of cellular senescence markers, apoptosis regulators, and pro-inflammatory factors in PVAT and thoracic aortas of aged mice.

Male and female SAMP8 mice, a strain known for signs of accelerated aging, at 1 (1M), 3 (3M), and 8 (8M) months of age were used (CEUA no 4393010223). Thoracic aortas samples were dissected and cut into 2-mm rings, with PVAT either intact (PVAT+) or removed (PVAT-), and mounted on a myograph for ex vivo vascular reactivity assays using norepinephrine (NE) and acetylcholine (ACh) (n=5-10/group). In 3M and 8M males, samples of PVAT and aortas were evaluated for mRNA expression by qPCR of cell cycle arrest markers (p16, p21, and p53), apoptosis regulators (Bcl-2, Bcl-xL, Bad, and Mcl-1), and factors related to the senescence-associated secretory phenotype (TNF- α , IL-1 β , IL-6, and PAI-2). Analysis was performed using the $2^{-\Delta\Delta Ct}$ method. Results were expressed as mean $\pm$ standard error of the mean (SEM). Two-way ANOVA followed by Tukey's post-hoc test was used for concentration-response curves. Unpaired t-tests were applied to qPCR data, with $p < 0.05$ considered statistically significant. In male aortas, PVAT increased contractile responses to NE at 1M, exerted an anticontractile effect at 3M, and lost this effect by 8M. PVAT modulated ACh-induced relaxation only at 1M, reducing the vasodilatory response. In female aortas, PVAT had a pro-contractile effect on NE at 1M, but no significant effect at 3M or 8M, and did not alter ACh-induced relaxation at any age. In male PVAT, mRNA expression of cell cycle arrest markers and senescence-associated factors was similar between 3M and 8M. However, the anti-apoptotic factor Mcl-1 was significantly reduced at 8M. No differences were found in the expression of other apoptosis-related genes. In the aorta, p16 expression increased and p53 expression decreased in 8M mice compared to 3M, with no changes in apoptotic or senescence-associated inflammatory markers. In conclusion, although senescence markers in female PVAT and aorta have not yet been evaluated, our results indicate that PVAT responses to aging differ by sex. These findings underscore the complexity of aging-related changes in vascular physiology and the role of biological sex in modulating PVAT function in SAMP8 mice. **Financial support:** CNPq grant 140163/2024-7.

06.031 **Sodium Nitrite Lowers sFlt-1 Levels, Increases NO Production and Endothelial Cell Migration in an *in vitro* Model of Preeclampsia in Hypoxic Conditions.** Zampieri GM, Kaihara JNS, Bueno-Pereira TO, Passeti LF, Sandrim VC IBB-Unesp-Botucatu, Dept Biophysics and Pharmacology, PPG in Biomolecular and Pharmacological Sciences, Brazil

Introduction: Preeclampsia (PE) is a hypertensive disease established worldwide as one of the main causes of maternal-fetal mortality and morbidity. PE is widely considered a two-stage disorder, where placental hypoxia (stage 1) results in an increase of oxidative stress and antiangiogenic factors such as soluble fms-like tyrosine kinase-1 (sFlt-1) and causes the systemic maternal syndrome (stage 2) evidenced by reduced nitric oxide bioavailability and endothelial dysfunction. Within this framework, the nitrate-nitrite-NO pathway is an alternative form of NO production, and nitrite specifically has been correlated with antihypertensive and angiogenic effects. To study its effects on the antiangiogenic aspect of PE, an *in vitro* model of the syndrome was conducted where human umbilical vein endothelial cells (HUVECs) were subjected to hypoxic conditions and then incubated with plasma from patients diagnosed with early-onset (EOPE) and late-onset (LOPE) preeclampsia. **Methods:** HUVECs were subjected to cobalt chloride (100 μ M) for 24 hours before they were incubated with EOPE (n=10) and LOPE (n=10) plasma and treated with nitrite (1, 10 and 20 μ M). The wound healing (WH) assay was performed to investigate cell migration. Enzyme-linked immunosorbent assay (ELISA) with the supernatant of the WH assay was used to quantify sFlt-1, and the NO production was assessed by the fluorescent probe DAF-FM. **Results:** All concentrations of nitrite increased cell migration in 8, 12 and 24 hours of incubation with EOPE plasma, and the highest concentration increased cell migration in 8 and 12 hours of incubation with LOPE plasma. Production of sFlt-1 was diminished by all concentrations of nitrite in the EOPE group, and nitrite 1 and 20 μ M diminished sFlt-1 in the LOPE group. Lastly, NO production was augmented by nitrite after incubation with plasma of both groups in hypoxic conditions. **Conclusion:** Nitrite lowered sFlt1 levels and increased NO production in hypoxic conditions in this *in vitro* PE model, augmenting HUVEC migration in both EOPE and LOPE, with a more expressive effect on the EOPE group. This demonstrates a positive influence of nitrite on the angiogenic balance, corroborating its therapeutic potential for cardiovascular disorders like PE. **Financial support:** Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), grant numbers 2021/12010-7 and 2023/10669-7. Approved by the Research Ethics Committee of the Botucatu School of Medicine (nº 6747398)

06.032 Evaluation of Cardiac Autonomic Tone in Rats with Heart Failure with Preserved Ejection Fraction. Oliveira CGA¹, Alflen L², Betat A^{1,2}, Lataro RM¹ ¹UFSC, Dept Physiological Sciences, Brazil, ²UFSC, Dept Pharmacology, Brazil

Heart failure with preserved ejection fraction (HFpEF) is defined by left ventricular diastolic dysfunction associated with preserved ejection fraction. It is a heterogeneous syndrome and is characterized by the presence of comorbidities such as obesity, arterial hypertension, and diabetes mellitus. Currently, the available therapies for HFpEF remain limited, partly due to the lack of well-established preclinical models for this disease. Sympathetic nervous system hyperactivation has been demonstrated in HFpEF, with elevated coronary plasma norepinephrine levels and exacerbation of chemoreceptor responses. However, the literature data about cardiac autonomic tone in HFpEF are still scarce, highlighting the need for characterizing autonomic dysfunction in this disease, especially in animal models, which will provide a more comprehensive understanding of the HFpEF pathophysiological mechanisms. The animal model of HFpEF, induced by the concomitant administration of a high-fat diet and N^ω-nitro-L-arginine methyl ester (L-NAME), was recently standardized in our laboratory and reproduces many of the features observed in patients with this condition. Hence, this study aimed to test the hypothesis that the HFpEF rats would present increased sympathetic and reduced cardiac parasympathetic cardiac tone. The study was approved by the Animal Research Ethics Committee (CEUA/UFSC N° 8460220224). Wistar rats (60 days) were used, and HFpEF was induced by administering a high-fat diet (standard balanced chow, roasted peanuts, milk chocolate, and cornstarch biscuits in a 3:2:2:1 ratio) for 5 weeks plus L-NAME (20 mg/kg/day) for 4 weeks. Control rats received the standard diet. Data are presented as mean ± SD (control vs HFpEF). Compared to control rats (n=5), rats subjected to L-NAME and high-fat diet treatment (n=3-5) showed increased retroperitoneal fat (p=0.0104), epididymal fat (p=0.006), and higher blood glucose levels (p=0.002). However, body weight gain was not different between the two groups over the five weeks of treatment (p=0.68). Additionally, rats exposed to L-NAME and high-fat diet treatment exhibited reduced exercise tolerance (running time: p=0.0016; distance: p=0.0035) and increased heart weight (p=0.0009), which was not associated with an increase in lung weight (p=0.45). Rats treated with L-NAME and a high-fat diet also exhibited increased mean arterial pressure (p=0.0007) without changes in heart rate (p=0.60). The cardiac autonomic tone analyses showed no differences in sympathetic tone (p=0.66) or parasympathetic tone (p=0.10). Therefore, it is concluded that the combination of a high-fat diet and L-NAME reproduces, in rats, many of the clinical features observed in HFpEF. However, in this HFpEF model, there was no alteration in cardiac autonomic tone despite the observed metabolic and cardiovascular dysfunctions.

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06.033 **Resveratrol Reduces Vascular Hypercontractility in Rats with Obesity and Metabolic Syndrome through Reducing Oxidative Stress, Visfatin and MMP-2 Activity.** Manoel LB¹, Blascke de Melo MM¹, Dourado TMH¹, Assis VO¹, Bueno EKP¹, Fazan Jr R², Tirapelli CR¹, Castro MM¹ ¹FMRP-USP, Dept Pharmacology, ²FMRP-USP

Introduction: Obesity is a global health problem associated with the secretion of pro-inflammatory adipokines and oxidative stress. These factors favor the development of metabolic syndrome and cardiovascular diseases, such as hypertension. Oxidative stress leads to arterial remodeling through various pathways, including increased expression of NFkB, a transcription factor for the matrix metalloproteinase (MMP)-2. Elevated MMP-2 activity facilitates maladaptive cardiovascular remodeling that may precede the onset of hypertension in animals or individuals with metabolic syndrome. The use of antioxidants, such as resveratrol, aims to mitigate cardiovascular dysfunctions in various experimental rodent models of cardiovascular issues. Given its potent antioxidant capacity, resveratrol is hypothesized to reduce oxidative stress and the increased activity of MMP-2, thereby preventing vascular dysfunction in rats with metabolic syndrome. By studying these mechanisms, we will help to provide insights into new therapeutic strategies for early intervention in vascular dysfunction associated with the metabolic syndrome. **Methods:** Sprague Dawley rats were subjected to 16 weeks of a standard or high sugar (HS) diet *ad libitum*. At the last 6 weeks, rats also received vehicle or resveratrol (10 mg/kg, 5% alcohol solution; via gavage). Systolic blood pressure (SBP) was assessed using the invasive pressure technique. Aortas were used for *in situ* zymography, Western Blot for detect visfatin and NFkB, and vascular reactivity to phenylephrine with and without the perivascular adipose tissue (PVAT). Plasma was used to measure triglycerides, glucose, malondialdehyde and nitrite. Statistical analysis: Two-way ANOVA followed by Tukey post-test (CEUA: 180/2022R1). **Results:** At the end of 16 weeks, SBP was similar between the groups. HS rats gained more weight and fat followed by higher feed consumption, higher plasma levels of glucose and triglycerides ($p < 0.05$ vs. Controls), without changes by resveratrol. Oxidative stress was observed in the plasma of HS rats, which was significantly reduced by resveratrol ($p < 0.05$). Resveratrol also enhanced plasmatic nitrite bioavailability in the HS ($p < 0.05$). An elevated expression of visfatin was detected in aortas of HS rats ($p < 0.05$), which was reverted by resveratrol ($p < 0.05$ vs HS). Similarly, NFkB seems to increase in aortas of HS rats and resveratrol tend to decrease it. There was a heightened in the arterial MMP-2 activity in the HS rats, with reduction caused by resveratrol ($p < 0.05$). Resveratrol also reduced aortic hypercontractility to phenylephrine in the presence or absence of PVAT in the HS rats ($p < 0.05$). **Conclusion:** Treatment with resveratrol for 6 weeks reduced oxidative stress, the increased MMP-2 activity and restored vascular changes caused by the chronic consumption of HS diet. Acknowledgements: CAPES, FAPESP and CNPQ.

06.034 Testosterone Modulates Autonomic and Cardiovascular Responses to Stress in a Rat Model of Gender-Affirming Hormone Therapy. Martins SCA, Belem IJA, Suzuki GMF, Correa FMA, Tostes RC, Santos JD FMRP-USP, Dept Pharmacology, Brazil

Introduction: Sex hormones have opposite roles in cardiovascular system homeostasis. While female-related hormones exert cardioprotective effects, androgens are linked to pro-hypertensive effects, which is related to autonomic nervous system (ANS) activation. Supraphysiological levels of androgens in cis men and cis women increase sympathetic tone and reduce parasympathetic outflow, increasing blood pressure levels. Transmasculine individuals use testosterone in gender-affirming hormone therapy (GAHT-T), which frequently achieves high circulating levels. Considering that transgender people frequently face stressful environments due to social stigma, we hypothesized that testosterone, in a GAHT-T experimental model, exacerbates pressor responses under stress. **Methods:** Eight-week-old female Sprague-Dawley rats were treated with testosterone cypionate (24 mg/kg/week; s.c.) or vehicle, for 8 weeks. A group of male rats was also treated with vehicle to evaluate of “male phenotype”. Following the treatment period, the animals had a polyethylene catheter inserted into the femoral artery for mean arterial pressure (MAP) and heart rate (HR) recording. Cardiovascular parameters and tail temperature were collected during the basal (10 min), restraint stress (60 min), and post-stress recovery periods (30 min). After stress exposure, the animals were euthanized, and skeletal muscle and adipose tissues were collected for body composition analysis. Ethics committee approval nº 1402/2025 (FMRP-USP). **Results:** Testosterone-treated female rats showed decreased abdominal and retroperitoneal adipose tissue mass, suggesting a reduced fat body mass, which mimics clinical findings in transmasculine individuals on GAHT-T. Testosterone did not change body mass index and skeletal muscle mass in female rats. Testosterone treatment increased heart mass in females, suggesting cardiac hypertrophy. Cardiovascular analysis revealed that testosterone increased HR in baseline conditions, with no change in MAP. Under stress restraint, however, testosterone-treated female rats showed increased MAP, resembling cardiovascular records of male rats. Interestingly, the decrease in HR after stress exposure was faster and more pronounced in testosterone-treated females. All groups exhibited a decline in caudal cutaneous temperature in the stress period, suggesting acute vascular adaptive responses, which evidence restraint-induced ANS activation. **Conclusion:** Our results suggest that testosterone induces “masculinizing” effects in female rats, validating the GAHT-T experimental model. Testosterone alters ANS-mediated cardiovascular responses in female rats. Testosterone promotes positive chronotropic effects in basal conditions, exacerbating pressor responses under restraint stress. Testosterone induces more intensive cardiovascular recovery after stress, which may be related to compensatory mechanisms. In a translational perspective, our results reveal new insights regarding side effects induced by GAHT-T, revealing potential mechanisms whereby testosterone impacts cardiovascular performance, especially in adverse contexts. In the last instance, these findings may contribute to GAHT-T optimization, improving cardiovascular health of transmasculine individuals. **Financial support:** FAPESP, CNPq

06.035 **Effects of the Hydrogen Sulfide (H₂S)-Releasing Non-Steroidal Anti-Inflammatory Drug (NSAID) ATB-346 on the Proliferation and Migration of Cultured Vascular Smooth Muscle Cells.** Silva

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Introduction: NSAIDs are among the most worldwide used drugs. However, marked gastrointestinal lesions (GTL) are an adverse effect that hinder adherence to chronic treatment. In this way, anti-inflammatory, antioxidant, and vascular effects (including angiogenesis) from hydrogen sulfide (H₂S) donors may represent useful characteristics in order to minimize GTL. In fact, it has been shown that the H₂S-releasing NSAID ATB-346, a H₂S-releasing naproxen (NAP) derivative (i.e., the thiobenzamide - TBZ ester of NAP), presented decreased incidence of GTL *in vivo*. However, it has not been addressed, to date, whether neovascularization is within the underlying protective mechanisms. This study, therefore, aimed to investigate the effects of ATB-346 on cultured vascular smooth muscle cells (VSMCs) proliferation and migration. **Methods:** Rat aortic VSMCs (A7r5) were treated with 0.1 to 10 μ M NAP, TBZ, or ATB-346, and 10 nM angiotensin II (ANG-II) for 48 h. Cell viability and damage after treatments were assessed by the reduction of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium (MTT) and lactate dehydrogenase (LDH) release, respectively. Cell proliferation and migration were assessed via sulforhodamine B protein staining (SRB) or biplanar scratch wound (SW) assays. The H₂S production by A7r5 homogenates was assessed using the PbS formation-based method in the presence of 1 mM AOAA and the CSE-selective inhibitor 30 mM β -ciano-L-alanine (BCA). Data were expressed as mean $\pm$ SEM; differences among the group means were considered significant for values of $P < 0.05$. **Results:** Cell viability was significantly increased after 48 h-treatments with either NAP (1 μ M: 129.3 $\pm$ 7.2%, * $P < 0.05$; 10 μ M: 128.8 $\pm$ 5.7%, * $P < 0.05$; N=4-5) or TBZ (0.1 μ M: 135.6 $\pm$ 2.4%, ** $P < 0.01$; 10 μ M: 134.1 $\pm$ 5.1%, ** $P < 0.01$, N=5). Cell proliferation measured by the SRB staining assay was significantly decreased after treatment with NAP (1 μ M: 74.8 $\pm$ 2.1%, *** $P < 0.001$; 10 μ M: 48.8 $\pm$ 2.6%, *** $P < 0.001$; N=4) or ATB-346 (10 μ M: 65.4 $\pm$ 2.0%, *** $P < 0.001$, N=4) when compared with the respective vehicle-treated groups (0.01% DMSO: 100.2 $\pm$ 4.5%, N=4; 0.1% DMSO: 100.0 $\pm$ 9.7%, N=3). Cell proliferation was increased after treatment with 10 nM ANG-II when compared with vehicle-treated group (111.7 $\pm$ 1.3 vs. 100.0 $\pm$ 0.6%, *** $P < 0.001$, N=5). Furthermore, a marked increase in wound closure measured by the SW assay was found after treatment with 10 nM ANG-II for 48 h in comparison with the vehicle-treated group (81.6 $\pm$ 2.1 vs. 69.2 $\pm$ 4.1%, ** $P < 0.01$, N=3). However, wound closure was decreased after co-treatment of 10 nM ANG-II with 0.1 or 1 μ M ATB-346 for 48 h (0.1 μ M ATB-346: 67.5 $\pm$ 3.0%, *** $P < 0.001$, N=4; 1 μ M ATB-346: 60.5 $\pm$ 3.1%, *** $P < 0.001$, N=5) in comparison with the respective vehicle-treated groups (0.001% DMSO: 83.7 $\pm$ 0.7%, N=6; 0.01% DMSO: 80.6 $\pm$ 2.6%, N=5). Endogenous H₂S production by A7r5 homogenates (13.8 $\pm$ 0.5 μ M/mg, N=4) was significantly reduced after incubation with either 1 mM AOAA (0.2 $\pm$ 0.2 μ M/mg, *** $P < 0.001$, N=4) or 30 mM BCA (6.1 $\pm$ 0.8 μ M/mg, *** $P < 0.001$, N=4). **Conclusions:** At the highest concentrations tested, ATB-346 exerts a protective effect against ANG-II-induced VSMCs proliferation. Ongoing experiments aim to investigate the mechanisms that underlie this protective response, as well as the role of endogenous H₂S. **Financial support:** This study was supported by the Brazilian National Council for Scientific and Technological Development – CNPq (process 125248/2024-0) and São Paulo Research Foundation – FAPESP (process 2023/13436-3).

06.036 **O-GlcNAcylation Increases the Activity of Matrix Metalloproteinase (MMP)-2 in Aortas Incubated with Glucosamine and in Rat Aortas with Metabolic Syndrome.** Bueno EKP¹, Manoel LB¹, Assis VO¹, Ferreira GM², Neves VGO¹, Blascke de Mello MM¹, Tostes RC¹, Castro MM¹ ¹FMRP-USP, Dept Pharmacology; ²FCF-USP, Dept Clinical and Toxicological Analysis

Introduction: alterations in glucose and lipid metabolism in metabolic syndrome (MS) corroborate cardiovascular diseases, such as hypertension, and post-translational modification (PTM) O-GlcNAcylation, which occurs by the enzymatic addition of O-GlcNAc to proteins. An increase in the activity of MMP-2, an endopeptidase present in vascular smooth muscle, is usually associated with maladaptive remodeling and vascular dysfunction. 72 kDa MMP-2 can be activated intracellularly by PTMs, such as phosphorylation and S-glutathiolation, in the same residues that O-GlcNAc can bind; however, the effect of O-GlcNAcylation on MMP-2 is still unknown. Thus, the aim of this study is to evaluate whether O-GlcNAcylation increases MMP-2 activity in aortas incubated *ex vivo* with glucosamine (GlcN) and in aorta of male rats with MS. **Methods:** prediction of O-GlcNAcylation sites in MMP-2 was performed *in silico*. Aortas from male Sprague-Dawley (SD) rats weighing 200 grams were incubated *ex vivo* with different concentrations of GlcN for 24 hours (CEUA-FMRP, 1078/2022). The aortas were used to evaluate the protein expression of O-GlcNAc and MMP-2 by Western Blotting, MMP-2 activity by gel and *in situ* zymography, and vascular reactivity by wire myography in the presence of phenylephrine (PE). In addition, 6-week-old male SD rats were fed a standard diet or a high-glycemic diet (energy density of 13.35 kJ/g) for 16 weeks (CEUA-FMRP, 180/2022R1). Aortas were used to assess O-GlcNAc levels and MMP-2 activity. One-way ANOVA followed by Dunnett's test, two-way ANOVA followed by Tukey's test or Student's t-test ($p < 0.05$) were used, as appropriate. **Results:** O-GlcNAcylation can occur in the catalytic site and in the propeptide, fibronectin-II-like, and hemopexin-like domains. Aortas incubated with GlcN at 10^{-12} M showed an increase in O-GlcNAc levels compared to the control. The activity of 64, 72 kDa, and the sum of MMP-2 isoforms (64+72+75 kDa) showed an increase in GlcN at 10^{-12} M by gel zymography (64 kDa MMP-2: 2.411 ± 0.3030 versus 1.623 ± 0.2400 ; $p = 0.0431$. 72 kDa MMP-2: 2.807 ± 0.2787 vs. 1.966 ± 0.1345 ; $p = 0.0300$. Total: 5.943 ± 0.5949 vs. 4.019 ± 0.2400 ; $p = 0.0144$; $n = 5$) and *in situ* (121.1 ± 1.997 vs. 100.0 ± 0.000 ; $p < 0.0001$; $n = 5$), while MMP-2 protein expression was increased in GlcN at 10^{-8} and 10^{-12} M by Western Blotting (GlcN at 10^{-8} M: 0.3710 ± 0.02792 vs. 0.2030 ± 0.04242 ; $p = 0.0108$. GlcN at 10^{-12} M: 0.4195 ± 0.04517 ; $p = 0.0009$; $n = 7-8$). Furthermore, the PE power for contraction showed a reduction in aortas incubated with GlcN at 10^{-12} M, as shown by the leftward shift of the curve (6.3367 ± 0.1291 vs. 7.0101 ± 0.0747 ; $p = 0.0002$; $n = 4-10$). Furthermore, characterizing the experimental model, rats subjected to a high-glycemic diet showed an increase in body mass and lipid profile, plasma glucose and triglycerides, as well as a reduction in total cholesterol. In addition, the aorta of rats with MS showed an increase in O-GlcNAc levels and MMP-2 activity (0.3416 ± 0.03967 vs. 0.2263 ± 0.02018 vs. 0.2231 ± 0.02777 ; $p = 0.0426$; $n = 7$). **Conclusion:** *ex vivo* treatment with GlcN and SM increased the O-GlcNAc profile in the aortas, contributing to an increase in MMP-2 activity. **Financial support:** CNPq e FAEPA.

06.037 **Effects of Metformin on Anti-Angiogenic Factors and Mitochondrial Function in an *in vitro* Model of Early and Late-Onset Preeclampsia.** Passeti LFP, Pereira DA, Bertozzi-Matheus M, Kaihara JSN, Bueno-Pereira TO, Santos KN, Zampieri GM, Nunes PR, Sandrim VC IBB-UNESP-Botucatu, Institute of Biosciences, Dept Biophysics and Pharmacology, Brazil

Introduction: Preeclampsia (PE) is one of the leading causes of maternal mortality worldwide, characterized by hypertension after the 20th week of pregnancy, with proteinuria and/or target organ dysfunction. When diagnosed before the 34th week, it is classified as early-onset PE (EOPE); equal or after, as late-onset PE (LOPE). PE involves spiral arteriole impairment, causing ischemia and poor placentation, which leads to release of anti-angiogenic factors like soluble fms-like tyrosine kinase-1 (sFlt-1) and soluble endoglin (sEng). About half of PE patients do not respond to conventional treatments, and underlying mechanisms remain unclear. Targeting these pathways with specific drugs is promising. Metformin (MET), used for type 2 diabetes, has shown benefits in hypertensive pregnancy disorders due to cardioprotective, anti-inflammatory, and antioxidant effects. This study evaluated MET effects in an *in vitro* PE model employing human umbilical vein endothelial cells (HUVECs) incubated with plasma from healthy pregnant women (Early Healthy Pregnancy [EHP] and Late Healthy Pregnancy [LHP]) and PE patients (EOPE and LOPE), evaluating anti-angiogenic factors, mitochondrial superoxide, and mitochondrial membrane potential.

Methods: HUVECs were treated with MET (1 μ M) and incubated for 24 hours with 20% (v/v) pooled plasma from EHP (n=5), LHP (n=5), EOPE (n=5) LOPE (n=5) patients. We assessed anti-angiogenic factors by ELISA for sFlt-1 and sEng in cell culture supernatants; mitochondrial superoxide by MitoSOX™; and mitochondrial membrane potential by JC-1, both via flow cytometry. **Results:** sFlt-1 levels were higher in EOPE than EHP (around 26,770pg/mL versus 19,000pg/mL); similarly, LOPE showed higher levels than LHP (around 24,000pg/mL versus 19,800pg/mL). EOPE+MET had lower sFlt-1 than untreated EOPE (around 22,000pg/mL versus 26,770pg/mL). sEng was higher in EOPE than EHP (around 6,700pg/mL versus 3,600pg/mL), and EOPE had higher sEng than LOPE (around 6,700pg/mL versus 4,330pg/mL). Mitochondrial superoxide showed no significant differences. Mitochondrial membrane potential was increased in LOPE versus LHP (around 0.75 versus 0.58 Aggregates : Monomers ratio). **Conclusions:** EOPE showed elevated anti-angiogenic factors, indicating greater severity than LOPE, aligning with existing evidence. MET treatment reduced these factors in endothelial cells exposed to PE plasma, suggesting a role in improving angiogenesis and potentially mitigating endothelial dysfunction. Further studies should explore MET's effects on these and other pathways with specific inhibitors. **Financial suport:** Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), grant nº 2024/13523-6, 2021/12010-7, 2024/01935-8, 2024/03439-8, 2024/10080-6, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), grant nº 308504/2021-6, and 444682/2024-4, and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) grant nº 88887.948965/2024-00. **Ethics:** Approval by the Ethics Committee: Research Ethics Committee of the Botucatu School of Medicine 6.853.434.

06.038 **A Model of Salt-Sensitive Hypertension with Early Renal Dysfunction: Investigating New Therapeutic Targets.** Pereira T¹, Araújo GVP², Brasil JPP¹, Esteves GAB¹, Santos AVZ², Filho HM¹, Morcillo LSL¹ ¹UFRJ, ²Unirio

Introduction: Salt-sensitive hypertension (SSH) is a prevalent, silent, and difficult-to-control condition associated with the progression of chronic kidney disease (CKD). This study aimed to establish an SSH model with early renal injury to further investigate the pathological mechanisms.

Methodology: Male Wistar rats (200–250 g; n=4–5/group; CEUA UFRJ (110/24) underwent uninephrectomy, followed by treatment with deoxycorticosterone acetate (DOCA, 8 mg/kg, 2x/week, for 28 days) with or without a high-salt diet (4% NaCl). The normotensive (NT) group received vehicle (soybean oil) only. Systolic blood pressure (SBP), urinary volume (UV), and urinary and plasma biomarkers were analyzed. **Results:** The DOCA-salt group showed a significant increase in SBP (154.7 ± 6.6 mmHg) compared to NT (114.0 ± 4.7 mmHg; $p < 0.01$), while the DOCA group (128.3 ± 8.6 mmHg) showed a non-significant upward trend ($p > 0.05$). UV was higher in the DOCA-salt group (79.2 ± 10.2 ml) compared to NT (18.6 ± 1.8 mL; $p < 0.001$) and DOCA (20.3 ± 6.7 ml; $p < 0.001$), with no difference between NT and DOCA. Proteinuria was higher in the DOCA-salt group (78.9 ± 4.8 mg/24h) than in NT (61.1 ± 3.6 mg/24h; $p < 0.05$), but not significantly different from the DOCA group (75.8 ± 1.5 mg/24h; $p > 0.05$). Albuminuria was significantly higher in the DOCA-salt group (0.028 ± 0.006 mg/mg) compared to NT (0.012 ± 0.006 mg/mg; $p < 0.05$) and DOCA (0.019 ± 0.006 mg/mg; $p < 0.05$). No statistically significant differences were observed in urinary creatinine (NT: 1.96 ± 0.31 ; DOCA: 2.43 ± 0.33 ; DOCA-salt: 2.94 ± 0.54 mg/dl), plasma urea (NT: 18.6 ± 1.8 ; DOCA: 20.3 ± 6.7 ; DOCA-salt: 19.2 ± 10.2 mg/dl), or plasma creatinine levels (NT: 1.57 ± 0.14 ; DOCA: 1.21 ± 0.22 ; DOCA-salt: 1.08 ± 0.12 mg/dl). **Conclusion:** The data demonstrate that the DOCA-salt model induces SSH, characterized by an increase in SBP, urinary output, and protein loss, including albumin, without impairing renal filtration function. These findings suggest that the model represents an initial pathological stage, making it suitable for investigating its mechanisms. **Financial Support:** Carlos Chagas Filho Foundation for Research Support from the State of Rio de Janeiro (FAPERJ: E-26/203.405/2023, E-26/210.441/2024, and E-26/210.561/2024), Brazilian Federal Agency for Support and Evaluation of Graduate Education (CAPES: 88887.014826/2024-00)

06.039 Sodium Nitrite Attenuates NLRP3 Associated Genes in a Hypoxia *in vitro* Model of Early-Onset Preeclampsia. Santos KN, Nunes PR, Pereira DA, Zampieri GM, Bueno-Pereira TO, Passeti LFP, Matheus MB, Sandrim VC IBB-Unesp- Botucatu, Dept Biophysics and Pharmacology, Brazil

Introduction: Preeclampsia (PE) is a critical gestational condition responsible for high maternal and fetal morbidity and mortality rates. It is characterized by hypertension, proteinuria, systemic inflammation, and endothelial dysfunction. Its pathophysiology involves impaired placental vascular development, leading to hypoxia, ischemia, poor perfusion, and release of endothelial-damaging factors such as reactive oxygen species (ROS). Inflammatory pathways, including the NLRP3 inflammasome, are activated, promoting the release of pro-inflammatory cytokines (IL-18 and IL-1 β). At the same time, nitric oxide (NO), an essential mediator of vascular regulation and inflammation, is reduced. In this context, the nitrate-nitrite-NO pathway emerges as a potential alternative NO source and regulator of inflammation. This study aims to investigate the effects of nitrite on the NLRP3 inflammasome pathway in endothelial cells (ECs) cultured with plasma from women with early-onset PE (EOPE), the most severe form (diagnosed before 34 weeks). The study also assessed whether these effects depend on NO conversion. **Methods:** Human umbilical vein endothelial cells (HUVECs) were pretreated with cobalt chloride (100 μ M) for 24 hours and assigned to three groups: Group 1 (n=6), EOPE plasma + sodium nitrite (20 μ M); Group 2 (n=6), EOPE plasma; and Group 3 (n=3), EOPE plasma + sodium nitrite + NO scavenger cPTIO (100 μ M, applied before nitrite). Treatments lasted 6 hours with 10% pooled plasma (n=10), nitrite, and cPTIO, as applicable. Total RNA was extracted using the Quick-RNA™ Miniprep kit (ZYMO R1055) and quantified with a BioDrop μ Lite+ spectrophotometer. cDNA was synthesized using 4 μ L LunaScript RT SuperMix (5x) from 1 μ g RNA. RT-qPCR reactions (10 μ L) contained 1 μ L cDNA (1.5 ng/ μ L), 5 μ L KiCqStart® SYBR® Green ReadyMix™ (SIGMA KCQS00-250RXN), 0.5 μ L of each primers, and 4.5 μ L nuclease-free water. Thermal cycling followed standard conditions, and fluorescence was recorded on a StepOnePlus Real-Time PCR System (Applied Biosystems). Six target genes (TLR4, NLRP3, ASC, Caspase-1, IL-18, IL-1 β) and one reference gene (HPRT1) were analyzed. Results: Compared with Group 2 (EOPE plasma alone), Group 1 (EOPE plasma + nitrite) showed downregulation of all target genes, with the following fold change values: TLR4 (2.2), NLRP3 (1.77), ASC (1.79), Caspase-1 (1.64), IL-18 (1.08), and IL-1 β (2.17). Conversely, relative to Group 1, Group 3 (EOPE plasma + nitrite + NO scavenger) exhibited upregulation of the same genes, with fold change values: TLR4 (3.3), NLRP3 (2.73), ASC (2.66), Caspase-1 (2.56), IL-18 (1.64), and IL-1 β (4.43). **Conclusions:** Nitrite reduced expression of genes linked to the NLRP3 pathway in ECs cultured with EOPE plasma. This effect appears dependent on NO conversion, as the NO scavenger reversed the downregulation. Despite this, nitrite showed a positive modulatory role in the inflammatory response, contributing to the understanding of its potential therapeutic benefits in preeclampsia. **Financial support:** Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), grant numbers 2021/12010-7, 2024/19683-5 Approval by Ethical Committee: Research Ethics Committee of the Botucatu School of Medicine 6.747.398

06.040 Renin-Angiotensin System and Abdominal Aortic Aneurysm: An Imbalance Between Circulating and Local Environments. Dugaich VF¹, Mestriner F¹, Dantas PB¹, Kovacs HZ¹, Jordani MC¹, Ribeiro MS¹, Becari C^{1,2} ¹FMRP-USP, Vascular Disease Lab, Dept Surgery and Anatomy, Brazil. ²FOB-USP, Dept Biological Science, Bauru, Brazil

Although systemic components of the renin-angiotensin system (RAS) are widely studied in cardiovascular diseases, the relevance of its local regulation remains underexplored in abdominal aortic aneurysm (AAA). This study investigated the differential expression of RAS components in plasma and aortic tissue of patients with and without AAA. Protein levels were quantified via ELISA, while gene and protein expressions in tissue were analyzed using qRT-PCR and Western blotting. In AAA patients, tissue levels of ACE and elastase-2 (ELA-2)—enzymes responsible for Ang II generation—were significantly elevated, whereas their plasma concentrations were reduced. Conversely, components of the protective axis such as Ang 1-7 showed an inverse pattern: decreased in tissue and elevated in plasma. ACE2 protein was decreased in both compartments. MAS receptor showed increased gene expression but decreased protein levels in tissue. Neprilysin, another contributor to Ang 1-7 formation, also demonstrated opposing expression between compartments. These results highlight a striking dissociation between circulating and local RAS profiles in AAA, reinforcing the notion that aneurysm pathophysiology is predominantly driven by tissue-specific mechanisms. Our findings question the reliability of plasma RAS components as standalone biomarkers for AAA progression and support the need for tissuetargeted diagnostic and therapeutic strategies.

06.041 **Effects of Carvedilol in the Cognition of Rats that Survived Sepsis.** Betat A¹, Bialeski MEG², Candido AJS², Caixeta LFAF², Oliveira CGA², Fernandes D³, Lataro RM² ¹UFSC, PPG Pharmacology, Brazil; ²UFSC, Dept Physiological Sciences, Brazil; ³ UFSC, Dept Pharmacology, Brazil

Introduction: Sepsis is considered a clinical syndrome resulting from organ dysfunction due to a dysregulated host response to infection and is the most common cause of mortality in critically ill patients. Despite the high prevalence and mortality rates of sepsis, there is still a shortage of studies addressing the repercussions that occur in the post-sepsis period. Post-sepsis psychiatric disorders, which include anxiety and depression, are highly prevalent complications secondary to sepsis, leading to a marked increase in long-term mortality among affected patients. A recent meta-analysis showed that the use of beta-blockers is associated with improved survival in patients with sepsis, improving lactate levels and heart rate control. Previous studies have also demonstrated that carvedilol exhibits potential neuroprotective effects, delaying memory decline in patients with impaired cognitive function. However, there is still a lack of evidence regarding beta-blocker treatment in the post-sepsis phase. The present study aimed to evaluate the effects of carvedilol treatment on the memory of rats surviving sepsis. **Methods:** The experimental protocol was approved by The Committee of Ethics in Animal Research (CEUA/UFSC; Protocol No. 4016210824). Sepsis was induced in adult male Wistar rats (300g, 90 days old), using the Cecal Slurry model. The effectiveness of the sepsis induction procedure was analyzed using the Score of Sepsis, blood lactate and glucose, and body temperature that were evaluated before induction of sepsis, 24 h, and 96 h after the intraperitoneal administration of the cecal suspension. The analyses were performed on rats that survived sepsis, treated (n=3) or not (n=2) with a beta-blocker (carvedilol, 60 mg/kg/day, drink water) for 8 weeks. Locomotion, recognition memory and work memory were assessed using the open field test, new object recognition test, and Y-maze test, respectively. **Results:** The Score of Sepsis was higher 24h after the sepsis induction (before 0±0 vs. 24 hours 13±1h; p<0,0001), indicating that the Cecal Slurry model was effective. The lactate levels returned to baseline after 96h (24 hours 5±1 vs. 96 hours 3±0,3; p<0,05). Blood glucose and body temperature were not changed 24h and 96h after the sepsis induction. The treatment with carvedilol had no effect on locomotion (Time in the center of the open field: post-sepsis 10±3 vs. post-sepsis+Carvedilol 23±11; p>0.05; Time in the periphery of the open field: post-sepsis 298±2 vs. post-sepsis+Carvedilol 277±11; p>0.05), recognition memory (Discrimination index: post-sepsis 0,6±0,4 vs. post-sepsis+Carvedilol 0,17±1; p>0,5), and working memory (Spontaneous alternation: post-sepsis 331±11 vs. post-sepsis+Carvedilol 334±264; p>0,5) in the rats survived that survived to sepsis. **Conclusion:** These preliminary data indicate that treatment with carvedilol did not change the cognition function of septic rats. **Financial support:** National Council for Scientific and Technological Development (CNPq/Brazil).

06.042 **Effects of a Single Dose of Methylmercury on Nitrosative Mechanisms Involved in the Regulation of Vascular Tone.** Caetano ESP, Tella SOC, Rebelo MA, Barros AC, Cunha MS, Santos JET FMRP-USP, Dept Pharmacology, Ribeirao Preto Medical School, University of Sao Paulo, Ribeirao Preto, Brazil

Introduction: Mercury exposure represents a significant public health issue in Brazil, particularly among riverine Amazonian populations, as it is associated with vascular dysfunction, including alterations in blood pressure, increased mortality risk from ischemic heart disease, atherosclerosis, and renal impairment, among other conditions. However, the pathophysiological mechanisms underlying these mercury-related comorbidities remain poorly understood, and effective treatment options are scarce. In light of this challenge, it is crucial to explore novel and effective pharmacological targets. The classical nitric oxide (NO) pathway has been extensively investigated in the context of mercury exposure and cardiovascular pathologies; however, the protein S-nitrosylation pathway may also play a critical role in the regulation of vascular tone. **Methods:** Male Wistar rats (200–250 g) were randomly assigned to four experimental groups: Control (H₂O), Nitrite (NO₂⁻), Methylmercury (MeHg), and Nitrite + Methylmercury (NO₂⁻ + MeHg). The Control and MeHg groups received water by gavage for five consecutive days; the NO₂⁻ and NO₂⁻ + MeHg groups received sodium nitrite by gavage at a dose of 15 mg/kg for five consecutive days, with the final dose given 40 minutes prior to euthanasia. Methylmercury was administered at a dose of 8 mg/kg to the MeHg and NO₂⁻ + MeHg groups on the fifth treatment day, 3 hours before euthanasia. Animals were anesthetized and euthanized by exsanguination. Plasma samples were collected for biochemical analysis, and the aorta was carefully isolated for vascular reactivity experiments. Aortic rings with intact endothelium were challenged with increasing concentrations of the α_1 -adrenergic agonist phenylephrine (10⁻¹⁰ to 10⁻⁴ M). Plasma nitric oxide metabolites (nitrate, nitrite, and nitrosylated species) were measured using ozone-based chemiluminescence. **Results:** In response to phenylephrine, the Nitrite group exhibited a significantly lower E-max (P = 0.003) compared to the MeHg-treated groups and a significantly higher pD₂ value (P = 0.0007) compared to the other groups. Regarding nitric oxide metabolites, the NO₂⁻ + MeHg group showed lower plasma nitrate and nitrite levels compared to the NO₂⁻ group (P = 0.029 and P = 0.04, respectively). In the analysis of nitrosylated species, the NO₂⁻ + MeHg group also demonstrated a significant reduction compared to the NO₂⁻ group (P = 0.005). **Conclusion:** Our findings demonstrate that sodium nitrite decreases vascular responsiveness to α_1 -adrenergic stimulation while increasing plasma levels of nitric oxide metabolites (nitrate and nitrite) and nitrosylated species. However, following a single dose of methylmercury, vascular responsiveness is enhanced, accompanied by reductions in nitric oxide metabolites and nitrosylated species, suggesting a possible impairment of protein nitrosylation mediated by sodium nitrite. **Financial support:** CNPq.

06.043 Antioxidant Effect of Ascorbic Acid on Heart Damage Associated with Doxorubicin in Murine Breast Carcinoma. Ferreira LPF^{1,2}, Cavalcante KDM^{1,2}, Cavalcante FAS¹, Silva ACA¹, Santos BLB³, Nascimento MCA¹, Leal BS², Silva MTBS⁴, Amaral DT⁵, Ferreira PMP², Dittz D^{1,2,3}. ¹UFPI, Antineoplastic Pharmacology Lab, Brazil; ²UFPI, PPG Pharmacology, Brazil; ³UFPI, PPG Pharmaceutical Sciences, Brazil; ⁴University of Porto, Institute of Biomedical Sciences Abel Salazar, Portugal; ⁵UFABC, Center for Natural and Human Sciences, Brazil

Introduction: Doxorubicin (DOX) is a cytotoxic anthracycline widely used in the treatment of breast cancer, with cardiotoxicity being one of its major adverse effects. The generation of reactive oxygen species contributes to both its antitumor efficacy and doxorubicin-induced cardiotoxicity (DIC). This study aimed to evaluate the effect of co-treatment with ascorbic acid (AA) and DOX on DIC.

Methods: Targets related to the antitumor and cardiotoxic effects of DOX were identified by literature review and data mining (KEGG, DrugBank, PubChem, and STRING). Molecular interactions between AA/DOX and cardiotoxicity targets were investigated by blind docking (AutoDock Vina, PyMol). The antioxidant activity of AA was tested against DPPH and ABTS•+ radicals. In animals with DIC treated with AA (2.5–100 mg/kg), ECG, cardiac histopathology, hepatic GSH, NAG, MPO, leukocytes, and body weight were analyzed. In breast cancer models with DIC treated with AA (2.5–100 mg/kg, oral), tumor and body weights were measured. In 4T1 cells, the cytotoxicity of DOX (0.15–24 µg/mL) + AA (3.1–400 µg/mL) co-treatment and AA was added in single concentrations, gradually, ranging from 10 µg/mL to 100 µg/mL, for 24 hours, followed by treatment with DOX in serial double dilution, starting at 24 µg/mL, for 24 hours, being evaluated by the MTT assay and Bliss model. **Results:** The EC50 values of AA for DPPH and ABTS•+ radicals were 13.3 µg/mL and 8.5 µg/mL, respectively. Data mining identified 14 proteins involved in both the antitumor and cardiotoxic effects of DOX, associated with cell proliferation (ERK, MAPK2, EGFR, FGFR1), differentiation (RASK, MAPK2), survival (HIF1A, HIF3A, MAPK2), and apoptosis (P53, Bax, mitochondrial ubiquitin ligase activator of NFκB1) in both tumor and cardiac tissues. Among these proteins, AA showed the lowest binding energies with NADPH-cytochrome P450 reductase (–8.069 kcal/mol) and mitogen-activated protein kinase (–7.862 kcal/mol). In DIC animal models, AA (especially at 2.5 and 25 mg/kg) prevented the DOX-induced increases in R–R', QT, and QRS intervals, and the reduction in heart rate. AA also attenuated cardiac tissue damage, weight loss, and leukocyte alterations induced by DOX. In the heart, AA reduced NAG and MPO activity. In animals with breast tumors and DIC, co-treatment with AA preserved the antitumor efficacy of DOX and mitigated weight loss. In 4T1 cells exposed to AA for 24 hours, the IC50 of 50% cell viability was 26.62 µg/mL. The cytotoxic action of DOX presented an IC50 of 12.37 µg/mL, compared to the mean of the negative control. The combination of AA and DOX demonstrated synergistic cytotoxicity in 4T1 cells at concentrations ≤ 25 µg/mL (AA) and ≤ 3 µg/mL (DOX). **Conclusion:** Co-treatment with AA prevents electrocardiographic and histological alterations associated with DIC, likely due to its antioxidant properties, while preserving the antitumor activity of DOX in breast carcinoma models. *In silico* analyses identified NADPH and MAPK as potential targets for AA-mediated cardioprotection.

06.044 **Dopaminergic Receptor Expression in the Aorta of *Chelonoidis carbonarius*: An Immunohistochemical Study.** Santos EXD¹, Souza VB¹, Britto-Júnior JB¹, Campos R¹, Nucci G^{1,2}, Schenka AA¹ ¹Unicamp, Dept Pharmacology, Faculty of Medical Sciences, Brazil ²ICB-USP, Dept Pharmacology, Brazil

Introduction: Dopaminergic signaling is increasingly recognized for its modulatory role in vascular function, but the distribution and potential roles of dopamine receptor subtypes in reptilian blood vessels are still poorly characterized. This study aimed to determine the expression and localization of dopamine receptors D1R, D2R, D3R, D4R, and D5R in the aorta of the tortoise *Chelonoidis carbonarius* using immunohistochemistry. **Methods:** Aorta samples (n=3) were collected and fixed in 10% neutral buffered formalin for 48 hours, followed by paraffin embedding and sectioning at 4 µm thickness. Sections were deparaffinized, rehydrated, and subjected to antigen retrieval in citrate buffer (pH 6.0). Immunostaining was performed using primary antibodies against each receptor subtype and visualized by the NovoLink™ Max Polymer Detection System with DAB as chromogen. Harris' hematoxylin was used for counterstaining. Appropriate positive controls (tortoise brain sections) and negative controls (omission of primary antibody) ensured staining specificity. CEUA/UNICAMP Authorization Number: 6441-1/2024 **Results:** The results demonstrated a heterogeneous pattern of dopamine receptor expression. D1R was undetectable in all aortic layers, indicating it is not involved in vascular regulation in this species. D2R was weakly positive in the endothelial cells of the tunica intima, but not in *vasa vasorum*, suggesting a minor role in intimal endothelium. Both D3R and D4R were moderately expressed in endothelial cells of both the intima and *vasa vasorum*. Interestingly, D4R was also present in smooth muscle cells of the tunica media, pointing to a dual role in both endothelial and contractile vascular function. D5R was faintly detected in the intima and *vasa vasorum*. **Conclusion:** These findings suggest that D2R, D3R, and D5R may contribute to endothelial modulation, while D4R might play a broader role, influencing both endothelial and smooth muscle functions. The complete absence of D1R highlights a distinct receptor distribution profile in *Chelonoidis carbonarius* compared to other vertebrates, underlining the evolutionary diversity in dopaminergic vascular modulation. **Financial support:** CAPES 88887.932791/2024-00 / FAPESP (2019/16805 - 4). **Keywords:** dopamine receptors, *Chelonoidis carbonarius*, aorta, immunohistochemistry, vascular regulation

06.045 **Therapeutic Potential of Glibenclamide and Metformin as Endothelial Protective Agents in a Preeclampsia *in vitro* Model.** Nunes PR, Pasetti LFP, Pereira DA, Bueno-Pereira TO, Sandrim VC IBB-Unesp-Botucatu, Dept Biophysics and Pharmacology

Introduction: Preeclampsia (PE) is a pregnancy-specific syndrome and one of the leading causes of maternal death worldwide. Approximately half of patients with PE do not respond to current therapies, and its pathophysiology remains poorly understood. Persistent endothelial inflammation promotes the secretion of several inflammatory mediators, which activate multiple important signaling pathways. Given that the underlying causes of PE are still not fully elucidated, targeting these pathways with specific drugs may represent a promising approach for future treatments. In this context, drugs such as glibenclamide (GB) and metformin (MET), commonly used to treat type 2 diabetes, have shown encouraging results in managing hypertensive disorders of pregnancy due to their cardioprotective, anti-inflammatory, and antioxidant properties. We aimed to evaluate the effects of treatment with GB and MET in an *in vitro* model of PE, based on the two-stage theory of the disease, incubated with plasma from pregnant women with PE concerning viability, cytotoxicity, oxidative stress, and cell migration. **Methods:** Human umbilical vein endothelial cells (HUVECs) were treated with glibenclamide (GB, 100 μ M) and metformin (MET, 1 μ M or 500 μ M), and incubated for 24 hours with 20% pooled plasma (n=10) from pregnant women with preeclampsia (PE). The following parameters were assessed: cell viability using the PrestoBlue® assay, cytotoxicity by measuring lactate dehydrogenase (LDH) release, nitric oxide (NO) levels using the DAF-FM® probe, reactive oxygen species (ROS) production via the DCFH probe, antioxidant capacity through the MTT assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide), and cell migration employing the wound healing assay. **Results:** Cell viability was increased on average by 15% by GB addition compared to the PE group, while neither drug affected LDH release. NO levels, expressed in arbitrary units (a.u) were also elevated in cells cultured with PE plasma and treated with GB (15,000 a.u. versus 12,000 of only PE plasma and PE+MET addition). In the same way, ROS levels were reduced in cells treated with MET (13,000 a.u.) and GB (10,000 a.u.) compared to PE (15,000 a.u.) group, whereas antioxidant capacity was enhanced in both treatment groups (16,6 and 25% on average, compared to PE). Additionally, cell migration was promoted by MET and GB (around 20%) after 8 and 12 hours of incubation. **Conclusions:** Treatment with GB and MET demonstrated protective effects on endothelial cells exposed to plasma from preeclamptic women. Both drugs reduced oxidative stress and enhanced antioxidant capacity, while GB specifically increased cell viability and nitric oxide levels. Furthermore, both treatments promoted cell migration, suggesting potential roles in vascular repair. These findings support the therapeutic potential of GB and MET in mitigating endothelial dysfunction associated with PE and warrant further investigation in preclinical and clinical settings. **Financial support:** Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), grant numbers 2024/13523-6, 2021/12010-7, 2024/01935-8, 2024/03439-8, 2024/10080-6, and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), grant numbers 308504/2021-6, and 444682/2024-4.

06.046 **Pharmacological Potential of Cafestol, a Bioactive Substance in Coffee, in Preventing Ischemia-Reperfusion-Induced Acute Kidney Injury.** Gomes DS^{1,2}, Romanelli MA^{1,2}, Gomes SPS^{1,2}, Brand ALM³, da Silva RMV³, Oliveira SSC⁴, Santos ALS⁴, Rezende CM³, Lara LS^{1,2} ¹UFRJ, ICB, Brazil. ²CPMP-IBCCF-UFRJ, ³IQ-CCMN-UFRJ, ⁴IMPG-UFRJ

Renal ischemia-reperfusion (I/R) is the leading cause of acute kidney injury (AKI) and is a relevant complication of kidney transplantation. Cafestol (CAF) is a natural diterpene extracted from coffee beans, and studies suggest that CAF inhibits pathways related to renal fibrosis. This work evaluated whether a single dose of CAF prevents I/R-induced AKI. Male Wistar rats (180-220 g) were divided into: (a)CTRL (N=14): sham surgery; (b)CTRL+CAF₅₀ (N=4) and (c)CTRL+CAF₇₅ (N=10) two hours before sham surgery, CAF was administered orally by gavage at a concentration of 50 and 75 mg/Kg respectively; (c)I/R: ischemia was induced by applying a non-traumatic vascular clamp to both renal arteries for 30 min, followed by clamp removal and 24h blood reperfusion (N=18); (d)I/R+CAF₅₀ (N=8) and (e)I/R+CAF₇₅ (N=10): two hours before renal ischemia, CAF was administered orally by gavage at a concentration of 50 mg/Kg or 75 mg/Kg, respectively(CEUA: 073/21). During 24 h of reperfusion, rats were placed in metabolic cages for urine collection. The rats were euthanized, blood and urine were stored for renal function analyses, and the kidneys were collected for biochemical and histological studies. The data was analyzed using One-way ANOVA with Sidak's post-test. A value of P<0.05 was regarded as significant. The results represent the mean ± SEM. The I/R rats developed AKI, as evidenced by kidney damage (space between tubules, glomerular segmentation, and marked collagen accumulation throughout the renal tissue) and a decline in function and Na⁺ handling: proteinuria (39±3.4 mg/dL CTRL vs. 86±3.8 mg/dL I/R), elevated plasma urea nitrogen (53±2.3 mg/dL CTRL vs. 74±3.3 mg/dL I/R) and plasma creatinine (0.3±0.02 mg/dL vs. 0.5±0.05 mg/dL I/R); reduced fractional Na⁺ excretion (0.68±0.07 % CTRL vs. 0.41±0.08 % I/R) and decreased in (Na⁺+K⁺)-ATPase activity (100±4.7 % CTRL vs. 68±9.5 % I/R). These observations were associated with a 52 % reduction in HIF-1α protein content and a 357 % increase in matrix metalloproteinase (MMP)-9 activity. In CTRL rats, CAF decreased the urine volume (15±0.6 mL/24h CTRL vs. 9±0.3 mL/24h CTRL+CAF₇₅) and glomerulus area (19 %) but increased MMP-9 protein content (43 %). The treatment with CAF (75 mg/kg) prevented the impairment of renal function caused by I/R, observed as a reduction in plasma urea nitrogen (16 %), proteinuria (40 %) and plasma creatinine (40 %). CAF also prevented the alteration of cortical Na⁺ transporters (68±9.5 % I/R vs. 109±6.9 % I/R+CAF₇₅ to (Na⁺+K⁺)-ATPase activity), and the changes associated to fibrosis caused by the I/R process: reduction in collagen deposition (55 % in cortex and 48 % in medulla), and partially prevention in HIF-1α protein content and MMP-9 activity increase. In conclusion, while the data revealed potential side effects associated with the treatment, CAF proved effective in mitigating the alterations induced by the I/R process. **Financial support:** Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro Award numbers: E-26/200.862/2021 and E-26/210.561/2024; Emendas Parlamentares do Governo Federal. CEUA/UFRJ: Comissão de Ética no Uso de Animais em Pesquisas da Universidade Federal do Rio de Janeiro

06.047 **SC1, a Novel AT1 Receptor Antagonist, Inhibits Angiotensin II-Mediated Vascular Responses and Lowers Blood Pressure in Rats with Chronic Kidney Disease.** Aires RS¹, Ferreira JG¹, Barbosa FM¹, Ferreira LFL², Anjos JV², Araujo AV¹ ¹Centro Acadêmico de Vitória, UFPE, Brasil; ²Lab Síntese Orgânica Medicinal, Centro de Ciências Exatas e da Natureza, UFPE, Brasil

Introduction: Renin–angiotensin system inhibitors play a key role in the treating of chronic kidney disease (CKD), although their use is often limited by adverse effects. SC1, a novel oxadiazol-5-one derivative and angiotensin II (AngII) type 1 (AT1) receptor antagonist, was developed to improve stability and solubility. This study aimed to investigate the cardiovascular effects of SC1 in an experimental CKD model. **Methods:** The experimental protocol was approved by the Animal Ethics Committee (CEUA/UFPE, nº 005/2023). Male Wistar rats (300-350 g) were randomly assigned to sham-operated or 5/6 nephrectomy (5/6Nx) groups. Fifteen days post-surgery, a subset of animals was euthanized for thoracic aorta isolation and vascular function assessment. Another subset received SC1 (1 µg/kg, i.v.) under anesthesia for evaluation of mean arterial pressure (MAP) and heart rate (HR). Results are presented as mean ± standard error of the mean. Data were analyzed using two-way ANOVA followed by the Holm-Sidak post hoc test, considering $P < 0.05$ as significant.

Results: Vascular reactivity experiments demonstrated that in endothelium-intact aortic rings, the 5/6Nx group exhibited a 29.7% reduction in maximal contractile response (Emax) to AngII compared with the Sham group (Sham: Emax = 0.91 ± 0.12 g, n = 5; 5/6Nx: Emax = 0.64 ± 0.15 g, n = 5). In endothelium-denuded rings, AngII-induced contraction was markedly attenuated in the 5/6Nx group, showing an 89.4% decrease in Emax relative to Sham (Sham: Emax = 1.32 ± 0.58 g, n = 4; 5/6Nx: Emax = 0.14 ± 0.05 g, n = 9). Pre-incubation with SC1 inhibited AngII-induced contraction in both endothelium-intact (21.4% reduction; Sham: Emax = 0.36 ± 0.11 g, n = 5; 5/6Nx: Emax = 0.24 ± 0.07 g, n = 7) and endothelium-denuded rings (89.4% reduction; Sham: Emax = 1.48 ± 0.58 g, n = 4; 5/6Nx: Emax = 0.11 ± 0.04 g, n = 9). No significant differences were observed in pCE50 values between groups in the absence (Sham: 3.84 ± 0.11 ; 5/6Nx: 4.09 ± 0.20) or presence of SC1 (Sham: 5.96 ± 2.03 ; 5/6Nx: 7.77 ± 4.47). Conversely, in endothelium-denuded rings, pCE50 increased by 36.1% in the 5/6Nx group compared with Sham in the absence of SC1 (Sham: 6.24 ± 2.18 ; 5/6Nx: 8.49 ± 3.27) and by 722.9% in its presence (Sham: 1.79 ± 0.59 ; 5/6Nx: 14.72 ± 7.86). Intravenous administration of SC1 reduced MAP by 13% in Sham rats (111 ± 7 mmHg to 97 ± 8 mmHg, n = 5) and by 21% in the 5/6Nx group (165 ± 16 mmHg to 130 ± 4 mmHg, n = 5), without affecting HR in either group (Sham: 403 ± 35 bpm vs. 390 ± 22 bpm; 5/6Nx: 385 ± 19 bpm vs. 392 ± 29 bpm).

Conclusion: The present findings demonstrate that SC1 reduces MAP without affecting HR in a rat model of CKD. SC1 also inhibited angiotensin II–induced vascular contraction in aortic rings from 5/6Nx rats. These results indicate that SC1 exerts antihypertensive effects and may represent a promising therapeutic candidate for the management of cardiovascular dysfunction associated with CKD. **Financial Support:** FACEPE, CNPq, CAPES. **Keywords:** Angiotensin 2. AT1 receptor antagonist. Chronic kidney disease. Vascular reactivity. Hypertension.

06.048 **Protective Effects of β -Caryophyllene, a CB₂ Agonist, on Renal and Vascular Alterations in Sepsis.** Dal-Secco D, Mariot LN, Simas A, Sordi R UFSC, Center of Biological Sciences, PPG Pharmacology, Brazil

Introduction: Sepsis is a life-threatening organ dysfunction caused by a dysregulated host response to infection, accounting for a large proportion of Intensive Care Unit admissions and significant costs to the Health System. No specific therapy exists for sepsis; treatment relies on supportive measures such as intravenous fluid resuscitation, vasopressor administration, oxygen supplementation, broad-spectrum antibiotics, and infection source control. Severe hypotension and sepsis-associated acute kidney injury result in decreased renal blood flow, glomerular filtration rate, and acute tubular necrosis. The endocannabinoid system has emerged as a potential therapeutic target due to its ability to modulate immune, cardiovascular and renal functions. Type 2 cannabinoid receptors (CB₂) have been implicated in atherosclerosis, ischemia-reperfusion injury, and fibrosis. Although evidence suggests a role for cannabinoid receptors in sepsis, the specific contribution of CB₂ receptors to severe hypotension and renal dysfunction remains unclear. Therefore, we investigated the effects of the CB₂ agonist β -caryophyllene on renal and vascular alterations associated with sepsis. **Methods:** Swiss mice were subjected to the cecal slurry-induced sepsis model via intraperitoneal injection of cecal content and were divided into two groups receiving either vehicle (peanut oil) or the CB₂ agonist β -caryophyllene (100 mg/kg) by oral administration (20 μ L). Control animals received sterile PBS intraperitoneally. Survival and clinical score were evaluated over seven days, while blood pressure, renal blood flow, and plasma markers (ALT and creatinine) were assessed 24 h after sepsis. The experimental protocol was approved by the Animal Ethics Committee of UFSC (CEUA / 7021210824). **Results:** The cecal slurry sepsis model reproduces clinical features of sepsis, including severe hypotension associated with reduced renal blood flow. Treatment with β -caryophyllene improved hypotension by 40% and renal blood flow by 55% in septic animals. In addition, β -caryophyllene reduced mortality rate by approximately 67%, clinical score by 65%, and renal and liver damage by 90% and 50%, respectively. **Conclusion:** This study enhances the understanding of the role of β -caryophyllene in sepsis-associated renal and vascular alterations, organ injury, and clinical score, highlighting its potential as a target for future therapeutic interventions. **Financial support:** FAPESC, CNPq, CAPES. Approval from the Animal Research Ethics Committee: CEUA / UFSC N° 7021210824.

06.049 Differently Expressed Genes Regulated by Metformin in an *in vitro* Model of Preeclampsia. Pereira DA, Passeti LFP, Nunes PR, Sandrim VC Unesp - Botucatu, Institute of Biosciences, Dept Biophysics and Pharmacology - Brazil

Introduction: Preeclampsia (PE) is the leading global cause of maternal and perinatal morbidity/mortality and is classified as early or late-onset PE according to the week of diagnosis (before or after 34, respectively). The pathophysiology of PE is not fully understood, but placental ischemia plays a key role, and the release of anti-angiogenic factors is associated with endothelial dysfunction observed in PE. Nevertheless, >40% of pregnant are nonresponsive to antihypertensive therapy. Metformin has shown potential in preventing or treating PE by decreasing endothelial dysfunction and inducing angiogenesis. However, molecular mechanisms underlying these effects are not fully known. We aimed to identify genes related to endothelial dysfunction regulated by metformin in Human Umbilical Vein Endothelial Cells (HUVECs) incubated with plasma from pregnant women with PE and treated with metformin. **Methods:** HUVECs were incubated with 20% pooled plasma (n=10) from pregnant women with early- or late-onset PE and with 1 μ M metformin hydrochloride (Merck – CAS Number: 1115-70-4) for 48h. Total RNA was extracted using Quick-RNA™ Miniprep kit (ZYMORESEARCH/R1055), according to manufacturer recommendation and quantified with spectrophotometer UV-Vis BioDrop μ Lite+. Reverse transcription was performed using LunaScript RT SuperMix Kit with 4 μ L SuperMix (5x) for which 1 μ g of cDNA. RT-PCR was performed in a total volume of 10 μ L; 1 μ L of cDNA (1.5ng/ μ L), 5 μ L of KiCqStart® SYBR® Green qPCR ReadyMix™ (SIGMA - KCQS00-250RXN), 0.5 μ L of each forward and reverse primer and 4.5 μ L of nuclease-free water. Thermal cycling was performed in standard conditions and fluorescence was recorded by StepOnePlus Real-Time PCR equipment (Applied Biosystems). **Results:** We performed the analysis in triplicate and investigated 17 target genes and 2 housekeeping genes. According to Reactome Pathways 2024, “Signaling by Interleukins” was the top ranked pathway for this set of genes. In HUVEC incubated with plasma from early-onset PE compared to late-onset, caspase 3 (CASP3) and endothelin-1 (END1) was downregulated and arachidonate 5-lipoxygenase (ALOX5) and Selectin E (SELE) was upregulated (fold change of 0.23, 0.12, 4.27 and 3.14, respectively). As for early-onset PE compared to early-onset treated with metformin, CASP3 was the only downregulated gene (fold change of 0.38). For late-onset PE compared to late-onset treated with metformin, Von Willebrand factor (VWF), ALOX5 and SELE were all upregulated by metformin (fold change of 2.92, 2.01 and 5.46, respectively) and VWF and SELE with a p-value <0.05. **Conclusions:** The results show that metformin is capable of regulating genes related to endothelial dysfunction and also inflammation in PE. Our research may help understanding the molecular mechanisms regarding metformin’s effects in PE and identifying potential new biomarkers of metformin use and response. **Financial suport:** Pró-reitoria de pesquisa – UNESP, public notice number 05/2024, Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), grant numbers 2024/13523-6, 2021/12010-7, 2024/01935-8, 2024/03439-8, and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), grant numbers 308504/2021-6, and 444682/2024-4. **Ethics:** Approval by the Ethics Committee: Research Ethics Committee of the Botucatu School of Medicine 6.853.434.

06.050 **Dependent on Concentration Procainamide is Either a Negative or a Positive Inotropic Agent in Guinea Pig Heart.** Sigler W^{1,2}, Oliveira AC¹ ¹ICB-USP, Dept Pharmacology, Institute of Biomedical Sciences, University of São Paulo; São Paulo, Brazil; ²Faculty Oswaldo Cruz, São Paulo, Brazil

Introduction: In a previous cellular electrophysiological work of ours, procainamide (PA) induced a decrease in the maximum upstroke velocity (V_{max}) of the cardiac action potential, in a lower range of concentrations. Increasing the concentrations disclosed a further effect of PA: an increase of the half decay time (HDT) of the cardiac action potential. Theoretically, these opposing effects could lead to also opposing effects on the cardiac inotropism. In this work, to clarify the impact of the mentioned opposing electrophysiological effects on the contraction of the heart muscle, the effects of PA on the heart muscle tension were studied, using a larger range of concentrations than that found in the literature. **Methods:** The *in vitro* preparation was the isolated right papillaris magnus of guinea pig's heart. The isolated muscle was mounted in a chamber filled with Tyrode solution kept at 37±0.5 °C and gassed with a mixture of O₂ (95%) and CO₂ (5%). The muscle contractions were evoked using a bipolar platinum electrode connected to a Grass electronic stimulator delivering supramaximal pulses of 1 ms duration and 1 Hz frequency. The muscle tension was measured using a Narco F50 isometric transducer connected to a Tektronix digital oscilloscope. The effects of PA on muscle tension were examined using concentrations from 0.7 mM to 5 mM. Within this range 10 different concentrations were applied cumulatively to the preparation, in 7 different experiments. Among the concentrations two were especially considered: a) the concentration at which the dose-response curves changed from a descending to an ascending direction; b) the concentration, in the ascending phasis of PA's dose-response curves that doubled the muscle tension. **Results:** A decrease of the muscle tension was initially observed, at lower concentrations of PA, followed by an increase in the muscle tension, in response to larger concentrations. Thus, the dose-response curve for the effects of PA on the muscle tension was typically hormetic, being a J-shaped biphasic curve. Mathematically, the dose-response curves were best correlated to a second degree polynomial of general equation: $Y=ax^2-bx+c$, with correlation coefficients between 0.8-0.9. The average concentration where the dose-response curves of PA turned from descending to ascending was 1.75 ± 0.22 mM (mean ±SE; n=7). The average concentration, in the ascending phasis of PA's dose-response curves, that doubled the muscle tension measured from the lowest point in the dose-response curves was 4.57 ± 0.66 mM (mean±SE; n=7). **Conclusions:** Hormetic dose-response relationships of PA effects on the muscle tension were clearly demonstrated, in the present work. To the authors knowledge this is a hitherto undescribed feature of procainamide pharmacology. A possible explanation is that underlying the descending phasis of the muscle tension dose-response curve, PA's main electrophysiological effect was the decrease in V_{max}, leading to negative inotropism. However, with the gradual increase in PA concentrations, the HDT was also affected and its increase by PA overcame the decrease in V_{max}, propitiating positive inotropism. **Financial support:** WS:FAPESP doctoral fellowship; ACO: FAPESP; CNPq Local animal research ethics committee protocol # 99, 08/19/2013.

06.051 **Effects of Hyperbaric Oxygen Therapy on the Remodeling of Intramural Coronary Arteries in Diabetic Rats.** Silva FS¹, Moraes MV², Abreu BJ³, Ishikawa U³, Oliveira Albuquerque ECS³, Medeiros KCP³, Farias NBS³, Araújo Júnior RF³, Carmo BA¹, Augusto dos Santos JR¹, Vidal TIR¹, Kennedy Feitosa E¹, Moacir FO⁴ ¹UFERSA, Dept Health Sciences,; ²UFRN, Dept Surgery; ³UFRN, Dept Morphology,; ⁴UFERSA, Dept Animal Sciences

Introduction: Diabetes mellitus (DM) is projected to affect over 1.3 billion people by 2050. It is a chronic disease that requires continuous glycemic control and management of its complications, particularly cardiovascular ones. Hyperbaric oxygen therapy (HBO) has been used as an adjunct treatment for diabetic wounds and has recently shown potential for improving ventricular function. However, its effects on coronary microcirculation in DM have not yet been described. **Objective:** To evaluate the effect of HBO on the remodeling of intramural coronary arteries in the left ventricle (LV) in a model of streptozotocin-induced (STZ) diabetes. **Methods:** Thirty-two male Wistar rats (60 days old, 230–270 g) were divided into four groups: control (CON, n = 8), hyperbaric oxygen (HBO, n = 8), diabetic (STZ, n = 10), and diabetic treated with HBO (STZ+HBO, n = 8). Diabetes was induced with a single dose of STZ (60 mg/kg, i.p.). Animals in the HBO and STZ+HBO groups were exposed to 100% oxygen in a pressurized chamber at 2.5 atmospheres absolute for 60 minutes/day, 5 days/week, for 5 weeks. Blood glucose and body mass were recorded weekly. At the end of the protocol, LV fragments were processed for histological analysis using hematoxylin-eosin and picrosirius red stains. Slides were coded by an external assistant to ensure blinding from microscopy acquisition (Leica DM750/ICC50 HD/LAS EZ v. 3.3.1) through final analysis using ImageJ v. 1.54p. Group comparisons were performed using ANOVA with Fisher's LSD post hoc test and linear regression slope comparison in GraphPad Prism v. 8.0.2, with a significance level of 5%. Variables analyzed included: number of intramural coronary arteries in the LV, cross-sectional area and diameter of vascular layers, coronary endothelial cell density, and immunoexpression of CD34 and VEGF. The study was approved by the Ethics Committee on Animal Use of the Federal University of Rio Grande do Norte (protocol no. 125.054/2018). **Results:** HBO treatment prevented significant changes in coronary microcirculation induced by DM, such as medial layer atrophy, luminal enlargement, and perivascular fibrosis ($P > 0.05$ STZ+HBO vs. CON). It also prevented endothelial cell rarefaction, normalized arteriolar VEGF expression, and restored capillary density (CD34+) in the myocardium of diabetic animals ($P > 0.05$ vs. CON). Additionally, the STZ+HBO group exhibited increased intramyocardial arteriole density compared to the other groups ($P < 0.05$), along with a shift in the microvascular diameter profile favoring smaller-caliber arterioles (~50–100 μ m). **Conclusion:** HBO prevented maladaptive coronary remodeling in experimental diabetes by normalizing VEGF expression, preserving the adventitial, muscular, and endothelial structure of arterioles, and maintaining capillary density. These findings demonstrate the cardioprotective potential of HBO in diabetic microangiopathy, support previous clinical observations of improved ventricular function, and provide a foundation for future clinical investigations targeting cardiovascular complications in diabetic patients.

07. Endocrine, Reproductive and Urinary Pharmacology

07.001 **6-Cyanodopamine Enhances Norepinephrine- and Dopamine-Mediated Contractions in Rat Isolated Seminal Vesicle.** dos Santos IAR, Pozzo CFSD, Ribeiro LC, Ramos MCC, de Nucci G UNICAMP, Dept Pharmacology, Faculty of Medical Sciences, Campinas, Brazil

6-Cyanodopamine (6-CYD) is released from isolated rat vas deferens and represents a novel epithelium-derived catecholamine, similar to 6-nitrodopamine (6-ND). 6-CYD also plays a significant role in the modulation of vas deferens contractility, as it enhances contractions mediated by noradrenaline, adrenaline, and dopamine. 6-ND is an endogenous mediator of rat seminal vesicles contractility. It has been shown to modulate smooth muscle contraction of the seminal vesicle and to enhance contractions mediated by the traditional catecholamines, primarily through the potentiation of norepinephrine-induced contractions and, to a lesser extent, those induced by dopamine. However, the effects of 6-CYD on seminal vesicle contraction remains unknown, as well as the possible influence of this endothelial catecholamine on the norepinephrine- and dopamine-mediated contractions in the seminal vesicle. This study aimed to investigate the effect of 6-CYD released by rat isolated seminal vesicles (RISV) on smooth muscle contractility mediated by norepinephrine and dopamine. For this purpose, RISV was incubated with 1 μ M and 100 nM of 6-CYD for 30 minutes and concentration-response curves to norepinephrine and dopamine were performed. A Student's t-test was conducted to compare Emax and pEC50 values, with $p < 0.05$ considered statistically significant. Pre-treatment with 100 nM 6-CYD significantly enhanced the Emax values for noradrenaline and dopamine. Additionally, 100 nM 6-CYD markedly shifted the concentration-response curve to norepinephrine to the left. 10nM 6-CYD didn't affect the contraction mediated by noradrenaline and dopamine. The potentiation of noradrenaline and dopamine contractions shows that 6-cyanodopamine has a synergic effect with traditional catecholamines. This is the first demonstration that 6-CYD modulates rat seminal vesicle contractility by changing the response to noradrenaline and dopamine. Ethical Committee: CEUA; Protocol No. 5987-1/2022 This study was financed, in part, by the São Paulo Research Foundation (FAPESP), Brasil. Process Number #2024/13928-6

07.002 6-Cyanodopamine Potentiates Electric Field Stimulation-Mediated Contractions in Rat Isolated Seminal Vesicle. Ribeiro LC, Dal Pozzo CFS, Ramos MCC, dos Santos IAR, de Nucci G Unicamp, Dept Pharmacology, Faculty of Medical Sciences, State University of Campinas, Campinas, Brazil

E-catecholamines are non-neurogenic catecholamines produced by endothelial and epithelial cells, such as 6-nitrodopamine and 6-cyanodopamine. 6-Cyanodopamine (6-CYD) is a novel endothelial catecholamine released from isolated rabbit heart and rat vas deferens. In the vas deferens, 6-CYD enhances electric field stimulation (EFS)-induced contractions and potentiates the contractile activity of traditional catecholamines (noradrenaline, adrenaline, and dopamine). Moreover, tetrodotoxin (TTX) does not affect the basal release of 6-CYD in the vas deferens, nor does it interfere with the potentiation mediated by 6-CYD. Since the effect of this novel catecholamine on rat seminal vesicles remains unknown, the aim of this study was to evaluate the effect of 6-CYD on EFS-mediated seminal vesicle contractions and to determine whether TTX modulates 6-CYD activity in this tissue. Rat isolated seminal vesicles (RISVs) were subjected to EFS at different frequencies (2 Hz, 4 Hz, 8 Hz, 16 Hz, and 32 Hz) in the presence and absence of 6-CYD (1 nM, 10 nM, and 100 nM). Additionally, RISVs were pre-incubated with 1 μ M TTX for 30 minutes, with or without different concentrations of 6-CYD. Pre-incubation with 100 nM 6-CYD significantly enhanced contractions at 8 Hz, 16 Hz, and 32 Hz. In contrast, 10 nM and 1 nM 6-CYD significantly increased contractions only at 32 Hz. Pre-incubation with 1 μ M TTX did not alter the enhancement in RISV contractions induced by 100 nM 6-CYD. These results indicate that 6-cyanodopamine plays a significant role in modulating seminal vesicle contractions and acts independently of neurogenic input, as its effect was not inhibited by tetrodotoxin. This is the first time that a cyanide-based molecule is described as a modulator of seminal vesicle contractions mediated by electric field stimulation. Ethical Committee: CEUA; Protocol No. 5987-1/2022 This study was financed, in part, by the São Paulo Research Foundation (FAPESP), Brasil. Process Number: 2024/13789-6

07.003 **6-Nitrodopamine as a Mediator of Prostate Contractility in Mouse and Man.** Pereira LGS¹, Dias DRM¹, Olivotti AB¹, Oliveira DL², Schenka AA², Souza VB², De Nucci², de Oliveira MG¹ ¹USF, Lab of Pharmacology, PPG Health Sciences, ²Unicamp, Dept Translational Medicine, PPG Pharmacology

Introduction: 6-Nitrodopamine (6-ND) is a novel endogenous catecholamine that exhibits distinct cardiovascular effects, including potent and long-lasting positive chronotropic and inotropic responses, as well as strong vasorelaxation. In the genito-urinary system, the basal release of 6-ND has been identified in the urinary bladder, vas deferens, seminal vesicles, and corpus cavernosum across multiple species, including mice, rats, rabbits, and humans. In the prostate, the release and effects of 6-ND are not described yet. **Aims:** To evaluate the release of 6-ND from both human and mouse prostate, and determine 6-ND effects on prostate contractility *in vitro*. **Methods:** Prostate fragments were obtained from patients with Bening Prostatic Hyperplasia (BPH) submitted to transurethral resection of the prostate. C57BL/6 mice (12 weeks old) prostate were also used. Catecholamine release was detected in Krebs-Henseleit solution before and after incubation with tetrodotoxin (TTX, 100 μ M, 30 min) by High-Performance Liquid Chromatography coupled with Mass Spectrometry (HPLC-MS; limit of quantification 0.1 ng/mL). *In vitro*, electrical field stimulation (EFS, 1-32 Hz) of both mouse and human prostates were performed in the absence and presence of TTX. Cumulative concentration-response curves to 6-ND were evaluated under basal tension and after pre-contraction with the muscarinic agonist carbachol (1 μ M). Immunohistochemistry to tyrosine hydroxylase (TH) were also performed. **Results:** The prostate from both mouse and human presented basal release of 6-ND, as detected in the Krebs solution by LC-MS. In the mouse prostate, incubation with TTX caused a significant reduction in 6-ND levels, while no significant changes were observed in human prostate. *In vitro*, EFS-induced frequency-dependent contractions in both mice and human isolated prostate tissues, however, human prostate contractions were TTX-resistant. Concentration-response curves to 6-ND demonstrated that it does not induce contractile responses in either mouse or human prostate. Instead, 6-ND promoted relaxation by about of 21 % and 20 % in mouse and human prostate, respectively. Positivity for TH was observed in prostate epithelium vascular endothelia and in stromal nodules. In contrast, mice prostate did not show TH expression in the epithelium, although TH was detected in nerves. These results are consistent with the effect of TTX, which reduced 6-ND release and EFS-induced contractions only in mice. **Conclusion:** This study will demonstrate, for the first time, that the human prostate epithelium, unlike that of mice, is a source of catecholamines, including 6-ND. Furthermore, 6-ND induces relaxation of prostate smooth muscle at very low concentrations, suggesting a potential additional pathway through which nitric oxide may modulate prostate contractility.

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07.004 **6-Nitrodopamine as a Novel Mechanism of Nitric Oxide-Mediated Prostate Relaxation.** Dias, JM¹, Curiel AA¹, De Nucci², de Oliveira MG¹ ¹USF, Lab of Pharmacology, PPG Health Sciences, Brazil
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Introduction: 6-Nitrodopamine (6-ND) is a newly identified catecholamine extensively studied in the cardiovascular system. However, its effects on lower urinary tract organs, such as the prostate, remain largely unexplored. Prostate smooth muscle relaxation is believed to be primarily mediated by nitric oxide (NO), which activates the enzyme soluble guanylyl cyclase (sGC), leading to the production of cyclic GMP (cGMP). However, the generation of 6-ND may represent an additional, yet undescribed, mechanism through which NO exerts its relaxant effects on the prostate. Aims: To investigate the effects of 6-ND on the relaxation of smooth muscle from isolated mouse prostate.

Methods: Prostates from 10-week-old C57BL/6 mice were isolated and mounted in a wire myograph. Contractions induced by electrical field stimulation (EFS) were assessed in the presence of 6-ND (10 nM), and concentration–response curves to noradrenaline were generated. In a separate set of experiments, concentration-response curves to 6-ND were constructed in tissues precontracted with the muscarinic agonist carbachol (1 μ M), in the presence or absence of either the nitric oxide synthase inhibitor L-NAME (100 μ M) or the GCs inhibitor ODQ (100 μ M). Additionally, in tissues preincubated with 6-ND (10 nM, 30 min), concentration-response curves to either the GCs activator BAY58-2667, the adenylyl cyclase activator forskolin, the phosphodiesterase (PDE)-5 inhibitor sildenafil, or the PDE-4 inhibitor rolipram were constructed. Data are expressed as mean $\pm$ SEM and analyzed by paired t-test. **Results:** 6-ND significantly reduced EFS-induced contractions at 16 and 32 Hz and caused a ~5-fold rightward shift in the noradrenaline concentration–response curve. In precontracted tissues, 6-ND induced a maximum relaxation of $\sim 21 \pm 5.1\%$ ($n = 7$), which was not significantly different in the presence of either L-NAME or ODQ. Moreover, forskolin induced $\sim 58 \pm 6.2\%$ relaxation ($n = 5$), which was significantly enhanced in the presence of 10 nM 6-ND ($\sim 90 \pm 5.7\%$, $n = 5$). No significant changes were observed for the response of BAY58-2667, sildenafil or rolipram. **Conclusion:** 6-ND induces relaxation of prostate smooth muscle at very low concentrations and enhances adenylyl cyclase-mediated responses, suggesting that its mechanism of action may involve direct activation or modulation of this enzyme. These findings point to a potential additional pathway by which nitric oxide may regulate prostate contractility. The underlying mechanisms of 6-ND–induced relaxation will be further investigated in ongoing experiments. **Financial support:** FAPESP 2023/09792-9; 2023/01376-6; 2025/20136-9.

07.005 TTP488 (Azeliragon) Enhances Bladder Function in Leptin-Deficient Obese Mice: The Role of RAGE in Diabetic Bladder Dysfunction. Oliveira AL, Medeiros ML, Antunes E FCM-Unicamp, Dept Pharmacology, PPG of Pharmacology

Abstract: Diabetic bladder dysfunction (DBD) is a highly prevalent complication of diabetes mellitus (DM), characterized by decreased sensation and increased capacity. In hyperglycemia, the generation of advanced glycation end products (AGEs) increases, resulting from the non-enzymatic glycation of dicarbonyl compounds, such as methylglyoxal, leading to protein dysfunction. AGEs interact with their receptor RAGE and trigger a cascade of intracellular events. However, little is known about the effects of RAGE blockade in DBD. TTP488 (azeliragon) is an orally bioavailable RAGE inhibitor capable of inhibiting the receptor-binding cascade. In this study, we investigated the effect of TTP488 on DBD in ob/ob leptin-deficient mice. Animal procedures and experimental protocols were approved by the Ethics Committee on Animal Use, State University of Campinas (CEUA-UNICAMP; Protocol No. 6366-1/2023). B6.V-Lep ob/JUnib (ob/ob) females received TTP488 (10 mg/kg) in drinking water for 15 days. C57BL6 females were used as lean controls. Serum levels of MGO and AGEs, as well as RAGE expression in the bladder, were assessed. Bladder function was evaluated using a void spot assay in filter paper and ex vivo contractility tests to cumulative concentration-response curves to the muscarinic receptor agonist carbachol (1 nM–100 μ M), non-cumulative curves to the P2X1 purinergic agonist α,β -methylene ATP (1–10 μ M), contractile response to the depolarizing agent potassium chloride (KCl, 80 mM), and Electrical field stimulation (EFS) (1–32 Hz). Second harmonic microscopy was employed to analyze collagen fibers. Antioxidant activity was assessed through the enzymes glutathione reductase (GR), glutathione peroxidase (GPX), and superoxide dismutase (SOD). Statistical differences between groups were determined using two-way ANOVA, followed by Bonferroni's multiple comparisons test. TTP488 treatment in ob/ob mice significantly ($p < 0.05$) reduced serum MGO and AGE levels without altering RAGE expression in the bladder. It also reduced significantly ($p < 0.05$) voided volume without affecting spot number and decreased significantly ($p < 0.05$) bladder hypercontractility in response to carbachol and electric field stimulation, while reducing the increased significantly ($p < 0.05$) bladder collagen and increased significantly ($p < 0.05$) antioxidant activity of GR, GPX and SOD observed in the ob/ob group. These findings suggest that TTP488 normalizes bladder MGO and AGE levels and attenuates voiding dysfunction in ob/ob mice, further supporting the critical role of the AGE-RAGE pathway in diabetes-associated bladder dysfunction. Fapesp Grant Number 23/09353-

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07.006 **Pharmacological Characterization of Probenecid in Urinary Bladder and Corpus Cavernosum from Human.** Leonardi GR, Moretti MB, Barros JVC, Lima JM, Antunes E, Mónica FZ FCM-Unicamp, Dept Pharmacology

Introduction: Lower urinary tract symptoms (LUTS) involve issues with storage, micturition, and post-micturition. These symptoms can significantly impact social, sexual, and financial quality of life and exhibit a strong correlation with erectile dysfunction. Drug repurposing is a strategy that aims to redirect substances already on the clinical setting in order to explore new therapeutic indications. Previous data from our group demonstrated that probenecid induces relaxation in both pig bladder and rat corpus cavernosum (CC), and enhances the potency (pEC_{50}) and maximal response (E_{max}) to classical agents in these tissues (data not yet published). The therapeutic concentration range of probenecid is 0.1–1 mM. **Methods:** Experiments were conducted on isolated human urinary bladder tissue obtained from patients with prostate cancer who underwent cystectomy, as well as on human corpus cavernosum tissue from patients undergoing gender reassignment surgery. Concentration-response curves (CRCs) and electric field stimulation (EFS) were carried out and the pEC_{50} and E_{max} values were determined. The data are expressed as mean + SD, N= number of specimens. All protocols were approved by the Ethic Committee (CAAE number: 88849718.9.0000.5404 and 28126219.2.0000.5467) and were financially supported by FAPESP (Grant: 2023/12406-3). **Results:** Probenecid induced a bladder relaxation with an E_{max} closely to 52%. Incubation with probenecid (1 mM; 30 min) promoted a leftward shift in the relaxing response induced by isoprenaline (pEC_{50} : 5.49 ± 0.30 vs 6.55 ± 0.55 , $n=5$, $p<0.01$; E_{max} : 64.30 ± 23.86 % vs 72.35 ± 15.50 %, $n=5$, $p>0.05$) but did not produce any difference in the relaxation induced by tadalafil (pEC_{50} : 5.03 ± 0.90 vs 3.77 ± 0.43 , $n=3$, $p>0.05$; E_{max} : 48.09 ± 34.10 % vs 63.86 ± 11.98 %, $n=3$, $p>0.05$). In human CC probenecid also induced relaxation with pEC_{50} : 4.74 ± 0.36 , $n=4$, and E_{max} of 82.77 ± 21.04 %, $n=3$. This response was not altered in the presence of the NOS inhibitor (L-NAME; 100 μ M). The pre-incubation of probenecid (100 μ M; 30 min) promoted a leftward shift in the relaxing response induced by tadalafil (pEC_{50} : 4.38 ± 0.20 vs 5.21 ± 0.35 , $n=3$, $p<0.05$; E_{max} : 72.91 ± 5.99 % vs 82.64 ± 17.18 %, $n=3$, $p>0.05$), but did not affect the responses induced by acetylcholine (pEC_{50} : 5.90 ± 0.35 vs 6.13 ± 0.30 , $n=4$, $p>0.05$; E_{max} : 64.76 ± 17.04 % vs 82.50 ± 13.82 %, $n=4$, $p>0.05$) or isoprenaline (pEC_{50} : 6.33 ± 0.50 vs 6.65 ± 0.50 , $n=4$, $p>0.05$; E_{max} : 57.00 ± 26.26 % vs 61.15 ± 28.87 %, $n=4$, $p>0.05$). **Conclusion:** Probenecid relaxes the human bladder and corpus cavernosum, enhances responses to agents that increase the levels of cGMP or cAMP and exhibits specific-species differences. Further studies are currently underway to elucidate the underlying mechanisms in each tissue and species. **Keywords:** probenecid, urinary bladder, corpus cavernosum, erectile dysfunction

07.007 Basal Release of 6-Cyanodopamine from Rat Isolated Seminal Vesicle and Its Role in Adrenaline-Mediated Contractions. Ramos MCC, Dal Pozzo CFS, Santos IAR, Ribeiro LC, De Nucci G Unicamp, Dep. of Pharmacology, IC Program, Brazil

Dopamine, norepinephrine, and epinephrine are well-established catecholamines that act as mediators of smooth muscle contraction in the male reproductive system. Recently, novel catecholamines containing nitro and cyano groups, such as 6-nitrodopamine (6-ND) and, more recently, 6-cyanodopamine (6-CYD), have been identified. Previous studies have demonstrated that 6-CYD is released by the rat vas deferens and modulates its contractility. However, the release and functional role of this novel catecholamine in the rat seminal vesicle remain uninvestigated. The present study aimed to evaluate the basal release of 6-CYD from rat isolated seminal vesicles (RISVs) and its impact on smooth muscle contractility mediated by adrenaline. To this end, the basal release of 6-CYD was quantified using tandem mass spectrometry in the presence and absence of tetrodotoxin (TTX, 1 μ M) and after chronic treatment with L-NAME. RISVs were also incubated with 1 μ M and 100 nM 6-CYD, and concentration–response curves to adrenaline were constructed. Basal release of 6-CYD from rat seminal vesicles was detected. Pre-treatment with 1 μ M TTX did not significantly alter 6 CYD basal release. Similarly, chronic treatment with L-NAME had no effect on 6-CYD levels. Functionally, pre-treatment with 1 μ M 6-CYD reduced the maximal contractile response to adrenaline, whereas 100 nM 6-CYD had no significant effect. 6-CYD is apparently the first endogenous mediator that contains a cyanide group attached to its molecule described in the seminal vesicle. The identification of this novel catecholamine and its role in adrenaline mediated contractions indicate that 6-cyanodopamine contributes to regulation of smooth muscle cells contractility. The identification of the cyano-catecholamines should expand the physiological importance of cyanide as opposed to its toxic role. This study was financed, in part, by the São Paulo Research Foudation (FAPESP) Brazil. Process Number #2024/20651-0. This study was approved by CEUA, Protocol No. 5987-1/2022.

07.008 **Lack of 6-Nitrodopamine, a Prostate Relaxant, Leads to Voiding Dysfunction in Mice.** Dias, DR¹, Dias JM¹, De Nucci², de Oliveira MG¹ ¹USF, Lab of Pharmacology, PPG Health Sciences, Brazil ²Unicamp, Dept Translational Medicine, PPG Pharmacology, Brazil

6-Nitrodopamine (6-ND) is a newly identified molecule known to modulate lower urinary tract organs such as the urinary bladder, vas deferens, and seminal vesicles. However, its effects on the prostate are not well understood. Reduction in 6-ND production can be achieved by inhibiting nitric oxide synthase (NOS), the enzyme responsible for NO synthesis, using pharmacological tools such as the inhibitor L-NAME or through genetic deletion of NOS isoforms. These approaches allow investigation of 6-ND's effects across different organs. It is believed that relaxation of prostate smooth muscle is primarily mediated by NO, which activates soluble guanylyl cyclase (sGC), leading to the production of cyclic GMP (cGMP). However, 6-ND generation may represent an additional, yet undescribed, mechanism by which NO exerts its relaxant effects on the prostate. **Aims:** To characterize the effects of inhibiting 6-ND synthesis and sGC activation on prostate contractile responses *in vitro* and voiding function *in vivo* in mice. **Methods:** Experiments were conducted using isolated prostates from adult wild-type (WT) C57BL/6 mice and knockout mice lacking all three NOS isoforms: endothelial, inducible, and neuronal (*e/i/nNOS*^{-/-}). WT mice were treated with L-NAME (0.5 mg/mL, 4 weeks) for chronic NOS inhibition. Isolated prostates were mounted in a myograph, and contractile responses were evaluated using electrical field stimulation (EFS, 1–32 Hz). Acute effects of NOS inhibition with L-NAME (100 μ M) and sGC inhibition with ODQ (100 μ M) were assessed by pre-incubation (30 min) before EFS. The void spot on filter paper (VSOP) assay was performed individually for 4 hours, after which urine spots were analyzed to determine urinary frequency and volume. Data are expressed as mean $\pm$ SEM. Results Acute NOS inhibition did not alter EFS-induced prostatic contractions, but chronic inhibition led to increased responses at 16 and 32 Hz ($p < 0.05$). The inhibition of sGC did not affect prostate contractility. Compared to WT mice, *e/i/nNOS*^{-/-} mice exhibited increased contractions at all frequencies ($p < 0.05$). In the VSOP assay, triple NOS knockout mice showed fewer micturition spots (2.5 ± 0.2 vs. 1 ± 0.44 ; $p < 0.05$) and reduced micturition frequency (0.67 ± 0.05 vs. 0.25 ± 0.11 events/hour; $p < 0.05$). Total urinary output was lower in knockout mice compared to WT (248 ± 29.4 vs. 172 ± 70 mL; $p < 0.05$); however, the volume per void was significantly higher (109 ± 11 vs. 125 ± 48 mL; $p < 0.05$). **Conclusion:** Prostatic hypercontractility attributed to the lack of NO and impairment of sGC/cGMP signaling may, in fact, reflect the absence of 6-ND, which may represent an additional mechanism by which NO causes prostate relaxation. Triple NOS knockout mice exhibit clear voiding dysfunction, possibly due to increased resistance to urine flow resulting from reduced prostatic relaxation. **Financial support:** FAPESP 2023/09792-9; 2023/01376-6; 2025/20136-9.

07.009 *Arthrospira platensis* Modulates Inflammatory Cytokines in the Corpus Cavernosum of Obese Rats: A Promising Strategy Against Obesity-Induced Erectile Dysfunction. Melo MB¹, Barros BC^{1,2}, Diniz AFA^{1,2}, Arruda RRA^{1,2}, Silva JMA^{1,2}, Ribeiro SB⁴, Lima CAS⁴, Sousa Filho JEC¹, Claudino BFO^{1,2}, Soares MFS^{1,2}, Araújo AA⁴, Silva BA^{1,2,3} ¹UFPB, Lab Functional Pharmacology, Institute of Research in Drugs and Medicines, Brazil ²UFPB, Postgraduate Program in Bioactive Natural and Synthetic Products, Health Sciences Center, Brazil ³UFPB, Dept Pharmaceutical Sciences, Health Sciences Center, Brazil ⁴UFRN, Lab Experimental Model of Intestinal Mucositis, PPG Pharmaceutical Sciences, Brazil

Obesity is a chronic low-grade inflammatory condition that disrupts homeostatic signaling across multiple systems, including the reproductive axis. Its pathophysiology involves the overproduction of pro-inflammatory cytokines—such as IL-1 β , TNF- α , and IL-6—alongside a decrease in anti-inflammatory mediators like IL-10. These imbalances contribute to endothelial dysfunction, oxidative stress, and hormonal disturbances, which are key factors in male reproductive impairments, including erectile dysfunction (ED). The corpus cavernosum (CC), essential for penile erection, is particularly vulnerable to inflammation-induced disruptions in smooth muscle relaxation and nitric oxide signaling. *Arthrospira platensis* (AP), a microalga with well-documented antioxidant and anti-inflammatory properties in various tissues (Souza, ILL. Rev. Mar. Drugs., v. 20, p. 467, 2022), has not yet been thoroughly investigated for its role in protecting reproductive tissues under obesogenic conditions. This study aimed to evaluate the preventive effects of AP supplementation on the expression of inflammatory cytokines in the CC of rats fed a hypercaloric diet, providing new insights into its therapeutic potential for mitigating obesity-induced inflammation in male reproductive tissues. Male Wistar rats were randomly divided into three groups (n=5): standard diet (SD), hypercaloric diet (HCD), and hypercaloric diet supplemented with AP 25 mg/kg/day (HCD+AP25), administered orally for eight weeks. At the end of the treatment period, tissue samples from the CC were collected for cytokine quantification by enzyme-linked immunosorbent assay (ELISA). The HCD group exhibited significant increases in IL-1 β , TNF- α , and IL-6 levels in CC compared to the SD group, while the HCD+AP25 group showed marked reductions in all these markers. Specifically, the levels of IL-1 β were reduced from 126.3 ± 10.1 to 46.74 ± 9.5 pg/mg. TNF- α levels decreased from 358.1 ± 9.8 to 164.4 ± 23.7 pg/mg. IL-6 levels were also significantly reduced, from 305.8 ± 33.9 to 178.9 ± 28.4 pg/mg. Moreover, AP supplementation significantly increased IL-10 levels in the CC (351.8 ± 32.2 vs. 86.4 ± 8.7 pg/mg). These results indicate that AP exerts a protective and immunomodulatory effect on the corpus cavernosum, attenuating the inflammatory cytokine profile, potentially contributing to the prevention of ED associated with obesity. Further studies are warranted to investigate the molecular pathways involved, particularly the role of JNK and NF- κ B signaling. All procedures were approved by the Ethics Committee on Animal Use of the UFPB (CEUA/UFPB), under protocol number 4499030624 / ID 023341. **Financial support:** CNPq, CAPES and FAPESQ-PB.

07.010 Probenecid Potentiates the Relaxant Effect of β -Adrenergic Agonists in the Prostate of Rats.

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Probenecid is a drug developed in the 1950s to serve as an adjuvant to penicillin in the treatment of tuberculosis. It acts as an inhibitor of organic anion transporters #1 and #3 (OAT1 and OAT3), as well as the urate transporter 1 (URAT1). Recent studies have renewed interest in the pharmacological repositioning of Probenecid for cardiovascular applications, where its benefits appear to be mediated through the modulation of multidrug resistance-associated proteins (MRPs), pannexin-1 (Panx-1), and TRPV2 channels. This study aimed to characterize the effects of Probenecid on rat prostate tissue, exploring its potential to enhance smooth muscle relaxation when combined with other pharmacological agents. *In vitro* experiments were conducted using prostate strips from rats, following approval by UNICAMP's Ethics Committee on Animal Use (CEUA – 6459-1/2024). The strips were mounted in organ baths containing Krebs solution (maintained at 37°C), and isometric force was recorded using a PowerLab 400™ system. After a 45-minute equilibration period and a subsequent 30-minute incubation period for Probenecid, concentration–response curves (CRCs) were generated for Isoproterenol (100 pM–100 μ M), Salbutamol (100 pM–100 μ M), and Sodium Nitroprusside (SNP; 100 pM–100 μ M). Relaxation responses were expressed as a percentage of the contraction induced by Phenylephrine (10 μ M). Maximum response (Emax) and potency (pEC50) values were determined. All data were presented as mean $\pm$ SEM (n), and statistical analyses were performed using unpaired Student's t-test or one-way ANOVA. A p value < 0.05 was considered statistically significant. Our results show that Probenecid incubation (1 mM, 300 μ M, 100 μ M for 30 minutes) significantly enhanced Isoproterenol-induced relaxation, as evidenced by increased pEC50 values at all concentrations (respectively: 6.59 $\pm$ 0.18 vs. 7.35 $\pm$ 0.18, N=5; 5.43 $\pm$ 0.07 vs. 4.86 $\pm$ 0.08, N=5; and 4.37 $\pm$ 0.07 vs. 4.79 $\pm$ 0.08, N=6; p < 0.05). A similar effect was observed with Salbutamol (respectively: 4.56 $\pm$ 0.07 vs. 5.08 $\pm$ 0.08, N=6; 4.27 $\pm$ 0.08 vs. 5.25 $\pm$ 0.07, N=6; and 4.37 $\pm$ 0.09 vs. 4.94 $\pm$ 0.08, N=5; p < 0.05), along with a significant increase in the Emax for all concentrations tested (respectively: 64.48 $\pm$ 5.01 vs. 89.72 $\pm$ 10.73, N=6; 42.34 $\pm$ 5.08 vs. 77.47 $\pm$ 18.70, N=6; and 72.79 $\pm$ 9.91 vs. 88.05 $\pm$ 9.90, N=5; p < 0.05). Sodium Nitroprusside (SNP), a nitric oxide donor, showed a significant increase in Emax only at the highest concentration of Probenecid (p < 0.05; Emax: 47.51 $\pm$ 12.82 vs. 64.02 $\pm$ 14.61, N=8). In conclusion, our findings suggest that Probenecid may act within the lower urogenital tract when combined with drugs currently approved for treating benign prostatic hyperplasia (BPH) and erectile dysfunction, for example. It could enhance therapeutic benefits by optimizing prostate function and potentially reducing the required dosage of conventional medications. This approach might lower the incidence of adverse effects without compromising therapeutic efficacy.

07.011 Intermittent Glucocorticoid Exposure Programs Dexamethasone Resistance and Alters Adult Metabolic Outcomes. Peixe CMS¹, Giusti-Paiva A², Rafacho A² ¹UFSC, PPG em Farmacologia, Brazil; ²UFSC, Dept. of Physiological Sciences, Brazil

Dexamethasone (DEX) is a potent glucocorticoid (GC) widely used for its anti-inflammatory and immunosuppressive properties across different stages of life. However, DEX administration is also known to induce significant metabolic side effects and is often regarded as a diabetogenic agent. Thus, we aimed to examine whether exposure to DEX early in life could worsen the metabolic disturbances triggered by a later DEX treatment in adulthood. Male rats were subjected to up to three treatment cycles beginning at postnatal days 30, 60, and 90. Each cycle consisted of five consecutive daily intraperitoneal injections of DEX (1.0 mg/kg) or saline (0.9% NaCl; 1 mL/kg). Animals were assigned to one of five groups: Control (saline throughout), 90 (DEX only at 90 days), 30+90 (DEX at 30 and 90 days), 60+90 (DEX at 60 and 90 days), and 30+60+90 (DEX at all three time points). Body weight and food intake were monitored throughout. On the final day of the last cycle, animals underwent a glucose tolerance test (ipGTT; 1.5 g/kg), followed by an insulin tolerance test (ipITT; 0.75 IU/kg) 24 h later. Animals were then euthanized, and blood and organs were collected. One-way ANOVA and Tukey's post hoc test were used. During the first cycle, DEX-exposed groups showed reduced weight gain ($p < 0.0001$). In the second cycle, 60+90 and 30+60+90 groups exhibited weight loss ($p < 0.0001$) and reduced food intake ($p = 0.01$). In the third cycle, all DEX-treated groups showed weight loss and hypophagia ($p < 0.0001$). In the ipGTT, all DEX groups exhibited glucose intolerance, with increased area under the curve (AUC, $p < 0.0001$). In the ipITT, 90, 30+90, and 60+90 groups had higher post-insulin glycemia and AUC ($p = 0.001$), suggesting reduced insulin sensitivity. The 30+60+90 group showed relatively lower AUC values than other DEX-treated groups. All DEX groups had reduced adrenal and spleen weights ($p < 0.0001$). Liver weight was increased in 90, 30+90, and 60+90 ($p = 0.002$). No significant changes were found in visceral adipose tissue mass. All DEX-treated groups had elevated plasma triglyceride levels ($p = 0.009$). Hyperinsulinemia was observed in the groups with one or two DEX exposures ($p = 0.001$), but insulin levels in the 30+60+90 group remained comparable to controls. The 90 group showed reduced corticosterone levels ($p = 0.04$), indicating hypothalamic-pituitary-adrenal axis suppression. In contrast, the 30+60+90 group exhibited corticosterone levels similar to control and tended to be higher than the 90 group ($p = 0.054$). Hepatic triglyceride ($p = 0.001$) and glycogen ($p = 0.009$) contents were elevated in the 90, 30+90, and 60+90 groups when compared to both control and 30+60+90. Pancreatic immunofluorescence revealed increased islet mass in 90, 60+90, and 30+60+90 groups ($p = 0.019$). Increased beta-cell mass was observed in 90 and 60+90 groups ($p = 0.014$), while alpha-cell mass remained unchanged across all groups. While repeated DEX exposure throughout life appeared to lessen some adverse metabolic effects of DEX in adulthood, this may occur at the cost of inducing GC resistance and reduced DEX responsiveness, raising concerns about the safety of intermittent DEX use. **Financial support:** CNPq

07.012 Acute Activation of the MGO-AGE-RAGE Axis Promotes Bladder Hypercontractility. Horioka CP, Oliveira AL, Medeiros ML, Antunes E FCM-Unicamp, Dept Translational Medicine – Pharmacology area

Methylglyoxal (MGO) is a highly reactive dicarbonyl compound, primarily formed under hyperglycemic conditions, such as in diabetes and obesity. Studies from our group have shown that chronic MGO administration induces bladder dysfunction. Here, we investigated whether *ex vivo* incubation with MGO in healthy mouse bladders would reproduce the hypercontractility observed *in vivo*. The experimental procedures and protocols were approved by the Animal Use Ethics Committee of the Universidade Estadual de Campinas (CEUA-UNICAMP; Protocol No. 6497-1/2024). Male C57BL6 mice (17 weeks old) had their bladders isolated and divided into two strips: one incubated with MGO (10 μ M) and the other with vehicle for 30 minutes. Bladder contractility was assessed in response to carbachol (1 nM–30 μ M) and electrical field stimulation (EFS, 1–32 Hz). We then tested co-incubation with anti-glycation agents: alagebrium (10 μ M), RAGE antagonist peptide (RAP, 1 μ M), and metformin (10 μ M). Data were expressed as the mean $\pm$ standard error of the mean (SEM), statistical difference between groups was determined by ANOVA follow Dunnett's multiple comparisons test; $p < 0.05$ was accepted as significant. Incubation with MGO induced significant ($p < 0.05$) detrusor hypercontractility in response to both carbachol and EFS. Co-incubation with alagebrium, RAP, and metformin significantly ($p < 0.05$) reversed this hypercontractility. These findings indicate that MGO acutely activates the MGO-AGE-RAGE axis, promoting detrusor hypercontractility and reinforcing this pathway as a potential therapeutic target for avoiding dysfunctions associated with glucotoxic stress. **FAPESP grant number: 2025/10412-1**

07.013 **Progression of Lower Urinary Tract Symptoms in the Face of Obesity: Interaction between H₂S and Cyclic Guanosine Monophosphate.** Souza ALC¹, Gonzaga L³, Alves MCO¹, Teixeira SA¹, Antunes E², Muscara MN¹, Costa SKP¹ ¹ICB-USP, Dept. of Pharmacology, São Paulo, Brazil. ²FCM-Unicamp, Dept. of Translational Medicine, São Paulo, Brazil. ³IB

Introduction: Obesity is a major public health concern, linked to diabetes, cardiovascular diseases, and, although less discussed, lower urinary tract symptoms (LUTS) which affect both sexes. Our previous research showed that inhibiting hydrogen sulfide (H₂S) production worsens LUTS. H₂S donors are known to maintain soluble guanylate cyclase (sGC) in a reduced state, which enhances nitric oxide (NO) signaling. However, the role of these pathways in the bladder smooth muscle of obese individuals is not well-understood. This study therefore investigated how obesity, induced by a high-fat diet in male and female mice C57BL/6, affects LUTS and the modulation of the H₂S and sGC pathways in the urinary bladder. **Objective:** Evaluate the role of H₂S and associated mechanisms in the potential urological complications of obesity, as well as to characterize the sGC-cGMP pathway in tissues from obese animals. **Methods:** Obesity was induced using a high fat diet (6,1kcal/g; PragSoluções Biociências, SP) during 9 weeks in C57BL/6 mice, male and female, starting at 28 days old (n= 6-12). The body weight gain from mice with a high fat diet (HFD) or normal diet (ND) was obtained weekly. Mechanic allodynia was performed using the von Frey test to evaluate the impact of obesity in nociception of the urogenital area (n = 6). The voiding pattern was assessed using the filter paper spot test to determine whether obesity caused any voiding dysfunction (n= 5-6). At the end of diet period, the animals were euthanized, the serum collected and cholesterol, triglycerides, high density lipoprotein (HDL) were dosed by commercial kits (n= 11-14). Antioxidant enzymes (superoxide dismutase - SOD, catalase - CAT, glutathione reductase - GR, glutathione peroxidase - GPx, and glutathione S-transferase - GST) was measured in bladder samples (n= 4-11). Protein assay was determined using Bradford method. The contractility of the bladder smooth muscle was evaluated through an isolated organ bath reactivity test, using the muscarinic agonist carbachol (CCh 0.1nM-1uM, n= 6). The protein expression of enzymes involved in H₂S synthesis and sGC was evaluated by Western blotting (n= 4-6). The results were expressed by mean ± standard error of the mean. **Results:** The results show that the HFD led to significant weight gain (P < 0.0001 vs male ND) and alterations in the lipid profile — total cholesterol, HDL, cholesterol and triglycerides (P < 0.0001 vs male ND and P < 0.001 vs female ND). The antioxidant activity increased only to GPx enzyme (P < 0.001 vs male ND and P < 0.5 vs male HFD), no alteration was observed in SOD, CAT, GR and GST activity. Also, there was no change in mechanical allodynia in animals HFD or in the contractility of bladder smooth muscle, However, there was a decrease in the voiding profile of HFD males compared to their ND counterparts and to HFD females (P<0.0001 vs male HFD and P<0.01 vs male HFD). A decrease in 3MST protein expression was observed in HFD males (P<0.05 vs male HFD), which explains the reduced generation of H₂S in the urinary bladder (P<0.001 vs male HFD) and sGC expression was reduced in HFD females, but not in males (P<0.001 vs female HFD). **Conclusion:** These results suggest a possible relationship between voiding dysfunction and the H₂S–sGC–cGMP pathway; however, they also highlight the complexity of the mechanisms involved in obesity-related urological complications and indicate the need for further investigation to fully understand these processes. **Acknowledgments:** CAPES; CNPq; FAPESP

07.014 Antioxidant Effects of Morosil® [*Citrus sinensis* (L.) Osbeck var. Moro] on the Reproductive System of Rats Treated with High Fat Diet. Rosa, GA; Oliveira, DA; Oliveira, LV; Lima, GF; Brazão, SC; Mendes, ABA; Bragança, LAR; Ribas, JAS; Santos, CM; Brito, FCF; Marostica, E. LAFE-UFF, Dep. Physiology and Pharmacology

Introduction: The obesity epidemic, a condition that leads to a chronic state of inflammation and oxidative stress, have been linked to the progressive increase in infertility rates (Afshin A. *N Engl J Med.* v.377, p. 13, 2017). Studies have identified more than 20 commercially available natural anti-obesity products and among them is the *Citrus sinensis* (L.) Osbeck extract (Morosil®), approved by ANVISA, with diverse pharmacological effects including antineoplastic, antidiabetic, anti-inflammatory and cardiovascular protective action, attributed to its important antioxidant activity (Grosso, G. *Oxid Med Cellular Long.* v1, p. 1, 2013). However, little is known about its impact on the male reproductive system. Thus, the aim of this study was to evaluate the antioxidant effects of Morosil® on the testes, epididymis and spermatozoa of rats treated with high fat diet. **Methods:** Adult male Wistar rats were randomly divided into 3 groups: CO (control -standart rodent chow + saline), HF (high fat diet - standart rodent chow 87.7%, cholesterol 2%, lard oil 10%, cholic acid 0,3% + saline) (Da Motta, NA. *J Pharmacol Sci.* v.123, p.47, 2013) and HM (HF + Morosil®, 150mg/kg, 15 days, via gavage). The animals were anesthetized with ketamine/xylasin (80/10mg.Kg⁻¹, i.p.), the testes and epididymis were removed, weighed and processed for morphological analysis or homogenate (CEUA 7582240224). Sperm were obtained from the cauda epididymis for sperm evaluation (progressive motility, vigor, membrane functionality, concentration and morphology). Oxidative stress in the testis was assessed by using TBARs assay, as well as SOD, catalase, GPx, NFkB expression by using western blotting. The values are mean±SEM, Student-Newman-Keuls, $P<0.05$. **Results:** HF increased the relative weight of the testes (CO: 0.395 ± 0.01 ; HF: $0.678\pm0.06^*$; HM: 0.363 ± 0.019 g), the tubular diameter (CO: 311.3 ± 3.7 ; HF: $338.8\pm3.8^*$; HM: 311.8 ± 3.9 µm), the tubular area (CO: 77393.8 ± 1534.7 ; HF: $90012.8\pm2156.1^*$; HM: 77890.9 ± 1734.9 mm²) and disrupted the seminiferous epithelium. Preliminary analysis also suggests an increase in inflammatory cells in the epididymis of the HF group, which seems decreased in the HM group. Deleterious effect of HF on the testis was accompanied by an increase in lipid peroxidation (MDA) (CO: 1.41 ± 0.23 ; HF: $4.02\pm0.81^*$; HM: 1.94 ± 0.58 nmol/mg protein), showing local oxidative stress, with consequent loss of sperm motility and membrane functionality. Sperm also showed defects in the head and tail as well as the vigor decrease after HF, but these two lasts were not recovered by Morosil®. All other parameters above showed improvement in the HM group when compared to HF. Morosil® increased catalase expression in the testes when compared to CO and HF, but not the expression of SOD, GPx and NFkB. **Conclusion:** Treatment with Morosil® for 15 days was able to reverse all deleterious effects of HF due to its antioxidant action, except tail defects and male gamete vigor, in part by decreasing peroxide concentration. Given the variety of active ingredients of *Citrus sinensis*, we can consider the perspective of its bioactive agents as drug candidates that could contribute to reducing the global rate of male infertility. **Financial support:** FAPERJ, CNPq, CAPES, PROPPI/UFF.

07.015 Evaluation of the Participation of Pannexin Channels -1 in Relaxation of the Corpus Cavernosum of Rats: Implications for the Treatment of Erectile Dysfunction - An *ex-vivo* study.

Barros JVC, Moretti MB, Leonardi GR, Lima JM, Passos GR, Antunes E, Mônica FZ UNICAMP, Faculty of Medical Sciences, Dept Translation Medicine, PPG Pharmacology, Brazil

Erectile dysfunction (ED) is defined as the inability to achieve and/or maintain a penile erection long enough to allow satisfactory sexual intercourse. Although it does not represent a life-threatening condition, ED can significantly impair quality of life, affecting social, sexual, and even financial aspects. Data from the 2015 and 2016 National Health and Wellness Surveys (NHWS), conducted in eight countries, revealed that 40.5% of men reported some degree of erectile dysfunction, with higher prevalence observed from the age of 40 onwards. Furthermore, approximately 70% of men with lower urinary tract symptoms (LUTS), such as urinary urgency, incontinence, and post-micturition discomfort, also exhibited ED, highlighting a strong association between these urological conditions. Pannexins are channel-forming proteins, among which Pannexin-1 (Panx1) stands out for its role in modulating purinergic signaling through ATP release, thereby contributing to the regulation of vascular tone. Panx1 is activated by stimuli such as membrane stretch and cell depolarization, which promote the activation of P2Y2 purinergic receptors (P2Y2R), recruitment of PKC α , and stimulation of TRPV4 channels, leading to increased Ca²⁺ influx and vasodilation. This project focuses on the characterization of Panx1 channels in the corpus cavernosum (CC) and their role in smooth muscle relaxation, a key process for penile erection. Experiments were conducted using CC tissues from rats. Western blot (WB) analysis was used to assess Panx1 expression, which was confirmed in the analyzed tissues. Concentration-response curves (CRCs) were also performed to determine potency (pEC₅₀) and maximal response (E_{max}) values to different relaxing agents. All experimental protocols were approved by the Ethics Committee (CEUA/UNICAMP – Protocol: 6559-1/2024). Statistical analyses were performed using the unpaired Student's T-test. Inhibition of Panx1 channels with 10Panx (50 μ M, 30 minutes) enhanced CC relaxation in response to acetylcholine (ACh) and sodium nitroprusside (SNP), as evidenced by a leftward shift in the relaxation curves (pEC₅₀: ACh: 5.61 $\pm$ 0.34 vs. 6.26 $\pm$ 0.30; N = 4, p<0,05; SNP: 5.55 $\pm$ 0.29 vs. 6.69 $\pm$ 0.38; N = 5, p<0,05). Regarding tadalafil (TDL), a significant increase in E_{max} was observed in the presence of 10Panx (control: 58.8% $\pm$ 13.8; 10Panx: 82.1% $\pm$ 10.7; N = 5, p<0,05), along with a trend toward a leftward shift in pEC₅₀, although without statistical significance. In conclusion, Panx1 channels are present in the rat corpus cavernosum, and their inhibition enhances relaxation responses to key agents such as ACh, SNP, and TDL, suggesting a functional role for these channels in the pathophysiology of erectile dysfunction. Further studies are needed to better elucidate the underlying pathways and molecular mechanisms involved.

07.016 **Toll-Like Receptor 4 (TLR4) as Mediator of High-Fat Diet-Induced Structural Remodeling and Inflammation in Mouse Epididymis: Mechanistic Link to Male Fertility Dysfunction?** Silva CP¹, Cruz Machado SS¹, Santiago MSA², Perobelli JE², Avellar MCW¹, ¹Unifesp-EPM, Dept. Pharmacology, Brazil; ²Unifesp-Baixada Santista, Dept. Marine Sciences, Brazil

Introduction: Obesity is a global health concern, recognized risk factor for systemic inflammation and male infertility. The effects of obesity-related metabolic and hormonal disturbances on the physiology of the epididymis, a reproductive tract organ where sperm maturation occurs, remain poorly understood. Here, we investigated the effects of high-fat diet (HFD)-induced obesity on tissue remodeling and inflammatory profile and the role of TLR4 signaling in mediating these events in the epididymis of wild-type (WT; C57BL/6J) and TLR4 knockout (TLR4KO) mice. **Methods:** Adult male mice (60 days) were housed individually for 4 weeks with free access to water and a standard diet (Chow; 3.5% Kcal/fat) or a HFD (42% Kcal/fat). Body mass, food and water intake were monitored. After euthanasia, testes, epididymis, and perigonadal adipose tissue were collected and weighed. Epididymides were fixed (4% PFA), paraffin-embedded, sectioned (8 μ m; 3 non-consecutive tissue sections/sample) and stained (H&E) for histopathologic and stereological analyzes. Stereology was performed using a Weibel grid overlaid on histological images to quantify the relative proportion (RP, %) of epididymal compartments (epithelium, interstitium and lumen) based on point counts; $RP = (\text{compartment points} / \text{total points}) \times 100$. Data were analyzed using Student's t-test ($p < 0.05$). **Results:** Compared to WT-Chow mice, WT-HFD showed a significant gain of body mass (1.18 ± 0.29 vs 4.04 ± 0.86 g, $p = 0.02$), with an increase in perigonadal adipose tissue mass (0.48 ± 0.02 vs 1.05 ± 0.15 g, $p = 0.01$), and decrease in relative testicular mass (0.44 ± 0.005 vs 0.39 ± 0.01 g, $p = 0.002$), with no change in epididymal mass (44.0 ± 1.92 vs 52.25 ± 3.09 mg, $p = 0.06$). However, in TLR4KO mice, HFD resulted in no significant change in body mass gain (1.64 ± 0.36 vs 2.46 ± 0.40 g, $p = 0.2$) or in testicular mass (absolute or relative mass, $p = 0.7$), with an increase in adipose (0.41 ± 0.009 vs 0.60 ± 0.05 g, $p = 0.02$) and epididymal tissue mass (47.0 ± 1.58 vs 54.0 ± 1.34 mg, $p = 0.01$) compared to TLR4KO-Chow. Histopathology showed more evident and significant alterations in WT-HFD epididymis, especially in corpus/cauda regions (increased frequency of epithelial cell vacuolization, clear cell number, luminal cells/debris, inflammatory infiltrates and enhanced interstitial vascularization) compared to WT-Chow. Stereological analysis of distal cauda epididymis of WT-HFD confirmed an increased RP of epithelial (8.34 ± 1.0 vs $11.72 \pm 0.82\%$, $p = 0.04$) and luminal compartments (68.29 ± 2.87 vs $78.11 \pm 2.26\%$, $p = 0.04$) and a decrease in interstitium (23.35 ± 2.34 vs $10.13 \pm 1.66\%$, $p = 0.005$). In contrast, histopathologic changes in TLR4KO-Chow and TLR4KO-HFD were more discreet, suggesting TLR4 absence protects against HFD-induced obesity. **Conclusion:** The results show that TLR4 deletion significantly attenuates epididymal tissue damage in response to HFD, highlighting its potential role in mediating inflammatory epididymal changes associated with HFD-induced obesity. The study may provide advances in the understanding of the molecular mechanistic links between TLR4 signaling, epididymitis, obesity and male fertility dysfunction. **Ethical Committee:** CEUA-Unifesp/8760240820. **Financial support:** CNPq, CAPES/CAPES-Print, FAPESP.

07.017 Pharmacological Characterization of the Newest Phosphodiesterase (PDE) Type 4 Inhibitor, Roflumilast in Isolated Detrusor from Pig Bladder: An *in vitro* Assay.

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Introduction: Lower urinary tract symptoms (LUTS) have multifactorial causes and, are frequently related to alterations in the bladder and bladder outlet. Clinical studies have shown that PDE5 inhibitors improve LUTS, even in the absence of erectile dysfunction. In women, this class of drugs has also been associated with improvement in bladder symptoms. These findings have sparked interest in the role of PDEs and their inhibitors in the pathophysiology and treatment of LUTS. In isolated pig bladder neck tissue, roflumilast increased hydrogen sulfide levels, an effect that was reduced in the presence of nitric oxide synthase and cystathionine gamma-lyase inhibitors. Additionally, roflumilast (0.6 nM) significantly enhanced isoproterenol-induced relaxation (Agis-Torres et al., Sci Reports, 2018). Because it is important to understand how a drug affects different regions of the bladder, this study aims to characterize the effect of roflumilast in isolated detrusor smooth muscle from male and female pig. **Methods:** In the first part, concentration response curves (CRCs) to roflumilast were carried in the absence (control) and in the presence of inhibitors of soluble guanylate cyclase (ODQ, 10 μ M), adenylate cyclase (SQ 22,526, 100 μ M) and nitric oxide synthase (L-NAME, 100 μ M). In some experiments, the urothelium was removed (U-). In the second set of experiments, CRCs to sodium nitroprusside (SNP), tadalafil, salbutamol, cilostazol, and riociguat were carried out in the absence and presence of roflumilast (100 nM). The maximal response (Emax) and potency (pEC50) values were determined. Data are presented as mean $\pm$ standard error, N= number of animals. Analyses were performed by unpaired Student's t-test, with $p < 0.05$. All the protocols were approved by the Ethics Committee (5585-1/2020). **Results:** Roflumilast relaxed detrusor smooth muscle with pEC50 and Emax values of 7.33 ± 1.43 and $57.4 \pm 37.3\%$; N = 6, respectively. The inhibitors, ODQ, SQ and L-NAME did not affect significantly ($P > 0.05$) the pharmacological parameters of roflumilast. Additionally, roflumilast did not interfere in the relaxations induced by SNP, tadalafil, salbutamol, cilostazol and riociguat. Surprisingly, the urothelium removal increased by 1,6-fold the Emax values of roflumilast (U-: $84.7 \pm 28.5\%$; N = 4 animals, in comparison with tissues with urothelium (U+: $52.15 \pm 15.92\%$; N = 4 animals). **Conclusion:** The effects of roflumilast on the detrusor muscle differ from its effects on the bladder neck in pigs. The enhanced relaxation observed in the absence of the urothelium suggests that the mucosa may be an important site of action for roflumilast. Further studies are needed to confirm this hypothesis.

07.018 **Effects of the Non-Phthalic Plasticizer DEHT on the Morphophysiology of the Male Genital System.** Lima LE, Silva Junior EP, Nogueira, LS, Lambertucci RH, Perobelli JE Unifesp, Experimental Toxicology, Brazil, Brazil

Introduction: Plasticizers are chemical additives widely used to enhance the flexibility and durability of plastic polymers. Due to increasing evidence linking phthalate plasticizers, such as di(2-ethylhexyl) phthalate (DEHP), to adverse reproductive and endocrine effects, their use has been progressively restricted by regulatory agencies, and alternatives like bis(2-ethylhexyl) terephthalate (DEHT) have been increasingly employed. However, the potential reproductive toxicity of this alternative plasticizer remains poorly understood. **Aim:** To evaluate the effects of subchronic oral exposure to a low dose of DEHT on the morphophysiology of the male genital system in adult rats, and to compare its potential reproductive toxicity to that of DEHP at a high dose. **Methods:** Male Wistar rats (n = 18; 60 days old) were randomly assigned to three groups: negative control (received only the vehicle, olive oil), positive control (received 250 mg/kg bw/day of DEHP), and DEHT (received 810 µg/kg bw/day of DEHT). Animals were orally exposed to the respective treatments once daily for 22 days. On postnatal day (PND) 82, animals were euthanized for assessment of biometric parameters, sperm quality (motility and morphology), and oxidative stress in testicular samples through biochemical assays, including total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI), thiobarbituric acid reactive substances (TBARS), and enzymatic activities of catalase and superoxide dismutase. Statistical analyses were performed using Jamovi software. Shapiro-Wilk normality test was adopted, followed by ANOVA with Tuckey's posteriori test. Statistical differences were considered significant when $p < 0.05$. This study was approved by the UNIFESP Animal Ethics Committee (CEUA nº 3434200624) and funded by the São Paulo Research Foundation (FAPESP). **Results:** Both DEHT and DEHP significantly reduced the mass of the seminal vesicle, both full and empty, indicating an impact on this androgen-sensitive accessory reproductive gland. DEHT did not affect sperm morphology, whereas DEHP induced a higher incidence of sperm morphological abnormalities, particularly in the head region, compared with the control group. No significant changes were observed in sperm motility, body weight gain, or food and water intake in either the DEHT or DEHP groups. Regarding biochemical parameters, DEHT increased catalase activity and promoted lipid peroxidation (TBARS), suggesting a redox imbalance. DEHP decreased superoxide dismutase activity, consequently increasing the total antioxidant status (TAS) and the oxidative stress index (OSI). **Conclusion:** Overall, these findings indicate that DEHT can exert adverse effects on male genital morphophysiology and testicular oxidative balance, even at a low dose relevant to environmental human exposure. These alterations were comparable to those observed in the group exposed to a high dose of DEHP. These preliminary results underscore the need for further investigation into the risks associated with alternative plasticizers, particularly regarding reproductive health. **Keywords:** Reproductive Toxicology; Oxidative Stress; Plasticizers.

08. Respiratory and Gastrointestinal Pharmacology

08.001 Methylglyoxal as a Key Mediator in the Exacerbation of Allergic Asthma in Leptin-Deficient Obese Mice. Medeiros ML, Oliveira AL, Antunes E FCM-Unicamp, Dept Pharmacology

Introduction and Aim: Obesity is a metabolic syndrome that has been associated with the worsening of several chronic inflammatory diseases, including allergic asthma of the Th2-high phenotype (1). Asthma, in turn, is characterized by chronic inflammation of the airways. Growing evidence indicates that obesity not only worsens asthma control and severity but also contributes to metabolic alterations that enhance the systemic inflammatory response (2). Among these alterations, the increase in circulating levels of methylglyoxal (MGO), a highly reactive dicarbonyl molecule, stands out. Elevated levels of MGO have been implicated in the pathophysiology of various chronic diseases (3), such as cardiopathies, systemic inflammatory processes, and pulmonary diseases (4, 5). We hypothesize that the increased systemic levels of MGO, observed under obese conditions, act as a potentiating factor in the exacerbation of airway inflammation in allergic asthma. Therefore, this study aims to evaluate the pulmonary inflammatory response in an experimental model of allergic asthma induced by ovalbumin (OVA) in obese mice, to elucidate the role of MGO in the interaction between obesity and asthma. **Methods:** Male isogenic mice C57BL/6J (lean) and B6.V-Lep^{ob}/Junib, ob/ob (obese), aged 4 weeks, were maintained for 14 weeks and Allergic sensitization was performed via two subcutaneous injections 100 µg of OVA. In the 12th week, mice were challenged intranasally with OVA (10 µg/50 µL), bronchoalveolar lavage (BAL) was performed by count total and differential cell. Levels of IL-4, IL-5, GLO, and RAGE in BAL fluid were measured using commercially available ELISA kits. Lung tissue was collected for analysis of reactive oxygen species (ROS), and serum samples were used to assess MGO levels. **Results:** After 14 weeks, obese mice exhibited serum MGO concentrations 6.7-fold higher than lean controls ($P<0.05$), accompanied by significant increases in glycemia and circulating RAGE levels ($P<0.05$). In contrast, GLO enzymatic activity remained unchanged between groups. OVA-induced allergic asthma in obese mice led to a marked increase in eosinophil infiltration in BAL, along with elevated levels of IL-4 and IL-5 compared to lean mice ($P<0.05$). Additionally, MGO exposure resulted in a 58% increase in ROS levels in lung tissue of obese asthmatic mice ($P<0.05$). **Conclusion:** This study demonstrates that obese asthmatic mice exhibit heightened airway inflammation and oxidative stress, alongside increased serum MGO and RAGE levels. These biomarkers may serve as potential targets for understanding and managing obesity-aggravated asthma.

08.002 Prevention of Gut Microbiota Imbalance: The Role of *Arthrospira platensis* in Hypercaloric Diet-Induced Dysbiosis. Claudino BFO^{1,2}, Arruda RRA^{1,2}, Diniz AFA^{1,2}, Melo MB¹, Sousa-Filho JEC¹, Colunga AAG⁴, Nobre CSN⁴, Silva BA^{1,2,3} ¹UFPB, Lab of Functional Pharmacology Prof. George Thomas, Institute of Research in Drugs and Medicines, Brazil ²UFPB, Postgraduate Program in Bioactive Natural and Synthetic Products, Health Sciences Center, Brazil ³UFPB, Dept Pharmaceutical Sciences, Health Sciences Center, Brazil, ⁴University of Minho, Fermentations Lab, Centre of Biological Engineering, Portugal

Obesity is a chronic metabolic condition associated with intestinal dysbiosis, characterized by an imbalance in the composition and diversity of the gut microbiota. Microbial alterations influence dietary energy extraction, systemic inflammation, and gut-brain axis modulation, contributing to the development and progression of the obesogenic phenotype. In this context, therapies aiming to restore intestinal eubiosis have shown promise for obesity prevention and management. *Arthrospira platensis* (AP), a filamentous alga with recognized antioxidant and anti-inflammatory properties, has gained interest as a potential microbiota modulator (Yu, T. *J Cell Mol Med.*, v. 24, p. 8603, 2020). This study evaluated the effects of a hypercaloric diet and AP supplementation 25 mg/kg on the gut microbiota composition of male Wistar rats. Animals were randomly assigned to four groups: standard diet (SD), standard diet with AP (SD + AP25), hypercaloric diet (HCD), and hypercaloric diet with AP (HCD + AP25). After eight weeks, fecal samples were collected and analyzed via 16S rRNA gene sequencing. A significant dysbiosis was observed in the HCD group compared to SD. At the phylum level, *Firmicutes* decreased (from 83.2% to 57.5%) while *Actinobacteriota* increased (from 7.6% to 39.07%). *Bacteroidota* showed no changes between the groups, remaining at low values. AP supplementation modulated this profile in the HCD + AP25 group, increasing *Firmicutes* to 63.7%, reducing *Actinobacteriota* to 19.4%, and elevating *Bacteroidota* to 11.7%. At the genus level, HCD induced a sharp increase in *Bifidobacterium* (from 1.2 to 37.4%) and complete loss of *Ligilactobacillus* (from 16.02 to 0%). With AP supplementation, *Bifidobacterium* decreased to 17.6%, and *Ligilactobacillus* reappeared (3.8%), indicating a partial restoration of the microbial balance. Phylogenetic analysis confirmed a loss of various *Firmicutes* genera and a rise in *Actinobacteriota*, particularly *Bifidobacterium*, in the HCD group. Alpha diversity indices showed significantly reduced microbial diversity in HCD, with the lowest Shannon and Simpson values across all groups, partially restored by AP. In conclusion, a hypercaloric diet induced marked intestinal dysbiosis in male Wistar rats, while *Arthrospira platensis* supplementation exerted protective and modulatory effects, favoring the recovery of gut microbial composition and diversity. **Financial support:** CAPES and Fermentations Laboratory of the University of Minho (Portugal). Ethical approval: CEUA/UFPB (protocol n°. 1664310523; ID 001937). Keywords: Obesity, Microalga, Prebiotics

08.003 **Anti-colitis Effect of Citral *in vivo* and *in vitro* models: Preventive Effect Mediated by Antioxidant and Anti-Inflammatory Action against Intestinal Damage.** Maycon T. Emílio-Silva; Vinícius P. Rodrigues; Antonio J. Ruiz-Malagon; Isabela G. Guidolin; Felipe L. Dario; Mariana M. Fioravanti; Luiz G. S. Branco; Alba Rodriguez-Nogales; Julio Gálvez; Clelia A. Hiruma-Lima Silva MTE^{1,2,3}, Rodrigues VP¹, Ruiz-Malagon AJ³, Guidolin IG¹, Dario FL¹, Fioravanti MM¹, Branco L², Rodriguez-Nogales A³, Galvez J^{3,4}, Lima CAH¹ ¹IBB-Unesp-Botucatu, Dept Structural and Functional Biology, Physiology sector; ²FORP-USP, Dept Basic and Oral Biology; ³University of Granada, Dept Pharmacology, Center for Biomedical Research, Granada, Spain. ⁴Instituto de Salud Carlos III CIBER de Enfermedades Hepáticas y Digestivas, Madrid, Spain

Introduction: Ulcerative colitis (UC) is a chronic condition characterized by inflammation of the colon and rectum caused by the overproduction of pro-oxidant species and an immune response that damages the gut epithelium. Currently, UC treatment focuses on symptom relief and achieving clinical remission. However, side effects have been reported. Therefore, searching for therapeutic alternatives is crucial for minimizing side effects and increasing remission periods. Thus, we investigated the effects of citral, a monoterpene, on dextran sulfate sodium (DSS)-induced gut inflammation in mice and examined the healing mechanism in human normal colon epithelial (NCM)-356 cells stimulated with lipopolysaccharide (LPS). **Methods:** Male C57BL/6J mice (4-6 weeks, n= 10 animals per group) were used for DSS 3%-induced UC for 5 days in drinking water. Concomitantly, oral treatment with citral (25, 100, and 300 mg/kg; p. o.) or vehicle (1% Tween 80, 10 mL/kg; p. o.) was administered. After induction, the animals were euthanized, and the colons were collected to evaluate neutrophil infiltration, lipid peroxidation, and antioxidant mechanisms. *In vitro*, NCM-356 cells were stimulated with LPS (10 ng/mL) for 48h. Cell viability, scratch wound-healing assay, and gene expression of inflammatory parameters, growth factors, and tight junction proteins were assessed. Statistical analysis was performed using one- or two-way ANOVA, followed by Tukey's post-test (p<0.05). **Results:** Acute treatment with citral (25, 100, and 300 mg/kg) exhibited a preventive effect by reducing the disease activity index (reduction of 59.05, 76.70, and 82.35%, respectively) and body weight loss compared with the DSS-vehicle group. This response was mediated by a significant reduction in myeloperoxidase (MPO) activity, thiobarbituric acid reactive species (TBARS) levels, and superoxide dismutase activity, indicating anti-inflammatory and antioxidant activity compared to the colitis group (p<0.05 vs. DSS-vehicle group). The monoterpene reversed LPS-induced intestinal epithelial disturbance in the scratch wound healing process under different conditions and possibly reduced *inos* expression. **Conclusion:** Thus, citral prevents damage and inflammation in the colons of mice. Its effects have been linked to the prevention of UC mediated by anti-inflammatory, antioxidant, and wound healing activities. **Financial support:** FAPESP grant number 2020/15225-1; 2021/11110-8; 2024/17177-5.

08.004 Investigation of the Antioxidant and Gastroprotective Activity of the Hydroethanolic Extract of *Plectranthus barbatus*. Casimiro LM¹, Ferreira P², dos Santos AC², da Silva LM¹ ¹UFSC, Center of Biological Sciences, Postgraduate Program in Pharmacology, Brazil. ²Univali, Postgraduate Program in Pharmaceutical Sciences, Brazil

Peptic ulcer's main clinical manifestation is localized epigastric pain, which is relieved by the ingestion of food and antacids, with the most serious complication being hemorrhage caused by stomach perforation. *Plectranthus barbatus*, popularly known as boldo and false-boldo, its popular use is for the treatment of indigestion, liver and gastrointestinal problems Objective: evaluate the gastroprotective effects of *Plectranthus barbatus*. **Methods:** For the development of the study, the dry extract obtained by macerating the leaves of *Plectranthus barbatus* and 70% ethanol was used. To achieve the results, various types of methods were used, including thin layer chromatography (TLC), and also 2,2-diphenyl-1-picrylhydrazyl (DPPH), for the determination of total phenols, pharmacological assays with male tracks of the Wistar species (CEUA/UNIVALI 35/17p.). Subsequently, the antiulcer activity was evaluated by exposing the gastric mucosa to 96% ethanol. **Results:** The determination of the antioxidant potential of the hydroalcoholic extract of *Plectranthus barbatus* (HEPB), demonstrated that this medicinal plant has potential in the reduction of free radicals. It was observed that HEPB promoted gastroprotection administered orally and intraperitoneally, as there was a reduction of ethanol-induced lesions by 96%. **Conclusion:** The study demonstrates that HEPB is able to protect the gastric mucosa of rats submitted to the ulcer model, which we suggest is related to the antioxidant activity of HEPB.

08.005 **Evidence of Biochemical Alterations of Oxidative and Inflammatory Damage in the Esophageal Mucosa of Rats with Autism Spectrum Disorder Induced by Valproic Acid.** Martins BSP¹, Souza Neto AE², Souza YAF², Nunes RKS², Longo B¹, Venzon L², Da Silva LM¹ ¹UFSC, Dept Pharmacology, Florianópolis, Santa Catarina, Brazil. ²Univali, Graduate Program in Pharmaceutical Sciences, Itajaí, Santa Catarina, Brazil

Introduction: Autism spectrum disorder (ASD) is a neurodevelopmental condition characterized by impairments in social interaction and repetitive behaviors. It is often associated with gastrointestinal comorbidities, such as gastro-oesophageal reflux and oesophagitis. There is growing evidence to suggest that inflammation and oxidative stress in the gastrointestinal tract may play a role in the pathophysiology of ASD. Therefore, this study investigated the presence of biochemical changes indicative of oxidative and inflammatory damage in the oesophageal mucosa of rats with ASD induced by prenatal exposure to valproic acid (VPA). **Methods:** Pregnant Wistar rats received VPA (600 mg/kg, i.p.) or saline on the 12.5th day of gestation. The offspring were evaluated for neurodevelopment in the first 10 postnatal days (eye opening to assess initial physical development, negative geotaxis test of motor coordination reflex, and swimming performance to assess motor control and muscle tone) and weaned on day 23. On the 60th postnatal day, a social interaction test was performed in three chambers to assess social behavior. At 70 days, the animals were euthanized, and the esophagus was collected for biochemical analysis. Markers of oxidative stress [malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), and glutathione S-transferase (GST)] and inflammation [myeloperoxidase (MPO)] were measured. (CEUA/UNIVALI: 002/22p1 and 034/22p). **Results:** Exposure to VPA resulted in impaired neurodevelopment and social interaction, particularly in males ($p < 0.05$). MDA levels in oesophageal tissue increased by 247% in males and 95% in females compared to controls ($p < 0.05$), indicating lipid peroxidation. SOD activity increased only in females exposed to VPA ($p < 0.05$), while GSH and GST showed no significant changes. MPO activity increased in both sexes ($p < 0.01$), suggesting an inflammatory infiltrate in the oesophageal mucosa. **Conclusion:** Prenatal exposure to VPA induces inflammatory and oxidative changes in the oesophageal mucosa of rats, as evidenced by increased levels of MDA and MPO. These results support the use of the VPA-induced ASD experimental model for studying gastrointestinal comorbidities, indicating oxidative and inflammatory processes as potential targets for managing these alterations in individuals with ASD. **Financial support:** CAPES, CNPq, FAPESC.

08.006 Toxicological Evaluation and Therapeutic Potential of the Ethanolic Extract of *Combretum leprosum* Mart. Stem Bark in an Ovalbumin-Induced Asthma Model in Rats. Oliveira MM¹, Souza ORB¹, Silva RLT¹, Sousa HG², Anjos BS², Leal PPMS², Alves MMM², Vieira Júnior GM², Oliveira AP¹, Oliveira RCM¹, Santos RF¹. ¹UFPI, PPG Pharmacology, Brazil, ² UFPI, Lab. of Natural Products, Brazil, ²UFPI, BioLeish - Advanced Research in Leishmania and Alternative Models, Brazil

Introduction: Asthma is a chronic inflammatory airway disease that affects millions of people worldwide. Despite the availability of conventional therapies, their limitations, including adverse effects and insufficient control of symptoms, highlight the need for new treatment options. *Combretum leprosum* Mart. (Combretaceae), a plant traditionally used in Brazilian folk medicine to treat a variety of diseases, including respiratory disorders, has demonstrated pharmacological properties that may be beneficial in asthma management. This study aimed to evaluate the acute toxicity of the ethanolic extract from the stem bark of *C. leprosum* (Cl-EtOH) in *Zophobas morio* larvae and its therapeutic effects in an ovalbumin (OVA) induced asthma model in rats. **Methodology:** Cl-EtOH was obtained from 1.901 kg of *C. leprosum* stem bark through ethanol maceration, followed by filtration, concentration, and lyophilization. Acute toxicity and melanin production were evaluated in *Z. morio* larvae exposed to increasing doses of Cl-EtOH (3, 30, 300, and 3,000 mg/kg). Experimental asthma was induced in rats (*Rattus norvegicus*) by intraperitoneal injections of a suspension containing OVA and aluminum hydroxide on days 0, 7, and 14, followed by nebulization with a 4% OVA solution for 5 minutes over 7 days. Rats were allocated into groups and received oral administration of saline, dexamethasone (1 mg/kg), or Cl-EtOH at doses of 50, 100, or 150 mg/kg, administered 30 minutes before each nebulization session. Tracheal reactivity to carbachol was measured, and inflammatory responses were assessed via bronchoalveolar lavage. Lung tissues were stained with hematoxylin and eosin for histological analysis. Differences were considered statistically significant when $p < 0.05$. The study was registered in SISGEN (R6B0934) and approved by the UFPI Animal Ethics Committee (783/2023). **Results:** The final yield of the Cl-EtOH extraction was 9.43% (179.3 g). In the acute toxicity test, Cl-EtOH was considered safe at the tested doses, with the median lethal dose (LD₅₀) estimated at 7,295 mg/kg. No significant melanization response was induced by any of the Cl-EtOH doses when compared to the negative control group. Asthmatic rats exhibited a significant increase in tracheal reactivity compared to non-asthmatic animals. The tracheal reactivity increase was significantly prevented by dexamethasone and all tested doses of Cl-EtOH when compared to asthmatic rats. The asthmatic group also exhibited significantly increased total and differential leukocyte counts compared to non-asthmatic animals. These increases were significantly reduced by treatment with dexamethasone or Cl-EtOH at doses of 100 and 150 mg/kg. The treatment with dexamethasone or Cl-EtOH at doses of 100 and 150 mg/kg prevented the thickening of the bronchial smooth muscle layer observed in the asthmatic group. **Conclusion:** This study demonstrated the safety of Cl-EtOH and its protective effects in an OVA-induced asthma rat model, providing scientific support for its traditional use. **Financial support:** Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

08.007 Reactivity Profile of the Colon in Sepsis Survivors: Sex-Related Functional Differences Involving Angiotensin Signaling Lima-Moura SA, da Silva-Santos JE UFSC, Lab Cardiovascular and Smooth Muscle Biology, Dept Pharmacology

Introduction: Sepsis is a clinical condition characterized by organic dysfunction resulting from the host's exacerbated inflammatory response to the infectious process. The intestine is also one of the organs affected by sepsis. However, a few studies have explored aspects related to intestinal tone, both in sepsis and survivors. During sepsis and septic shock, there is clinical and experimental evidence that RAS signaling is dysfunctional. However, little is known about angiotensin signaling in the gut of sepsis survivors, and sex-associated differences in this signaling remain unexplored. This study explored the hypothesis that angiotensin signaling in colonic smooth muscle is disordered in sepsis survivors. **Methods:** Female and male Swiss mice (3–4 months old) were anesthetized with isoflurane (5% induction and 3% maintenance) for induction of sepsis by the cecal ligation and puncture (CLP) model. Sex- and age-matched control groups and survivor groups were used at 15, 30, and 60 days (named S15, S30, and S60, respectively) after the CLP surgery. For testing, animals were anesthetized (ketamine/xylazine, 100/20 mg/kg, i.p.) and euthanized to remove their distal colon. Colon segments from each group (~1.5 cm in length) ($n \geq 6$ /group) were mounted in organ baths under a basal tone of 0.5 g and bubbled with atmospheric air at a controlled temperature (37 °C). The spontaneous movement and the reactivity to 120 mM KCl, 30 μ M acetylcholine, and cumulative concentrations (100 pM–3 μ M) of angiotensin I and II were recorded using force transducers. Results were evaluated as mean $\pm$ standard error of the mean contraction (g/g tissue) and subjected to one- or two-way ANOVA followed by Sidak's test. **Results:** There were no differences between the amplitude of the spontaneous movements developed by our experimental groups. However, we found an augmented frequency of movements in the S60 female group (~23 waves/min) compared with the control (~12 waves/min), which was not presented in the colon of the S60 male group. Additionally, the phasic contraction evoked by ACh in the colon of female mice was reduced from 34.1 ± 2.8 g/g in control to 20.6 ± 3.3 g/g in the S15 group, while the tonic response was increased from 14.7 ± 1.2 to 21.4 ± 2.7 g/g only in the S60 group. The colon of male mice that survived sepsis presented unaltered responses to AI and AII, compared with those from control groups, regardless of the evaluation period. However, the AI-induced contractile responses were reduced by ~60 and 40% in the colon of the S15 and S30 female groups, respectively, due to a significant rightward shift and depressed $E_{\max}$ of the cumulative concentration curves. A similar profile of depressed responses to AII was found in the colon of the S15 group. Moreover, the $E_{\max}$ of AII remained reduced in the S30 group (27.3 ± 2.9 g/g), compared with the age-matched control group (36.8 ± 3.6 g/g). **Conclusion:** Our research discloses that the colon of females, but not from male mice that survived the septic insult, display impaired responses to acetylcholine and reduced reactivity to angiotensin I and II. While our results do not allow us to speculate the mechanisms involved in this sex-related difference, our study reinforces the current literature, indicating that females are more susceptible to developing intestinal disorders. The long-lasting consequences of our findings on the gastrointestinal function of sepsis survivors deserve further investigation. **Financial support and ethical approval:** CAPES, 001; CNPq 312637/2021-7 e 403615/2023-2. Approved by CEUA/UFSC (3404210225).

08.008 Protective Effect of Lauric Acid on Lung Remodeling: Modulation of ROCK1 and α -SMA in Asthmatic Rats Martins AMO¹, Figueiredo IAD¹, Felício IM¹, Oliveira LN¹, Souza MLB², Alves AF^{1,2}, Vasconcelos LHC^{1,3}, Cavalcante FA^{1,3} ¹PPgPNSB/CCS/UFPB; ²PIBIC/UFPB; ³DCB/CCS/UFPB

Introduction: Asthma is a chronic inflammatory disease that also promotes airway remodeling, involving structural changes associated with activation of the RhoA/ROCK pathway. Previous studies have shown that lauric acid (LA) prevents tracheal hyperreactivity in a murine model of asthma. This study aimed to investigate whether LA modulates ROCK1 positivity and prevents lung remodeling in asthmatic rats. **Methods:** Experimental protocols were approved by the Ethics Committee on Animal Use (No. 9310040522) of UFPB. Wistar rats were randomized into six groups: control (CG), asthmatic (AG), asthmatic treated with lauric acid at 25, 50 or 100 mg/kg (LAG25, LAG50, LAG100), and an asthmatic group treated with dexamethasone 5 mg/kg (ADEXAG). Asthma was induced by intraperitoneal sensitization and intranasal challenges with ovalbumin for 21 days; controls received saline 0.9%. LA was administered daily at the respective doses through intragastric gavage. All animals were euthanized on day 22, and lungs were collected, fixed in 10% formalin, and processed for histological and immunohistochemical analyses. Slides were stained with hematoxylin-eosin and Masson's trichrome for morphological evaluation and with antibodies against α -smooth muscle actin and ROCK1 for immunohistochemical analysis. Image acquisition was performed under optical microscopy at 40x magnification. **Results:** Histological analysis of lung parenchyma stained with hematoxylin and eosin revealed preserved morphology in the CG. AG animals exhibited intense peribronchiolar inflammatory infiltrate and reduced luminal areas. Treatment with LA at 25 mg/kg partially reduced these alterations, while LAG50, LAG100, and ADEXAG preserved normal pulmonary architecture. Complementing these findings, analysis of pulmonary tissue stained with Masson's trichrome demonstrated extracellular matrix (ECM) deposition. The AG showed intense ECM accumulation, particularly in peribronchovascular regions. Treatment with LA at doses of 25, 50, and 100 mg/kg, as well as ADEXAG, prevented these ECM alterations, maintaining a distribution pattern similar to that of the CG. Immunohistochemical analysis of lung sections stained for α -smooth muscle actin (α -SMA) revealed intense α -SMA positivity in the AG, especially in inflammatory regions. Treatment with LA at doses of 25, 50, and 100 mg/kg, as well as ADEXAG, prevented these increases, maintaining α -SMA levels similar to the CG. Similarly, immunohistochemical staining for ROCK1 showed that the AG exhibited intense and diffuse ROCK1 positivity in bronchiolar regions and inflammatory infiltrates. Treatment with LA at 25 mg/kg (LAG25) resulted in partial ROCK1 positivity in bronchiolar regions. In contrast, LA at 50 and 100 mg/kg, as well as ADEXAG, effectively prevented these alterations, preserving staining patterns comparable to the CG. **Conclusion:** Lauric acid at doses of 50 and 100 mg/kg reduces airway inflammation, ECM deposition, and positivity of α -SMA and ROCK1 in asthmatic rats, preventing airway remodeling. These effects likely involve inhibition of the RhoA/ROCK pathway, supporting LA as a potential asthma therapy. **Financial support:** PPgPNSB/CCS/UFPB, FAPESQ, CAPES and CNPq. **Keywords:** Asthma, Lauric acid, Airway remodeling, RhoA/ROCK pathway

08.009 Uncovering the Gastroprotective Properties of Araucaria Brown Propolis and its Active Compound, Abietic Acid. Cury BJ¹, Silva HFT², Makowieski LP², Jerônimo DT², Silva LM², Silva TFQ², Longo B¹, Santos MR¹, Cruz AB², Bastos JK³, Santos MFC⁴, Silva LM¹ ¹UFSC, Dept Pharmacology, Postgraduate Program in Pharmacology, Brazil. ²UNIVALI, Postgraduate Program in Pharmaceutical Sciences, Brazil. ³FCF-USP, Brazil ⁴University of Franca, Research Center in Exact and Technological Sciences, Brazil

Peptic ulcer disease (PUD) primarily affects the stomach and duodenum and is strongly associated with *Helicobacter pylori* infection. Additionally, PUD is also correlated to the prolonged use of nonsteroidal anti-inflammatory drugs (NSAIDs). Although several pharmacological therapies are available for PUD treatment, they are all based on gastric acid suppression, such as histamine H₂ receptor antagonists and proton pump inhibitors (PPIs). However, the long-term use of these drugs is associated with adverse effects, including nutrient malabsorption, increased risk of fractures and neurological disorders. Therefore, it is necessary to seek safer and more effective therapeutic alternatives. Propolis is a natural product produced by bees, characterized by a wide chemical diversity and promising biological properties. Araucaria propolis, has a brown color, is mainly found in southern Brazil, and is rich in phenolic compounds and diterpenes, such as abietic acid (AA). This study investigated the gastroprotective effects of Araucaria brown propolis hydroalcoholic extract (HEBPA) and its isolated compound, abietic acid (AA). The extract's anti-ulcer activity was evaluated using acidified ethanol-induced gastric ulcers and pylorus ligation to assess gastric antisecretory activity. Additionally, the study examined histological, oxidative, and inflammatory parameters, and the extract's anti-*Helicobacter pylori* and cytotoxic effects. The 100 mg/kg oral dose of HEBPA promoted gastroprotection by 69.63% ($P < 0,01$) by increasing antioxidant defenses (GSH, CAT, and SOD) and reducing MPO activity and MDA levels in ulcerated mucosa. This gastroprotective effect was not due to antisecretory activity, but rather involved non-protein sulfhydryl compounds, alpha-2 adrenergic receptors, prostaglandins, and nitric oxide, as evidenced by the abolition of the effect with L-NAME, NEM, indomethacin, or yohimbine pre-treatment. HEBPA increased mucin stained by PAS in the gastric mucosa and prevented histological damage, reducing edema and inflammatory infiltrate. Additionally, HEBPA promoted *in vitro* fibroblast (L929 cells) proliferation at 1 µg/mL, but showed no antibacterial activity against *H. pylori* (MIC > 1000 µg/mL). In addition, AA at 17 mg/kg reduced by 44.82% ($p < 0,001$) the ethanol induced- ulcers. These findings contribute to validate the anti-ulcer effect of Brazilian Araucaria brown propolis, highlighting its potential as a natural resource for developing new gastric ulcer treatments. Notably, AA does appear to be a key bioactive compound responsible for this effect. This study was supported by CAPES. All experimental procedures were approved by the Animal Experimentation Ethics Committee (CEUA) of UNIVALI under protocol number 029-19p.

08.010 Sex-Specific Differences in Gastric Ulcer Healing in Normotensive and Spontaneously Hypertensive Rats: Preliminary Findings. Rizzon-Santos M, Willrich CH, Fontoura LB, Cury BJ, Longo B, Moura, SAL, De Oliveira IM, Casimiro LM, De Amorim GES, Prediger RD, Da Silva-Santos JE, Da Silva LM. UFSC, Dept Pharmacology, Florianópolis

Introduction: Gastric ulcers are lesions that affect the gastric mucosa and result from an imbalance between the stomach's protective and aggressive factors. On the other hand, systemic arterial hypertension (SAH) is considered a multifactorial disease characterized by a persistent elevation of blood pressure (BP) levels. Although they are different diseases, both gastric ulcers and SAH pose a serious threat to public health. While it is known that hypertension can influence the damage and repair of the gastric mucosa, the mechanisms responsible for this relationship are not yet fully understood. Against this backdrop, this study investigated possible differences in the gastric healing process between normotensive rats and spontaneously hypertensive rats (SHR), as well as the existence of sexual dimorphism in this process. **Methods:** Wistar Kyoto (WKY) and SHR rats of both sexes were included in the study. Before ulcer induction, mean arterial pressure (mmHg) and heart rate (BPM) were measured. The animals were divided into groups and subjected to an ulcer model induced by 80% acetic acid. Lesion area, gastric mucosal blood flow, and oxidative stress parameters, including reduced glutathione (GSH) and malondialdehyde (MDA) levels, were assessed. In parallel, histological analyses were performed on the ulcerated tissue. Approval number 8247201123. **Results:** As expected, SHR males and females exhibited significantly higher mean arterial pressure and heart rates than the WKY group ($p < 0.0001$). However, regarding the healing process of gastric ulcers, only SHR males exhibited a lesion area that was 38% larger than that of males in the WKY group ($77 \pm 5 \text{ mm}^2$). No differences were found in the lesion area of WKY and SHR females; however, WKY females showed a significantly larger lesion area than males of the same strain ($p < 0.01$). SHR males exhibited significantly increased mucosal blood flow under both ulcerated and non-ulcerated conditions when compared to WKY males. In contrast, SHR females did not show any difference in mucosal blood flow under ulcerated conditions relative to WKY females. However, under non-ulcerated conditions, SHR females demonstrated significantly higher mucosal blood flow than WKY females ($p < 0.0001$). Notably, ulcerated SHR females exhibited a marked reduction in mucosal blood flow compared to their own non-ulcerated counterparts ($p < 0.001$). In both sexes and strains, decreased GSH availability, elevated MDA levels, and microscopic tissue damage were observed. **Conclusion:** Taken together, our data reveals important differences in the spontaneous healing process of gastric ulcers between normotensive and hypertensive rats, as well as the existence of sexual dimorphism. Further analyses are needed to elucidate the pathophysiological mechanisms involved in this process. **Financial support:** CAPES, CNPq.

08.011 **Lauric Acid Modulates Nitric Oxide and Cyclooxygenase Pathways to Prevent Tracheal Hyperresponsiveness in Asthmatic Wistar Rats.** Oliveira LN¹, Figueiredo IAD¹, Martins AMO¹, Felício IM¹, Vasconcelos LHC^{1,2}, Cavalcante FA^{1,2} ¹PPgPNSB/CCS/UFPB; ²DCB/CCS/UFPB

Introduction: Asthma is a chronic inflammatory disease characterized by airway obstruction and lung inflammation, affecting over 300 million people worldwide, with prevalence ranging from 1% to 29%. Lauric acid (LA) has been shown to reduce tracheal hyperreactivity in asthmatic rats (Figueiredo, IAD. Pharm., v. 18, p. 221, 2025). Nitric oxide (NO•) and eicosanoids are key mediators in asthma, regulating smooth muscle contraction in the airways (Van den Berg, MPM. Curr. Opin. Pharmacol., v. 40, p. 126, 2018; Martin, SA. J. Lipid Res., v. 57, p. 1126, 2016). This study aimed to evaluate the involvement of nitric oxide, cyclooxygenase, and lipoxygenase pathways as targets of LA, highlighting its *in vitro* mechanism to prevent tracheal hyperresponsiveness in asthmatic Wistar rats. **Methods:** Experimental protocols were approved by the Ethics Committee on Animal Use (No. 9310040522) of UFPB. Wistar rats were divided into three groups (n = 5): control (CG), asthmatic (AG), and asthmatic treated with lauric acid (25 mg/kg; LAG25). Asthma was induced by intraperitoneal sensitization and intranasal challenges with ovalbumin for 21 days; controls received saline 0.9%. Treatments were administered intragastrically by gavage throughout the sensitization period. Animals were euthanized on day 22. To investigate mechanisms related to asthma pathogenesis, tracheal rings were pre-incubated with indomethacin 10⁻⁵ M, a COX inhibitor; zileuton 10⁻⁵ M, a 5-LOX inhibitor; and L-NAME 3 x 10⁻⁴ M, a non-selective nitric oxide synthase inhibitor. Contractile reactivity was assessed by comparing E_{max} and EC₅₀ values across groups. **Results:** In CG, contractile responses to CCh remained unchanged by L-NAME, indomethacin, or zileuton, showing no significant changes in efficacy or potency. In AG, both L-NAME and indomethacin significantly reduced contractile efficacy (E_{max} decreased from 146.3 ± 5.6% to 107.9 ± 3.4% with L-NAME and 64.0 ± 5.8% with indomethacin; *p* < 0.05), without affecting potency. In LAG25, similar reductions in contractile efficacy were observed with L-NAME and indomethacin (E_{max} decreased from 106.0 ± 9.0% to 76.1 ± 4.1% and 62.6%, respectively, *p* < 0.05). Conversely, zileuton significantly reduced contractile reactivity in AG (E_{max} from 146.3 ± 5.6% to 90.4 ± 6.9%; *p* < 0.05), but did not affect responses in LAG25. **Conclusion:** These results indicate that lauric acid reduces tracheal hyperresponsiveness in asthmatic rats by modulating the nitric oxide and cyclooxygenase pathways, while the lipoxygenase pathway seems not to be directly involved. This mechanism may be associated with the protective effects of lauric acid in asthma, opening new possibilities for its therapeutic application. **Financial support:** PPgPNSB/CCS/UFPB, FAPESQ, CAPES and CNPq. **Keywords:** Lauric acid, Asthma, Tracheal hyperresponsiveness, Inflammatory mediators.

08.012 *In silico*, *in vitro* and *in vivo* Approach for Evaluation of the Ethyl Acetate Fraction of *Platonia insignis* Mart. in a Model of Ovalbumin-Induced Asthma. Souza ORB¹, Oliveira MM¹, Barbosa BS¹, Nascimento ER², Moreira RA², Freitas SDL², Oliveira AP¹, Oliveira RCM¹, Santos, RF¹ ¹UFPI, PPG Pharmacology, Brazil. ²UFPI, Lab. Natural Products, Dept Chemistry, Brazil

Asthma is a chronic respiratory disease characterized by inflammation, excessive secretion of mucus and bronchial contraction of the smooth muscle. Although glucocorticoids are the primary drug treatment, their side effects often make long-term therapy difficult. In this context, medicinal plants have emerged as promising alternatives for asthma management. *Platonia insignis* Mart. (Clusiaceae), popularly known as bacurizeiro, has confirmed anti-inflammatory, antioxidant and vasorelaxant properties. It is rich in bioactive compounds, with morelloflavone being the main constituent. This study aimed to investigate the antiasthmatic activity of the ethyl acetate fraction of *P. insignis* branches (Pi-AcOEt_g) using *in vivo*, *in vitro* and *in silico* approaches in an ovalbumin-induced asthma model (OVA). **Methods:** Male rats from *Rattus norvegicus* Wistar (180-220g) were used. Asthma was induced with ovalbumin (OVA) and aluminum hydroxide on days 0, 7 and 14, followed by daily nebulization of the OVA for 7 days. The experimental groups included asthmatic and control animals. The relaxing effect of Pi-AcOEt_g was evaluated in isolated tracheal rings pre-contracted with carbachol (CCh). The potential involvement of ATP-sensitive potassium channels (K_{ATP}) was evaluated using glibenclamide. Tracheal reactivity was evaluated in animals treated with Pi-AcOEt_g (v.o.), by means of concentration-response curves to CCh. The lungs were dissected, fixed in 10% formaldehyde and stained with hematoxylin-eosin for histological analysis. Protocols approved by CEUA/UFPI (509/18) and SISGEN (A8DC804). Molecular docking was performed with AutoDock Vina 1.2.3 software to determine the interactions of the ligand with the target. **Results:** the induction of asthma by tracheal reactivity test was confirmed in non-asthmatic animals (log CE₅₀= 0.19 ± 0.04/ Emax= 115%), sensitized (log CE₅₀= 0.24 ± 0.06/Emax= 174%) and asthmatic (log CE₅₀= 0.74 ± 0.14/Emax = 231%)* with statistically significant difference (*p < 0.05). Pi-AcOEt_g relaxed, in a concentration-dependent manner, the trachea of non-asthmatic animals (log CE₅₀ 0.98 ± 0.19), with reduced potency when compared to asthmatic animals (log CE₅₀ 2.54 ± 0.52)*. In the relaxing effect in the presence of glibenclamide (log de CE₅₀ = 2.26 ± 0.17)* there was a significant deviation of the curve to the right in relation to the control curve (log CE₅₀= 0.98 ± 0.19). In muscle reactivity, the tracheae of asthmatic animals treated with Pi-AcOEt_g at doses of 12.5 (log CE₅₀ = 0.17± 0.02)*, 25 (log CE₅₀ = 0.22 ± 0.04)* reduced this response when compared to asthmatic animals (log CE₅₀ = 0.74 ± 0.14) there was no difference in dose of 50 mg/kg (logCE₅₀ = 1.09 ± 0.19). Histologically, there was a reduction of bronchial smooth muscle hyperplasia and/or hypertrophy, alveolar wall thickening and infiltrated inflammatory cells in animals treated with Pi-AcOEt_g (v.o). The molecular docking indicated affinity prediction of morelloflavone by K_{ATP} target site. **Conclusion:** Pi-AcOEt_g showed promising potential in the treatment of bronchial asthma, evidencing protective effect against lung damage in asthmatic and spasmolytic rats, being morelloflavone possibly the substance responsible for this effect.

08.013 **Modulation of Pulmonary Inflammation and the Redox Pathway *in vitro* and *in vivo* by Fumaric Esters.** Mineiro PCO¹, Cardoso AOP², Figueiredo-Júnior ATF¹, Mota EC¹, Amorim CS¹, Valença HM¹, Gomes ACCA³, Serra SSS³, Silva PL³, Takiya CM³, Lima LM², Moraes JA², Valença SS², Deus MLD² ¹ICB-UFRJ, ²UFRJ, Postgraduate Program in Immunology and Inflammation, Federal University of Rio de Janeiro, ³IBCCF-UFRJ

Introduction: Chronic Obstructive Pulmonary Disease (COPD), marked by chronic lung inflammation and emphysema, is the third leading cause of death globally, with no effective treatment. Molecules with antioxidant and anti-inflammatory properties, such as dimethyl fumarate (DMF), show therapeutic potential for inflammatory diseases by activating the Keap-1/Nrf2 pathway, enhancing gene expression of detoxifying enzymes. This study investigates the protective effects of DMF and its metabolite, monomethyl fumarate (MMF), against chronic lung inflammation induced by cigarette smoke *in vitro* and porcine pancreatic elastase (PPE) in mice. **Methods:** *In vitro*, human lung epithelial cells (PC9) were treated with MMF (10, 30, 100 µM) and exposed to 5% cigarette smoke extract (EFC) to assess cell viability (1h and 24h), oxidative stress (ROS), lipid peroxidation (MDA), and nitrite production. *In vivo*, female C57BL/6 mice received oral DMF (30, 100 mg/kg) for 32 days during and after emphysema induction by intranasal 3 U PPE. We analyzed ROS levels, bronchoalveolar lavage (BAL) cell counts, lung histology, and expression of oxidative stress proteins (SOD1, HO-1, MMP9, nitrotyrosine). Data are presented as mean ± S.E.M., analyzed by ANOVA with Bonferroni post-test. **Results:** *In vitro*, EFC reduced cell viability after 24h and increased ROS, nitrite, peroxynitrite, and MDA levels. MMF, at all concentrations, reversed these effects, demonstrating potent antioxidant activity. *In vivo*, PPE-induced emphysema elevated oxidative stress, lung inflammation, macrophage counts, alveolar diameter, MMP-9, and nitrotyrosine expression, while reducing HO-1. DMF treatment (30, 100 mg/kg) prevented or reversed these changes, restoring biochemical, cellular, and histological parameters. **Conclusion:** DMF exhibits potential to mitigate oxidative stress and promote lung tissue repair, supporting its pharmacological repositioning for COPD treatment. These findings highlight fumarates as promising therapeutic agents for inflammatory lung diseases via Nrf2/NF-κB pathway modulation. CEUA: CCS-UFRJ (016/22) **Financial support:** CAPES

08.014 Lauric Acid Reduces Oxidative Stress to Prevents Tracheal Hyperresponsiveness in Asthmatic Wistar Rats. Felício IM¹, Figueiredo IAD¹, Martins AMO¹, Oliveira GNM¹, Oliveira LN¹, Alves BFL², Golzio AMFO^{1,3}, Vasconcelos LHC^{1,4}, Cavalcante FA^{1,4} ¹PPgPNSB/CCS/UFPB, ²PIBIC/UFPB, ³DTA/CTDR/UFPB, ⁴DCB/CCS/UFPB

Introduction: Asthma is a chronic inflammatory disease characterized by oxidative stress, resulting from an imbalance between reactive oxygen and nitrogen species and the antioxidant defense system. Lauric acid (LA) has demonstrated a preventive effect on asthma-related changes in animal models (Vasconcelos, LHC. *Oxid. Med. Cell. Longev.*, v. 2020, 2020; Figueiredo, IAD. *Pharm.*, v. 18, p. 221, 2025). Based on these findings, the present study investigated the mechanism of action of LA in modulating oxidative stress markers and tracheal hyperresponsiveness in a murine model of asthma, including the effects of NADPH oxidase inhibition (apocynin) and superoxide dismutase (SOD) mimetic activity (tempol). **Methods:** All procedures were approved by the Ethics Committee on Animal Use of UFPB (No. 9310040522). Wistar rats were divided into four groups (n = 5): control (CG), asthmatic (AG) and asthmatic treated with lauric acid (25 mg/kg; LAG25). Asthma was induced over 21 days by intraperitoneal sensitization with ovalbumin on the first three days, followed by intranasal challenges, while control animals received saline. Treatments were administered orally by gavage throughout the sensitization period. Animals were euthanized on day 22. To explore redox-related mechanisms, tracheal rings were pre-incubated with apocynin (10^{-4} M), a NADPH oxidase inhibitor, or tempol (10^{-3} M), a SOD mimetic, followed by cumulative concentration-response curves to carbachol (CCh). Contractile reactivity was assessed by comparing E_{max} and EC_{50} values across groups. Lungs were collected, frozen at -20°C , and homogenized in 10% KCl (1:1, w/v). Oxidative stress markers were evaluated in lung homogenates: lipid peroxidation by TBARS assay, nitrite levels via the Griess reaction, reduced glutathione (GSH) with DTNB, and SOD activity by NBT reduction inhibition. All analyses were performed in duplicate. **Results:** In CG, the cumulative concentration-response curve to CCh was not significantly altered by apocynin or tempol, showing no differences in efficacy or potency. In AG, both apocynin and tempol significantly reduced the contractile efficacy ($E_{max} = 146.3 \pm 5.6\%$ to $78.8 \pm 3.1\%$ and $102.1 \pm 7.6\%$, respectively; $p < 0.05$), without affecting potency. In LAG25, apocynin reduced the contractile efficacy ($E_{max} = 106.0 \pm 9.0\%$ to $73.9 \pm 6.8\%$; $p < 0.05$), while tempol did not significantly alter the contractile response. In lung homogenates, AG showed increased MDA levels (3.8 ± 0.4 mg/g) compared to CG (1.9 ± 0.3 mg/g), an effect prevented in LAG25 (2.3 ± 0.4 mg/g). Nitrite concentrations were elevated in AG (136.3 ± 13.7 $\mu\text{M/g}$) versus CG (81.5 ± 14.3 $\mu\text{M/g}$), but prevented in LAG25 (65.8 ± 9.1 $\mu\text{M/g}$). GSH levels were reduced in AG (8.4 ± 0.6 $\mu\text{g/g}$) compared to CG (12.3 ± 1.3 $\mu\text{g/g}$), and although increased in LAG25 (10.5 ± 0.7 $\mu\text{g/g}$), this was not statistically different from either CG or AG. SOD activity was lower in AG (36.2 ± 8.0 U/ μg) relative to CG (74.4 ± 7.3 U/ μg), and restored in LAG25 (74.1 ± 11.6 U/ μg). **Conclusion:** These findings suggest that LA may attenuate tracheal hyperresponsiveness in asthma by modulating oxidative stress and NADPH oxidase-related pathways. This mechanism may contribute to the protective effects of coconut oil in asthma. **Keywords:** Asthma, Lauric acid, Airway remodeling, Oxidative stress.

09. Natural Products and Toxinology

09.001 **Action of Marimastat, a Synthetic Broad-Spectrum Metalloprotease Inhibitor, on the *in vitro* coagulotoxicity of *Lachesis muta* (South American Bushmaster) Venom.** Pessoni JG¹, Guldoni LF¹, Oliveira IN¹, Proença-Hirata VS¹, Silva NJ², Torres-Bonilla KA³, Hyslop S³, Sousa EP⁴, Morais-Zani K⁴, Floriano RS¹ ¹Unoeste, Lab. of Toxinology and Cardiovascular Research, Graduate Program in Health Sciences, Brazil, ²PUC-Goiás, School of Medical and Life Sciences, Brazil, ³UNICAMP, Dept Pharmacology, Faculty of Medical Sciences, Brazil, ⁴IBu, Lab. of Pathophysiology, Graduate Program in Toxinology, Brazil

The genus *Lachesis* includes the largest snakes in the Viperidae family, found in Central and South America. It comprises four species: *L. stenophrys*, *L. melanocephala*, *L. acrochorda*, and *L. muta*, which can occasionally cause human envenomation. In Brazil, *L. muta* is exclusively located in the Amazon River basin and the Atlantic Forest, and it is one of the most venomous snakes responsible for bites in the northern region of the country. Marimastat, a broad-spectrum metalloprotease inhibitor, has shown a satisfactory interaction with snake venom metalloproteases, which makes it a promising pharmacological agent to replace antivenom when it is unavailable or to enhance its neutralization. In this work, we assessed the metalloprotease inhibitory action of marimastat (MMT) as an alternative pharmacological tool to anti-*Bothrops/Lachesis* antivenom (AV) on the coagulant effects of *Lachesis muta* venom (LMv). Enzymatic activity was assayed using azocasein as substrate. Factor X activation and thrombin-like activity were assessed using chromogenic substrates (S-2238 and S-2765); minimum coagulant dose (MCD) and fibrinogenolytic activity were assayed in human citrated plasma (CAAE protocol no. 43560620.0.0000.5493). MMT (0.03 mM) was determined as the minimum inhibitory concentration to abolish the caseinolytic activity of LMv. The MCD of LMv was determined in 7.5 mg of venom/ml, with MMT (0.03 mM) being ineffective in preventing clotting formation, whereas AV (antivenom:venom ratio of 1:3 v/w) avoided the clotting formation in 240 sec of monitoring ($p < 0.05$, $n = 3$). The thrombin-like activity of LMv was significantly delayed only by AV (1:3) ($p < 0.05$, $n = 3$), and both agents failed to prevent factor X activation by this venom; LMv did not induce prothrombin (factor II) activation. LMv (7.5 mg/ml) exhibited a- and b-chain fibrinogenolysis. MMT (0.03 mM) reduced in $68 \pm 1.2\%$ ($p < 0.05$, $n = 3$) the venom-induced a-chain fibrinogenolysis only at 5 min of incubation, with AV (1:3) being ineffective; in addition, MMT (0.03 mM) abolished the b-chain fibrinogenolysis at 5 min of incubation and significantly delayed this alteration at 30 and 90 min of incubation in $42 \pm 4.1\%$ and $36 \pm 3.9\%$, respectively ($p < 0.05$, $n = 3$), whereas AV (1:3) produced superior b-fibrinogenolysis inhibition at 30 ($69 \pm 1.1\%$) and 90 ($65 \pm 2.1\%$) min of incubation. MMT (0.03 mM) did not show additive protection when combined with AV (1:3) at any of these approaches. In conclusion, LMv exhibits haemostatic activity *in vitro*. MMT by itself or combined with AV does not appear to be a plausible pharmacological strategy to prevent haemostatic disorders caused by LMv. However, the drug has prominently reduced the a- and b- fibrinogenolysis of LMv.

09.002 The Antioxidant Potential of Extra Virgin Olive Oil on the Prefrontal Cortex of Rats Exposed to a High-fat Diet. Lena MC, Peres, AM, Raphaelli GB, Dantas RM, Ferreira AC, Silveira ACS, de Souza LCP, Santos, ES, Harttfeil MHB, Dalmaz C, Bast RKSS UFRGS, Dept Biochemistry, Brazil

Introduction: Exposure to high-fat diets (HFD) can negatively affect brain function, triggering increased oxidative stress and inflammation. Oxidative stress is characterized by excessive production of reactive oxygen and nitrogen species (ROS/RNS) and reduced antioxidant capacity, resulting in biomolecular damage and increased levels of free radicals. These alterations may directly influence cognitive function by impairing neurogenesis and synaptic plasticity, affecting memory in animals exposed to this diet. The rodent brain is especially susceptible to the effects of chronic HFD consumption, particularly the prefrontal cortex (PFC), a brain region involved in decision-making, reward processing, and motor, cognitive, affective, and social behaviors. Considering this scenario, extra virgin olive oil (EVOO), known for its antioxidant and neuroprotective properties, may represent a potential therapeutic strategy to counteract the effects induced by HFD in the PFC. **Objective:** This study aimed to evaluate the impact of EVOO supplementation on short-term memory and the antioxidant response in the PFC of adult male and female rats exposed to HFD. **Methods:** At postnatal day 21, Wistar rats (CEUA-UFRGS 41855) were randomly allocated into four groups: (1) standard chow (SC), (2) SC+EVOO, (3) HFD, and (4) HFD+EVOO. EVOO was administered daily via oral gavage (1.3 mL/kg body weight). Caloric intake, body weight, and body mass index (BMI) were monitored weekly throughout the experimental period. Locomotor activity was evaluated using the open field test. Short-term memory was assessed through the object recognition (OR) test, performed over two consecutive days. On the first day, animals were habituated to the apparatus. On the second day, during the training session, they explored two identical objects for 5 minutes. One hour later, one object was replaced with a novel one, and animals were again allowed to explore for 5 minutes. The total exploration time was recorded, and the discrimination index (%) was calculated as: (time exploring the novel object / total time exploring both objects) × 100. In the medial PFC (mPFC), the activities of antioxidant enzymes—glutathione peroxidase (GPx), catalase (CAT), and superoxide dismutase (SOD) — as well as ROS levels were quantified. **Results:** Caloric intake increased overtime in both sexes ($p < 0.0001$). HFD-fed animals showed greater weight gain ($p = 0.038$ for males; $p < 0.0001$ for females) and increased BMI ($p = 0.017$ for males; $p = 0.023$ for females). No changes in locomotor activity were detected ($p > 0.05$). In the OR test, HFD+EVOO males demonstrated improved object discrimination ($p = 0.044$). In females, HFD reduced SOD ($p < 0.001$), CAT ($p = 0.011$), and ROS production ($p < 0.001$). EVOO supplementation in HFD females increased SOD ($p < 0.001$), GPx ($p = 0.030$), and decreased ROS ($p = 0.025$). In males, EVOO also elevated GPx activity ($p = 0.049$). **Conclusion:** Given the findings, it was possible to verify that females were more vulnerable to the effects of HFD, since they presented a deficit in short-term memory. In addition, EVOO supplementation demonstrated a potential antioxidant effect in the PFC of HFD-fed animals.

09.003 **Mechanisms Involved in Hemostatic Disorders Caused by *Bothrops leucurus* and *Bothrops erythromelas* Envenomation.** Rodrigues MAF¹, Paulesini ES¹, Felipe AGC¹, Carneiro IB¹, Lima JAF², Lima PHV³, Sant'Anna SS⁴, Morais-Zani K^{1,4} ¹IBu, Lab Fisiopatologia,; ²IBu, Lab Ecologia e Evolução, Instituto Butantan; ³IBu, Lab Farmacologia, Instituto Butantan; ⁴IBu, Lab Herpetologia, Instituto Butantan

Bothrops snakes are related to 87% of ophidian accidents reported in Brazil, and the majority of snakebites that occur in the Northeast region of the country are associated with this genus. *Bothrops erythromelas* and *Bothrops leucurus* species are of medical relevance in Northeast Brazil due to the incidence of Bothropic related snakebites in the region. The coagulotoxic mechanisms of *Bothrops* venom are known to cause hemostatic disorders, mainly due to the action of disintegrins, metalloproteases (SVMPs) and serine proteases (SVSPs). The only specific treatment against ophidic accidents is the antivenom serum. The antiothropic antivenom is prepared using the venom pool of five species of the genus, that does not contemplate *B. erythromelas* and *B. leucurus* venoms. In addition, there are few studies that characterize the composition and functional mechanisms of these two snake venoms in comparison with other snakes of the same genus, such as *Bothrops jararaca*. Therefore, the present study aims to characterize and investigate the mechanisms involved in hemostatic alterations caused by *B. erythromelas* and *B. leucurus* envenomation. For the following steps, pools of equal parts of venom from 10 individuals of each species, provided by Butantan Institute, with a concentration of 1 mg/mL of venom diluted in saline 0.9%, were used (SISGEN AC75E7B). The compositional profile of both venoms was achieved via SDS-PAGE and RPHPLC. The activities of toxins such as SVMPs, SVSPs and phospholipase A2 (PLA2) on casein, azocoll and NOBA substrate were evaluated as enzymatic profiling. Additionally, the coagulotoxicity of said venoms was assessed via minimum coagulant dose (MCD) on human plasma, thrombin-like and Kallikrein-like activities (on S-2238 and S-2302 substrates, respectively) and factor II, X and XII activation, using the substrates S-2238, S-2765 and S-2302, respectively. The assays related to enzymatic activity and coagulotoxicity were run in triplicate, and the results were first evaluated via spectrophotometry (except for MCD, that was executed via coagulometer). *B. erythromelas* and *B. leucurus* chromatographic profiles exhibited 8 and 12 major peaks, respectively, and the SDS-PAGE with 15% acrylamide concentration confirmed the compositional profiles obtained by HPLC. SVMP, SVSP and PLA2 activity were observed in both venoms, with the first two being higher in *B. leucurus*, and SVSP corresponded to approximately 10% of proteolytic activity on casein in both cases. Factor II, XII and X activation, and Kallikrein-like activity were also observed in both venoms, with the first two being higher in *B. erythromelas*, whereas thrombin-like was only quantifiable in *B. leucurus*. MCD assay indicated a more coagulant effect for *B. erythromelas*, even though the other species demonstrated a more coagulotoxic profile, indicating the need for further research regarding the mechanisms of action of *B. leucurus* and *B. erythromelas* venoms in hemostatic disorders.

09.004 Evaluation of the Toxicity of Venom from the Epidermal Mucus of the Catfish *Pterodoras granulosus* in the Alternative Zebrafish Model. Iwanichen J, Silva EM, Lima C, Ferreira MVAL IBu

Introduction: Fish represent the largest group of vertebrates, comprising 35,000 species, of which approximately 2,000 are venomous. In Brazil, catfish species are among the most abundant venomous fish that can cause accidents in humans, which commonly cause local effects, such as pain and edema. These fish species present a risk due to the venom present in their spines and toxins in the mucus covering their bodies. Despite the high frequency of these accidents, notably in fishers, there is a scarcity of studies on the pathophysiology and toxins involved, hindering prevention and treatment strategies. It is known that the catfish *Pterodoras granulosus*, also known as “armed”, is responsible for many of these injuries. However, the effects of the epidermal mucus, which acts as a protective dermal barrier in the fish, in experimental models are scarce. For this purpose, the zebrafish (*Danio rerio*) emerged as a promising model for toxicity research, following the principles of the 3Rs (reduction, replacement, and refinement) and supporting the elucidation of the adverse effects of fish toxins. Therefore, this project aims to evaluate the acute toxicity of the *P. granulosus* epidermal mucus using the zebrafish model during its embryo-larval phase. **Methods:** To this end, was provided by Dr. Emanuel Marques da Silva the mucus of the fish in 0.9% saline solution, quantified proteins by the Bradford protein assay (equivalent to the toxin content), determined their electrophoretic profile in an 8–16% Novex WedgeWell gel, evaluated the toxicity of 3, 6, and 12.5 µg/mL of mucus by the Fish Embryo Toxicity test (OECD 236) up to 96 hours post-fertilization (hpf), and the behavior analysis of 3 µg/mL at 72 hpf using the Zebrafish system. **Results:** The amount of protein recovered from the mucus of a pool of animals was 0.434 mg/mL. The electrophoresis showed that the most prominent bands are between 6–38 kDa. The toxicity test indicated increased mortality rates compared to the control, notably at 96 hpf for the concentrations of 3 and 12.5 µg/mL, where the mortality was 45 and 52.5%, respectively. In contrast, in the control, the mortality was 16.7%. All tested mucus concentrations also presented sublethal and teratogenic effects, manifested from 48 hpf in the lower concentration and 24 hpf in the two highest treatments. The most common developmental alterations recorded were yolk and pericardial edemas, developmental delay, uninflated swim bladder, curved tail, and scoliosis, which were dose-dependent and more prominent in the 12.5 µg/mL group. Locomotor analysis indicated that larvae exposed to the mucus had a significant decrease ($p < 0.0001$) in the distance moved (10,000 mm) compared to the control (190,000 mm). **Conclusion:** This is the first study evaluating the toxic effects of the *P. granulosus* epidermal mucus in zebrafish. Our work highlights the potential toxic effects of the mucus in the early development of the zebrafish, marked by increased mortality and sublethal effects in treated animals compared to controls. Moreover, the mucus considerably affects the ability of the larvae to move during the behavioral test. Altogether, the results point to a high toxicity of the *P. granulosus* mucus toxins and evidence that zebrafish can be efficiently used to test toxins derived from other species.

09.005 **Antiparasitic and Cytotoxic Potential of *Justicia secunda* Extract: Perspectives for the Control of *Schistosoma mansoni*.** Baptista B¹, Canossa IC¹, Fragelli BDL^{1,2}, Rodolpho JMA¹, Valentini MB³, Ataíde AMS⁴, Allegretti SM³, Silva CYY⁴, Silva MN⁴, Monteiro MC⁵, Anibal FF¹ ¹UFSCar, Lab of Inflammation and Infectious Diseases, Brazil, ²UFSCar, Center for the Development of Functional Materials, Brazil, ³UNICAMP, Dept Animal Biology, Brazil, ⁴UFPA, Lab of Liquid Chromatography, Brazil, ⁵INCT-PROBIAM-UFPA Pharmaceuticals Amazônia, Brazil

Schistosomiasis mansoni, caused by *Schistosoma mansoni*, affects 250 million people worldwide and remains a serious public health problem. Currently, it is treated exclusively with praziquantel, raising concerns about the emergence of resistant strains. In this context, there is a need to explore alternative treatments, such as herbal medicines containing promising bioactive compounds, including the ethanolic extract of *Justicia secunda* leaves, which possesses antioxidant, anti-inflammatory, antibacterial, and antiviral properties. This study aimed to evaluate the cytotoxicity of the ethanolic extract of *J. secunda* leaves and its potential antiparasitic effect in a murine model. In the *in vitro* assays, the extract was tested for its effect on cellular metabolic activity through mitochondrial function using the MTT colorimetric assay in NIH/3T3 fibroblast cells, at ten concentrations ranging from 7.8 to 2000 µg/mL. At 1000 µg/mL, cell metabolism viability was 47.28% ($p < 0.05$) compared to the negative control, while at 2000 µg/mL, viability was 4.39% ($p < 0.01$). Nitric oxide (NO) production was quantified using the Griess reaction, which measures nitrite ion levels based on a standard curve. At 2000 µg/mL, NO synthesis reached 31.38 mM ($p < 0.01$) compared to the negative control, indicating a potential induction of cellular oxidative stress. For the *in vivo* assay, Balb/c mice were infected with 80 *S. mansoni* cercariae and divided into four groups: negative control, positive control, praziquantel-treated, and extract-treated (*J. secunda*, 550 mg/kg, orally for 8 consecutive days). Efficacy was assessed by fecal egg count using the Kato-Katz method and recovery of adult worms by hepatic portal and mesenteric perfusion. The extract-treated group showed a 23.81% reduction in egg count compared to the positive control, while the praziquantel-treated group showed a 37.43% reduction. In worm count, the extract reduced the burden by 23.96% compared to the positive control, while praziquantel achieved a 55.76% reduction ($p < 0.05$). These results indicate that the extract exhibits cytotoxicity at high concentrations *in vitro*, but the tested dose *in vivo* was insufficient to produce a significant effect on egg production and worm recovery, suggesting that higher doses may be necessary. **Financial support:** São Paulo Research Foundation (FAPESP), Process No. 2024/21995-5 **CEUA:** 7393230924

09.006 **Antiproliferative Activity of Hinokinin in Luminal Breast Cancer: An *in silico* and *in vitro* Approach.** Santos DC¹, Nagata KS¹, Bertolin LR, Mello LP, Ferreira EA, Sciani JM¹, Longato GB¹ ¹USF, Lab of Natural Products, Graduate Program in Health Sciences, Brazil. ²Mackenzie Presbyterian University, School of Engineering, Brazil

Breast cancer is the most common type of cancer among women in Brazil. Among its subtypes, the luminal subtype is the most prevalent and is characterized by the overexpression of hormone receptors. Natural compounds with bioactive properties have been studied over time with the aim of causing fewer adverse effects and improving patients' quality of life. In this context, the lignan hinokinin, a natural product obtained from the leaves of the species *Hydrocotyle leucocephala*, has been described in the literature as having antiproliferative and anti-inflammatory activity without causing genotoxicity. This study aimed to investigate the interaction of hinokinin with estrogen receptors and its antiproliferative activity in the luminal breast cancer cell line (MCF7), both alone and in combination with the chemotherapeutic agent docetaxel. An in-silico study indicated that the molecule interacts with estrogen receptors (ER) alpha and beta, with a binding energy of -8 ΔG Kcal/mol, and showed that the molecule complies with all pharmacokinetic parameters of Lipinski's rules. Based on previous evidence of the antiproliferative activity of hinokinin in two-dimensional MCF7 cell cultures, the present study evaluated its behavior at concentrations of 10 μM , 20 μM , and 40 μM in three-dimensional cell cultures that more accurately mimic a tumor microenvironment. Subsequently, cell viability was measured using CellTiter-Glo and a luminescence reader. Hinokinin exhibited an IC_{50} of 25 μM and altered the tumor necrotic zone, supporting the previously observed antiproliferative activity. The combination of hinokinin with docetaxel showed a synergistic effect, reducing the IC_{50} of docetaxel from 18 μM to 0.3 μM , suggesting a potentially beneficial interaction in promoting antitumor activity. Additionally, results from the apoptosis assay supported this synergistic effect: compared to the negative control (DMSO), the combination of hinokinin and docetaxel led to a 29.75% reduction in cell viability and a 500% increase in the early apoptotic cell population, highlighting the impact of hinokinin in enhancing the chemotherapeutic response. In conclusion, hinokinin shows promising antiproliferative activity, induces cell death through apoptosis and has favorable hydrophilic-lipophilic balance, making it a good drug candidate. This molecule is predicted to bind to ER-alpha and ER-beta receptors, which are responsible for stimulating and inhibiting breast cell proliferation, respectively. **Financial support:** MackPesquisa 231010

09.007 *In vitro* Modulation of Oxidative Stress by a Topical Formulation Containing Red Propolis. Munhoz LLS¹, Pichinelli-Noronha JJ², De Sá, SG³, Severino AM⁴, Favaro-Trindade CS³, Ambrósio CE², Martins DS¹ ¹FZEA-USP, Lab. of Immunohistochemistry and Experimental Physiology, PPG Animal Biosciences, Brazil ²FZEA-USP, Lab. of Cell Culture and Gene Therapy, PPG Animal Biosciences, Brazil ³FZEA-USP, Lab. of Encapsulation and Functional Foods, PPG Food Engineering, Brazil ⁴ESALQ-USP, Multiuser Center for Biochemistry and Instrumental Analysis, Interunit PPG in Integrated Food Systems, Brazil

Introduction: The search for alternative treatments for wound healing, particularly in cases of burn injuries, has given rise to a surge of interest in natural products due to their biocompatibility and therapeutic potential. In this context, Olivem®1000, a commercial emulsifier derived from olive oil, and red propolis extract from Alagoas, Brazil (SISGEN no. A305815), have emerged as promising candidates for topical application. The present study was conducted with the objective of evaluating the *in vitro* modulatory effects of a topical formulation containing red propolis on cellular oxidative stress. **Methods:** L929 fibroblasts were cultivated and treated with formulations conditioned at 40 µg/mL (IC₅₀ of the red propolis extract). The cells were exposed for predetermined time intervals. Molecular analysis was performed by RT-qPCR to assess the expression of antioxidant-related genes (HO-1, GCLC, GCLM) after 24 hours. Biochemical assays conducted included the quantification of superoxide dismutase (SOD) activity at 12, 24, 48, and 72 hours, as well as lactate dehydrogenase (LDH) release at 24 hours. **Results:** These assays were used to evaluate membrane integrity and the potential for cellular toxicity. The LDH assay showed a substantial increase in enzyme release in both the OL (Olivem®1000 formulation) and OLRP (Olivem®1000 + red propolis formulation) groups in comparison to the untreated control group. The OLRP group exhibited the highest levels of enzyme release ($p < 0.05$), suggesting the potential cytotoxic effects associated with red propolis bioactives. Statistical analysis of SOD activity showed no statistically significant differences among the groups at any of the measured time points, suggesting that the enzymatic antioxidant pathway was not subject to modulation. At the molecular level, both OL and OLRP treatments significantly downregulated GCLM and HO-1 gene expression ($p < 0.001$). Furthermore, the GCLC expression was significantly reduced only in the OLRP group in comparison to the untreated ($p < 0.01$) and OL groups ($p < 0.05$). **Conclusion:** The present findings suggest that, despite the widespread recognition of red propolis for its antioxidant potential, its use in topical formulations at the IC₅₀ concentration may induce cytotoxic effects, as indicated by LDH release, and suppress key antioxidant genes. The lack of effect on SOD activity suggests that the responses occur predominantly at the transcriptional level. Overall, the data highlight the complexity of cellular responses to bioactive compounds and emphasize the importance of concentration-dependent evaluations. Further investigations, including dose-response studies and recovery assays, are required to optimize the formulation for safe and effective topical application. Ethical Committee: CEUA nº 4424210723 **Financial support:** Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), Brasil, Finance Code 001.

09.008 **Metalloproteases Phenotypic Diversity in *Bothrops jararaca* Venom.** Paulesini ES¹, Galizio NC¹, Vidueiros JP², Sant'Anna SS², Lima JAF³, Pimenta DC³ Gonçalves LRC¹, Morais-Zani K¹ ¹IBu, Lab of Pathophysiology; ²IBu, Lab of Herpetology; ³IBu, Lab of Biochemistry and Biophysics

Snake venoms are the most studied animal toxins. In Brazil, the *Bothrops* genus is responsible for 90% of snakebites, with their venom having three main activities: proteolytic or necrotizing, coagulant and hemorrhagic. The resulting clinical aspects of the envenoming from the bites of this genus can be characterized by important local manifestations that although rarely lethal, can cause severe damage, potentially leaving victims unable to work. As with other vipers, one of the main toxins of the venom are the metalloproteases (SVMP). These enzymes are involved in several pathophysiological processes, such as edema development, degradation of extracellular matrix components, hemorrhage, and depletion of factors of the coagulation cascade. In the *Bothrops jararaca* species, this toxin family corresponds to about 25% of the venom. However, in the present study, using high-performance liquid chromatography (HPLC), electrophoresis, and mass spectrometry (MALDI-TOF) for venom profiling, we observed that certain individuals of this species exhibit reduced levels of SVMPs or specific SVMP subclasses, which could imply variability in the pathophysiology of envenomation. Based on SVMP abundance, 54 *B. jararaca* individuals were classified into four venom phenotypes: (1) typical SVMP profile, (2) low total SVMP abundance, (3) low abundance of SVMP-PI, and (4) low abundance of SVMP-PIII. As expected, *in vitro* proteolytic assays showed reduced activity in venoms with lower SVMP-PIII levels, particularly in type II and type IV collagenolytic assays (azocoll and IV collagen–fluorescein conjugate). Interestingly, clotting time in human plasma incubated with a Minimum Coagulant Dose (2 µg/mL) of venom exhibited marked individual variability, which was not correlated with SVMP content. This individual variation did not seem to impact recognition by the antivenom produced by the Butantan Institute, as demonstrated by western blot analysis. Similarly, no significant differences were observed in hemorrhagic potential among the phenotypes when measuring the hemorrhagic halo induced by 0.5 µg of venom, an interesting finding, given that SVMPs, especially SVMP-PIII, are traditionally considered the main toxin responsible for hemorrhage. The same was observed in an edematogenic assay measuring the formation of edema in mice paw using 2 µg of venom. We hope our work may contribute to a better understanding of how these toxins, even in lower-abundance, might still play key roles in envenoming or how they may synergize with other toxins present in the venom, besides highlighting possible isoforms of these SVMPs that may have pharmacological or biotechnological potential.

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09.009 Omega-3 Fatty Acid Nanoemulsion Attenuates Inflammatory and Myotoxic Effects Induced by *Bothrops asper* Venom in Mice. Almeida CX¹, Lima RO¹, Lemos G¹, Calmasini FB¹, Escalante T², Purgatto E³, Moreira V¹ ¹Unifesp-EPM, São Paulo, PPG Farmacologia, Brasil, ²Universidade da Costa Rica, Faculdade de Microbiologia, Instituto Clodomiro Picado, Costa Rica, ³FCF-USP, PPG Sistemas Integrados em Alimentos, Brasil

Accidents caused by *Bothrops asper* snake venom (Bav) lead to severe clinical outcomes, including both local and systemic damage. Locally, Bav induces intense inflammation, edema, hemorrhage, and necrosis, while systemic effects are mainly characterized by myotoxicity, coagulopathy, and cardiovascular alterations. Although antivenom therapy is effective in neutralizing systemic effects when administered promptly, it has limited efficacy in controlling local tissue damage, highlighting the need for complementary treatments. In this context, omega-3 polyunsaturated fatty acids (PUFAs), particularly EPA and DHA, are known for their anti-inflammatory, cardioprotective, and immunomodulatory properties, making them promising as supportive therapy for Bav-induced toxicity. This study evaluated the protective effect of a fish oil nanoemulsion (FON), rich in EPA and DHA, on local (leukocyte recruitment) and systemic (cardiac injury and smooth muscle dysfunction) alterations induced by Bav in mice. Male Swiss mice (8 weeks old – CEUA 3995011223) were intraperitoneally (i.p.) injected with Bav (2.5 mg/kg) or saline. One hour later, they received FON (6.0 µg/g DHA and 10.2 µg/g EPA, i.p.) or saline. On days 7 and 14 post-envenomation, peritoneal exudate, blood, and bladder tissues were collected. Total leukocyte counts in the exudate were determined using a Neubauer chamber. Plasma CK-MB levels, a marker of cardiac injury, were measured with a commercial kit. Bladder detrusor muscle (BDM) contractility was assessed *in vitro* in response to cumulative concentrations of carbachol (1 nM–10 µM). Bav+FON mice showed a significant reduction in leukocyte influx ($1.1 \pm 0.1 \times 10^6$ cells/mL; $p < 0.05$) compared to Bav+Sal ($2.7 \pm 0.3 \times 10^6$ cells/mL). CK-MB levels were elevated in Bav+Sal mice (1769 ± 39 U/L) but were significantly reduced in Bav+FON (1120 ± 49.2 U/L; $p < 0.05$), suggesting cardiac protection. BDM contractility was reduced by 65% in Bav+Sal mice at day 14, whereas Bav+FON animals showed a 26% improvement ($p < 0.05$), indicating partial functional recovery. In conclusion, treatment with omega-3 fatty acids via fish oil nanoemulsion reduced both local leukocyte migration and systemic markers of myotoxicity and muscle dysfunction in Bav-injected mice. These findings support the use of EPA and DHA as adjunctive agents in the treatment of *Bothrops asper* envenomation, offering protection beyond that provided by conventional antivenom therapy.

09.010 Cytotoxicity of Lysine/Arginine-Substituted M-pontx-dq3a [1–15] Analogues and Effect Against *Trypanosoma cruzi* Y Strain. Magalhães EP¹, Edson EA², Martins BVB¹, Reis MP³, ¹Silva FRC, da Silva Júnior PN¹, Sampaio TL^{1,3}, Oliveira VX⁴, Menezes RRPPB^{1,3}, Martins AMC^{1,2,3} ¹UFC, PPG in Pharmaceutical Sciences, Brazil. ²UFC, PPG in Development and Technological Innovation in Medicines, Brazil. ³UFC, Clinical and Toxicological Analysis Dept, Brazil, ⁴UFABC, Natural and Human Sciences Center, Brazil

Chagas' disease (CD), caused by *Trypanosoma cruzi*, is endemic in South and Latin America. Benznidazole (Bz) is the only available treatment, with limited efficacy and known toxicity. So, there is a need to develop new effective and safe antichagasic drugs. Dinoponeratoxins (DNTXs) is a group of antimicrobial peptides (AMP) whose trypanocidal effect was described. Among the DNTXs, M-PONTX-Dq3a is promisor peptide. AMP presents physicochemical and structural properties that can be modulated, leading to compounds with enhanced biological properties. So, M-PONTX-Dq3a peptide was fragmented and tested. The M-PONTX-Dq3a [1–15] fragment presented better anti-*T. cruzi* effect, when compared to parental peptide. So, this study aims to synthesize and evaluate cytotoxicity and trypanocidal effect of M-PONTX-Dq3a [1-15] analogues substituted with lysine (K) or arginine (R). [K³,K⁴]; [K³,R⁴]; [K³,R⁷] and [K³,R¹⁰] M-PONTX-Dq3a [1-15] analogues were synthesized in solid-phase (Fmoc), and physicochemical properties (Liquid charge – z; hydrophobicity – H, and hydrophobic moment – μ H) were calculated in Heliquest and Pepcalc servers. Cytotoxicity was assessed in LLC-MK2 cells (10⁵ cells/mL) treated during 24 hours with peptides (50 – 0.78 μ M) through MTT assay, being calculated percentual of viable cells and CC₅₀ (concentration able to reduce cell viability in 50%). After, trypanocidal effect was measured in trypomastigote forms (10⁶ parasites/mL), obtained by infection of host cells, after incubation with peptides (50 – 0.78 μ M). The percentage of viable parasites was determined in Neubauer's chamber, with calculation of LC₅₀ (concentration that kill 50% of trypomastigote forms). Selectivity index (SI = CC₅₀/LC₅₀) was calculated. Bz was used as the reference drug. Experiments were performed in triplicate (n=3), results expressed as mean $\pm$ standard error mean, and compared by One-way ANOVA (Dunnet's post-test). Among the peptides tested, [K³,K⁴] M-PONTX-Dq3a [1-15] was the least cytotoxic. In trypomastigote forms, all peptides reduced parasites viability between 50 and 3.0 μ M, with LC50 values of 0.14 [K³,K⁴]; 3.76 $\pm$ 1.09 [K³,R⁴]; 9.07 $\pm$ 2.3 [K³,R⁷] and 2.80 $\pm$ 0.65 [K³,R¹⁰] (Bz LC50 = 161.40 $\pm$ 31.80). Furthermore, [K³,K⁴] M-PONTX-Dq3a [1-15] presented as the most selective peptide (SI > 133), followed by [K³,R¹⁰] (SI = 11.48); [K³,R⁴] (SI = 6.19), and [K³,R⁷] (SI = 5.22) (Bz SI = 3.10). In conclusion, M-PONTX-Dq3a [1-15] analogues showed with higher trypanocidal effect and selectivity than the reference drug, highlighting the [K³,K⁴] M-PONTX-Dq3a [1-15] compound, a promisor biotechnological tool in the context of antichagasic drug development. Authors thank CNPq, CAPES and FUNCAP for their financial support.

09.011 **Gamma-Oryzanol as a Natural Modulator of Hepatic Lipid Metabolism Caused by Obesogenic Diet.** Siqueira JS¹, Palacio TLN¹, Cruzeiro J¹, Garcia JL¹, Aiello G², D'Amato A², Correa CR¹ ¹Unesp-Botucatu, Medical School, Graduate Program in Pathology, Brazil ²University of Milan, Dept Pharmaceutical Sciences, Doctoral Program in Pharmaceutical Sciences, Italy

Introduction: Obesogenic diets, rich in sugar and fat, increase the hepatic influx of lipids, leading to the accumulation of fatty acids and triacylglycerol (TG). This lipid overload is positively associated with hepatic steatosis, inflammation, oxidative stress, mitochondrial dysfunction and insulin resistance. Gamma-oryzanol (ORY), a bioactive compound naturally found in rice bran oil, has emerged as a promising candidate for preventing obesity-related disturbances. **Objective:** This study aimed to investigate the protective effect of ORY on biological processes and diseases associated with hepatic proteomic changes in rats fed a high sugar–fat diet (HSF). **Methods:** Male Wistar rats were fed a control (CTRL), HSF, or HSF+ORY (0.5% w/w) diet for 30 weeks. Liver samples were analyzed by label-free quantitative proteomics. Ingenuity Pathway Analysis (IPA) was used from the differentially expressed proteins to predict the most significantly modulated biological pathways and disease functional modules based on protein interaction networks. In parallel, biochemical parameters were also assessed: hepatic and plasma TG (mmol/g liver and mg/dL), HOMA-IR, malondialdehyde (MDA, nmol/mg protein), carbonylated proteins (CBO, nmol/mg protein), TNF- α , and IL-6 (pg/g protein). The differences among the experimental groups were determined by a one-way ANOVA followed by Tukey's post-hoc test, considering a α of 5%. **Results:** Label-free quantitative proteomics identified 2,194 proteins in the liver. HSF feeding significantly increased TG (hepatic and plasma), MDA, CBO, TNF- α , IL-6, and insulin resistance ($p < 0.05$). Proteomic analysis predicted activation of lipid flux ($z = 1.320$), fatty acid concentration ($z = 1.096$), TG concentration ($z = 1.105$), hepatic steatosis ($z = 1.547$), oxygen consumption ($z = 2.159$), mitochondrial dysfunction ($z = 1.342$), oxidative stress ($z = 1.455$), glycolysis ($z = 2.588$), and insulin resistance ($z = 0.592$). Conversely, pathways such as fatty acid beta-oxidation ($z = -1.038$), glutathione conjugation ($z = -2.441$), and HDL concentration ($z = -1.387$) were suppressed. The IPA results suggest that ORY treatment preserved these alterations ($p < 0.05$), with predicted downregulation of cholesterol ($z = -0.545$), fatty acids ($z = -0.364$), TG ($z = -0.716$), oxygen consumption ($z = -2.063$), oxidative stress ($z = -0.447$), glycolysis ($z = -1.941$), and inflammation ($z = -1.387$). These predictions were corroborated by biochemical analyses, in which ORY significantly reduced TG (hepatic and plasma), MDA, CBO, TNF- α , IL-6, and insulin resistance ($p < 0.05$). **Conclusion:** These findings support the potential of gamma-oryzanol as a natural therapeutic agent capable of modulating hepatic lipid metabolism, mitochondrial function, and inflammatory responses resulting from obesogenic diet consumption. **Ethics Committee Approval:** 1451/2024. **Financial support:** FAPESP (2022/16108-4); CAPES (88887.891854/2023-00).

09.012 Screening of Snake Venoms for the Identification of Possible Novel Therapeutical Targets and Toxins with Schistosomicidal Potential.

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Schistosomiasis is a neglected tropical disease caused by flatworms of the genus *Schistosoma*. Treatment of the disease relies only on a partially effective drug, praziquantel. Therefore, new therapeutic alternatives are needed. Snake venoms have lethal activity against different organisms, ranging from bacteria to higher vertebrates. Several venoms have been shown to be active against protozoa, however, data on the activity of venoms against helminths are scarce. In the search for new antischistosomal compounds, we tested here the *in vitro* effects of crude venoms from eight *Bothrops* snake species, from *Crotalus d. terrificus* and venom fractions against two developmental stages of *Schistosoma mansoni*: post-infectious larvae (schistosomula) and adults. We obtained adult *S. mansoni* worms by perfusion of infected hamsters (CEUAIB approved protocol 8859090919). We monitored mortality, motility, pairing rates and oviposition of adult worms over 72 h of incubation with the venoms. Furthermore, we assessed the viability of adult and schistosomula worms by ATP quantification. We used scanning electron microscopy (SEM) to examine morphological changes after incubation with *B. jararacussu* venom and EdU assays to measure the effect of venom against parasite stem cells proliferation. We subjected *B. jararacussu* venom to size-exclusion chromatography and analyzed the effects of protein fractions on parasite viability. We found that adult worms incubated with each venom at concentrations of 100 and 50 µg/mL showed a reduction in parasite viability, motility, pairing, and egg laying. Schistosomula incubated with each venom at concentrations of 3.12, 6.25, 12.5, 25, and 50 µg/mL showed a significant dose and time-dependent decrease in viability. Adult and schistosomula showed the most significant changes after exposure to *B. jararacussu* venom, which was therefore chosen for further assays. *B. jararacussu* venom resulted in tegumental damage of adult worms, as shown by SEM. EdU assays revealed a reduction in worm stem cell proliferation when incubated with *B. jararacussu* venom. RNA-Seq revealed that male adults incubated with *B. jararacussu* venom presented alterations in genes related to lipid metabolism and protein kinases pathways. Seven fractions of *B. jararacussu* venom were investigated for antischistosomal activity. After incubation of schistosomula and adult worms with each fraction at 25 or 50 µg/mL, we observed that fraction 4 significantly reduced the viability of these parasites. In conclusion, the anthelmintic activity of snake venoms and their components against *S. mansoni* is promising, justifying further investigations.

09.013 **Evaluation of Cytotoxicity and Mechanisms of Anti-Inflammatory Action of the Hydroethanolic Extract from *Fridericia chica* Leaves in *in vitro* Assays.** Figueiredo FF¹, Arunachalam K¹, Cruz TCD², Muller JAI¹, Serio BFD¹, Martins DTO¹ ¹UFMT, Pharmacology Area, Graduate Program in Health Sciences, Brazil, ²UFMT, Virology Area, Graduate Program in Health Sciences, Brazil

Introduction: *Fridericia chica* (Bonpl.) L.G. Lohmann (Bignoniaceae) is a native, non-endemic climbing plant found across all Brazilian biomes. In Mato Grosso, it is popularly known as *crajiuru*. The leaf infusion is traditionally used to treat various ailments, particularly inflammation, ulcer and wound healing, and infections [1]. Previous studies have isolated flavonoids, anthocyanidins, and phytosterols from its leaves. The hydroethanolic extract of *Fridericia chica* leaves (EHFc) has demonstrated anti-inflammatory, anti-ulcer, and antimicrobial activities, among others, with low toxicity in *in vivo* and *in vitro* assays. **Objective:** To evaluate the cytotoxicity and potential anti-inflammatory mechanisms of EHFc in *in vitro* experimental models. **Methods:** EHFc was obtained by macerating leaf powder in 70% ethanol. Cytotoxicity was assessed in CHO-K1 and RAW 264.7 cells using the Alamar blue assay. To investigate anti-inflammatory mechanisms, RAW 264.7 cells were stimulated with LPS (1 µg/mL), followed by indirect measurement of nitric oxide (NO) levels and quantification of pro- and anti-inflammatory cytokines. **Results:** EHFc exhibited no cytotoxicity in CHO-K1 and RAW 264.7 cells (IC₅₀ > 400 µg/mL). The extract significantly ($p < 0.001$) reduced nitrite levels at all tested concentrations, with the highest inhibition (47.9%) observed at 5 µg/mL. EHFc (1, 5, and 20 µg/mL) also markedly decreased IL-1 β levels by 45.5%, 54.3%, and 55.6%, respectively ($p < 0.001$). For TNF- α , reductions of 10.7% ($p < 0.01$) and 6.8% ($p < 0.05$) were observed at 1 and 5 µg/mL, respectively. Additionally, EHFc increased ($p < 0.05$) IL-13 levels only at the lowest concentration (1 µg/mL). **Conclusions:** The anti-inflammatory activity of EHFc involves inhibition of NO production and modulation of cytokine release. **Financial support:** INAU/CNPq (Grants: 421733/2017-9 and 381529/2024-0), PROAP/CAPES, and CNPq (Grant: 314936/2021-1). **Reference:** [1] Freitas Figueiredo, F., Damazo, A.S., Arunachalam, K., Silva, M.J.D., Pavan, E., da Silva Lima, J.C. and de Oliveira Martins, D.T., 2023. Evaluation of the gastroprotective and ulcer healing properties by *Fridericia chica* (Bonpl.) LG Lohmann hydroethanolic extract of leaves. Journal of Ethnopharmacology, 309, p.116338.

09.014 Apigenin Administration Protects Against Obesity-related Muscle Atrophy but Fails to Avert Obesity in Middle-aged Rats Treated with a High-Calorie Diet. Stein DJ^{1,2}, de Castro JM^{1,2}, Melo AS^{1,2}, Silveira BL^{1,2}, Martins IAS^{1,2}, Marçal MM^{1,2}, Dal Bosco T^{1,2}, Keingeski MB^{3,4}, de Oliveira ECL^{3,4}, Alvares-da-Silva MR^{3,4}, Ayres LR^{5,6}, Kowalewski LS^{5,6}, Russo MKB^{5,6}, Krause M^{5,6}, Torres ILS^{3,4} ¹HCPA, Lab Pain Pharmacology and Neuromodulation: Preclinical Investigations, ²UFRGS, Postgraduate Program in Medicine: Medical Sciences, Faculty of Medicine, ³UFRGS, Postgraduate Program in Gastroenterology and Hepatology, Porto Alegre, Brazil ⁴HCPA, Experimental Lab Hepatology and Gastroenterology, ⁵UFRGS, Lab Inflammation, Metabolism and Exercise, and Lab Cellular Physiology, Dept Physiology, ⁶UFRGS, Postgraduate Program in Human Movement Sciences, School of Physical Education, Physiotherapy and Dance

Introduction: The increasing prevalence of obesity in the aging population is associated with greater morbidity and mortality, making it a global health concern. In this sense, flavonoids that increase NAD⁺ levels, such as apigenin (Api), are advocated for the treatment of this condition. Aim: To provide preliminary evidence on the effects of Api administration in middle-aged rats induced to obesity via a high-calorie diet (HCD). **Methods:** Forty-seven male Wistar rats (15 months old) were assigned to 5 groups according to body weight, high-calorie diet (HCD) or standard diet, and treatment with Api or vehicle. Obesity was induced throughout 88 days of exposure to an HCD associated with a daily dose of Api (50 mg/kg, oral). Blood, muscle, and adipose tissue were collected for analysis of adiposity, skeletal muscle tissue, and biochemical markers. Project approved by CEUA/HCPA under #2021.0208. **Results:** After an 88-day HCD, rats exhibited increased body weight gain (~14.3%), higher visceral and subcutaneous fat (~28.0 and ~38.9%, respectively), hyperglycemia (~17.5%), and hypertriglyceridemia (~15.5%). In the gastrocnemius, HCD-fed rats exhibited muscle loss (~13.3%) and tissue fibrosis (P<0.05), collectively suggesting sarcopenia. Preemptive Api treatment did not prevent parameters associated with obesity, nor did it influence genes related to the browning of subcutaneous adipose tissue. Conversely, Api administration prevented hypertriglyceridemia and muscle loss triggered by obesity. Still on skeletal muscle, Api increased total p70S6K levels (~56.1%; P<0.05), a key enzyme for muscle protein synthesis, thereby sustaining cell anabolism and counteracting tissue atrophy. **Conclusion:** The current results indicate that Api may successfully address age- and obesity-related sarcopenia in obese middle-aged rats, notably through p70S6K anabolic signaling. These findings encourage future research on the muscular effects of Api in experimental contexts of obesity and aging.

09.015 Colilen IBS® Attenuates Inflammation and Alleviates Allodynia in DSS-induced Colitis in C57BL/6 Mice. Santos LBS, Brito MSC, Rech KS, Martins FG1, Werner MFP UFPR

Introduction: Colilen IBS® is a phytomedicine containing polysaccharides from *Aloe vera*, resins from *Boswellia serrata*, and polyphenols from *Matricaria chamomilla* and *Melissa officinalis*. It is indicated for the treatment of irritable bowel syndrome. This study investigated the therapeutic potential of Colilen IBS® in a murine model of colitis. **Methods:** Colitis was induced in male and female C57BL/6 mice (25g) using 3% Dextran Sulfate Sodium (DSS). Animals were treated orally with vehicle (CMC, 1mL/kg) or Colilen IBS® 500 mg/kg for 7 days. The disease activity index (DAI) was monitored daily by assessing parameters such as weight loss, blood presence, and stool consistency. Mechanical allodynia was assessed using von Frey filaments (0.008 - 0.16 g), and animal welfare was analyzed through the nest-building test. On day 8, mice were euthanized, and their colons were collected for myeloperoxidase (MPO) assay. **Results:** On days 7 and 8, the male DSS group exhibited weight loss of 6.8% and 16.6%, respectively, while in females, the reduction on day 8 was 10.9%. The DSS group also showed increased DAI in both males and females on day 8, reaching scores of 7.6 ± 0.7 for males and 6.3 ± 0.4 for females. Treatment with Colilen IBS® was unable to effectively prevent weight loss and DAI increase. DSS increased mechanical allodynia in males from day 4 (181.8%) and in females on day 8 (225.8%) in response to 0.07g filament, compared to the control group ($27.5 \pm 7.5\%$ and $22.5 \pm 12.8\%$, respectively). In male mice, Colilen IBS® reduced allodynia from day 4 (74.2%) compared to the DSS group ($77.5 \pm 7.9\%$). In females, the antiallodynic effect was observed only on day 8 (57.2%) compared to the DSS group ($73.3 \pm 11.4\%$). Moreover, DSS shortened colon length by 30.9% and 27.3% in males and females, respectively, compared to the control group (9.7 ± 0.2 and 9.9 ± 0.2 cm), but Colilen IBS® did not prevent this change. Regarding animal welfare, the male DSS group showed a 58.0% decrease in nest formation compared to the control group (5.0 ± 0.0), while the female DSS group showed no differences compared to controls. DSS increased colonic MPO levels by 172.9% and 198.5% in males and females, respectively, compared to the control group (10.6 ± 1.6 and 6.9 ± 0.9 O.D./mg of protein). This increase was reduced by 68.8% in males and 49.0% in females following Colilen IBS® treatment compared to DSS (28.9 ± 2.1 and 10.6 ± 1.9 O.D./mg of protein). **Conclusion:** The experimental colitis model was successfully induced in both sexes, with the development of clinical and behavioral symptoms. DSS reduced nest-building behavior in males but not in females. Colilen IBS® prevented the increase in MPO levels in both sexes and was more effective in reducing mechanical allodynia in males compared to females. Taken together, these findings suggest that Colilen IBS® may have significant anti-inflammatory and analgesic effects and demonstrate that the development of DSS-induced colitis symptoms in male and female C57BL/6 mice presents temporal differences. **Financial support:** CAPES-Finance Code 001; CNPq 308816/2023-4.

09.016 Malvidin's Activity in Dextran Sulfate Sodium (DSS)-Induced Inflammatory Bowel Disease.

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Introduction: Inflammatory bowel diseases (IBD), including ulcerative colitis (UC) and Crohn's disease (CD), affect millions worldwide and show a high incidence in Brazil. Although UC and CD differ in anatomical localization and clinical features, both share chronic mucosal inflammation driven by genetic, hormonal, and immunological factors. Pro-inflammatory cytokines and reactive oxygen species are central to IBD pathogenesis. Standard therapies often have limited efficacy and significant adverse effects, prompting exploration of alternative agents. Phenolic anthocyanidins such as malvidin exhibit potent anti-inflammatory and antioxidant effects in preclinical gastrointestinal models. Malvidin, a predominant anthocyanidin in purple fruits, thus emerges as a promising candidate for IBD treatment. This study aimed to assess the reparative potential of malvidin in a dextran sulfate sodium (DSS)-induced colitis model. **Objective:** To evaluate the reparative activity of malvidin in a dextran sulfate sodium (DSS)-induced murine model of inflammatory bowel disease in male C57BL/6 mice. **Methods:** Male C57BL/6 mice (8 weeks old, n = 20) were allocated to four experimental groups. Colitis was induced in ten animals by administration of 2.5% DSS in drinking water for five days. Subsequently, mice received oral gavage of either vehicle (water, 10 mL/kg) or malvidin (5 mg/kg) for seven consecutive days. Control groups (n = 10) were treated with vehicle or malvidin alone over the same period. Animals were monitored daily to calculate the Disease Activity Index (DAI) based on body weight loss, stool consistency, and fecal occult blood. On day 13, animals were euthanized, and colonic length was measured. Fecal specimens were collected to quantify lipocalin-2 (Lcn-2) by ELISA. Data were analyzed by one-way ANOVA with Dunnett's post hoc test, and significance was set at $p < 0.05$. All procedures were approved by the University of Calgary Animal Care Committee (protocol AC21-0152). **Results:** Malvidin treatment significantly prevented DSS-induced colon shortening compared to vehicle ($p < 0.05$). Fecal lipocalin-2 concentrations were also markedly reduced in the malvidin group, whereas DAI scores did not differ significantly among groups. **Conclusion:** Oral administration of malvidin accelerated recovery from DSS-induced colitis in male mice, primarily through attenuation of inflammatory biomarkers. Further investigations are warranted to elucidate the molecular mechanisms underlying these protective effects. **Keywords:** Malvidin. Inflammatory Bowel Disease. Inflammation. **Financial support:** FAPESP and CNPq. **Ethical Committee:** University of Calgary Animal Care Committee, protocol number AC21-0152

09.017 **Evaluation of Hematological and Biochemical Effects of Apitoxin and Melittin** Vila JRM¹, Oliveira AMB¹, Leonor MGR¹, Misaca RC¹, Silva GSA¹, Dallazen JL¹, Santoro ML², Picolo G¹ ¹IBu, Lab Pain and Signaling, São Paulo, Brazil; ²IBu, Central Animal Facility, São Paulo, Brazil

Introduction: Chronic pain is a multifactorial disease that affects 20% of the worldwide population, and is one of the leading causes of suffering, since it interferes with daily routine. Although several pharmacological treatments exist, none fully reverse the condition and may cause side effects. Due to this fact, the search for new analgesic compounds or strategies to control chronic pain is essential. The use of toxins as a source of new analgesic compounds occurs since antiquity and among these toxins, the use of apitoxin and its main peptide, melittin, is reported. Results from our group confirmed the potential of both apitoxin and melittin, when applied as pharmacopuncture, in the control of chronic pain and also in the delay of its onset. However, toxicity has been described for both compounds. Based on that, the aim of this study was to evaluate the possible toxic effect of apitoxin or melittin over whole blood and plasma samples, when applied by this technique. **Methods:** Pharmacopuncture, a technique where the administration of pharmacologically active substances is performed at acupoints, was performed using apitoxin (1 mg) or melittin (0.6 mg), administered subcutaneously at the E36 acupoint (located approximately 1 mm lateral to the tibial tuberosity, 0.5 mm above the medial malleolus, with the needle inserted a depth of approximately 0.5 mm) or intraperitoneally, allowing comparison between administration routes. Animals underwent five treatment sessions over a 7-day period. To evaluate the potential hematological toxicity of the compounds, blood samples were collected from the mandibular gingiva of the rats (CEUA Nº 7218300924) at baseline and 7 days after each treatment. On each collection day, a complete blood count (CBC) was performed to detect changes in blood cells suggestive of hemolysis. Additionally, plasma from the collected blood was analyzed for biochemical parameters. The resulting CBC data were analyzed using a Two-Way Repeated Measures ANOVA (One Factor Repetition) in GraphPad Prism software, comparing treated animals to the Naive group by collection day. **Results and Conclusion:** Regarding the CBC, the statistical analyses indicated significant differences at specific points in the groups treated with intraperitoneal administration however, since these differences did not persist in the following days, which would be expected given the continuation of the treatment, it is possible to conclude that the changes observed throughout the study are not indicative of chronic hemolysis resulting from the treatment. Furthermore, the melittin acupoint group showed no significant variations compared to the naive group, suggesting that this treatment route did not impact the analyzed parameters of these animals. Additionally, no changes were observed in the biochemical parameters analyzed. In conclusion, the injection of Apitoxin or Melittin in the acupoint demonstrates to be a safe and attractive therapy by the absence of hematological and biochemical changes. **Supported by:** FAPESP/CETICS (2013/07467-1), FAPESP (2024/04901-7) PIBIC Program/CNPq, Butantan Foundation

09.018 **Determination of the Effective Dose for Neutralizing the Coagulant Activity of *Bothrops matogrossensis* Snake Venom by Antivenom.** Carneiro IB¹, Galizio NC¹, Sousa EP¹, Rodrigues MAF¹, Felipe AGC¹, Sant'anna SS², Morais-Zani K^{1,2} ¹IBu, Lab Fisiopatologia; ²IBu, Lab Herpetologia

Introduction: Snakebite envenomation is a significant global public health issue and is classified by the WHO as a Neglected Tropical Disease. Annually, up to 2.7 million cases are reported, with approximately 138,000 deaths. In Brazil, over 32,000 cases were recorded in 2023, with around 86% caused by *Bothrops* snakes. These venoms are complex mixtures containing toxins such as metalloproteases, serine proteases, phospholipases A₂, and L-amino acid oxidases, which produce severe local and systemic effects. Antivenom therapy is the only specific and effective treatment, produced in Brazil by public institutions like the Butantan Institute. *In vitro* studies have shown that certain toxic activities, such as PLA₂, SVMP, and SVSP, require doses much higher than those recommended to achieve partial neutralization. **Objectives:** This work aims to determine the effective dose (ED₅₀) of antiothropic antivenom for *in vitro* neutralization of the coagulant activity of *B. matogrossensis* venom. **Methods:** Venom protein profile was analyzed by 15% SDS-PAGE; the coagulant activity of *B. matogrossensis* venom was assessed through the determination of its Minimum Coagulant Dose (MCD) and subsequently the ED₅₀ of antiothropic antivenom for neutralization of this activity was also determined. **Results and Discussion:** The electrophoretic profile of *B. matogrossensis* showed a band composition typical of *Bothrops* venoms. The MCD was determined to be approximately 3.77 µg/mL, inducing clotting of human plasma in 60 seconds. A double MCD (7.54 µg/mL) reduced the clotting time to 24.9 seconds. Serum neutralization assays revealed that 81.88 µg of antivenom per µg of venom was required to triple the clotting time, corresponding to 10.86 µg of antivenom per µg of venom (ED₅₀). Given that the antivenom used contains 26 µg of protein per µL, this ED₅₀ is about twice the neutralization capacity stated by the manufacturer for the neutralization of the lethal activity of *B. jararaca* venom. The observed *in vitro* requirement suggests lower efficacy in neutralizing coagulant activity compared to systemic lethality.

09.019 Effect of Bergamot Leaf Extract on Renal Antioxidant System and Proteinuria of Obese Rats.

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Introduction: The chronic consumption of high sugar-fat (HSF) diets is one of the main contributors to the development of obesity in the 21st century. One of the key consequences of obesity is oxidative stress (OS), a condition characterized by reduced antioxidant defense. OS plays a central role in the progression of kidney damage, starting with proteinuria. Natural compounds with antioxidant properties have been studied to increase antioxidant capacity and protection against OS. Among them, Citrus bergamia, commonly known as bergamot, has emerged as a promising candidate. Notably, extracts obtained from bergamot leaves have shown a higher concentration of flavonoids compared to other parts of the plant, suggesting they may represent a promising treatment strategy. **Objective:** To investigate the effect of bergamot leaf extract on the renal antioxidant system and proteinuria in obese rats. **Methodology:** Male Wistar rats were randomized into two groups: Control (C; n = 10) and High Sugar and Fat diet (HSF; n = 20). After 20 weeks, obesity induction was confirmed by measuring body weight (g) and triglycerides levels (TG, mg/dL). The HSF group was then redistributed into two groups (n=10): HSF and HSF supplemented with bergamot leaf extract (HSF+Lf). The supplementation of bergamot leaf (50mg/kg) was administered daily by gavage for 10 weeks. At the end of 30 weeks the following parameters were analyzed: caloric intake (kcal/day), adiposity index (AI, %), the activity of the antioxidant enzymes superoxide dismutase (SOD, U/mg protein/minute) and catalase (CAT, pmol/g protein/minute), as well as proteinuria assessed by the protein/creatinine ratio (PROT/CREAT). Statistical analyses included Student's t-test ($\alpha = 0.05$). **Results:** At week 20, a significant difference in TG levels was observed between groups ($p < 0.001$), with higher levels detected in the HSF group. After 10 weeks of treatment, completing 30 weeks of experiment, no significant differences between the two groups were found in caloric intake ($p = 0.921$) or adiposity index ($p = 0.153$). However, significant increases were observed in antioxidant enzymes activity in the HSF+Lf group compared to the HSF group: SOD activity (U/mg protein/min; HSF: 72.87 ± 12.60 vs. HSF+Lf: 86.34 ± 15.45 ; $p = 0.032$) and CAT activity (pmol/g protein/min; HSF: 61.42 ± 12.97 vs. HSF+Lf: 75.19 ± 17.98 ; $p = 0.049$). Furthermore, a significant difference was found in PROT/CREAT between the two groups (HSF: 17.46 ± 3.64 vs. HSF+Lf: 13.86 ± 2.96 , $p = 0.017$). **Conclusion:** Supplementation with bergamot leaf extract increased renal antioxidant enzyme activity and decreased proteinuria in obese rats. These results suggest benefits of bergamot leaf against renal obesity-related oxidative stress. **Financial support:** FAPESP (2025/01524-0).

09.020 Ability of Suramin to Antagonize Some Activities of American Rattlesnake Venoms. Rocha-Junior JRS¹, Nogueira-Souza PD¹, Pinheiro AN¹, Gomes da Costa PI¹, Strauch MA², Melo, PA¹ ¹ICB-CCS-UFRJ, Lab Farmacologia das Toxinas, Rio de Janeiro, RJ, Brazil; ²ICBS-UFRRJ, Depto de Ciências Fisiológicas, Seropédica RJ, Brazil

Introduction: Snakebite is a worldwide public health problem, including American continent. The recommended treatment is the specific serotherapy that in some cases are low effective in neutralizing tissue inflammatory process that involves phospholipase and proteolytic activities. Suramin is a polysulfonated naphthyl urea and a polyanion that has been described in literature with various abilities against venom enzyme activities, as well tissue damage. Rattlesnakes across the American continent is involved in snakebites among humans and animals. These venoms contain crotoxin, a phospholipase A2 component, and other active enzymes that induce tissue degradation, angiorrhesis and edema. These effects are ascribed to the direct action of venom enzymes on the substrates which are the snake venom target, resulting in tissue damage. **Objective:** The present project aims to test the ability of suramin to inhibit phospholipase, hyaluronidase and proteolytic activities of some crotalid rattlesnake venoms on *in vitro* assay protocols. **Methods and Results:** In the phospholipase A2 assay, five different venoms: *Crotalus durissus terrificus*, *Crotalus viridis viridis*, *Crotalus atrox*, *Crotalus horridus horridus* and *Crotalus adamanteus* (From Oklahoma State University, OK, USA and Instituto Vital Brazil, Niterói, RJ, Brazil) and crotoxin. They were tested with egg yolk substrate at different concentrations alone, and the concentration of 10 µg/mL were chosen to be preincubated with suramin (0.1 – 10 µM) and the reaction with the substrate occurred for 30 minutes. The *in vitro* effect of suramin on proteolytic activity was evaluated through the degradation of azocasein by those five crude venoms and this test was performed with preincubation of 10 µg/mL of *Crotalus atrox* venom with 100 and 300 µM of suramin. To evaluate the ability of the substance in antagonize the hyaluronidase activity of venom of *Crotalus* sp. (10 or 30 µg/mL) with suramin (1-7 µM), preincubation occurred in the same solution of venom containing hyaluronic acid and the degradation of the substrate was quantified after 1 hour of reaction. The results of those enzymatic assays were obtained through the absorbance of different samples that were measured using a spectrophotometer. **Conclusions:** These Suramin antivenom data appears to be a promising drug for the treatment of local effects of rattlesnake venoms and may be used as a complement to serotherapy. Further *in vivo* experiments are still needed to assess survival, myotoxicity and hemorrhage.

09.021 *In Vitro* Investigation of the Anti-*T. cruzi* Potential of Natural Products Isolated from *Artemisia umbelliformis*. Carvalho YAM¹, Karoutzou O², Cezar IS^{1,3}, Neves MVG^{1,3}, Silva JW¹, Mikros E², Skaltsounis LA², Soares MBP^{1,3}, Meira CS^{1,3} ¹FIOCRUZ-BA, PPG Biotechnology in Healthy and Investigative Medicine, Brazil, ²National and Kapodistrian University of Athens, Greece, ³ SENAI CIMATEC-BA, Brazil

Introduction: Chagas disease, caused by the protozoan *Trypanosoma cruzi*, is a zoonotic infection endemic to 21 countries in the Americas and affects approximately 6 million people. In its chronic phase, the disease may progress with potentially fatal cardiac, gastrointestinal, or neurological complications. Current treatment relies on benznidazole or nifurtimox, drugs with limited efficacy in the chronic stage and associated with significant adverse effects. Given this scenario, the search for new therapies is urgent, and natural products have emerged as promising sources of bioactive compounds with trypanocidal potential. **Objective:** This study aimed to evaluate the anti-*Trypanosoma cruzi* potential of two natural compounds, telekin (AU_04) and 5-deoxy-5-hydroperoxy-telekin (AU_05), isolated from *Artemisia umbelliformis*. **Methods:** The cytotoxicity of AU_04 and AU_05 was assessed using the Alamar Blue assay in H9c2 cells exposed to different concentrations. Trypanocidal activity was determined by incubating *T. cruzi* trypomastigotes (Y strain) with the compounds, and parasite viability was estimated using a Neubauer chamber. Ultrastructural changes were analyzed by transmission electron microscopy (TEM) and scanning electron microscopy (SEM) after parasite exposure to the compounds for 24 h. Finally, mitochondrial reactive oxygen species (ROS) production was quantified by flow cytometry using the MitoSOX probe. **Results:** Both compounds showed selectivity for *T. cruzi*, demonstrating greater cytotoxicity against trypomastigote forms compared to H9c2 cells. AU_04 exhibited an IC₅₀ of 8.5 µM and a CC₅₀ of 12.67 µM, while AU_05 showed an IC₅₀ of 3.8 µM and a CC₅₀ of 4.3 µM, indicating higher potency but lower selectivity than AU_04. SEM analysis revealed ultrastructural alterations in trypomastigotes, including cell body shrinkage, formation of membrane projections and blebs, leakage of intracellular content, and deformities in the undulating membrane and flagellum. TEM images showed Golgi complex dilation, endoplasmic reticulum disorganization, and discontinuity of the nuclear envelope. Flow cytometry indicated MitoSOX positivity in treated forms, suggesting mitochondrial superoxide production as a possible mechanism underlying the compounds' action. **Conclusion:** Compounds AU_04 and AU_05 demonstrated strong trypanocidal activity, supporting their potential as effective candidates for new Chagas disease therapies. **Financial Support:** FAPESB.

09.022 Potential of Myristicin in Reversing Multidrug Resistance in Chemotherapy Treatments.

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Chemotherapy for cancer treatment faces a barrier that is multidrug resistance (MDR), and there is no effective response to treatment. There are several mechanisms involved in this resistance, but one of them is the increase in the expression of some carrier proteins, such as P-glycoprotein (P-gp), which causes the efflux of the chemotherapy drug into the extracellular medium, making treatment difficult. Molecular docking studies recently published by our research group have shown that myristicin, a natural compound isolated from nutmeg, is able to bind to P-gp and potentially inhibit its extruder activity, letting the chemotherapy drug act on the cell without having its efflux. In addition, the compound has been shown to fit into important pharmacokinetic parameters, which denote the druglikeness potential of the compound. The present study aims to investigate, *in vitro*, the effects of myristicin on the mechanism of MDR reversal. For this purpose, NCI/ADR-RES ovarian lineage cells that are resistant to multiple drugs and overexpress P-gp were used, which were treated with myristicin in association with the chemotherapy drugs cisplatin and docetaxel, in a three-dimensional cell culture model, which more reliably mimics a tumor, by inducing cell-cell and cell-matrix interactions and allowing the arrangement of cells in layers, conferring a biological barrier to the diffusion of drugs. The action of myristicin on the efflux of rhodamine-123 by P-gp was also investigated. Results showed that myristicin 1mM potentiated the effect of chemotherapeutic agents, a result demonstrated by the reduction of IC₅₀ values (minimum concentration necessary to inhibit 50% of cell viability) found for them when associated with myristicin, which reduced by 33% for cisplatin (from 215 to 145 μ M) and 75% for docetaxel (from 15 to 4 μ M). The results of the efflux test showed that after 90 minutes about 80% ($p < 0.001$) of the cells treated with myristicin maintained their intracellular rhodamine content, as well as the positive control, demonstrating to inhibit the action of P-gp. Myristicin is a promising molecule to be considered in adjuvant chemotherapy. The evaluation of the association between myristicin and chemotherapeutic agents in the mechanism of programmed cell death and cell cycle is ongoing. This study was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP). Keywords: Natural products. Myristicin. Multidrug resistance (MDR). P-glycoprotein (P-gp). Chemotherapy. Financial support: FAPESP 2024/05656-6

09.023 **Galactomannan- and Silver Nanoparticle-Based Dressing Accelerates Wound Healing *in vitro* and *in vivo* Experimental Models.** Silva FA¹, Silva ELES¹, Lucena LCP¹, Flor JF¹, Almeida JH¹, Silva EC¹, Nunes DM², Albuquerque PBS², Lima LRA², Rodrigues NER², Correia ACC², Ferro JNS¹
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Introduction: Tissue repair is a complex and dynamic process involving multiple cellular and molecular events. Polysaccharide-based biomaterials, such as galactomannan (GC), when combined with antimicrobial agents like silver, have emerged as promising therapeutic strategies to accelerate wound healing. **Aim:** In this context, we investigated the healing potential of a dermal dressing composed of GC extracted from *Cassia grandis* L.f. and silver nanoparticles (AgNPs) synthesized via green methods using an aqueous extract of *Anadenanthera colubrine* (Vell.) in both *in vitro* and *in vivo* models. **Methods:** The dressing were physicochemical characterized and evaluated *in vitro* for cell viability (MTT assay) and fibroblast migration. *In vivo* evaluation was performed using an excision wound model in *Mus musculus* (C57BL/6, male, 10–12 weeks, CEUA-12/2023), divided into four groups: Untreated (NT), Treated with Collagenase (COL 0.6 U/g), GC Dressing (CG 0.8%), and GC+AgNPs Dressing (CG@AgNPs 50 mg/mL). Wound closure was assessed macroscopically, and histopathological analysis included cellular profile and extracellular matrix (ECM) deposition on days 4, 7 and 12 post-wounding. Water and food intake, granulation tissue characteristics, reepithelialization, and crust formation were also evaluated. Statistical significance was considered at $p < 0.05$ using GraphPad Prism. The green synthesis and characterization method for AgNPs and the production of the dermal dressing were registered under patents BR1020230279180 and BR1020250027852, respectively. **Results:** L929 fibroblasts maintained viability after 24 hours of treatment, showing $94.4 \pm 4.04\%$ (GC 0.8%) and $88.7 \pm 3.3\%$ (AgNPs 81 $\mu\text{g/mL}$) viability. Furthermore, GC (0.8%), AgNPs (27 $\mu\text{g/mL}$), and CG@AgNPs (50 mg/mL) enhanced fibroblast migration compared to control, with CG@AgNPs showing the greatest increase: 29.7% (6h), 44.3% (12h), and 46.4% (24h). Consistent with *in vitro* findings, animals treated with GC@AgNPs showed a 34.25% increase in wound closure compared to NT, with histological findings revealing clear epithelial margins, reduced leukocyte infiltration, and increased fibroblast presence at day 12. Additionally, ECM deposition was significantly higher in the GC@AgNPs group on day 12. Reepithelialization increased by 23.33%, and crust formation decreased by 37.5% in this group compared to NT. No changes were observed in water/food intake, wound weight, or animal body weight following CG@AgNPs treatment. **Conclusion:** Overall, AgNP incorporation into GC enhanced the wound healing process by positively modulating fibroblast activity. These findings highlight the therapeutic potential of this dressing formulation, with further studies underway to better understand the observed effects and advance product development for broad accessibility. **Funding:** FAPEAL and FACEPE. **Keywords:** Medicinal plant. Nanomaterials. Dermal dressings. Tissue remodeling.

09.024 Influence of the Geographical Variability on the Neurotoxic Potential and Reactivity with Commercial Antivenom of Venoms from Four Subspecies of *Crotalus durissus* (Viperidae-Crotalinae) Distributed in Brazil Demico PJ¹, Azevedo SNS¹, Gonçalves de Lima IG¹, Sant'Anna SS², Morais-Zani K³, Floriano RS¹ ¹Unoeste, Lab. of Toxinology and Cardiovascular Research, Graduate Program in Animal Science, Brazil, ²IBu, Lab. of Herpetology, Brazil, ³IBu, Lab. of Pathophysiology, Brazil

Envenomation by *Crotalus* snakes is characterized by potent neurotoxic and myotoxic effects, primarily mediated by crotoxin, a complex composed of a basic and weakly toxic phospholipase A2 (PLA2) subunit and an acidic non-toxic subunit. Recent studies have elucidated the influence of geographical variability on the PLA2 composition in the venoms of *C. durissus terrificus* and *C. d. collilineatus*, revealing greater isoform complexity among specimens from central-west and southeast regions of Brazil. This study evaluated the impact of geographical variability on the neuromuscular activity of *C. d. collilineatus* (CDC), *C. d. ruruima* (CDR), *C. d. terrificus* (CDT), and *C. d. cascavella* (CDV) (rattlesnake subspecies native to Brazil), including the reactivity of these venoms with the commercial anti-*Crotalus* antivenom (AV) and the inhibition of PLA2 activity by varespladib (VPL). PLA2 activity was measured using a colorimetric assay with 3-octanoyloxybenzoic acid as the substrate. The mouse phrenic nerve-diaphragm (PND) preparations were mounted in a Ugo Basile SRL myographical system under 1 g tension. Muscle responses were elicited by supramaximal stimulation via the motor nerve (0.1 Hz, 0.2 ms, ≥ 6 V), recorded using a DY1 transducer coupled to a DataCapsule-Evo, and analysed with LabScribe4 software (CEUA/UNOESTE protocol no. 8798/2024). Results were expressed as the mean $\pm$ SD (n=4), with $p < 0.05$ considered statistically significant. CDR venom exhibited the higher PLA2 activity ($76 \pm 2 \Delta A425\text{nm}/\text{min}$), while CDV venom showed the greatest sensitivity to VPL (0.3 mM), resulting in $\sim 97\%$ inhibition of PLA2 activity ($p < 0.05$ vs. venom alone). CDC venom (10–100 $\mu\text{g}/\text{ml}$) produced initial neuromuscular facilitation followed by complete blockade within 120 min of incubation. In contrast, CDV venom (10–100 $\mu\text{g}/\text{ml}$) caused more rapid neuromuscular blockade at all concentrations, completing within 80–110 minutes of incubation. CDR venom induced complete blockade only at 100 $\mu\text{g}/\text{ml}$ after ~ 110 min of incubation. AV (antivenom:venom ratio 1:1.5 v/w) provided partial protection against CDT and CDV venoms (30 $\mu\text{g}/\text{ml}$), preventing 90% blockade in both. However, AV showed limited reactivity against CDC and CDR venoms (30 $\mu\text{g}/\text{ml}$). VPL (0.3 mM) effectively prevented 90% blockade by CDT and CDV venoms (30 $\mu\text{g}/\text{ml}$) and also avoided 50% blockade by CDC venom (30 $\mu\text{g}/\text{ml}$); VPL did not affect CDR venom (30 $\mu\text{g}/\text{ml}$)-induced blockade. VPL was more effective than AV in neutralizing the neuromuscular effects of CDT, CDV, and CDC venoms. In conclusion, the venoms of *C. durissus* subspecies exhibit considerable variation in their neuromuscular effects *in vitro*, revealing a convincing influence of their geographical distribution. Furthermore, the commercial antivenom shows inconsistent cross-reactivity against these venoms, and CDR venom appears resistant to both AV and varespladib. **Financial support:** São Paulo Research Foundation (grant no. 2023/11341-5).

09.025 **Agro-Industrial Waste Upcycling: Polysaccharides from Passion Fruit Peels as Protective Shields Against the Toxicity of 5-Fluorouracil.** Schiebel CS¹, Oliveira NMT¹, Braga LLVM¹, Silva KS¹, Abboud KY², Cordeiro LMC², Maria-Ferreira, D¹ ¹FPP, Instituto de Pesquisa Pelé Pequeno Príncipe, PPG Biotecnologia Aplicada à Saúde da Criança e do Adolescente, Brazil; ²UFPR, PPG Ciências-Bioquímica, Brazil

Introduction: Intestinal mucositis is a frequent and debilitating side effect of chemo- and radiotherapy, causing pain, severe diarrhea, and nausea. Chemotherapy drugs such as 5-fluorouracil (5-FU) can damage the intestinal barrier, triggering inflammation and mucosal injury (DAHLGREN et al., 2021). Treatment options remain limited, and many patients do not respond adequately, making mucositis a persistent challenge that affects treatment adherence (VILLA et al., 2015). Therefore, new therapies focusing on natural substances have been explored. Among these, polysaccharides from different sources have gained attention due to their biological activities (YU et al., 2018). Polysaccharides from fruit by-products offer a sustainable, cost-effective strategy for preventing and treating chemotherapy-induced intestinal mucositis while valorizing agro-industrial waste. Here, we investigated the potential effect of a polysaccharide extract derived from the biomass residues of yellow passion fruit (*Passiflora edulis*), termed FBP (Fruit By-Product), in a model of 5-FU-induced toxicity in human epithelial colorectal adenocarcinoma cells (Caco-2).

Methods: Caco-2 cells were treated with control (DMEM/F12), 5-FU (25 mg/mL), FBP (30, 100, 150 or 300 µg/mL) or FBP (150 µg/mL) + 5-FU (25 mg/mL). Cell viability was determined using PrestoBlue. A scratch assay was performed to investigate the healing potential of FBP. Mitomycin C (MMC) was used to inhibit cell proliferation and analyze the mechanism of FBP on wound healing. The integrity of the epithelial barrier was investigated using a permeability assay in transwell plates. Oxidative stress was investigated by measuring intracellular levels of reactive oxygen species (ROS), along with indirect assessments of antioxidant defenses such as reduced glutathione (GSH). Apoptosis was measured by flow cytometry and immunofluorescence was performed to analyze tight junction proteins and barrier structure. **Results:** FBP is non-toxic to Caco-2 cells and promoted wound healing compared to control (48 hours: 76% vs. 55%). MMC inhibited cell proliferation, but FBP (150 µg/mL) still promoted intestinal epithelial healing (48 h: 63% vs. 52%). 5-FU caused cellular toxicity and decreased metabolic activity from 58% at 2 hours to 34% at 6 hours compared to control (100%). FBP prevented this decrease and maintained metabolic activity at 94% and 87%, respectively. In addition, FBP protected cells from increased paracellular permeability in 2 hours (FBP: 3.225 ± 0.4450 ; 5-FU: 5.141 ± 0.1154 µg Evans Blue/h/cm²) as well as oxidative stress and 5-FU-induced apoptosis. These results were related to the maintenance of protein distribution (claudin and occludin) at the epithelial interface. **Conclusion:** FBP was able to protect Caco-2 cells from the toxicity induced by 5-FU. **Financial support:** Capes (001), Instituto de Pesquisa Pelé Pequeno Príncipe, and CNPq (302195/2022-0, 404717/2016-0, 310332/2015-0 and 307314/2018-9). References: DAHLGREN, D. *Front. Pharmacol.*, v. 12, p. 1–12, 2021.; VILLA, A. *Expert Opin. Pharmacother.*, v. 17, n. 13, p. 1801–1807, 2016. YU, Y. *Carbohydr. Polym.*, v. 183, p. 91–101, 2018.

09.026 Impact of Marine Animal Extracts on Neuronal Viability and Inflammation in Alzheimer's Disease Models. Alves MM¹, Vigerelli H², Kitahara MV³, Kerkis I⁴, Sciani JM⁵ ¹IBu, PPG Sciences – Toxinology, Lab. of Biochemistry, Brazil, ²IBu, Center of Excellence in Target Discovery, Brazil, ³USP, Center for Marine Biology, Brazil, ⁴IBu, Lab. of Genetics, Brazil, ⁵USF, Lab. of Natural Products, Brazil

Alzheimer's disease (AD) is the most common neurodegenerative disease, accounting for ~ 70% of dementia cases. Its etiology is closely linked to the aggregation and deposition β -amyloid peptides (A β) in the brain. A β triggers chronic neuroinflammation via microglial activation, promoting the release of pro-inflammatory mediators and reactive oxygen species (ROS), contributing to neuronal death. Drugs available do not cure the disease, highlighting the urgent need for new therapeutic strategies. Marine organisms are a rich and underexplored source of new bioactive molecules, with potential for drug development. This study aimed to evaluate the effects of extracts from six marine invertebrates - *Echinometra lucunter* (EL, echinoderm), and cnidarians *Zoanthus sociatus* (ZS), *Exaiptasia diaphana* (ED), *Palythoa caribaeorum* (PC), *Tubastraea tagusensis* (TT) and *Eudendrium carneum* (EC) - in an *in vitro* AD model, assessing their impact on cell viability, ROS production, cytokine release and antioxidant activity, using two experimental models: SH-SY5Y cells differentiated into neuron-like cells (single culture) and transwell coculture of neuron-like (SH-SY5Y) cells and U251 cells. In the single culture model, cells were treated with the extracts following A β 42 exposure, and then were evaluated for cell viability (MTT assay and live/dead staining with calcein AM and ethidium homodimer-1/fluorescent microscopy), ROS levels (DCFH-DA staining/flow cytometry), and antioxidant capacity (ABTS assay). In the coculture model, SH-SY5Y viability (MTT) and interleukin-6 (IL-6) release (Cytometric Bead Array/flow cytometry) were measured after A β 42 exposure and extract treatment. The antioxidant potential of extracts was evaluated using the DPPH assay. Molecules present in the extracts were identified by mass spectrometry by comparison to the GNPS database. In the single culture model, extracts from EC, ED, EL, PC, and ZS significantly reduced A β 42-induced cytotoxicity (MTT) and all extracts improved viability in live/dead staining. ZS significantly reduced ROS levels, while ZS and TT exhibited antioxidant activity. In the coculture model, EC and ZS reduced A β 42-induced cytotoxicity, while ED, EL, PC, and TT significantly decreased IL-6 release. Antioxidant activity by DPPH was observed for ED, EL, PC, and ZS. Mass spectrometry analysis revealed bioactive compounds potentially responsible for the observed effects. Notably, carnosic acid—identified in the ED extract—is known for neuroprotective effects via ROS reduction and anti-lipid peroxidative action, aligning with our findings. A flavonoid identified in ZS, recognized for its antioxidant properties, may explain its ability to reduce oxidative stress and cytotoxicity. In conclusion, marine invertebrates extracts — particularly ED and ZS—demonstrate promising neuroprotective effects by modulating oxidative stress and inflammation in A β 42-induced AD models. These findings highlight the potential of marine-derived molecules as candidates for developing novel AD therapies.

09.027 Apigenin Promotes Ulcer Healing in a Chronic Acetic Acid-Induced Murine Model. Bezerra MCF¹, Cechinel Filho V², Colodel EM³, Figueiredo FF¹, Rodrigues DW², Silva WDM³, Cavasani JPS³, Martins DTO¹ ¹UFMT, Pharmacology Area, Graduate Program in Health Sciences, Brazil, ²Univali, Center for Chemical and Pharmacological Investigations, Graduate Program in Pharmaceutical Sciences, Brazil ³UFMT, Veterinary Pathology Lab, Graduate Program in Veterinary Sciences, Brazil

Introduction: Gastric ulcer is a chronic disease characterized by injury to the gastric mucosa, resulting from an imbalance between aggressive and protective factors. It affects 5–10% of the global population, with the primary etiological factors being *Helicobacter pylori* infection and prolonged use of non-steroidal anti-inflammatory drugs (NSAIDs). Current treatments for peptic ulcers depend on the severity of complications but typically involve *H. pylori* eradication using antibiotics and/or proton pump inhibitors. Previous studies have demonstrated that apigenin exhibits anti-inflammatory and gastroprotective effects in animal models. This study investigates whether apigenin promotes the healing of chronic gastric ulcers. **Methods:** Animal experiments were approved by the Animal Ethics Committee of UFMT (Protocol No. 23108.074098/2023- 33). Swiss mice were randomly divided into six groups (n = 5–8 per group): sham (distilled water, p.o.), vehicle (distilled water, p.o.), apigenin (1, 5, and 20 mg/kg, p.o.), and standard drug (omeprazole 30 mg/kg, p.o.). Except for the sham group, ulcers were induced by applying 20 µL of 99.5% acetic acid to the serosal surface of the stomach. After seven days of treatment, the stomachs were excised, and the ulcer area (mm³) was measured. Tissue samples were collected for histopathological analysis and homogenized to assess myeloperoxidase (MPO) activity, malondialdehyde (MDA), and reduced glutathione (GSH) levels. Additionally, pro-inflammatory (TNF-α, IL-1β) and anti-inflammatory (IL-10) cytokines were quantified. **Results:** Apigenin significantly reduced (p < 0.01) the ulcerated area by 94.96%, 97.46%, and 95.20% at doses of 1, 5, and 20 mg/kg, respectively. Histopathological analysis revealed a 39.5% reduction in edema and mucosal damage, along with a 56.5% decrease in leukocyte infiltration at 5 mg/kg. Furthermore, mucus production increased by 75% at 20 mg/kg, reticular fibers increased by 40% at 1 mg/kg, and elastic fibers increased by 62.5% at 5 mg/kg. Apigenin also significantly decreased (p < 0.001) MPO activity by 57.85%, 74.42%, and 47.50% at 1, 5, and 20 mg/kg, respectively. GSH levels increased (p < 0.001) by 76.26%, 78.01%, and 77.40%, while MDA levels decreased (p < 0.001) by 86.13% (1 mg/kg), 93.08% (5 mg/kg), and 90.00% (20 mg/kg). TNF-α concentrations were reduced by 64.56% (1 mg/kg, p < 0.001), 71.90% (5 mg/kg, p < 0.001), and 49.86% (20 mg/kg, p < 0.01). IL-1β levels also decreased (p < 0.05) by 41.53% (1 mg/kg), 34.23% (5 mg/kg), and 45.76% (20 mg/kg). At doses of 1 and 5 mg/kg, apigenin reduced IL-10 concentrations by 57.38% and 76.71% (p < 0.001), respectively. **Conclusion:** Apigenin exhibits a healing effect on chronic gastric ulcers in mice, attributed to its mucogenic, antioxidant, and anti-inflammatory properties. **Financial support:** CAPES, PROAP CAPES 2024, CNPq, INCT-INAU II/CNPq, and PROPesq/UFMT.

09.028 **Proteomic and Functional Analysis of *Acanthophis antarcticus* Venom: An Integrative Approach for Biochemical Characterization.** Falla MVA¹, Sousa EP³, Morais-Zani K³, Valladao R¹, Santos NG¹, Galizio NC³, Rodrigues MS², Almeida HIF¹, Rocha ARL¹, Moyses MN¹, Lebrun I¹, Spancer P², Pimenta DC¹, Coelho GR¹ ¹ IBu, Laboratório Bioquímica e Biofísica, ²IPEN, ³ IBu, Lab Fisiopatologia

Introduction: *Acanthophis antarcticus* is a snake endemic to Australia, belonging to the family Elapidae but historically classified within the family Viperidae due to morphological similarities with vipers. Its venom exhibits neurotoxic, hemorrhagic, and hemolytic effects, and although envenomation cases are rare, they represent a public health concern. Most studies on its components have focused on characterizing three-finger toxins (3FTxs) and phospholipases A₂ (PLA₂). However, no previous work has integrated proteomic and functional analyses, limiting a comprehensive understanding of this species' venom. Therefore, the present study aimed to combine these approaches to broaden knowledge of the venom of this medically important species. **Methods:** In this study, crude venom was fractionated by reverse-phase high-performance liquid chromatography (RP-HPLC), and the collected fractions were subsequently digested and subjected to proteomic identification by mass spectrometry. Functional assays evaluated activities previously reported in the literature, such as PLA₂ and L-amino acid oxidase (LAAO). Additionally, for the first time in this species, novel analyses were performed, including the assessment of fibrinogenolytic activity by SDS-PAGE and the detection of serine protease inhibitors in the venom, using a fluorogenic substrate and trypsin as the enzyme (analyses conducted with both crude and boiled venom). **Results:** The analyses revealed the presence of toxins from nine distinct families, with 3FTxs and PLA₂ being the most abundant. Additional bioactive molecules were also identified, including Kunitz-type inhibitors (KTI), cysteine-rich secretory proteins (CRISP), metallopeptidases (SVMP), LAAO, nerve growth factor (NGF), natriuretic peptides (NP), and nucleotidases (Nsase). Functional assays confirmed the expected activities of PLA₂ and LAAO and, for the first time in this species, demonstrated fibrinogenolytic activity, characterized by the selective degradation of the fibrinogen α-chain. Trypsin inhibition was also observed in both crude and boiled venom, providing the first evidence of serine peptidase inhibitors in this species, a feature common among Elapidae snakes. **Conclusion:** This study presents, for the first time, the integration of proteomic and functional analyses of *A. antarcticus* venom, identifying nine toxin families, highlighting the high abundance of 3FTxs, and reporting the novel detection of fibrinogenolytic activity and serine peptidase inhibition, thus expanding our understanding of the biochemical and functional diversity of this venom.

09.029 **Evaluation of a Polysaccharide from *Acrocomia corumbaensis* (Bocaiuva) in DSS-Induced Colitis in Mice.** Brito MSC¹, Taporoski AK², Rech KS¹, Santos LBS¹, Martins FG¹, Cordeiro LMC², Werner MFP¹ ¹UFPR, Pharmacology, ²UFPR, Biochemistry and Molecular Biology

Introduction: Ulcerative colitis (UC) is a chronic inflammatory bowel disease characterized by weight loss, abdominal pain, diarrhea, and rectal bleeding. The polysaccharide extracted from the edible fruit of bocaiuva (BFS), an acetylated glucomannan (Man:Glc ratio 4.3:1, 24% acetylation degree, Mw = 1.82×10^5 g/mol), has been shown to protect the mucosa by forming a physical barrier in an ethanol-induced gastric ulcer model [1]. Here, we investigated the effect of BFS in an UC model. **Methods:** UC was induced in female and male C57BL/6 mice with 3% dextran sulfate sodium (DSS) in drinking water for 7 days. Animals were treated orally with vehicle (water) or BFS (1, 10 or 100 mg/kg) for 7 days. Weight loss, rectal bleeding, and stool consistency were monitored daily to calculate disease activity index (DAI). Abdominal mechanical hypersensitivity was assessed using von Frey filaments (0.008, 0.02, 0.04, 0.07, 0.16g) on days 2, 4, 6, and 8. On day 7, elevated plus maze (EPM), open field (OF) and nest-building tests were performed to assess animal welfare. On day 8, estrous cycle phase of female mice was determined. Then, animals were euthanized, colons were excised, measured and homogenized for myeloperoxidase (MPO) assay. **Results:** On days 7 and 8, DSS group exhibited weight loss of 4.5% and 12.6% in females, and 6.7% and 16.6% in males. DAI started increasing around day 5 and peaked at day 8, reaching scores of 6.1 ± 0.4 and 8.0 ± 0.4 for females and males, respectively. Treatment with BFS did not improve these UC symptoms. DSS promoted shortening of colon length in female (27%) and in male (31%) when compared to control groups (9.9 ± 0.2 ; 9.8 ± 0.2 cm). Notably, BFS 1 mg/kg increased in 20% the colon length of female mice when compared to DSS group. DSS induced an increase in mechanical hypersensitivity in females (311%) and males (318%) when compared to control group (22.5 ± 8.0 ; 27.5 ± 8.5) in response to 0.07 g von Frey filament, for example, in day 8. Treatment with BFS (100 mg/kg) reduced hypersensitivity by 66% in females and 51% in males compared to the DSS group. Nesting activity was reduced by 57.5% in DSS male group in comparison to control group (5.0) and BFS 100 mg/kg increased the score in 115.1% when compared to DSS group. DSS females showed 50% less time spent in open arms than control (6.9 ± 0.80) and males showed a reduction in time spent in open arms (84%), open arms entries (74%), closed arms entries (29%) and head dips (67%) compared to control group (8.4 ± 1.1 ; 4.4 ± 0.6 ; 17 ± 0.9 ; 3.7 ± 0.7). BFS 100 mg/kg in males increased time spent in open arms (307%) and open arms entries (294%). DSS increased colonic MPO levels by 218% in females and 143% in males when compared to control groups (9.2 ± 1.2 ; 10.6 ± 1.6 D.O./mg of protein). BFS 100 mg/kg reduced MPO levels in 63% in females and 73% in males. No estrous cycle phase-dependent variations were detected in females in this study. **Conclusion:** BFS did not improve remission rates in UC but demonstrated significant analgesic and anti-inflammatory effects, with partial welfare improvement. License Number of Ethics Committee: CEUA/BIO - UFPR: approval number 1628. **Financial support:** CAPES-Finance Code 001. CNPq 308816/2023-4. References: [1] TAPOROSKI, A. K. Master's dissertation, UFPR, 2021.

09.030 **Neuroprotective Potential of *Randia formosa* Fruit Extract in a Murine Model of Aluminum-Induced Neurotoxicity.** Okoh VI¹, Campos HM¹, Ferreira PYO¹, Uchenna N¹, Silva GNM², Leite JA¹, Costa EA¹, dos Santos FCA¹, Gil ES², Ghedini PC¹ ¹UFG, Institute of Biological Sciences, Goiânia, GO, Brazil; ²UFG, Faculty of Pharmacy, Goiânia, GO, Brazil

Introduction: Blackberry jam fruit (BJF) (*Randia formosa* (Jacq.) K. Schum), an Amazonian superfruit, has shown promising *in vitro* antioxidant and neuroprotective properties. Given the growing interest in functional foods with therapeutic potential, we evaluated the *in vivo* neuroprotective effects of a hydroalcoholic extract obtained from BJF, aiming to expand the scientific understanding of its pharmacological benefits. **Aim:** To assess the antioxidant and neuroprotective effects of BJF in a murine model of aluminum-induced neurotoxicity. **Methods:** Male Swiss mice were randomly assigned to five groups (n = 10 per group): I) Control, II) Induced, III) BJF-1, IV) BJF-30, and V) BJF-100. In the first treatment, Groups I and II received distilled water (dH₂O), while Groups III, IV, and V received BJF at doses of 1, 30, and 100 mg/kg, respectively. One hour after the initial treatment, Groups II through V received AlCl₃ (100 mg/kg in dH₂O, pH 7.4), whereas Group I received dH₂O again as vehicle control. All treatments were administered orally (10 µL/10 g body weight) once daily for 45 consecutive days. Animals had free access to food and water throughout the experimental period. Behavioral assessments—including the chimney test, open field test, and step-down passive avoidance test—were conducted to evaluate motor coordination, exploratory behavior, and memory, respectively. Following euthanasia, hippocampal tissues were collected and analyzed for oxidative stress biomarkers: lipid peroxidation (LPO), protein carbonyl content (CP), superoxide dismutase (SOD), catalase (CAT), acetylcholinesterase (AChE), butyrylcholinesterase (BuChE), and glutathione peroxidase (GPx). Amyloid-β deposition was evaluated by immunohistochemistry. All experimental procedures were approved by the Animal Ethics Committee of the Federal University of Goiás (Protocol No. 053/2016). Data were analyzed using one-way ANOVA followed by Tukey's post hoc test. Statistical significance was considered at p < 0.05. **Results:** Chimney and open field tests revealed no significant changes in locomotion or exploratory behavior across groups (p > 0.05). In the step-down test, BJF-30 and BJF-100 significantly increased latency time compared to the AlCl₃ group (p < 0.05), while BJF-1 did not differ significantly (p > 0.05). Biochemically, AlCl₃ reduced SOD, CAT, and GPx activities (p < 0.05), which were restored by BJF at 30 and 100 mg/kg (p < 0.05). Elevated AChE and BuChE activities in the AlCl₃ group were significantly attenuated by BJF (30 and 100 mg/kg, p < 0.05). Similarly, increased LPO and CP levels induced by AlCl₃ were significantly reduced in BJF-treated groups (p < 0.05). Immunohistochemistry revealed increased amyloid-β deposition in the AlCl₃ group, which was markedly reduced in all BJF-treated groups (p < 0.05). **Conclusion:** BJF exhibits significant neuroprotective effects *in vivo*, mitigating oxidative stress, cholinergic dysfunction, and amyloid-β accumulation in a model of aluminum-induced neurotoxicity. These findings highlight the potential of BJF as a nutraceutical candidate for preventing neurodegenerative disorders. **Financial support:** CNPq.

09.031 **Effect of Galactomannan and Silver Nanoparticles-Based Wound Dressing on the Cutaneous Wound Healing Assessed by Raman Spectroscopy.** Silva EC¹, Oliveira JNSR², Silva FA¹, Lucena LCP¹, Nunes DM³, Correia ACC³, Albuquerque PBS³, Lima LRA³, Rodrigues NER³, Souza ST², Ferro JNS¹ ¹UFAL, Lab Biologia Celular, ²UFAL, Grupo de Óptica e Nanoscopia, ³UPE, Lab Bioprospecção e Etnofarmacotoxicologia Aplicada e de Biologia Celular

Introduction: The wound healing process occurs in a well-coordinated way between the cellular and molecular components. Being a complex process, the search for new treatment is uninterrupted, and several studies evaluate the potential of nanotechnological materials. Hence, evaluating adequate tissue repair is expensive and time-consuming. Alternatively, Raman spectroscopy (RS) can be used as a quantitative and qualitative approach, indicating the sample fingerprint at a molecular level, and it has increasing applications for biological tissue analysis. **Methods:** Furthermore, this study aims to evaluate the cicatricial potential of a wound dressing incorporated with galactomannan obtained from *Cassia grandis* L.F. (GC) and silver nanoparticles (AgNPs) synthesized from the aqueous extract of *Anadenanthera colubrina* (Vell.) using RS as the primary analysis tool. Thus, the excisional cutaneous wound model in C57BL/6 mice (male, 10-12 weeks, CEUA-12/2023) was performed in 3 groups: Healthy Skin (HS), Non-Treated Wound (NT), or treated with the wound-dressing (CG@AgNPs). On the 12^o day after wound induction, the animals were euthanized, and the biopsy was prepared for RS acquisition, (XploRa system (Horiba, Japan) 532nm, 20 mW, calibrated with 520 cm⁻¹ silicon band, 60 spectra per sample). The Principal Component Analysis (PCA) was performed to discriminate between the groups according to band intensity. There was a significant increase in extracellular matrix components (EMC) in CG@AgNPs when compared with NT, revealed by vibrational peaks related to fibronectin, elastin, and laminin (1090 cm⁻¹, 1247 cm⁻¹, 1307 cm⁻¹, respectively). **Results:** The group CG@AgNPs has an attenuation of peaks associated with collagen degradation (853 cm⁻¹, 1453 cm⁻¹ and 1668 cm⁻¹), indicating the reorganization of collagen fibers. Furthermore, characteristic peaks related to proline and hydroxyproline were noticed at 853 and 875 cm⁻¹, respectively, and phenylalanine at 1007 cm⁻¹ were found on the skin of group CG@AgNPs. Supporting that, 1453 cm⁻¹ and 1668 cm⁻¹ bands regain their intensity, similar to HS. Additionally, mice treated with CG@AgNPs had a bigger re-epithelialization when compared to NT, according to a higher intensity on keratin band (1307 cm⁻¹). **Conclusions:** Finally, corroborating histopathological analyses of the group, where greater wound healing and deposition of EMC was observed, the analyses obtained by RS fortify the presence of EMC contributing to the wound biochemical recovery and promoting tissue repair by the biodegradable dermal dressing. Analyses will be performed to better characterize the molecules involved in the healing effect, enabling advances in the molecular understanding of this treatment.

09.032 **Anti-Obesogenic and Anti-Hyperglycemic Effect of Citral: Preventive Effect Against the Development of Metabolic Endotoxemia in Obese C57BL/6J Mice Challenged with Lipopolysaccharide.** Fioravanti MM, Emílio-Silva MT, Assunção R, Dario FL, Trinas JG, Guidolin IG, Hiruma-Lima CA Unesp- Botucatu

Introduction: Obesity is a growing global public health problem. Consumption of a high-fat diet (HFD) leads to inflammation, progressive increase in adipose tissue and systemic lipopolysaccharides (LPS) levels, and metabolic disorders such as hyperglycemia. These changes disrupt the balance in metabolic and inflammatory homeostasis. Current treatments for obesity are expensive and have many adverse effects. Citral (CT) is a natural product with proven anti-inflammatory properties. Thus, we aimed to compare the protective effect of CT in obese C57BL/6J mice with LPS-induced metabolic endotoxemia. **Methods:** Initially, adult male C57BL/6J mice (n=8–10 per group) were fed with a standard diet (SD) or HFD for 16 weeks. Concomitantly, daily oral treatment with CT (300 mg/kg) and its vehicle (1% Tween 80 at 10 mL/kg) was performed as follows: SD+vehicle (SD), HFD+vehicle (HFD), and HFD+CT (CT). In a second experiment, four groups were elaborated: SD+vehicle (SD10), HFD+vehicle (HFD10), HFD+vehicle+LPS (HFD+LPS), HFD+LPS and CT (300 mg/kg) (CT+LPS). Metabolic endotoxemia was induced by intraperitoneal injection of LPS at a dose of 10 µg/animal once a week for 10 weeks, in conjunction with an HFD and treatments. Body weight gain (g) and caloric intake (g/day) were assessed twice a week. The fasting blood glucose levels and oral glucose tolerance test (OGTT) were performed after 16 and 10 weeks, respectively. All animals were euthanized, and morphometric analyses were performed on adipose tissues. In addition, serum LPS levels and adiposity index were calculated, through the sum of total adipose tissues, resulting in a value that was divided by the body mass of the corresponding mice x100. Statistical analysis was performed using Kruskal-Wallis or one- or two-way ANOVA, followed by Tukey's post-test ($p<0.05$) (CEUA 1202130125). **Results:** The HFD diet for 16 weeks significantly increased body weight by 44.78% ($p<0.001$) and adiposity index by 5x compared to the SD group ($p<0.001$). However, treatment with CT (300 mg/kg) was able to reverse this profile, promoting a significant reduction in body mass gain of 14.29% ($p<0.05$) and 25.93% in the adiposity index compared to the HFD group ($p<0.05$). Treatment with CT had an antihyperglycemic effect by significantly reducing basal blood glucose levels by 29.41% compared to the HFD group ($p<0.01$). Nevertheless, when evaluated the metabolic endotoxemia induced by LPS together with HFD intake, CT treatment was unable to reverse body mass gain and hyperglycemia in mice after 10 weeks. However, it was able to reduce serum LPS levels by 47.60% ($p<0.05$). **Conclusions:** Our data show that treatment with CT (300 mg/kg) promotes an anti-obesogenic and anti-hyperglycemic effect, significantly reducing body mass, adiposity index, and blood glucose in mice fed a high-fat diet. However, it did not prevent weight gain and hyperglycemia under LPS-induced endotoxemia, despite reducing serum LPS levels. **Financial support:** FAPESP (2024/15790-1) and CNPq (311957/2020-0).

09.033 Coagulotoxicity *in vitro* of Two South American Snake (Viperidae: Crotalinae) Venoms and Inhibition by Marimastat and Commercial Antivenoms. Gonçalves de Lima IG¹, Proença-Hirata VS¹, Souza-Gomes GC¹, Demico PJ¹, Giuffrida R¹, Silva NJ², Torres-Bonilla KA³, Hyslop S³, Sant'Anna SS⁴, Morais-Zani K^{4,5}, Vaiyapuri S⁶, Floriano RS¹ ¹Unoeste, Lab. of Toxinology and Cardiovascular Research, Graduate Program in Health Sciences, Brazil, ²PUC-Goiás, School of Medical and Life Sciences, Brazil, ³Unicamp, Dept Pharmacology, Faculty of Medical Sciences, Brazil, ⁴IB, Lab. of Herpetology, Brazil, ⁵IB, Lab. of Pathophysiology, Brazil, ⁶University of Reading School of Pharmacy, Reading, United Kingdom

In Brazil, *Bothrops* snakes are responsible for most snakebites, while *Lachesis* envenomations are rarer and often occur in remote northern areas, where medical assistance is limited, heightening the risk of complications. Polyvalent antivenom therapy is used for both snake types, and Marimastat, a metalloprotease inhibitor, has been tested against various Viperidae venoms. In this work, we have evaluated the efficacy of marimastat, a broad-spectrum metalloproteinase inhibitor, in counteracting the coagulotoxic effects of *Lachesis muta* and *Bothrops alternatus* (South American Viperidae snakes) venoms *in vitro*. Enzymatic activity was assayed using azocasein as substrate, and changes in absorbance were determined by a multiwell plate reader. Prothrombin time (PT) and activated partial thromboplastin time (aPTT) were assayed using rat citrated plasma and commercial kits with a Quick Timer Coagmaster 4.0 (CEUA/UNOESTE protocol no. 8143 and 8244). Marimastat (0.03 and 0.3 mM) significantly inhibited the caseinolytic activity of *L. muta* and *B. alternatus* venoms in 77.6% and 72.2%, respectively. In rat citrated plasma, marimastat (0.03 mM) alone failed in neutralizing the procoagulant action of *L. muta* venom (1 mg/ml) on both of PT [26.7±0.9 s (control) vs. 11.7±0.4 s (venom) and 12.7±0.9 s (venom + marimastat); $p<0.05$ compared to control, $n=5$] and aPTT [24±1.3 s (control) vs. 8.3±0.4 s (venom) and 9±0.7 s (venom + marimastat); $p<0.05$ compared to control, $n=5$], whereas the commercial antivenom (anti-*Bothrops/Lachesis*) at an antivenom:venom ratio of 1:3 (v/w) effectively avoided the PT [21±0.7 s (venom + antivenom); $p<0.05$ compared to venom alone, $n=5$] and aPTT [29.7±1.8 s (venom + antivenom); $p<0.05$ compared to venom alone, $n=5$] alterations caused by this venom. On the other hand, marimastat (0.3 mM), as a stand-alone pharmacological tool, effectively prevented the procoagulant effect seen with *B. alternatus* venom (1 mg/ml) on both of intrinsic [aPTT: 24 ± 1.3 s (control) vs. 7.7±0.9 s (venom) and 16.7±0.4 s (venom + marimastat); $p<0.05$ compared to venom alone, $n=5$] and extrinsic [PT: 26.7±0.9 s (control) vs. 10.3±0.4 s (venom) and 24.3±1.6 s (venom + marimastat); $p<0.05$ compared to venom alone, $n=5$] pathways. However, the antivenom anti-*Bothrops* produced mild protection against the *B. alternatus* venom-induced aPTT alteration [10±1.3 s (venom + antivenom), $n=5$]. Marimastat did not produce an additive protection when combined with these commercial antivenoms. In conclusion, these findings reveal a variation of the inhibition produced by marimastat on the coagulotoxicity of *L. muta* and *B. alternatus* venoms, exceeding the neutralization induced by antivenom only for *B. alternatus* venom.

09.034 Involvement of K⁺ Channels in the Tocolytic Action of (S)-8-formyl-3',5-Dihydroxy-7-Methoxy-6-Methylflavanone and 3'-Formyl-3,4',6'-Trihydroxy-2'-Methoxy-5'-Methylchalcone, a Flavonoid Mixture from *Piper montealegreanum* Yuncker (Piperaceae): *in vitro* and *in silico* Study. Souza MLB¹, Oliveira LN², Martins AMO², Felício IM², Figueiredo IAD², Silva JLV³, Santos BVO⁴, Cavalcante FA^{2,5} ¹PIBIC/CNPq/UFPB; ²PPgPNSB/CCS/UFPB; ³FMO; ⁴UACEN/UFCG; ⁵DCB/CCS/UFPB

Introduction: Uterine smooth muscle dysfunctions can impair female reproductive health, particularly among adolescents and young women (Bafor, J Med Food, v.24, p.541, 2020). Natural products represent a promising source for development of new therapeutic agents. Furthermore, previous studies have reported that (S)-8-formyl-3',5-dihydroxy-7-methoxy-6-methylflavanone (PMT1) and 3'-formyl-3,4',6'-trihydroxy-2'-methoxy-5'-methylchalcone (PMT2), a mixture of formylated flavonoids (PMTmix) from *Piper montealegreanum* (Piperaceae) (Yuncker, Hoeh, v.2, p.21, 1972), exhibit spasmolytic effects in rat aorta; guinea pig trachea (Alves, master's dissertation, 2004) and rat uterus (Oliveira, LN. SBFTE Proc, v.1, p.12, 2024). Based on these findings, this study aimed to investigate the potential involvement of K⁺ channels in the tocolytic action of PMTmix using *in vitro* and *in silico* assays. **Methods:** Female rats were pretreated 24h with diethylstilbestrol (1mg/kg) to synchronize the estrous cycle. Following euthanasia, the uterine horns were suspended in isolated organ baths and isometric contractions were recorded (n=5). PMTmix (1–729 µg/mL) was tested in the absence and presence of selective K⁺ channel blockers: 4-aminopyridine (4-AP, selective K_V blocker), glibenclamide (K_{ATP} blocker), and tetraethylammonium (TEA⁺, BK_{Ca} blocker). For the *in silico* study, molecular docking simulations were performed using DockThor and analyzed with Chimera1.14. The structures PMT1 (CID 162897155) and PMT2 (CID 25155519) were downloaded from PubChem, while the channel proteins BK_{Ca} (ID 6V3G) and K_V4.3 (7W3Y) were obtained from ProteinDataBank. The output included conformers and affinity predictions (Aff). Experimental protocols were approved by Animal Use Ethics Committee/UFPB (7660011018). Statistically analysis was performed using the unpaired *t*-test, with significance level (*p*<0.05). **Results:** PMTmix relaxation curve (E_{max}=100.13.2%; EC₅₀=49.28.8µg/mL; *p*<0,05) was shifted to the right in the presence of selective K⁺ channel blockers, including 4-AP (E_{max}=88.02.8%; EC₅₀=101.411.8µg/mL; *p*<0,05), glibenclamide (E_{max}=97.36.6%; EC₅₀=143.023.3µg/mL; *p*<0,05) and TEA⁺ (E_{max}=92.73.9%; EC₅₀=218.839.4µg/mL; *p*<0.05), with a reduction in the relaxant potency of PMTmix of approximately 2, 3 and 4 times, respectively. This finding suggests that the tocolytic effect of PMTmix may be mediated by the positive modulation of potassium channels. Regarding the *in silico* studies, PMT1 (Aff= -6.7kcal/mol), PMT2 (Aff= -6.7kcal/mol) and 4-AP (Aff= -6.2kcal/mol) interacted with K_V4.3, as well as PMT1 (Aff= -7.2kcal/mol), PMT2 (Aff= -7.1kcal/mol) and TEA⁺ (Aff= -6.7 kcal/mol) interacted with BK_{Ca}. Docking simulations confirmed favorable interactions of PMT1 and PMT2 with K_V4.3 and BK_{Ca}, with binding affinities comparable to reference ligands. **Conclusion:** These findings suggest that PMTmix exerts tocolytic effect in rat uterus by positively modulating K_V, K_{ATP} and BK_{Ca} channels. These results support its potential as a candidate for the treatment of uterine contractility disorders. **Financial support:** FAPESQ-PB, PpPNSB, CCS/UFPB, PIBIC/CNPq.

09.035 **Antioxidant and Anticholinesterase Effect of Guarana Seed Extract** (*Paullinia cupana* Kunth).
Piristrello BS, Souto AF, Mendes FR UFABC

Introduction: Guarana (*Paullinia cupana* Kunth) is a plant rich in catechins and known for its stimulant effects attributed to its alkaloids, mainly caffeine. Considering that oxidative stress is strongly associated with the progression of neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD), natural compounds with antioxidant properties have been investigated as preventive strategies or as complementary treatments to current therapies. AD, for example, is treated with acetylcholinesterase (AChE) inhibitors, while PD involves mechanisms related to tyrosinase and the accumulation of neuromelanin. In addition, lipid peroxidation, marked by increased levels of malondialdehyde (MDA), contributes significantly to neurodegeneration. In this context, this study aims to evaluate the antioxidant potential of guarana seed extract, its inhibitory capacity on the enzymes AChE and tyrosinase, and its effects on spontaneous lipoperoxidation. **Methodology:** Guarana seed powder was obtained from Santos Flora, duly registered on the SISGEN platform (#A415A1E), and used in the preparation of a hot aqueous extract. The antioxidant activity of the extracts was evaluated using the DPPH and ABTS methods previously validated with the use of trolox. The *in vitro* anticholinesterase activity was assessed using the spectrophotometric method developed by Ellman, in which each repetition of the AChE experiments included a group treated with 0.1 µg/mL of neostigmine as a positive control to ensure better assay replicability. In addition, the inhibitory activity on the tyrosinase enzyme and the effect of the extract on spontaneous lipoperoxidation were evaluated. The DPPH and ABTS radical scavenging values, as well as the enzymatic and lipoperoxidation inhibition data, were used to calculate EC₅₀/IC₅₀ values by logistic regression. **Results and Discussion:** We observed the following values of EC₅₀ in antioxidant assays: 80.05 µg/mL (DPPH) and 19.00 µg/mL (ABTS). Regarding the extract's activity on spontaneous lipoperoxidation, an IC₅₀ = 28.69 µg/mL was obtained. In enzyme inhibition tests, guarana showed an IC₅₀ = 163.29 µg/mL for AChE and 108.27 µg/mL for tyrosinase. Guarana is rich in polyphenols, and one of its main components is catechins; thus, high antioxidant activity was expected. Besides, caffeine was described as an inhibitor of AChE, an effect found in our study. Considering that AD is currently treated with AChE inhibitors, the results obtained suggest that guarana may represent a potential candidate for AD treatment, although further studies are needed to confirm its therapeutic use. Recent studies have explored the use of tyrosinase inhibitors in the treatment of PD due to their role in regulating dopamine and neuromelanin levels in the brain. Despite the lack of previous studies evaluating guarana's activity on this enzyme, our results indicated a significant inhibitory effect, suggesting a new and promising target for future investigations. **Conclusion:** The guarana extract showed significant activity in all tests performed, standing out particularly for its potent antioxidant action and its ability to inhibit spontaneous lipoperoxidation. These results reinforce the therapeutic potential of guarana, especially in the context of neurodegenerative diseases associated with oxidative stress, although further studies are necessary to deepen the understanding of its mechanisms and clinical applications. **Financial support:** Delightex Pte.

09.036 **The Anti-Inflammatory Activity of Extra Virgin Olive Oil in the Hypothalamus And Hippocampus of Rats Exposed to a High-Fat Diet.** Harttfeil-Bajerski MH, Peres AM, Dantas RM, Ferreira AC, Silveira ACS, Machado DN, Souza LCP, Raphaelli GB, Lena MC, Netto CA, Dalmaz C, Bast RKSS UFRGS Porto Alegre, Lab Pesquisa da Programação Neurobiológica do Comportamento Alimentar (PROCAL), PPG Ciências Biológicas: Bioquímica, Brazil

Introduction: The chronic consumption of a High- Fat Diet (HFD) triggers signaling pathways related to inflammation, neuronal stress, and gliosis, particularly hypothalamic arcuate nucleus (ARC), a region responsible for regulating energy intake and expenditure. In addition, HFD exposure leads to structural changes in the hippocampus, including synapse and dendritic spine loss and decreases the number of glial cells. This inflammatory process leads to increased production and release of inflammatory cytokines, including interleukin (IL)-1 β , tumor necrosis factor (TNF)- α , and IL-6. In this sense, extra virgin olive oil (EVOO) supplementation, the primary fat source in the Mediterranean diet, with its particular composition rich in bioactive compounds could be an interesting therapeutic approach in a chronic HFD exposure model. EVOO has antioxidant and anti-inflammatory properties, contributing to the prevention of neurodegeneration, obesity and diabetes. This anti-inflammatory effect of EVOO is attributed to oleocanthal, a phenolic component, whose structure has a similar molecular geometry to ibuprofen, exhibiting cyclooxygenase (COX)-inhibiting activity. **Objective:** To evaluate the effects of EVOO supplementation in rats exposed to HFD on inflammation in the ARC of the hypothalamus and hippocampus. **Methods:** At 21 days, male and female Wistar rats (CEUA-UFRGS 41855, n = 30) were allocated into groups: (1) Standard chow (SC); (2) SC+EVOO; (3) HFD; (4) HFD+EVOO. EVOO was gavaged daily (dose 1.3mL/Kg body weight). Consumption and body weight were monitored weekly. Interleukin (IL)-6, IL-1 β , tumor necrosis factor (TNF)- α levels were measured by ELISA in the ARC and hippocampus. Immunoreactivity of ionized calcium-binding adaptor molecule 1 (IBA-1), a protein expressed in microglia, was measured in the ARC and CA1 region. **Results:** Food consumption increased over time ($p < 0.001$). HFD-males presented higher body weight ($p = 0.001$) and HFD+EVOO females decreased body weight ($p = 0.002$). In the ARC, HFD+EVOO group showed lower levels of IL-6 ($p = 0.006$ for males), IL-1 β ($p < 0.001$ for males, $p = 0.044$ for females) and TNF- α ($p = 0.002$ for males, $p = 0.046$ for females). In the hippocampus, EVOO supplementation significantly lowered IL-6 ($p = 0.001$), IL-1 β ($p = 0.001$), and TNF- α ($p < 0.001$) levels in HFD-fed females. Regarding IBA-1 protein, Regarding IBA-1, EVOO supplementation ($p < 0.001$) significantly lowered IBA-1 immunoreactivity in the ARC. In CA1, the immunoreactivity of IBA-1 was lower in HFD+EVOO males ($p = 0.048$) and females ($p = 0.050$). **Conclusions:** EVOO supplementation has shown promise, attenuating the metabolic consequences and inflammatory context caused by HFD exposure. **Financial support:** CAPES, FAPERGS e CNPq.

09.037 **Methyl Cinnamate Down-Regulates Epithelial Mesenchymal Transition Through Modulating NF- κ B/p65 Pathway in Alveolar Epithelial Cell.** Ferreira EGA, Barros ABB, Fidelix MSP, Silva GRS, Silva JP, Barreto E UFAL, Lab Biologia Celular

Introduction: Epithelial-to-mesenchymal transition (EMT) is a physiological process crucial for tissue repair, but when dysregulated, it contributes to the pathogenesis of several fibrotic diseases. EMT involves the loss of epithelial characteristics and the gain of a mesenchymal phenotype, enhancing cell motility and extracellular matrix production. This leads to tissue remodeling, excessive fibrous accumulation, and organ dysfunction, as observed in pulmonary fibrosis. Thus, agents that modulate EMT are promising for antifibrotic therapies. Methyl cinnamate (MC), a methyl ester of trans-cinnamic acid found in basil (*Ocimum basilicum*) essential oil, has known antioxidant and anti-inflammatory properties, but its effects on EMT remain unclear. This study aimed to investigate the effect and mechanisms of MC on EMT induced by transforming growth factor β 1 (TGF- β 1) in A549 alveolar epithelial cells. **Methods:** A549 cells were cultured in DMEM with 10% FBS, 1% penicillin/streptomycin, and 2 mM L-glutamine at 37 °C, 5% CO₂. MC cytotoxicity (1–30 μ M) was evaluated via MTT assay. EMT was induced using TGF- β 1 (10 ng/mL) with or without MC (10 μ M) for 24 h. Morphological changes were assessed by microscopy and morphometric analysis. Gene expression of epithelial and mesenchymal markers was analyzed by qPCR. Immunofluorescence was used to assess mesenchymal marker expression, Smad3 phosphorylation, and NF- κ B/p65 nuclear translocation. Data were analyzed using one-way ANOVA and Tukey's test ($p < 0.05$). **Results:** MC showed no cytotoxicity. TGF- β 1 induced EMT-like morphological changes, confirmed by reduced roundness and circularity, increased vimentin expression, mesenchymal gene upregulation, and enhanced migration at 24 and 48 h. TGF- β 1 also increased phospho-Smad3 and NF- κ B/p65 nuclear translocation by 3- and 1.5-fold, respectively. MC (10 μ M) attenuated these effects, reducing vimentin expression by 53%, partially restoring epithelial morphology, and reducing migration at time 24 h and 48 h by 35% and 23%, respectively. MC lowered mesenchymal gene expression but did not restore epithelial marker levels. It did not affect Smad3 phosphorylation but inhibited NF- κ B/p65 nuclear translocation by 31%. **Conclusion:** Our findings suggest that MC suppresses TGF- β 1-induced EMT in A549 cells, likely by inhibiting NF- κ B/p65 nuclear translocation, indicating its potential as an antifibrotic agent. **Financial Support:** CNPq, CAPES, BioproFar-BA, FAPEAL.

09.038 **Neuroprotective Effect of *Cannabis sativa* Oil-Rich in Delta-9-tetrahydrocannabinol (Δ^9 -THC) *in vitro* Epilepsy Model Is Modulated by Ionotropic Glutamatergic Receptors.** Tavares BLC¹, Aquino DN², Andrade GLR³, Marreiro VS², Ramalho IGS², Nunes VRV², Cipriano MSFL^{1,2}, Diniz MFFM², Donato MF⁴ ¹UFPE, PPG Ciências Farmacêuticas, Brazil, ² UFPB, PPG em Produtos Naturais e Sintéticos Bioativos, Brazil, ³UFPB, Brazil, ⁴UNILA, PPG em Biociências, Brazil

Introduction: The Endocannabinoid System (ECS) is a key modulatory axis of the central nervous system, crucial for synaptic plasticity and neuronal homeostasis. Within this framework, the ECS has been shown to regulate complex neurophysiological functions, including neurocognitive processing, nociception, and mood regulation. Neuromodulator effects occur through the regulation of excitatory neurotransmission, mainly for glutamatergic signalling. In this context, L-glutamate induces excitotoxicity that is involved in the pathophysiology of several neurological disorders, including epilepsy. Preclinical and clinical studies have demonstrated that phytocannabinoid-based therapies significantly improve the quality of life in patients with treatment-resistant conditions, such as Dravet Syndrome when compared to conventional treatments. **Objective:** Investigation of the pharmacological mechanisms of full-spectrum *Cannabis sativa* Oil-rich in delta-9-tetrahydrocannabinol (Δ^9 -THC) with a cellular epilepsy *in vitro* model. **Methodology:** Initially, *in silico* toxicity prediction using the ProTox 2.0 tool was employed to focus on the Δ^9 -THC mechanism, followed by an *in vitro* cytotoxicity MTT assay in an epilepsy model (SH-SY5Y cell line) treated with full-spectrum *Cannabis sativa* oil rich in Δ^9 -THC (ABRACE THC Gold oil 15 mg/mL). The neuroprotective mechanisms of Δ^9 -THC in the presence of pharmacological glutamate blockers were available. **Results:** *In silico* analysis predicted an 84% probability that Δ^9 -THC has low cytotoxicity and a 99% likelihood of interaction with the mitochondrial membrane potential. Moreover, *in vitro* studies demonstrated that Δ^9 -THC maintained cell viability at low concentrations, with an 88.92% survival rate at 0.5 μ g/mL. After excitotoxicity injury by L-glutamate, the viability ranged from 86.04% to 96.47% in the presence of Δ^9 -THC, suggesting a neuroprotective effect. In addition, the combination of the CNQX and MK-801 with Δ^9 -THC increased cell viability to 82.21% and 88.49%, respectively. **Conclusion:** Full-spectrum *Cannabis sativa* oil-rich in Δ^9 -THC demonstrated significant neuroprotective activity and low cytotoxicity at subtoxic concentrations in the cellular epilepsy model. Neuroprotection appears to be mediated via ECS in addition to the blockade of NMDA and AMPA/kainate ionotropic glutamatergic receptors. These findings are relevant for future research advancing the development of effective pharmacotherapies for neurotoxicity-related neuropathology. **Financial support:** Funded by Associação Brasileira de Apoio Cannabis Esperança (ABRACE), CNPq and FAPESQ-PB.

09.039 **Methyl Cinnamate Attenuates TGF- β 1-Induced Fibrotic Responses in Fibroblast via Inhibition of the Smad3 Pathway.** Barros ABB, Ferreira EGA, Fidelix MSP, Silva GRS, Silva JP, Santana JR, Barreto E UFAL, Lab Cell Biology

Introduction: Fibrosis is characterized by excessive deposition of extracellular matrix (ECM) components during tissue repair or regeneration following injury. It affects various organs, including lungs, heart, liver, and kidneys, leading to structural disruption and impaired function. Transforming growth factor-beta 1 (TGF- β 1) plays a pivotal role in fibrosis by promoting fibroblast proliferation and migration, myofibroblast differentiation, and ECM deposition. These effects occur mainly via the Smad-dependent signaling pathway, which regulates the expression of profibrotic genes. Targeting this pathway is a potential strategy to control fibrosis. Recent studies suggest that plant-derived compounds can modulate fibrotic processes. Methyl cinnamate, a methyl ester of hydroxycinnamic acid, exhibits anti-inflammatory and antioxidant activities, but its anti-fibrotic potential remains underexplored. This study aimed to evaluate the anti-fibrotic effects of methyl cinnamate in TGF- β 1-stimulated fibroblasts *in vitro*. **Methods:** L929 murine fibroblast cell line were cultured in DMEM with 10% FBS and 2 mM L-glutamine, maintained at 37°C with 5% CO₂. Cell viability was assessed by MTT assay after 24-hour exposure to methyl cinnamate (0.1–100 μ M). Migration was evaluated in TGF- β 1 (10 ng/mL)-stimulated fibroblasts treated with 1 or 10 μ M methyl cinnamate. Morphological changes were visualized using crystal violet staining. Immunofluorescence assays were performed to evaluate the expression of F-actin fibers, fibronectin, laminin, phosphorylated Smad3 (phospho-Smad3), and the proliferation marker Ki-67. Data were analyzed by one-way ANOVA and Tukey's test ($P < 0.05$). **Results:** Methyl cinnamate (0.1–10 μ M) did not affect viability, but 100 μ M reduced it by 46%. TGF- β 1 increased migration by 64%, which was reduced by 47% with 10 μ M methyl cinnamate. Treated cells showed decreased stress fiber elongation and reduced fibronectin and laminin expression by 29% and 23%, respectively. Ki-67 expression dropped by 57%, indicating antiproliferative activity. Mechanistically, methyl cinnamate inhibited TGF- β 1-induced nuclear translocation of phospho-Smad3. **Conclusion:** These findings demonstrate that methyl cinnamate suppresses fibrotic responses in fibroblasts, including migration, proliferation, ECM production, and cytoskeletal reorganization, likely via inhibition of the Smad3 pathway. Thus, methyl cinnamate emerges as a potential anti-fibrotic agent for further investigation.

Financial Support: CNPq, CAPES, BioproFar-BA, FAPEAL.

09.040 *Spondias tuberosa* Arruda Extract Attenuates Liver Damage in Streptozotocin-Induced Diabetic Animals by Modulating Oxidative Enzymes. Silva CPC¹, Sena V¹, Pires LAB¹, Freitas L¹, Araujo RFA¹, Abreu BJA¹, Araujo IGA², Medeiros IA², Santos, DD³, Gil CD³ Medeiros KCP¹ UFRN, Dept Morphology, Brazil, ²UFPB, Dept Health Sciences, Brazil. ³Unifesp-EPM, Dept Morphology and Genetics, SP, Brazil

Introduction: Diabetes mellitus (DM) is a chronic metabolic disorder characterized by persistent hyperglycemia resulting from impaired insulin secretion and/or action, ultimately leading to long-term complications. Conventional pharmacological therapies for DM are often associated with significant adverse effects, thereby underscoring the need for alternative treatments. Natural products have emerged as promising therapeutic agents in this context. *Spondias tuberosa* Arruda, commonly known as umbuzeiro, has been traditionally employed in folk medicine for the management of digestive disorders, infections, and diabetes. Given its pharmacological potential, the present study aimed to evaluate the antidiabetogenic effects of *Spondias tuberosa* extract (ExSt) in streptozotocin-induced diabetic rats, through clinical, biochemical, and histopathological assessments, as well as the analysis of oxidative stress-related molecular markers. **Methods:** 52 Wistar rats, approved by the ethics committee of the UFRN (319.056/2022) were divided into the following experimental groups: CT (non-diabetic control), DM (diabetic), EX (non-diabetic treated with ExSt), DM/EX (diabetic treated with ExSt), and DM/IN (diabetic treated with insulin). DM was induced by streptozotocin (i.p. 50 mg/kg), ExSt (500 mg/kg, orally) and NPH insulin (10 IU, s.c) were administered daily for 30 days following DM induction. **Results:** Biochemical analyses revealed that diabetic animals exhibited marked hyperglycemia, polydipsia, polyphagia, and significant body weight loss. Elevated serum levels of hepatic enzymes (AST and ALT), along with pronounced histopathological alterations in hepatic and pancreatic tissues, were also observed. Treatment with ExSt resulted in significant reductions in AST and ALT levels, attenuation of hepatic glycogen depletion, and preservation of pancreatic tissue architecture. Additionally, modulation of oxidative stress-related enzymes suggest potential mechanisms underlying the therapeutic effects of ExSt in experimental diabetes. **Conclusion:** Although ExSt does not normalize blood glucose levels, it improves the morphology of liver damage and reduces oxidative stress. These findings contribute to the broader understanding of the complex pathophysiology of type 1 diabetes and support the exploration of plant-based therapeutic alternatives derived from Brazil's diverse flora.

Financial support: CNPQ

09.041 *Boswellia serrata* Dry Extract with Intestinal Anti-Inflammatory Properties Also Accelerates Gastric Ulcer Healing in Rats. Oliveira IM¹, Serafim FGS², Breguez L², Venzon L², Santos AC², Longo B¹, Nunes RK¹, Gutiérrez MV³, Roman-Junior WA⁴, Silva LM¹ ¹UFSC, Center of Biological Sciences, PostGraduation Program of Pharmacology, Brazil ²Univali, Post-Graduation Program of Pharmaceutical Sciences, Brazil ³University of Sonora, Dept Chemistry, Biology and Agricultural Sciences, Mexico ⁴Unochapecó, Post-graduation Program of Health Sciences, Brazil

Introduction: *Boswellia serrata* Roxb. ex Colebr. is a medicinal plant traditionally used in oriental medicine. Its resin is rich in boswellic acids, which exhibit anti-inflammatory, antioxidant, and immunomodulatory properties. These bioactive compounds have demonstrated therapeutic potential in chronic inflammatory conditions, particularly those affecting the gastrointestinal tract. This study aimed to evaluate the gastric healing and intestinal anti-inflammatory effects of *Boswellia serrata* dry extract (BSDE) in preclinical models of chronic gastric ulcer and ulcerative colitis in Wistar rats. **Materials and Methods:** Chronic gastric ulcers were induced using 80% acetic acid. After two days, animals were treated orally with BSDE (30, 100, or 300 mg/kg), omeprazole (40 mg/kg), or vehicle for seven days, twice daily. After euthanasia, the ulcer area, mucin content, and oxidative stress markers (GSH, SOD, CAT, GST, MPO, MDA) were assessed. Ulcerative colitis was induced by rectal administration of 8% acetic acid, followed by once-daily administration of BSDE (100 or 300 mg/kg), dexamethasone (2 mg/kg), or vehicle for three days before and after the colitis. Colonic damage was evaluated macroscopically and histologically, along with mucin staining and oxidative stress biomarkers. Animal procedures were approved by the CEUA/UNIVALI (Protocol No. 024/22). **Results:** BSDE at 100 and 300 mg/kg accelerated gastric ulcer healing, improved antioxidant status, and modulated mucin production. In the colitis model, BSDE reduced macroscopic and histopathological damage, preserved crypt architecture, decreased inflammatory infiltration and edema, and enhanced mucin staining. Both doses of the extract reduced MDA levels, while 100 mg/kg also increased GSH levels in colonic tissue. **Conclusions:** BSDE demonstrates gastric healing actions and intestinal anti-inflammatory effects, likely mediated by its antioxidant and mucosal protective mechanisms. These findings support the traditional use of *Boswellia serrata* in gastrointestinal disorders and suggest its potential as a phytotherapeutic agent. **Funding:** CNPq/CAPES.

09.042 **Antivenom potential of *Jatropha mutabilis* (Pohl) Bail. (Euphorbiaceae) against *Bothrops leucurus* venom.** Veras BO, Souza FS, Ximenes RM UFPE, Lab. of Ethnopharmacology and Phytochemistry, Brazil

The genus *Jatropha* is traditionally recognized worldwide for its medicinal properties, particularly in the treatment of snakebites. *Jatropha mutabilis* (Pohl) Bail., commonly known as “pinhão” or “pinhão-de-seda” in the semi-arid region of Northeast Brazil, is a medicinal plant traditionally used as an antidote for snake envenomation. The latex exuded from its stems and branches is either ingested or applied topically to the bite site for this purpose. This study aimed to evaluate the antivenom potential of *J. mutabilis* latex against *Bothrops leucurus* venom by assessing its efficacy in neutralizing venom-induced toxic effects. The investigation sought to support the ethnopharmacological use of this species and explore its potential as a natural and accessible therapeutic option for envenomation treatment. Latex from *J. mutabilis* (Jm) was collected in situ by making incisions in the stems and branches of selected specimens at Vale do Catimbau National Park, Brazil (SISBIO permit no. 80,236-1). A voucher specimen was deposited in the IPA Herbarium (no. 95,334). The antivenom activity of the latex was evaluated *in vitro* by testing its ability to inhibit key venom enzymes, including proteases, hyaluronidase, phospholipase A₂, and DNase, as well as hemolytic, fibrinolytic, and fibrinogenolytic activities. Coagulation parameters—total plasma coagulation time, prothrombin time (PT), and activated partial thromboplastin time (aPTT)—were measured using a commercial plasma kit. Interactions between the latex components and venom proteins were analyzed by SDS-PAGE. The *in vivo* efficacy of the latex was assessed in Swiss mice (CEUA protocol no. 0019/2024, UFPE) for its ability to reduce nociceptive, edematogenic, and hemorrhagic effects induced by *B. leucurus* venom, using three treatment protocols. The latex fully inhibited protease, hyaluronidase, phospholipase A₂, and fibrinolytic activities at a venom-to-latex ratio of 1:4 (w/w). DNase and fibrinogenolytic activities were completely inhibited at a 1:1 ratio, and indirect hemolytic activity was neutralized at a 1:8 ratio. The latex also attenuated the venom's procoagulant effects, significantly reducing PT and restoring aPTT levels. SDS-PAGE analysis revealed precipitation of venom proteins above 52 kDa, suggesting complex formation. In animal models, oral administration of the latex (50, 100, and 200 mg/kg) either before, after, or pre-incubated with the venom (ratios of 1:0.25, 1:2, and 1:16, w/w) significantly reduced edematogenic and hemorrhagic effects at all doses and ratios. The nociceptive response was also reduced in pre-treated and pre-incubation protocols. These results underscore the antivenom potential of *Jatropha mutabilis* latex, supporting its traditional use and highlighting its promise as a natural therapeutic agent for snakebite envenomation.

09.043 **Luteolin Ameliorates Valproic-Acid Autism-Like Behaviors in Rats through the Attenuation of Neuroinflammation, Oxidative Stress, and Neural Damage.** Longo B¹, Nunes RKS¹, De Siqueira MCB², Ferreira PC², Cazarin CA², De Queiroz e Silva TF², Dos Santos AC², Venzon L², Da Silva LM², Pagliochi AC², Sievers J², De Souza MM², Da Silva LM¹. ¹UFSC, Dept Pharmacology, ²Univali, Graduate Program in Pharmaceutical Sciences

Introduction: Autism spectrum disorder (ASD) is characterized by deficits in social interaction, restrictive interests, and repetitive behaviors. In scenarios where pharmacological treatments for ASD are limited, flavonoids such as luteolin may offer a promising alternative due to their antioxidant, anti-inflammatory, and neuroprotective properties. Thus, the objective of this study was to examine the effects of luteolin on neurobehavioral changes in an experimental model of ASD induced by prenatal exposure to valproic acid (VPA). **Methods:** Pregnant rats received VPA (600 mg/kg, i.p.) or saline on gestational day 12.5. After birth, male offspring were evaluated for neurodevelopment from day 7 to 18. On postnatal day 23, the offspring were treated orally with vehicle (water plus 1% Tween, 1 ml/kg) or luteolin (10, 30, and 100 mg/kg). On postnatal day 60, social interaction, object recognition index, elevated plus maze, and open field tests were performed. Oxidative and inflammatory parameters were assessed in the cortex and hippocampus, and histological analyses were performed on the hippocampus. (CEUA/UNIVALI: 034/22p).

Results: Animals prenatally exposed to VPA showed poorer performance in neurodevelopmental tests compared to the control group ($p < 0.05$). Similarly, 60 days postnatally, the vehicle group showed decreased social interaction time ($p < 0.0001$), object recognition index ($p < 0.001$), and dwell time in open arms ($p < 0.01$) and impaired locomotor and exploratory activity ($p < 0.01$). In contrast, luteolin treatment increased social interaction time and reversed behavioral deficits observed in the object recognition, elevated plus maze, and open field tests caused by prenatal VPA exposure ($p < 0.05$ vs. vehicle group exposed to VPA). In addition, luteolin treatment increased levels of glutathione in the hippocampus, reduced malondialdehyde and myeloperoxidase levels in the cortex and hippocampus, and attenuated hippocampal damage compared to the VPA-exposed vehicle group. **Conclusions:** Luteolin attenuated the neurobehavioral disorders induced by prenatal VPA exposure, making it a promising therapeutic source in the search for treatments that improve central changes associated with ASD. **Financial support:** CAPES.

09.044 **Regenerative Effects of Citral in Human Epithelial Cells and Colon Organoids.** Fagundes FL¹, Aal MCE², Luo X³, Okhovat A³, Castillo F³, Delella FK², Villablanca EJ³, Hiruma-Lima CA¹ ¹Unesp-Botucatu, Dept Structural and Functional Biology, sector of Physiology, Lab Biological Assays with Natural Products, Brazil ²Unesp-Botucatu, Dept Structural and Functional Biology, sector of Morphology, Interactions and Cancer Biology Lab, Brazil ³Karolinska Institutet and University Hospital, Dept Medicine, Division of Immunology and Respiratory Medicine, Stockholm, Sweden

Introduction: Inflammatory bowel disease (IBD) is a chronic and relapsing condition that affects 5 million people worldwide, with a growing incidence in developing countries. Pharmacological treatment consists of immunosuppressive agents, such as glucocorticoids and JNK inhibitors. These drugs have significant side effects and do not promote sustainable mucosal healing. Citral is a monoterpene present in plant species, such as lemon grass (*Cymbopogon citratus*), with anti-inflammatory and analgesic effects; however, there is no evidence indicating its regenerative potential. **Materials and Methods:** Caco-2 cells purchased from the Rio de Janeiro Cell Bank were cultivated in DMEM/F12 with 10% fetal bovine serum for 5 days. After reaching 85% confluence, the cells were transferred to a 96 well plate at a density of 1×10^4 cells/well and treated with citral (10, 20, 40, 80, and 100 μ M) for 24, 48, or 72 h. To investigate the effects of citral on the migration of epithelial cells, the scratch assay was performed in a 12 well plate for 48 h. The monoterpene response was also investigated in colon organoids derived from C57BL/6J male mice (Approval 6778-2020). Crypts were isolated from the distal intestine and cultivated in WENR medium for three days and then ENR for two additional days. On the fifth day, single-cell suspensions were generated, and the cells were seeded at a density of 1×10^4 cells/well and treated with citral for 5 days (10, 20, 40, 80, and 100 μ M). The organoid number, size, and area were analyzed using the IncuCyte live cell imaging system. Bulk RNA sequencing and immunofluorescence were performed to evaluate the possible mechanism of action of citral. The data was expressed as mean $\pm$ standard error of the mean and the statistical significance was determined using (Anova) followed by Dunnett test **Results:** Citral treatment increased the cell viability in 24 (20, 40, 80 and 100 mM) and 48 hours (40 and 80 mM) when compared to non-treated cells (n=4). The opposite effect was observed at 80 mM after 72 h, where the molecule decreased proliferation by 40% compared to the vehicle. Citral 40 mM accelerated wound closure at 12, 24, and 48 h compared to the control, with no difference among the intervals analyzed. The monoterpene kept the organoid formation in the lowest and intermediate concentrations (10, 20, 40 mM) and decreased the organoid counting, size and area with highest concentrations (80 and 100 mM) when compared to non-treated organoids **Conclusion:** Citral treatment showed a proliferative potential in the Caco-2 cells confirmed by the scratch assay. The molecule also maintained organoid growth at levels like those of the untreated group. Together, these results suggest that citral is a novel compound that promotes mucosal healing and should be further investigated through *in vivo* assays. **Financial support:** CAPES (88887.689603/2022-00), Fapesp (2022/02730-5; 2024/01427-2), CNPq (311957/2020-0).

09.045 **Assessment of *Bothrops jararacussu* Venom-Induced Cardiotoxicity in an Isolated Heart (Langendorff) Model.** Almeida RACS¹, Santos JS¹, Amorim LS¹, Bueno GS¹, Melo RL¹, Monteiro-Machado M², Melo PA², Olivares EL¹, Strauch MA¹ ¹UFRRJ. Lab Fisiologia e Farmacologia Cardiovascular. Depto de Ciências Fisiológicas, Instituto de Ciências Biológicas e da Saúde. ²UFRJ. Lab Farmacologia das Toxinas, Programa de Pós-Graduação em Farmacologia e Química Medicinal, Instituto de Ciências Biomédicas

Introduction: Snakebites represent a significant global health burden, particularly in tropical regions of Africa, Asia, and Latin America, where they frequently result in severe morbidity (including amputations and permanent disabilities) and approximately 125,000 annual deaths according to WHO estimates. Envenomation by Crotalid snakes (family Viperidae) induces complex pathophysiological effects, ranging from prominent local tissue damage (edema, hemorrhage, and necrosis) to systemic manifestations including coagulopathy, acute kidney injury, and cardiovascular collapse. Notably, cardiovascular shock has been identified as a major cause of mortality following envenomation by snakes of the genus *Bothrops*. Despite its clinical relevance, the cardiotoxic mechanisms of *Bothrops* venoms remain poorly understood, particularly their direct effects on myocardial contractility and cardiomyocyte integrity. **Methods:** All experimental procedures were conducted in accordance with ethical guidelines (CEUA/ICBS/UFRRJ Protocol No 23083.056074/2024-17). Hearts were excised from euthanized male Wistar rats (250-300 g; n = 5 / Group) and immediately mounted on a Langendorff perfusion apparatus. Following a 30-minute stabilization period with oxygenated Krebs-Henseleit buffer (37°C, pH 7.4), baseline left ventricular developed pressure (LVDP) was recorded for 20 minutes. The hearts were then randomly assigned to three experimental groups receiving *Bothrops jararacussu* venom at different concentrations: 2.5 µg/mL (low dose), 5.0 µg/mL (medium dose), and 10.0 µg/mL (high dose). Hemodynamic parameters were continuously monitored throughout the experiment. **Results:** LVDP decreased significantly from baseline (100%) values by 62.45% ± 9.28, 56.45% ± 6.14, and 60.24% ± 8.32 in the 2.5, 5.0, and 10.0 µg/mL groups (p < 0.05), respectively. The survival time of perfused hearts showed an inverse relationship with venom concentration: cardiac function was maintained for 60 minutes in the 2.5 µg/mL group, compared to 25 minutes in the 5.0 µg/mL group and only 10 minutes in the 10.0 µg/mL. These results demonstrate the acute hemodynamic effects and the duration of cardiac viability following venom perfusion. **Conclusion:** The present study provides direct experimental evidence of the cardiotoxic potential of *B. jararacussu* venom in an isolated heart model. Our findings demonstrate that venom exposure induces: (1) significant decreases in ventricular pressure development, and (2) concentration-dependent reductions in cardiac viability time. These observations suggest that the venom contains components that directly affect myocardial contractility and cardiac endurance. **Financial Support :** FAPERJ; CNPQ.

09.046 **Investigation of the Protective Effect of Polysaccharides Extracted from Tamarillo (*Solanum betaceum*) in an Ulcerative Colitis Model.** Braga LLVM¹, Schiebel CS¹, Sauruk KS¹, Maia MHS¹, Fernandes ACVU¹, Nascimento GE², Issa TA³, Gois MB³, Cordeiro LMC², Maria-Ferreira D¹ ¹FPP-IPPPP, Programa de Pós-Graduação em Biotecnologia Aplicada à Saúde da Criança e do Adolescente; ²UFPR, Programa de Pós-Graduação em Ciências-Bioquímica, Faculdade de Ciências da Saúde, ³UFR

Introduction: Inflammatory bowel disease (IBD) includes chronic inflammatory diseases of the gastrointestinal tract, such as ulcerative colitis (UC) and Crohn's disease. Although the etiology is not fully understood, genetic factors, epithelial barrier dysfunction and changes in the intestinal microbiota play an important role. UC affects the colon and rectum and causes persistent inflammation and symptoms such as bloody diarrhea. Treatment aims to induce remission and promote healing of the mucosa. Common medications include aminosalicylates (5-ASA), corticosteroids, thiopurines and tumor necrosis factor-alpha (TNF- α) inhibitors, which improve outcomes. However, adverse effects (e.g. headaches, nausea, fatigue) and treatment failure, which affect up to a third of patients, remain a challenge. Natural polysaccharides have shown anti-inflammatory and healing potential. Polysaccharides from the pulp of tamarillo (*Solanum betaceum*) have known bioactive properties, but their effects on UC have not been investigated so far. **Methods:** The polysaccharides of tamarillo pulp (STWA) were extracted and tested in female BALB/c mice. The study was approved by the Institutional Animal Care and Use Committee (CEUA, protocol 067-2022). UC was induced with dextran sulfate sodium (DSS), and STWA was administered at a dose of 10, 30 or 100 mg/kg. Disease activity and body weight were monitored daily. At the endpoint, the large intestines were removed and measured. The markers of oxidative stress and inflammation were determined. Histological analysis was performed to determine tissue changes. **Results:** Treatment with STWA at a dose of 100 mg/kg significantly prevented weight loss (-7.08% vs. -15.67% in the DSS group) and reduced the disease activity index in DSS-induced colitis. The length of the colon was preserved (8.65 vs. 7.1 cm) and occult blood in the stool was reduced. Lower doses (10 and 30 mg/kg) showed partial effects; biochemical and histologic analyses were performed only at 100 mg/kg. STWA improved antioxidant defenses, increased GST activity (1077.00 vs. 923.46 nmol/min/ μ g protein) and reduced lipid peroxidation (1.37 vs. 2.61 μ M H₂O₂/ μ g protein). TNF- α levels decreased (407.10 to 264.50 pg/mg), while IL-10 increased (218.50 to 571.40 pg/mg). Histology showed a reduced infiltrate, improved mucosal and submucosal structure and a lower histopathologic score. STWA also restored goblet cell count (221,170 vs. 92,854) and mucin content and preserved epithelial integrity. **Conclusions:** STWA showed a protective effect in a mouse model of UC by modulating oxidative stress and inflammation and preserving the structure of colon tissue. **Financial support:** This work was supported by Capes (Finance Code 001), the Instituto de Pesquisa Pelé Pequeno Príncipe, and CNPq (Grant numbers: 302195/2022-0 to DMF and 404717/2016-0, 310332/2015-0 and 307314/2018-9 to LMCC). **References:** Ordas, I. The Lancet, 380, 1606–1619, 2012. Kobayashi, T. et al. Nat. Rev. Dis. Primers, 6, 74, 2020.

09.047 **Cockroaches** (*Nauphoeta cinerea*) as an Experimental Model for Screening Coralsnake Venoms. Oliveira IN¹, Oliveira RS¹, Dias DRC², Guimarães MP³, Vinadé LHC⁴, Dal Belo CA^{1,3}, Silva NJ Jr⁵, Hyslop S¹ ¹FCM-UNICAMP, Depto de Farmacologia, PPG Farmacologia, Brazil; ²UFES, Depto de Estatística; ³EPPEN-Unifesp-Osasco, Depto Multidisciplinar, ⁴Unipampa-São Gabriel, ⁵PUC-GO, PPG em Ciências Ambientais e Saúde

Introduction: Historically, the biological activities of coralsnake (*Micrurus* spp.) venoms have been assessed in rodents (mice and rats) and avian (chick) preparations *in vivo* and *in vitro*. In contrast, few studies have examined the usefulness of invertebrates for such studies, despite advantages such as the ease of handling, the low cost of reproduction and maintenance, and the lack of a need for approval by ethics committees. The lobster cockroach (*Nauphoeta cinerea*) has been used to study the neurotoxicity of pesticides and scorpion and toad venoms, but few studies have examined their suitability for screening snake venoms. In this work, we used cockroach preparations to screen the venoms of three coralsnake species (*Micrurus dumerilli carinicauda*, *Micrurus spixii* and *Micrurus surinamensis*). **Methods:** Cockroaches obtained from a local supplier were used in three assays (skeletal neuromuscular junction, semi-isolated heart and locomotory activity) as previously described (Rosa et al., 2023, J Exp Biol 226, jeb245239; Ferreira et al., 2024, Pestic Biochem Physiol 205, 106167). The data were expressed as the mean±SD and statistical comparisons were done using one-way ANOVA followed by the Tukey test, with $p < 0.05$ indicating significance. All data analyses were done using Prism software v.5 (GraphPad). **Results:** *Micrurus surinamensis* venom (150 mg) caused no neuromuscular failure, whereas this same dose of *M. d. carinicauda* and *M. spixii* venoms caused 85-90% blockade after 60 min ($n=5$ each). In semi-isolated heart preparations, *M. surinamensis* venom (300 mg) was not cardiotoxic after 60 min, whereas the venoms of *M. d. carinicauda* (300 mg) and *M. spixii* (150 mg) caused progressive bradycardia with complete cardiac arrest after 65 min ($n=6$) and 25 min ($n=10$), respectively. In the locomotion assay, *M. surinamensis* venom (300 mg) increased the distance travelled (from 643 ± 200 to 1399 ± 292 cm; $n=10$; $p < 0.05$) but did not affect the number or duration of stops during travelling; the venom did not kill the cockroaches during a 60 min observation. *M. d. carinicauda* venom (300 mg; $n=10$) increased the distance travelled (from 522 ± 190 to 896 ± 210 cm; $p < 0.05$), but did not alter the number or duration of stops; cockroaches died after 15.4 ± 4.9 min. *M. spixii* venom (300 mg; $n=10$) did not affect the distance travelled or the number of stops, but increased the total duration of the stops (from 15 ± 2.6 to 42.6 ± 12.7 min; $p < 0.05$); cockroaches died after 14.3 ± 5.9 min. **Conclusion:** These results indicate that cockroaches provide a useful alternative animal model for screening the biological activities of coralsnake venoms and allow distinction among venoms. The use of cockroaches is compatible with the replacement of vertebrates with invertebrates in the 3Rs of animal experimentation.

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09.048 **Acute Toxicity Evaluation and Gastroprotective Activity of the Flavone Chrysin in Female Rodents.** Pereira MC¹, Pereira QC², Santos RC³ ¹USF, School of Medicine, Brazil; ²USF, Graduate Program in Health Sciences, Brazil; ³USF, Graduate Program in Health Sciences, Brazil

Introduction: Chrysin is a polyphenolic compound belonging to the flavonoid class, specifically classified as a flavone. It is naturally found in honey, propolis, and *Passiflora caerulea* L., and exhibits antioxidant and anti-inflammatory properties, with potential therapeutic effects in gastrointestinal disorders. Previous studies have demonstrated chrysin's gastroprotective activity in male mice; however, data on its effects in females remain scarce. **Objective:** Evaluate the gastroprotective potential of the flavone chrysin in ethanol-induced gastric ulcer and assess its acute toxicity profile in female mice (OECD 425 protocol). **Methods:** In the acute toxicity test, female mice received a single oral dose of chrysin (2000 mg/kg) and were monitored over 14 days for behavioral changes, body weight, and biochemical parameters. For the gastroprotection assay, female mice were ovariectomized and divided into groups with or without 17 β -estradiol hormone replacement. After 14 days, the animals were treated with chrysin (10, 50, or 100 mg/kg), and absolute ethanol was administered to induce gastric lesions. Euthanasia was then performed for lesion assessment and biochemical analysis. Antioxidant enzymes such as glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), and myeloperoxidase (MPO) activity were evaluated, along with gene expression of IL-10 and MUC-5aC. Statistical analysis was performed using one-way ANOVA followed by Dunnett's or Student's T-tests, with $p < 0.05$ considered significant. Data was expressed as mean $\pm$ standard error. Analyses were conducted using GraphPad Prism 8. This study was approved by the Animal Use Ethics Committee of Universidade São Francisco (protocol nº 004.03.2020). **Results:** The 2000 mg/kg dose did not produce behavioral or biochemical alterations, indicating safety at a single dose. The 10 mg/kg dose was the most effective in reducing gastric lesions. Chrysin significantly reduced MPO activity in both estradiol-treated and untreated groups, suggesting an anti-inflammatory effect. Increased GSH and CAT activity was observed in hormone-deprived females, while SOD remained unchanged. MUC-5aC expression was elevated in the estradiol-treated group, with no significant changes in IL-10 expression. **Conclusion:** Chrysin was shown to be safe at high doses and effective in promoting gastric protection in female mice, with its mechanisms of action influenced by hormonal status. These findings reinforce chrysin's therapeutic potential and highlight the importance of including both sexes in preclinical studies to elucidate hormone-dependent pharmacological responses. **Keywords:** Chrysin, acute toxicity, gastroprotection, female mice, estrogen. **Funding:** FAPESP (Grant nº 2019/12510-0)

09.049 **Brown Propolis Produced by *Apis mellifera* Bees from Crateús, CE Prevents Tracheal Hyperreactivity in Wistar Rats with Allergic Asthma.** Alves BFL¹, Martins AMO³, Felício IM³, Oliveira LN³, Figueiredo IAD³, Souza MLB¹, Silva SO², Cordeiro KM², Silva TMS⁴, Cavalcante FA^{3,5} ¹UFPB, PIBIC, Brazil; ²UFPB, PIVIC, Brazil; ³ UFPB, CCS, PPgPNSB, Brazil; ⁴UFRPE, DCM, Brazil; ⁵UFPB, DCB, CCS, Brazil

Introduction: Asthma is a heterogeneous disease primarily characterized by airway obstruction due to reduced airway lumen diameter. This condition is accompanied by increased mucus production caused by goblet cells hyperplasia and hypertrophy, along with smooth muscle layer thickening and tissue remodeling (Hammad, H. Cel., v. 184, p. 1469, 2021). Approximately 10% of individuals with severe asthma do not respond to usual pharmacotherapy (Bourdin, A. Jour. Aller. Clin. Immu.: Pract., v. 12, p. 809, 2024). In this context, the search for new therapeutic alternatives arises. Natural products have emerged as promising, including bee-derived compounds such as brown propolis produced by *Apis mellifera* bees from Crateús – CE (Brazil). This study aimed to evaluate the preventive effect of brown propolis on tracheal reactivity of Wistar rats with ovalbumin-induced allergic asthma. **Methods:** Rats were randomly assigned to one of the following groups: control (CG), asthmatic (AG), asthmatic treated with brown propolis at 125, 250, or 500 mg/kg (APG125, APG250, APG500), or asthmatic treated with dexamethasone at 5 mg/kg (ADEXAG5). Asthma was induced by ovalbumin sensitization for 21 days. Non-asthmatic animals received 0.9% saline instead of ovalbumin (adapted from Galvão, JGF. Inf. Res., v. 66, p. 1117, 2017). Propolis was administered daily at the respective doses through intragastric gavage. After euthanasia, the tracheal rings were suspended in isolated organ baths and isometric contractions were monitored (n = 5). The results were analysed in GraphPad Prism®. Experimental procedures were approved by the Ethics Committee on the Use of Animals of UFPB (nº 8719020621). **Results:** Regarding contraction via pharmacomechanical coupling, an increase in contractile efficacy to carbachol was observed in AG ($E_{max}=139.8 \pm 6.8\%$) when compared to CG ($E_{max}=100\%$), and this increase was prevented in APG125, APG250, APG500 and ADEXAG5 ($E_{max}=99.8 \pm 9.7\%$; $77.1 \pm 3.2\%$; $93.1 \pm 4.6\%$ and $105.3 \pm 9.1\%$, respectively). Similarly, in the evaluation of the participation of the pharmacomechanical coupling of relaxation, a decrease in the relaxing efficacy and potency of aminophylline was also observed in AG ($E_{max}=85.1 \pm 1.4\%$; $EC_{50}=1.0 \pm 0.05 \times 10^{-3} \text{ M}$). The decrease in efficacy was prevented in APG125, APG250 and APG500 ($E_{max}=104.3 \pm 1.9\%$; $101.1 \pm 0.5\%$ and $96.1 \pm 2.9\%$, respectively), but not in ADEXAG5 ($E_{max}=90.4 \pm 0.9\%$), whilst the potency decrease was only prevented in APG500 ($EC_{50}=8.7 \pm 0.9 \times 10^{-4} \text{ M}$). **Conclusion:** It is possible to suggest that treatment with brown propolis produced by *Apis mellifera* bees from Crateús – CE prevents tracheal hyperresponsiveness in the ovalbumin-induced allergic asthma model in Wistar rats by negatively modulating the pharmacomechanical component of contraction and positively modulating the relaxation component. **Financial support:** PPgPNSB/CCS/UFPB, FAPESQ, CAPES and CNPq. **Keywords:** Brown propolis, Asthma, Tracheal reactivity.

09.050 *Arthrospira platensis* Prevents Intestinal Reactivity Induced by Obesity by Modulation of Oxidative Stress and Nitric Oxide Pathways: A Promising Nutraceutical Strategy Sousa Filho JEC¹, Arruda RRA^{1,2}, Melo MB¹, Diniz AFA^{1,2}, Silva JMA^{1,2}, Claudino BFO^{1,2}, Barros BC^{1,2}, Soares MFS^{1,2}, Silva BA^{1,2,3} ¹UFPB, Lab Functional Pharmacology Prof. George Thomas, Institute of Research in Drugs and Medicines, Brazil ²UFPB, PPG Bioactive Natural and Synthetic Products, Health Sciences Center, Brazil ³UFPB, Dept Pharmaceutical Sciences, Health Sciences Center, Brazil

Obesity has become a major global public health issue and is associated with several metabolic and inflammatory comorbidities, including dyslipidemias, hypertension, and gastrointestinal disorders, such as alterations in intestinal motility. In this context, the search for safe and effective therapeutic alternatives has gained prominence. *Arthrospira platensis* (AP), is a cyanobacterium with recognized nutraceutical potential, noted for its composition rich in proteins, vitamins, minerals, and bioactive compounds that confer antioxidant, anti-inflammatory, and lipid metabolism-modulating properties. Recent studies suggest that AP may prevent obesity-related complications by modulating oxidative stress and tissue redox signaling (Diniz, A. F. A. *J. Funct. Foods*, v. 115, p. 106116, 2024). This study aims to investigate the preventive mechanisms of AP on changes in relaxant reactivity in male Wistar rat ileum induced by obesity. Animals were randomly allocated into four experimental groups (n=5): 1 – standard diet (SD); 2 – standard diet supplemented with AP 25 mg/kg (SD+AP25); 3 – hypercaloric diet (HCD); and 4 – hypercaloric diet supplemented with AP 25 mg/kg (HCD+AP25). Diets and supplementation were offered for eight weeks, with AP delivered via intragastric gavage. The isolated ileum of the rats was mounted in organ bath chambers, and the tissue's relaxant reactivity was monitored. The pCE₅₀ was evaluated as a parameter of potency. Results were expressed as mean ± standard deviation and analyzed using one-way ANOVA followed by Tukey's post hoc test (p < 0.05, n = 5). Pharmacological analysis revealed that the HCD group showed reduced relaxant potency to verapamil (pCE₅₀= 4.8±0.6) compared to SD (pCE₅₀= 10.1±0.6) and HCD+AP25 (pCE₅₀= 11.5±0.3), with no difference between the latter two, indicating a preventive effect of AP. To investigate underlying mechanisms, tempol—a superoxide dismutase (SOD) mimetic—was used and induced a leftward shift in the concentration-response curve (pCE₅₀= 13.5±0.1), suggesting antioxidant participation mediated by SOD. Evaluation of the nitric oxide (NO) pathway using the non-selective nitric oxide synthase inhibitor L-NAME showed a reduction in relaxant potency (pCE₅₀= 8.2±0.4) compared to the HCD+AP25 group without inhibitor, suggesting the involvement of NO in AP's preventive effect. These findings indicate that AP may downregulate peroxynitrite formation—a reactive compound generated by the interaction between NO and superoxide anion—thus preserving intestinal smooth muscle function. In conclusion, AP supplementation prevented hypercaloric diet-induced impairment of ileal relaxant reactivity through mechanisms involving oxidative stress modulation and NO/SOD signaling. These results support the potential of AP as an adjuvant agent in preventing intestinal dysfunctions associated with obesity. All procedures were approved by the Ethics Committee on Animal Use (CEUA/UFPB), number 1664310523/ID001937. **Financial support:** CNPq, CAPES.

09.051 **Development and Characterization of a Food Gel Based on *Mauritia flexuosa* Mesocarp.** Teixeira MC¹, Amorim VR¹, Oliveira JHMX¹, Santos WB¹, Almeida AAC¹, Porto JCS¹, Pereira-Freire JA², Silva JN³, Ferreira PMP¹ ¹UFPI, Lab Experimental Cancerology (LabCancer), Dept Biophysics and Physiology; ²UFPI, Dept Nutrition; ³UFPI, Dept Chemistry

Introduction: The increased incidence of chronic diseases, such as hypertension, diabetes and dyslipidemia, associated with imbalance of endogenous antioxidants, have emphasized functional foods as natural therapeutic support strategies. *Mauritia flexuosa* (buriti), a native Brazilian fruit, is rich in bioactive compounds with recognized chemopreventive, anti-inflammatory, and hypolipidemic properties. Objective: To develop a food gel based on freeze-dried buriti mesocarp and evaluate its physicochemical, microbiological, and functional properties. Methodology: About one hundred and fifty fruits were collected in Água Branca, Piauí, Brazil, 2018. Afterwards, it was performed selection of fruits, cleaning, separation, lyophilization, packaging, refrigeration, and pulverization (SisGen #A690444). The gel was formulated using 150 g of freeze-dried buriti mesocarp powder, 348 g of carbogel, 1.5 g of powdered ginger, and 222 mL of deionized water, yielding 32 sachets of 20 g each. Fourier-transform infrared spectroscopy (FTIR) was used for chemical characterization, alongside analyses of pH, density, moisture, yield, protein content (Kjeldahl method), ash, total and reducing sugars (Lane-Eynon method), vitamin C (Tillmans method), titratable acidity, and water activity. Microbiological assessments included quantification of fungi, yeasts, mesophilic and Gram-negative bacteria in selective media, according to RDC nº 12/2001. Stability was evaluated for 30 days under thermal variations. Statistical analysis was performed using one-way ANOVA followed by the Student-Newman-Keuls test ($p < 0.05$) in GraphPad Prisma. FTIR data were analyzed in OriginLab. **Results:** FTIR confirmed the presence of flavonoids, carotenoids, and phenolic acids such as lutein, quercetin, and protocatechuic acid. The gel exhibited stable acidic pH (3.05 - 3.38), a density of 1 g/mL, and a yield of 400% (5 g of powder produced 20 g of gel). The moisture showed 74.2% of humidity, 0.84% of ash content, 0.24 g/100 g of crude protein, 12% of total sugars, reducing sugars of 8.7%, 23.9 mg/100 g of vitamin C, and titratable acidity around 1.1 g/100 g (citric acid). Water activity was 0.91 (within safe limits). Growth of fungi or yeasts was not observed, and bacterial load remained $<10^3$ CFU/g, without detection of coliforms or *Salmonella*. The gel-maintained color, odor, and flavor throughout the stability test without relevant changes. **Conclusion:** The food gel is stable, safe in a microbiological point of view and has functional antioxidant compounds, representing a promising complementary strategy of metabolic disorders. Financial Support: This was partially supported by FAPEPI and CAPES (Financial code 001).

09.052 **Cytotoxic and Anti-inflammatory Potential of the Extract of *Oenocarpus bacaba* Mart.** Da Silva, LB^{1,2}; Figueiredo, RO^{3,4}; Gomes, PWP⁴; Figueiredo, PLB⁴; Lopes, DCF²; Pinto, LC² ¹UNIFAMAZ, ²UFPA, Lab. Experimental Neuropathology, Brazil, ³UFPA, PPG Chemistry, Brazil, ⁴UEPA, Lab. Natural Products Chemistry, Brazil

Introduction: The Amazon has the largest biodiversity in the world, and its flora has huge potential for discovering phytotherapeutic that can contribute as adjuvant therapeutic tools. Bacabeira (*Oenocarpus bacaba* Mart.) is a native palm tree with great socioeconomic importance due to the production of its fruit pulp, in addition to being known for its antioxidant potential. The objective was to investigate the anti-inflammatory potential in a lipopolysaccharide (LPS) model induced in murine macrophages and cytotoxic potential of *Oenocarpus bacaba* ethanolic extract (EOB) in tumor cell. **Methods:** The chemical composition of the EOB extract was investigated using liquid chromatography-tandem mass spectrometry (LC-MS/MS). To evaluation anti-inflammatory activity, first, the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay was performed to define three concentrations that did not alter the cell viability of the murine macrophage (RAW 264.7) after treatment with EOB in a concentration-response curve (0.19-100 µg/ml) for 24 hours. Based on the results, a nitrite assay was performed with the treatment in the RAW 264.7 cell at the three EOB concentrations for 24 hours, previously stimulated with lipopolysaccharide (LPS, 0.5 µg/mL) for 2 hours. Dexamethasone (10µM) was used as a positive control for its anti-inflammatory potential, and 0.01% DMSO was used as a negative control. Nitrite levels were measured in the supernatant of treated cultures using the Griess assay. Furthermore, the MTT assay was also used to assess cytotoxic activity in gastric cancer (AGP01), lung carcinoma (A549), and skin melanoma (SK-MEL-19) cells and in normal fibroblast cell (MRC-5) from the 200 µg/ml curve of EOB for 72 hours. All assays were quantified by spectrometry, and statistical analysis was performed using GraphPad Prism version 10.1 for one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons and nonlinear regression was performed to determine the 50% inhibitory concentration (IC₅₀). **Results:** In LC-MS/MS, the chemical composition of the EOB extract revealed the presence of quinic and caffeic acids, isoquercitrin, and isorhamnetin 3,4'-diglycosides. Based on the results, concentrations (0.78, 0.39, and 0.19 µg/ml) that did not have changed cell viability were selected for analysis of anti-inflammatory potential. EOB significantly reduced nitrite production in an LPS-induced inflammation model in all concentrations tested compared to the positive control ($p < 0.01$). As for EOB, it showed moderate cytotoxic activity in the AGP01 with IC₅₀ of 33.45 (29.57-37.84) and greater selectivity when compared to MRC5 with an IC₅₀ of 37.8 (30.35-47.08). The A549 cell was also cytotoxic with an IC₅₀ of 43.67 (37.04-51.48), and less prominent activity was observed in the SK-MEL-19 cell line with IC₅₀ of 91.04 (73.41-112.9). **Conclusion:** This way, the results demonstrated that *Oenocarpus bacaba* Mart. extract had a significant cytotoxic effect on gastric tumor cells compared to normal cells. Moreover, to a significant anti-inflammatory effect, even at low concentrations, suggesting that this compound may be a promising source for the development of future therapies. **Financial support:** CNPq

09.053 Exploring the Toxicity, Antioxidant, and Anticholinesterase Potential of *Ilex paraguariensis*

Extract. Penha MEF¹, Morales-Lima G^{1,2}, Gray, NE², Lago, JHG¹, Teodorov, E³, Mendes FR¹ Affiliation: ¹Centro de Ciências Naturais e Humanas, UFABC, Santo André, SP, Brazil ²Oregon Health and Science University, Dept Neurology, United States ³Centro de Matemática, Computação e Cognição, UFABC, Santo André, SP, Brazil

Introduction: *Ilex paraguariensis* A. St.-Hil. is a native South American plant traditionally consumed as chimarrão, tereré, or mate tea. Beyond its cultural significance, it has a central nervous system stimulant effects and rich phytochemical profile. Its main bioactive compounds include purine alkaloids, saponins, and phenolic compounds such as flavonoids, tannins, and chlorogenic acid, known for their antioxidant and anti-inflammatory properties. This study aimed to evaluate the potential anticholinesterase and antioxidant activity of hot aqueous extract of *Ilex paraguariensis* (IP) leaves, investigate its capacity to activate the Nrf2 pathway, and its safety profile using toxicity assessment tests in the embryo-larval stage of zebrafish (*Danio rerio*). **Methods:** The total phenolic content of IP was determined using the Folin–Ciocalteu method and the main phytochemical classes identified by nuclear magnetic resonance (NMR). Antioxidant activity was assessed through DPPH and ABTS radical scavenging assays, and the anticholinesterase potential was assessed by Ellman’s method. In addition, toxicity was investigated in 6h post-fertilization embryo-larvae zebrafish, according to OECD 236, and differences in survival between treatments (15.625–250 µg/mL of IP) were assessed using a chi-square test. HepG2-ARE cells, a hepatocellular carcinoma-derived cell line stably expressing a firefly reporter under the control of the antioxidant response element (ARE), were used to evaluate Nrf2 pathway modulation by IP. After determining the non-cytotoxic concentration range of the extract, the cells were treated with IP extract (20-500 µg/mL) for 24h, and the Nrf2-ARE pathway induction was assessed by measuring the luminescence emission in the presence of a luciferase substrate. **Results:** NMR analysis revealed the presence of alkaloids, fatty acids, and phenolic compounds. Total phenolic content was equivalent to 1265.1mg of gallic acid/g extract. Antioxidant activity showed EC₅₀ values of 0.034 µg/mL (DPPH) and 22.17 µg/mL (ABTS). Acetylcholinesterase inhibition presented an IC₅₀ of 0.75 mg/mL. No significant difference in survival was observed in embryo-larvae zebrafish at 96h post-exposure at any tested concentration of IP extract compared to the control (chi-square test, p = 0.501), indicating the absence of detectable toxicity under the conditions tested. The extract treatment was not cytotoxic to HepG2-ARE cells at the tested concentrations and promoted Nrf2 induction at 500 µg/mL (Kruskall Wallis, Dunn post hoc, p<0.05). **Conclusion:** The results indicated that the aqueous extract of IP possesses a high phenolic content and strong *in vitro* antioxidant activity, with Nrf2 pathway induction as demonstrated in a cell model, indicating activation of cellular antioxidant defenses. In addition, the extract showed moderate acetylcholinesterase inhibition and absence of toxicity in zebrafish, supporting its potential as a safe source of bioactive compounds for neuroprotective applications.

09.054 Characterization and Evaluation of Biological Activities of the Polysaccharide obtained from *Anadenanthera colubrina* (Vell.) Brenan Seeds.

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Introduction: *A. colubrina* (Vell.) Brenan (Fabaceae), known as angico, is a species widely found in the Caatinga biome, traditionally used in medicinal practices and religious rituals. Extracts from its bark and leaves have demonstrated anti-inflammatory, antinociceptive, antidiarrheal, wound healing, antimicrobial, antioxidant, and insecticidal activities, described in *in vitro* and *in vivo* assays. Despite its widespread use, the chemical and biological properties of the polysaccharide extracted from its seeds remain unexplored. This study aimed to extract and characterize the polysaccharide present in the seeds of *A. colubrina* (Poli-angico), as well as to evaluate its antioxidant, cytotoxic, and wound-healing activities. **Methods:** Poli-angico was extracted using a 0.1 M NaCl, precipitated with ethanol (46%, 70%, or 95%) and washes with absolute ethanol and acetone. Characterization included Fourier Transform Infrared Spectroscopy with Attenuated Total Reflectance (FTIR-ATR), monosaccharide composition by High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection, zeta potential by Dynamic Light Scattering, and thermal behavior by Thermogravimetric Analysis and Differential Scanning Calorimetry. Antioxidant activity was evaluated against DPPH· and ABTS·⁺ radicals. Cytotoxicity was assessed using the MTT assay, and wound healing potential by scratch assay in murine fibroblasts (L929). **Results:** Ethanol precipitation at 46% (v/v) yielded the highest extraction efficiency (15.02 ± 2.27%) and total carbohydrate content (93.98 ± 3.85%), with low phenolic content (0.99 ± 0.39 mg/g GAE) and proteins (0.56 ± 0.11 mg/mL). The FTIR-ATR spectrum revealed bands typical of polysaccharides, including hydroxyl (-OH) stretching (3282 cm⁻¹), asymmetric C-H stretching (2924 cm⁻¹), carbonyl (C=O) stretching (1638 cm⁻¹), N-H bending (1542 cm⁻¹), as well as C-O, C-C, and C-O-C stretching (1399 cm⁻¹, 1240 cm⁻¹, and 1065 cm⁻¹). Monosaccharide analysis indicated a predominance of glucose (37.79%), arabinose (21.87%), galactose (19.49%), and glucuronic acid (5.61%), characterizing an arabinogalactan-type heteropolysaccharide. Zeta potential was -19.2 mV, suggesting moderate colloidal stability. Thermal analysis indicated high stability, with melting point 149.68 °C and major degradation above 300 °C. Poli-angico exhibited antioxidant activity with IC₅₀ values of 45.49 ± 0.48 µg/mL (DPPH·) and 71.45 ± 1.16 µg/mL (ABTS·⁺). In the MTT assay (24 h), Poli-angico (1.5–200 µg/mL) did not affect cell viability. In the scratch assay, Poli-angico (50 µg/mL) significantly enhanced fibroblast migration, accelerating wound closure by 4.6% (6 h), 8.0% (12 h), and 13.8% (24 h) compared to the control. **Conclusion:** Poli-angico exhibits antioxidant activity and potential to accelerate the wound healing process. Although the results are preliminary, further studies are required to better understand the observed effects and advance the development of a wound healing product. **Financial support:** PPG Saúde e Desenvolvimento Socioambiental.

09.055 Hepato and Nephroprotective Effects of *Petiveria alliacea* L. in a Preclinical Model of Metabolism Associated Fatty Liver Disease. Higashijima AN, Santa Clara-Amorim RC, Kluck AJ, Silva GR, Lívero FADR UFPR, Lab. of Cardiometabolic Pharmacology, PPG Pharmacology, Brazil

Metabolism-Associated Fatty Liver Disease (MAFLD) is characterized by hepatic steatosis, which may progress to cirrhosis and hepatocellular carcinoma. MAFLD affects up to 30% of the global adult population and is recognized as a multisystemic liver disorder influenced by both genetic and environmental factors, including epigenetic alterations, high-fat diets, obesity, insulin resistance, alcohol consumption, smoking, gut dysbiosis, and endocrine disruption. Despite its growing prevalence, currently, there is no specific treatment for MAFLD; lifestyle modifications, including diet and physical activity, with or without pharmacological management targeting the underlying causes, are the primary recommendations. In this context, ethnopharmacological studies offer a promising alternative approach to understanding and treating MAFLD. One plant of interest is *Petiveria alliacea*, commonly known as "guiné," "tipi," or "erva-de-alho" and traditionally used for its analgesic, hypoglycemic, diuretic, antipyretic, and anthelmintic properties. *P. alliacea* has been shown through phytochemical studies to contain flavonoids, alkaloids, and saponins. However, its metabolic effects had not been pharmacologically investigated. This study evaluated the hepato- and nephroprotective effects of *Petiveria alliacea* in a MAFLD preclinical model [approved under protocol No. 1536/2024 by the Ethics Committee on Animal Use (CEUA/BIO-UFPR)] combining dyslipidemia, alcohol consumption, and type 1 diabetes. Male Wistar rats ($n = 34$) received a high-fat (0.5% cholesterol) and 5% ethanol liquid diet *ad libitum* for five weeks. Diabetes was induced by streptozotocin (60 mg/kg, i.p.). A basal group ($n = 7$) was not exposed to these factors. In the last two weeks, animals were treated daily by gavage with hydroethanolic extract of *P. alliacea* (30, 100, or 300 mg/kg), or insulin (6 IU) combined with simvastatin (2.5 mg/kg) as a positive control, while the negative control and basal groups received the vehicle alone. After treatment, blood samples were collected for biochemical analysis [glucose, cholesterol, triglycerides, aspartate and alanine aminotransferase (AST and ALT), urea and creatinine], and liver and kidneys were harvested for relative weight and histopathological evaluation. The 300 mg/kg dose of *P. alliacea* partially reversed hyperglycemia, hypercholesterolemia, hypertriglyceridemia, and liver weight gain, and fully normalized ALT and AST levels. It also prevented increases in urea, creatinine, and kidney weight. Histologically, liver tissue damage was partially reversed. In contrast, the positive control reduced glucose, cholesterol, and triglycerides but had only partial effects on AST, ALT, and creatinine. These findings suggest that *P. alliacea* exerts relevant hepato- and nephroprotective actions in this multifactorial MAFLD model, warranting further investigation into its mechanisms of action.

09.056 **Hemodynamic Alterations Caused by Venom of the Semiaquatic Coralsnake (*Micrurus surinamensis*) in Anesthetized Rats.** Talone SC^{1,2}, Carvalho WA^{1,2}, Matos-Silva H³, Silva Jr NJ², Hyslop S¹ ¹Unicamp, Faculdade de Ciências Médicas, Depto de Farmacologia, PPG Farmacologia, Brazil; ²PUC-GO, PPG em Ciências Ambientais e Saúde, Brazil. NUPMA-UNIRV-Campus Goiânia, Núcleo de Pesquisa da Faculdade de Medicina, Brazil

Introduction: Coralsnake (*Micrurus* spp.) venoms can cause hypotension (Floriano *et al.*, 2020, *Toxicon* 186, 58) and affect cardiac function (Gaspar *et al.*, 2023, *Cardiovasc Toxicol* 23, 132). Venom of the Amazonian semiaquatic coralsnake (*Micrurus surinamensis*) is cardiotoxic in guinea-pigs (Reis *et al.*, 2021, *Cardiovasc Toxicol*, **21**, 462), but the mechanisms involved are unknown. Here, we examined the hemodynamic alterations caused by this venom in rats and some of the mediators involved. **Methods:** Male Wistar rats were anesthetized with isoflurane and cannulated for hemodynamic measurements, and the parameters were monitored with LabChart v.7 software. Lyophilized venom was dissolved in 0.9% NaCl for use. A71915 (NPR-A natriuretic peptide (NP) receptor antagonist; 70 µg/kg), 4-aminopyridine (4-AP; voltage-gated K⁺ channel blocker; 3.5 mg/kg), atropine (non-selective muscarinic receptor antagonist; 2 mg/kg), and L-NAME (non-selective nitric oxide (NO) synthase inhibitor; 20 mg/kg) (Sigma-Aldrich) were injected i.v. in 100 µl of 0.9% NaCl 15-20 min before venom (A71915 was given 1 min before venom). The experimental protocols were approved by an institutional Committee for Ethics in Animal Use (CEUA/UNICAMP, protocol no. 6146-1/2022). The data were expressed as the mean±SD and statistical comparisons were done using one-way ANOVA followed by the Tukey test, with p<0.05 indicating significance. All data analyses were done with GraphPad Prism software, v.8. **Results:** Venom doses of 0.1 mg/kg (n=2) and 0.3 mg/kg (n=4) caused minimal hemodynamic alterations, whereas 1 mg/kg and 3 mg/kg caused irreversible hypotension and death (survival times: 16±5 min (n=4) and 6.3±1.7 min (n=4), respectively). These doses caused ventricular arrhythmia, partial atrioventricular block, progressive bradycardia and bradypnea. The changes in mean arterial blood pressure with 1 mg/kg were biphasic (basal: 107±17 mmHg; hypotension: 50±5 mmHg; recovery: 103±18 mmHg, n=4), followed by a progressive decline to death. Pretreatment with A71915 or L-NAME potentiated the hypotension and lethality of 1 mg/kg (survival times: 10±3 min (n=5) and 7.3±3.0 min (n=4), respectively), without affecting the cardiac alterations. Atropine did not affect the hemodynamic response or survival time (11±7 min, n=4). 4-AP delayed but did not prevent death (survival time: 42±17 min, n=5; p<0.05 compared to all other groups). The mean time between venom injection and hypotension (6.3±2.3 min; n=5) was unaffected by A71915 (5.0±2.3 min, n=5), atropine (8.0±3.4 min, n=4) or L-NAME (5±2.5 min, n=4), but was prolonged by 4-AP (34±17 min, n=5; p<0.05 compared to all other groups). **Conclusions:** *Micrurus surinamensis* venom was lethal at ≥1 mg/kg and caused marked hypotension that was modulated by NO, NP and voltage-gated K⁺ channels, but not by acetylcholine. This is the first detailed investigation of the mediators involved in hypotension caused by a coralsnake venom.

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09.057 *Arthrospira platensis* Prevents Endometrial Damage and the Increase of Pro-Inflammatory Cytokines in the Uterus with Primary Dysmenorrhea. Silva JMA^{1,2}, Soares MFS^{1,2}, Arruda RRA^{1,2}, Diniz AFA^{1,2}, Ribeiro SB³, Lima CAS³, Melo MB¹, Sousa-Filho JEC¹, Sousa, TR¹, Barros BC^{1,2}, Alves, AF⁴, Araujo, AA³ Silva BA^{1,2,5} ¹UFPB, Lab Functional Pharmacology Prof. George Thomas, Institute of Research in Drugs and Medicines, Brazil ²UFPB, Postgraduate Program in Bioactive Natural and Synthetic Products, Health Sciences Center, Brazil ³UFRN, Lab Experimental Model of Intestinal Mucositis, Postgraduate Program in Pharmaceutical Sciences, Brazil ⁴UFPB, Dept Biomedical Sciences, Health Sciences Center, Brazil ⁵UFPB, Dept Pharmaceutical Sciences, Health Sciences Center, Brazil

Primary dysmenorrhea (PD) is the most common gynecological dysfunction affecting women of reproductive age worldwide, being characterized by uterine hypercontractility and exacerbation of the inflammatory response mediated by prostaglandins and pro-inflammatory cytokines (Itani, R. et al. *Korean J Fam Med.* v. 43, p. 101-108, 2022). Previous studies have shown that *Arthrospira platensis* (AP), a blue-green cyanobacteria with antioxidant and anti-inflammatory effects (Brito, AF. et al. *Sci Rep.* v. 10. p. 1-8, 2020), prevents the increase in contractile reactivity and the decrease in relaxing reactivity in the uterus with this experimental disease model. Thus, the aim of this study was to further elucidate the protective mechanisms of AP against the morphological and inflammation alterations in the uterus induced by PD. For this, virgin female Wistar rats were randomly allocated in three groups: control (CG), primary dysmenorrhea (PD) and PD supplemented with 100 mg/kg of AP (PD + AP100). Uterine samples were processed for histopathology with hematoxylin-eosin to quantify endometrial area, and pro-inflammatory cytokines were measured with enzyme-linked immunosorbent assay from uterine horn homogenates. CG animals showed intact and planar endometrium without lesions. In contrast, PD animals displayed prominent apical papillary projections, partially prevented in the PD + AP100 group. Endometrial area was significantly reduced in the PD group ($40.0 \pm 0.4 \mu\text{m}^2$) compared to CG ($95.3 \pm 0.5 \mu\text{m}^2$), while AP supplementation prevents partially this reduction (μm^2). Levels of Interleukin 1 β , Tumor Necrosis Factor α and Interleukin 6 were elevated in PD animals (193.5 ± 8.4 pg/mL; 386.3 ± 39.2 pg/mL; 363.3 ± 13.2 pg/mL, respectively) compared to CG (76.3 ± 5.2 pg/mL; 240.8 ± 10.6 pg/mL; 248.3 ± 17.2 pg/mL, respectively). These increases were fully prevented in the PD + AP100 group (76.6 ± 15.1 pg/mL; 224.1 ± 13.6 pg/mL; 149.8 ± 25.8 pg/mL, respectively). The findings indicate that AP exerts significant immunomodulatory effects in the context of PD, contributing to the preservation of endometrial structure and reduction of uterine pro-inflammatory cytokine levels. Thus, AP supplementation emerges as a potentially effective and safe adjunct strategy for the management of PD. All experimental procedures were approved by the Ethics Committee on Animal Use of the Federal University of Paraíba, under protocol nº 2080110522 (ID: 002002). Financial Support by CNPq and CAPES.

Keywords: inflammation; histopathological; microalgae supplementation.

09.058 **Modulation of Acute Inflammation by trans-Cinnamic Acid via Neutrophil Recruitment and Downregulation of ICAM-1.** Fidelix MSP, Santos SLD, Santana JR, Barros ABB, Ferreira EGA, Silva GRS, Silva JP, Barreto E UFAL Lab Biologia Celular

Introduction: Inflammation is an adaptive response of vascularized tissues to harmful stimuli, involving immune cell recruitment and activation to restore tissue integrity. Neutrophils are the first leukocytes to migrate to inflamed sites, where they act via degranulation and oxidative burst. However, excessive neutrophil recruitment can exacerbate tissue damage during deregulated acute inflammation. Refractoriness or severe adverse effects to drugs currently used is common and the cause of many preventable adverse reaction hospitalizations. This makes the search for new compounds with anti-inflammatory capacity needed. Natural products, such as *trans*-cinnamic acid (t-CA), have shown anti-inflammatory and antioxidant properties. This study aimed to evaluate the effects of t-CA on carrageenan-induced acute inflammation *in vivo* and on neutrophil-endothelium interactions *in vitro*. **Methods:** Adult Swiss mice (25–35 g; CEUA n°67/2014; unpublished data of *in vivo* assays done previously, within CEUA's validity) were treated intraperitoneally with t-CA (1, 10, or 30 $\mu\text{mol/kg}$) and submitted to carrageenan-induced pleurisy. After 4 h, pleural exudate volume, leukocyte infiltration, and CXCL-1 and TNF- α levels were measured in bronchoalveolar lavage fluid. For *in vitro* assays, human neutrophils were isolated from peripheral blood, and chemotaxis toward CXCL-1 (100 ng/mL) was assessed using Boyden chambers. Human endothelial EA.hy926 cells were pretreated with t-CA (10 μM) before TNF- α (50 ng/mL) stimulation. Neutrophil adhesion to EA.hy926 cells was evaluated microscopically after 2 h. ICAM-1 expression was analyzed by qPCR and flow cytometry. Cell viability was assessed by MTT assay. **Results:** t-CA (10 and 30 $\mu\text{mol/kg}$) significantly reduced pleural exudation (by 44% and 48%), total leukocyte count (by 46% and 60%), neutrophil influx (by 46% and 52%), and CXCL-1 (by 32% and 33%) and TNF- α (by 39% and 45%) levels. t-CA (1–100 μM) had no cytotoxic effect and did not affect CXCL-1-induced neutrophil chemotaxis in Boyden chamber assays. TNF- α increased neutrophil adhesion to endothelial cells by fivefold, which was significantly inhibited by t-CA (10 μM). Additionally, t-CA reduced TNF- α -induced ICAM-1 expression in EA.hy926 cells (by 54%). **Conclusion:** *trans*-Cinnamic acid attenuates acute inflammation by reducing proinflammatory mediator levels and neutrophil infiltration *in vivo*, and by impairing neutrophil adhesion to activated endothelium via downregulation of ICAM-1 expression. These findings highlight the therapeutic potential of t-CA in controlling neutrophil-driven inflammation. **Financial support:** CNPq, CAPES, BioproFar-BA, FAPEAL.

09.059 *Citrus sinensis* (L.) Osbeck var. Moro Attenuates Deleterious Effects on Liver of Hypercholesterolemic Rats. Bragança LAR¹, Brazão SC¹, Lima GF¹, Mendes ABA¹, Pereira NCA¹, Proença AB^{2,3}, Alexandre-Santos B^{2,3}, Frantz EDC^{2,3}, Motta NAV¹, Brito FCF¹ ¹FF, Lab Experimental Pharmacology, Dept. of Physiology and Pharmacology, Brazil. ²UFF, Lab Exercise Sciences, Dept. of Physiology and Pharmacology, Brazil. ³UFF, Research Center in Morphology and Metabolism, Dept. of Morphology

Introduction: Hypercholesterolemia is an important risk factor for several cardiovascular and metabolic diseases, as it triggers oxidative stress and inflammation. The liver is a vital metabolic organ that functions as the main axis of lipid and protein homeostasis. *Citrus sinensis* (L.) Osbeck var. Moro (CSM) presents different pharmacological effects, including antineoplastic, antidiabetic, hypolipemic, anti-inflammatory and antioxidant. In this study we have investigated the pharmacological effects of morosil®, a freeze-dried extract of CSM, in the liver of hypercholesterolemic Wistar rats. **Methodology:** The animal protocols were approved by the Ethics Committee for Experimental Research of UFF (CEUA/UFF 7474011123). Adult male Wistar rats were randomly divided into three groups (n= 6-8, each group): control group (C) fed standard chow diet, hypercholesterolemic diet group (HC) and hypercholesterolemic diet group + morosil® (MORO), for 12 weeks. On the 10th week, morosil treatment (150mg/kg/day, gavage) was performed once a day, totalizing 15 days. The animals were euthanized by cervical dislocation under ketamine and xylazine anesthesia. The rat blood was removed by cardiac puncture for posterior biochemical analysis. The livers were removed, weighed and stored at -80°C for posterior analysis. Data were analyzed using one-way ANOVA followed by a post-hoc Bonferroni Multiple Comparison Test, P<0.05. **Results:** The HC diet promoted an increase in serum levels of total cholesterol (C: 103.90±6.15 x HC: 170.40±16.13 mg/dL), LDL (C: 41.91±6.76 x HC: 107.0±15.96 mg/dL) and uric acid (C: 2.55±0.05 x HC: 3.10±0.17 mg/dL), as well as leads to increased hepatic levels of total cholesterol (C: 13.31±1.58 x HC: 145.70±8.88 mg/dL) and triglycerides (C: 90.23±1.18 x HC: 196.80±22.47 mg/dL). When we evaluate liver function, HC diet leads to increased alkaline phosphatase (C: 28.29±1.67 x 42.75±2.15 U/L), ALT (C: 34.00±3.16 x HC: 150.00±32.51 U/L) and AST (C: 68.75±3.47 x HC: 185.40±25.07 U/L) serum levels. These effects were associated with increased IL-6 gene expression in HC group, as well as reduced SOD and Catalase gene expression in the liver (p>0.05). On the other hand, morosil® subacute administration was able to improve some metabolic and inflammatory parameters, demonstrated by reduced serum uric acid levels (MORO: 2.63±0.09 mg/dL), hepatic levels of triglycerides (MORO: 140.90±5.89 mg/dL) and IL-6 gene expression (p>0.05). **Conclusion:** These results suggest that morosil® extract can play an important role in hepatic inflammation and metabolism under hypercholesterolemic conditions. This work is still in progress and further analysis needs to be carried out to better evaluate morosil® effects. **Financial support** – CNPq, CAPES, PROPPI-UFF, FAPERJ.

09.060 *Arthrospira platensis* Prevents KCl-Induced Uterine Hypercontractility in Primary Dysmenorrhea through Redox and Prostanoid Modulation. Soares MFS^{1,2}, Silva JMA^{1,2}, Arruda RRA^{1,2}, Diniz AFA^{1,2}, Barros BC^{1,2}, Melchiades MKN^{1,2}, Melo MB¹, Santos-Filho JEC¹, Sousa TR¹, Silva BA^{1,2,3} ¹UFPB, Lab Functional Pharmacology Prof. George Thomas, Institute of Research in Drugs and Medicines, ²UFPB, Postgraduate Program in Bioactive Natural and Synthetic Products, Health Sciences Center, ³UFPB, Dept Pharmaceutical Sciences, Health Sciences Center

Introduction: Primary dysmenorrhea (PD) is characterized by cyclic pelvic pain associated with menstruation and has a multifactorial etiology. Among the main mechanisms is the overproduction of prostaglandin F₂ α , which increases uterine contractility, reduces blood flow, and causes tissue hypoxia. In addition, oxidative stress contributes to smooth muscle hyperreactivity and amplification of nociceptive signaling. In this sense, *Arthrospira platensis* (AP) is a cyanobacterium with well-established antioxidant and anti-inflammatory properties. Previous studies in our laboratory showed that AP supplementation prevented increased uterine contractility in rats with PD (data not shown). Therefore, this study aimed to evaluate the functional mechanisms of AP supplementation in preventing uterine hypercontractility in an experimental model of PD in Wistar rats. **Methods:** Virgin female Wistar rats (*Rattus norvegicus*), weighing between 150-200 g, were maintained under controlled conditions of temperature (22 $\pm$ 2 $^{\circ}$ C), light/dark cycle (12 h/12 h), with free access to water and food. PD was induced by subcutaneous administration diethylstilbestrol for 10 days, with a dose of 2.5 mg/kg on days 1 and 10 and from days 2 to 8 a dose of 1 mg/kg, and oxytocin, intraperitoneally, at a dose of 1 IU/kg, on the eleventh day. The AP used in this study was obtained in powdered form (lot n^o 17J11-B004-020541) from (Infinit farma, Hong Kong, China). The product was diluted in saline solution and administered by gavage at a dose of 100 mg/kg/day for 8 weeks. Animals were distributed into four groups (n = 5/group): Control (CG), AP-supplemented (AP100), PD model, and PD treated with AP (PD+AP100). Uteri were isolated and mounted in organ baths for isometric recording of contractile activity. Concentration–response curves to KCl (60 mM) were generated to obtain maximal response (E_{max}) and potency (pEC₅₀). Results were expressed as mean $\pm$ standard deviation and analyzed using one-way ANOVA followed by Tukey's post hoc test (p < 0.05). **Results:** The PD group showed significantly increased contractility (E_{max} = 325 $\pm$ 5.8%; pEC₅₀ = 1.5 $\pm$ 0.03) compared to controls (E_{max} = 100%; pEC₅₀ = 1.9 $\pm$ 0.05). AP supplementation normalized contractile parameters (E_{max} = 111.5 $\pm$ 3.2%; pEC₅₀ = 2.2 $\pm$ 0.15). To investigate the underlying mechanisms, the following pharmacological tools were used: indomethacin (COX inhibitor), L-NAME (NOS inhibitor), apocynin (NADPH oxidase inhibitor), and tempol (SOD mimetic). Results indicate that AP reduces prostanoid overproduction, restores nitric oxide bioavailability, decreases superoxide generation, and enhances SOD antioxidant activity. These synergistic effects contributed to normalizing uterine reactivity and preventing PD-associated hypercontractility. **Conclusion:** daily supplementation with *Arthrospira platensis* exerts a protective effect against uterine dysfunction induced by PD via modulation of prostanoid pathways and redox balance, highlighting its potential as a safe and multifunctional nutraceutical. **Ethics approval:** CEUA/UFPB (n^o 1659111022) **Funding:** CNPq and CAPES.

09.061 **Immunomodulatory Effects of a Peptide Derived from *Phoneutria nigriventer* Venom, on PBMCs co-cultured with Glioblastoma: Preliminary Data and Ongoing Translational Studies.** Silva MVR¹, Santos NBD¹, Sciani J², Vitorino-Araujo JL³, Verinaud L³, Carneiro CRD¹ ¹Unicamp, Faculty of Pharmaceutical Sciences, Campinas, SP, Brazil ²USF-Bragança Paulista, Brazil ³FCMSCSP, Division of Neurosurgery, Dept Surgery, Brazil. ⁴Unicamp, Dept Structural and Functional Biology, Institute of Biology, University of Campinas, Campinas, SP, Brazil

Introduction Glioblastoma (GBM) is the most aggressive and lethal primary brain tumor, characterized by an immunosuppressive microenvironment that poses a major challenge to the development of effective immunotherapies. LW-9 is a synthetic peptide derived from *Phoneutria nigriventer* venom, previously identified as a potential immunomodulator with activity on macrophages, dendritic cells, and NK cells. Prior work from our group has shown that the low-mass peptide LW-9 modulates myeloid polarization and function, enhances NK effector features, and promotes dendritic-cell maturation with a Th1-skewing capacity, implicating NF- κ B/JAK-STAT engagement with modulation by mTOR/Rho-ROCK/iNOS and immunometabolic rewiring (retinol/nicotinamide). This study aims to investigate the effects of LW-9 on peripheral blood mononuclear cells (PBMCs) from healthy donors co-cultured with human glioblastoma cells.

Methods Peripheral blood mononuclear cells (PBMCs) from healthy donors (CAAE: 66995422.0.0000.5404) were co-cultured in direct contact with human GBM cells (NG97) and treated with synthetic LW-9 within the activity window previously established by our group. Flow cytometry was intentionally restricted to a minimal readout panel (CD19, CD3, CD86) to capture B-cell and T-cell compartments and quantify costimulatory activation (CD86) in the non-T/non-B fraction. For transcriptional profiling, total RNA was isolated; cDNA was synthesized with the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems; random primers); and qPCR (SYBR) targeted IL6, TNF, IFNG, IL10, antigen-presentation/costimulation (HLA-DRA, CD80, CD86), and pathway sentinels (RELA/NFKB1A for NF- κ B, STAT1 for JAK-STAT, NOS2 for iNOS; exploratory markers related to mTOR/Rho-ROCK as feasible). Complementary label-free LC-MS/MS proteomics surveyed enrichment of antigen-processing/presentation and cytokine-signaling programs and screened immunometabolic signatures. **Results** In PBMC-GBM co-culture, LW-9 yielded reproducible activation patterns consistent with the group's prior single-compartment systems: (i) a shift toward higher CD86 frequency/intensity within PBMCs (notably in the CD3⁺/CD19⁻ fraction) accompanied by upregulation of HLA-DRA, CD80 and CD86 transcripts, coherent with NF- κ B/JAK-STAT engagement and modulatory influences of mTOR/Rho-ROCK/iNOS; (ii) increased IFNG and IL6 transcripts indicating activation across innate-adaptive interfaces and a Th1-leaning milieu; and (iii) proteomic enrichment of antigen-presentation and cytokine-signaling. **Conclusion** Early PBMC-GBM findings support LW-9 as a multi-target immunomodulator acting across innate and adaptive compartments, consistent with NF- κ B/JAK-STAT dependency, modulation by mTOR/Rho-ROCK/iNOS, and immunometabolic realignment.

Keywords: *Phoneutria nigriventer*, LW-9, glioblastoma, PBMCs, immunomodulation, translational research. **Financial support:** FAPESP (2018/23559-7; 2022/03543-4).

09.062 **Dual Antinociceptive and Cardioprotective Actions of *Vasconcellea cundinamarcensis* Cysteine Proteases in a Chemotherapy-Induced Toxicity Model.** Lima KFN^{1,2}, Sousa FM¹, Mendes NO¹, Silva ACA², Gomes LS², Lopes MTP⁴, Ferreira PMP⁶, Rodrigues GA³, Silva WC³, Almeida FRC², Silva MTB⁵, Silva IS⁶, Dittz D^{1,2} ¹UFPI, Lab de Farmacologia Antineoplásica, Brasil; ²UFPI, PPG em Farmacologia, Brasil; ³UFPI, Depto de Química, Brasil; ⁴UFMG, Depto de Farmacologia, Brasil; ⁵Universidade do Porto, Depto de Imunofisiologia e Farmacologia, Portugal; ⁶UFPE, Depto de Fisiologia e Farmacologia, Brasil.

Introduction: Neuropathic pain and cardiotoxicity are relevant adverse effects of most antitumor agents, including oxaliplatin (OXA) and 5-fluorouracil (5-FU). Currently, these conditions are treated with analgesics and β -blockers, respectively; however, such therapies show limited efficacy and do not prevent the toxic effects of chemotherapy. In this context, substances derived from natural products have been investigated as alternatives to mitigate these toxicities. Our group has demonstrated that the proteolytic fraction P1G10, obtained from the latex of *Vasconcellea cundinamarcensis* and rich in cysteine proteases, exhibits antitumor activity in murine models of melanoma, breast, and colon cancer, as well as anti-inflammatory activity in trinitrobenzenesulfonic acid (TNBS)-induced colitis. However, its protein nature limits oral absorption; to overcome this limitation, a formulation with β -cyclodextrin (β CD@P1G10) was developed. This study evaluated the effects of P1G10 and β CD@P1G10 in murine models of chemotherapy-induced neuropathic pain and cardiotoxicity. **Methods:** Neuropathic pain was induced in Balb/c mice with oxaliplatin (6 mg/kg, i.p.) for 4 consecutive days. From day 9, animals were treated daily by oral gavage (p.o.) with P1G10 or β CD@P1G10 (0.1, 1.0, 10.0 mg/kg) for 11 days. Mechanical nociceptive threshold (MNT) was measured on alternate days from day 9 using the von Frey test. To investigate the mechanism of action of β CD@P1G10, the involvement of the L-arginine/nitric oxide and purinergic pathways was assessed using agonists (L-arginine, ATP) and antagonists (L-NOARG, BBG). Cardiotoxicity was induced in Balb/c mice with 5-FU (30 mg/kg, i.p.) for 7 days; subsequently, animals were treated with P1G10 or β CD@P1G10 (0.1, 1.0, 10.0 mg/kg, p.o.). On day 8, electrocardiography (ECG) was performed to assess cardiac function. Additionally, levels of reduced glutathione (GSH), myeloperoxidase (MPO), N-acetyl- β -D-glucosaminidase (NAG), and creatine kinase MB (CK-MB) were measured in the heart and/or liver. **Results:** Treatment with P1G10 or β CD@P1G10 (0.1, 1.0, 10.0 mg/kg, p.o.) was performed after cardiotoxicity induction. On day 8, ECG analysis was conducted to evaluate cardiac function. Furthermore, GSH, MPO, NAG, and CK-MB levels were analyzed in the heart and/or liver. Oral treatment with free P1G10 or β CD@P1G10 produced an antinociceptive effect at 0.1 mg/kg (P1G10) and at 0.1, 1.0, and 10.0 mg/kg (β CD@P1G10). The antinociceptive effect of β CD@P1G10 was reversed by pretreatment with L-arginine (600 mg/kg, i.p.), while pretreatment with β CD@P1G10 (0.1 mg/kg, p.o.) reduced ATP-induced hypernociception (100 nmol, intraplantar). Additionally, oral treatment with free P1G10 or β CD@P1G10 prevented 5-FU-induced cardiac toxicity, as evidenced by improved ECG parameters, reduced oxidative stress, and decreased inflammation. **Conclusion:** P1G10 and β CD@P1G10 exert antinociceptive and cardioprotective effects against toxicities caused by oxaliplatin and 5-FU, and complexation with β -cyclodextrin enhances the efficacy of the proteolytic fraction.

09.063 **Investigation of the Antiobesogenic Action of Myrcene in Obese C57BL/6J Mice and in 3T3-L1 Cells.** Assunção R¹, Pinto TS², Andrade AFC², Emílio-Silva MT¹, Fioravanti MM¹, Dario FL¹, Guidolin IG¹, Aquino MLA¹ Fogaça MS¹, Zambuzzi WF², Hiruma-Lima CA¹ ¹Unesp, Dept Structural and Functional

Introduction: Projections indicate that by 2025, 31% of Brazilian adults will be obese; therefore, treatments are necessary for this condition. Myrcene (My) is a monoterpene with anti-inflammatory properties. Our aim was to assess the activity of My in preadipogenic 3T3-L1 cells (*in vitro*) and C57BL/6J male mice (*in vivo*) and to determine its potential as a treatment for obesity. **Methodology:** Adult male C57BL/6J mice (CEUA 5700170524) were divided into five experimental groups (n=10 per group): a control group fed a standard diet (SD) and an obese group fed a high-fat diet (HFD). The remaining groups, which received HFD, were treated orally with a vehicle (Tween 80 1%) or My at doses of 3.75, 7.5, or 11.25 mg/kg once daily for 17 weeks. The body mass and adiposity index of the animals were evaluated and we also investigated the effects of My on preadipocyte 3T3-L1 cells. The cells were stimulated with My (0.39–100 µg/mL) to evaluate cellular viability and the expression of genes associated with cell survival (Src), cell cycle (Cdk2/Cdk4/p15), differentiation (leptin/PPAR-γ), and inflammation (IL-1β/TNF-α). The *in vitro* assay was performed in sextuplicate. One-way ANOVA followed by Tukey's post-hoc test was performed (p<0.05). **Results and Discussion:** HFD intake caused a significant increase in body weight (1,76x) in the second week following induction compared to the control group (p<0.05). This response was maintained until the end of 17 weeks, resulting in increased adiposity index (3,57x), indicating the development of obesity. Treatment with My (11.25 mg/kg) reduced body mass gain (0,15%) and consequently decreased the adiposity index (23%) at the end of 17 weeks. *In vitro* stimulation with My did not affect the viability of 3T3-L1 cells. However, evaluation of the gene expression of inflammatory mediators revealed that treatment with My resulted in a significant increase in IL-1β (2,63x) and TNF-α (1,54x) expression. In addition, treatment of cells with My increased the expression of PPAR-γ (6,67x) and leptin (4,91x), suggesting an increase in the differentiation process compared to control cells. Furthermore, treatment with My increased the expression of the proliferation gene (SRC – 15,39x) and the cell cycle genes [Cdk-2 (31,77x), Cdk-4 (2,45x), and P15 (5,21x)] compared to the vehicle. **Conclusion:** My treatment at a dose of 11.25 mg/kg had an antiobesity effect after 17 weeks of HFD intake in C57BL/6J mice. It is evident that My acts in the adipose tissue of animals, protecting against fat accumulation. However, it has been demonstrated that My can exert its effects through the mediation of an increase in pro-inflammatory mediators and cell differentiation in 3T3-L1 cells.

09.065 **Effects of *Rosmarinus officinalis* Extract on Human Neutrophil Extracellular Traps Formation and Neutrophil Apoptosis** *ex vivo*. Seito LN1, Tappin MR2, Pádua TA1, Costa TEMM1, Rosas EC1, ¹Fiocruz-Farmanguinhos, Dept Pharmacology, Lab Applied Pharmacology, Brazil; ²Fiocruz-Farmanguinhos, Dep Analytical Methods, Analytical Methods Service, Brazil.

Introduction: Neutrophil extracellular traps (NET) formation is an important innate immune defense mechanism against pathogens. However, in certain conditions the uncontrolled NET formation is involved in different pathophysiologic events that can lead to chronic diseases or even death. Aim: In the present study we tested a standardized extract obtained from the leaves of *Rosmarinus officinalis* on the NET formation (NETosis) *ex vivo*. **Methods:** The standardized extract was prepared and subjected to gas chromatography to determine the triterpenic acid content, which was 16.8% oleanolic acid, 6.2% betulinic acid, 48.9% ursolic acid and 18.6% micromeric acid. The *R. officinalis* standardized extract (ROSE) was assessed for cytotoxicity and Phorbol 12-Myristate 13-Acetate (PMA)-induced NETosis in human peripheral polymorphonuclear cells (PMN). The human PMN cells were obtained from healthy donors after approval by the institutional Ethics Committee (Certificate Presentation of Ethical Appreciation 56727722.6.0000.5248). PMN cells were isolated from peripheral blood after Polymorphprep isolation media centrifugation at 500 x g for 35 minutes at 20°C. The PMN cell layer was collected and processed for red blood cell lysis. PMN cells were resuspended in RPMI-1640 supplemented with 5% of fetal bovine serum, and 10⁵ cells/well in 96-well plates were pretreated with ROSE (0.93-46.5 µg/mL) or vehicle (maximum 1% DMSO) for 1 or 5 hours at 37°C and 5% CO₂ before Lactate Dehydrogenase (LDH) cell viability assay. For NET formation assessment in microplate, the PMN cells were pretreated for 1 h before 5 nM PMA stimulus and fluorescence reading at 490 nm (excitation) and 537 nm (emission) wave lengths for SYTOX Green at 1, 2, 3 and 4 hours after stimulus. Additionally, we assessed the possible effect of ROSE on neutrophil apoptosis by Flow Cytometry after Annexin V-FITC and Propidium Iodide (PI) staining. NET formation was also observed and registered by fluorescence microscopy with ROSE-pretreated PMN cells after 3 hours of PMA stimulation and staining with SYTOX-Green and Hoechst 34580 for extracellular and nuclear DNA respectively. **Results:** The ROSE was not cytotoxic on human PMN after 1 hour of incubation and reduced the cell viability by only 11% after 5 hours of incubation at the highest concentration. Thus, we tested the effect of ROSE on the PMA-induced NETosis microplate assay in the same concentration range and found that the extract dose-dependently impaired NETosis with a maximum inhibitory effect of 78% at 46.5 µg/mL. Since the cells were not undergoing NETosis after PMA stimulus, we further evaluated the fate of PMN cells incubated with only ROSE by Flow Cytometry. We found that neutrophils were dose-dependently undergoing apoptosis in a rate of 54% at 46.5 µg/mL after 5 hours of incubation. The fluorescence microscopy corroborate the findings with round blue and green nuclei of treated cells, with only a few NETs formed. **Conclusion:** The treatment of PMN with ROSE seems to engage the cells to undergo apoptosis even after PMA stimulus for NETosis. However, additional assays are necessary for complete elucidation of the process. Support: Programa Inova-Fiocruz, Farmanguinhos-Fiocruz.

09.066 ***In Silico* Screening admetSAR Profiles from Brazilian Red Propolis.** Silva JLV¹, Cruz RO¹, Borini GB², Berretta AA², Cavacante FA³, Oliveira TKB¹, Oliveira PLV¹, Correia ACC⁴ ¹FMO, Lab. Práticas Funcionais, Brasil, ²Apis Flora Industrial e Comercial Ltda, Depto. P&D&I, Brasil, ³UFPB, Lab. Farmacologia Funcional, Brasil, ⁴UPE, PPG Saúde e Desenvolvimento Socioambiental, Brasil

Introduction: Red propolis is a resinous mixture produced by bees from various plant sources. In Brazil, red propolis, which is abundant in the Northeast, is rich in polyphenols and bioactive compounds from *Dalbergia ecastophyllum* (L.) Taub. (Fabaceae), which is its main source. This species, known as “rabo-de-bugio”, has roots and bark traditionally used to treat inflammation. Although there are studies on red propolis, research on the biological activities of its chemical constituents is scarce. **Aim:** To analyse absorption, distribution, metabolism, excretion and toxicity profiles from Brazilian red propolis by *in silico* screening. **Methods:** A red propolis extract was prepared and analyzed by the Research, Development & Innovation Department at Apis Flora Industrial e Comercial Ltda. Chemicals were identified using HPLC and smiles were downloaded from PubChem. The *in silico* screening of the ADMET profiles of the compounds was performed using the online ADMET-Simulator v1.0. Data were expressed as a percentage of probability (%). **Results and Discussion:** The red propolis extract showed medicarpin, vestitol, neovestitol, guttiferone, and oblongifolin A as the majority compounds. Regarding the absorption, distribution, metabolism, and excretion (ADME) properties, only medicarpin and vestitol demonstrated blood-brain barrier penetration (54.3-89.6%). In contrast, all compounds showed human intestinal absorption (95.8-98.5%) and Caco-2 permeability (66.0-86.8%). Guttiferone and oblongifolin A had low CYP inhibitory promiscuity (55.8-74.5%), but medicarpin, vestitol, and neovestitol showed high promiscuity (77.4-88.1%). Based on the toxicological analysis, the compounds were theoretically not carcinogenic or mutagenic, were weak hERG inhibitors, and were classified as Category III for acute oral toxicity (500-5,000 mg/kg). The compounds exhibited a lethal dose 50% (LD50) in rats ranging from 2.23 to 3.13 mol/kg. **Conclusion:** The majority of compounds found in red propolis show potential ADME properties and a positive toxicological prediction. The data are preliminary but essential to provide a basis for future *in vitro* and *in vivo* biological analyses.

09.067 **Effects of Prodigiosin on Zebrafish *in vitro* Outcomes.** Teixeira SA, Rezende-Teixeira P, Jimenez PC², Machado-Neto JA, Machado-Santelli GM, Costa-Lotufo LV, ¹ICB-USP, ²Unifesp, Dept Marine Science, Brazil

Introduction: Prodigiosin (PG) is a microbial tripyrrole alkaloid pigment, produced by bacteria, which has promising antineoplastic activities in several human carcinoma types. Considering its impending therapeutic use, it is also important to evaluate the potential environmental impacts of PG, particularly considering the inefficiency of wastewater treatment plants in fully removing antineoplastic drugs from hospital wastewater. In this study, *in vitro* effects of PG were investigated using zebrafish (*Danio rerio*) liver cell line (ZFL). **Objectives:** The objectives of these study were: (i) to assess the environmental impact of PG on a vertebrate model species, and (ii) to observe *in vitro* effects to better understand how they can be extrapolated to *in vivo* outcomes. **Methods:** All *in vitro* exposures were performed utilizing the ZFL cell line, (ATCC CRL-2634), derived from the liver of healthy adult zebrafish as described in Damasceno et al. (Environmental Advances 8, 100208, 2022). The *in vitro* endpoints assessed were cell viability, morphology, cell cycle, and enzymatic activities. The cytotoxicity of PG to ZFL cell lines was examined through two distinct tests: the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) reduction assay and the trypan blue (TB) exclusion test. Morphological alterations induced by PG on ZFL were analysed by immunofluorescence. ZFL cell cycle analysis was analysed by flow cytometry (10000 events). Enzymatic activities (Acetylcholinesterase-AChE, Lactate Dehydrogenase-LDH, catalase-CAT, Glutathione Reductase-GR and Glutathione S-Transferase-GST) were determined spectrophotometrically in triplicates, using 96-well microplates. The unit used to quantify enzyme activity was the weight of substrate hydrolysed per minute per mg of protein. PG was added to the plate wells at 0.1 to 620 $\mu\text{g.L}^{-1}$ to MTT assay and at concentrations of 16, 32, 81 and 162 $\mu\text{g.L}^{-1}$ for the other assays. The incubation time used to assays was 24, 48 and 72h after addition of PG at 28°C. **Results:** The *in vitro* 72h-IC₅₀ of PG was 68 $\mu\text{g.L}^{-1}$ and 24h-TB assay confirmed a significant reduction in viable cell counts at PG concentrations of 81 and 162 $\mu\text{g.L}^{-1}$. Regarding the effects of PG on cell morphology, immunofluorescence analysis showed that PG induces changes in cell morphology in a concentration-dependent manner. An increased population of subG1 cells, suggesting DNA fragmentation, and alterations in cell morphology were also observed at 81 $\mu\text{g.L}^{-1}$ of PG after 24h treatment. The biomarker trials in ZFL cells revealed that CAT and GR levels were similar across all PG treatments (16, 32, and 81 $\mu\text{g.L}^{-1}$). On the other hand, GST values, were significant lower in the at PG concentrations of 32 and 81 $\mu\text{g.L}^{-1}$. LDH levels were similar across all groups, while AChE activity, although not significant, was reduced by 42 and 29% at 32 and 81 $\mu\text{g.L}^{-1}$, respectively. **Conclusion:** Our results show that *in vitro* models using ZFL cells can provide insights into cellular and biochemical toxicity mechanisms, but they are insufficient to fully predict *in vivo* effects, particularly for systemic and developmental endpoints, emphasizing the advantage of integrated approaches to assess the environmental and toxicological hazard of anticancer drugs. Financial support: CAPES, CNPq, FAPESP.

09.068 Effect of the Lipophilic and Hydrophilic Fractions of Pequi Oil on the Extracellular Matrix During Cutaneous Repair. Matsnaka DL, Silva MEU, Mimura KKO e Oliveira SM UFMT

Cutaneous wounds are among the major clinical and public health challenges worldwide, as their treatment requires therapies capable of promoting rapid and functionally effective repair while maintaining adequate control of inflammation and fibrosis. Pequi (*Caryocar brasiliense*), a fruit native to the Brazilian Cerrado biome, is widely recognized for its rich composition of bioactive compounds, including carotenoids, flavonoids, and unsaturated fatty acids, which exhibit potent antioxidant and anti-inflammatory properties. These attributes make pequi oil an attractive natural source for developing novel therapeutic formulations for skin repair. This study aimed to investigate the effects of the lipophilic (PL) and hydrophilic (PH) fractions of pequi oil on the wound-healing process in vivo. BALB/c mice were subjected to standardized full-thickness excisional cutaneous lesions and divided into three experimental groups: Control (untreated), PL, and PH (daily topical application of each fraction). Each group was analyzed on days 3, 7, and 14 post-injury (n = 5 per group). After euthanasia, skin samples from the wound area were collected and processed for complementary histological, morphometric, and molecular analyses. Picrosirius staining under polarized light revealed that, at day 14, animals treated with PL exhibited a pronounced reduction in both type I and type III collagen fibers within the dermis, whereas those treated with PH showed collagen levels comparable to the Control group throughout the experimental period. This finding suggests that the PL fraction attenuates extracellular matrix remodeling during the late phases of repair, potentially preventing excessive fibrosis. The Multiplex cytokine assay demonstrated a significant decrease in TGF- β 1 expression in skin homogenates at day 3 in both PL and PH groups, indicating that both fractions are capable of influencing growth factor signaling. Western blot analysis revealed no significant differences in MMP2 protein levels among groups at any time point, suggesting that the modulation of wound healing by pequi oil fractions is not mediated by changes in extracellular matrix degradation through this enzyme. Taken together, these results indicate that pequi oil fractions modulate the wound-healing cascade primarily through early downregulation of TGF- β 1 expression, contributing to tissue organization, without altering MMP2 activity. However, the lipophilic fraction interfered with collagen deposition at later stages, pointing to a potential antifibrotic effect and therapeutic relevance for conditions characterized by excessive scarring. Overall, these findings reinforce the therapeutic potential of *Caryocar brasiliense* oil as a bioactive agent for wound management, supporting its inclusion in future translational research focused on natural compounds for regenerative medicine. The study was conducted by two Pharmacy students under the supervision of researchers specialized in histology and immunology and is classified at technology readiness level (TRL) 4.

10. Cancer Pharmacology

10.001 **Assessment of Novel HDAC Inhibitors and Venetoclax in a Cellular Model of Oral Cancer.** Guimarães de Souza PO¹, Carlos JAEG¹, Tavares MT^{2,3,4}, de Sá RE^{1,5}, Costa-Lotufo LV¹, Parise-Filho R², Machado Neto JA¹. ¹ICB-USP, Dept. of Pharmacology, São Paulo, Brazil; ²FCF-USP, São Paulo, Brazil; ³Dana-Farber Cancer Institute. Dept. of Cancer Biology, Boston, United States; ⁴Harvard Medical School, Dept. of Biological Chemistry and Molecular Pharmacology, Boston, United States; ⁵UFDFar, Parnaíba, Brazil

Introduction: Oral cancer is one of the most prevalent malignancies and, in its advanced stages, presents limited therapeutic options. Recently, our research group developed a series of novel hybrid inhibitors targeting both histone deacetylases (HDAC) and kinases, which demonstrated synergistic activity with venetoclax, a BCL2 inhibitor that has transformed the treatment landscape of hematologic malignancies (Carlos *et al.*, Cell Death Discov. 6;11(1):147, 2025). However, the effects of these hybrid inhibitors in solid tumors, including oral cancer, remain largely unexplored.

Objectives: To investigate the effects of novel hybrid HDAC-kinase inhibitors in a model of oral cavity cancer. **Methods:** HSC-3 cells were exposed to vehicle or graded concentrations the hybrid inhibitors 4d and 4f, the pan-HDAC inhibitor vorinostat, the BCL2 inhibitor venetoclax, and the reference drug 5-fluorouracil (5-FU). Cell viability was assessed using the MTT assay (72 h), while clonogenic potential was evaluated through the colony formation assay (9 days). The half maximal inhibitory concentration (IC₅₀) values were determined by nonlinear regression analysis. **Results:** In HSC-3 cells, all compounds reduced cell viability. The IC₅₀ values and maximum efficacy observed were 4.2 µM and 80.8% for compound 4d, 3.2 µM and 78.6% for 4f, 11 µM and 70.1% for vorinostat, 10.9 µM and 70.4% for venetoclax, and 89.1 µM and 66.0% for 5-fluorouracil (5-FU). The results of the colony formation assay corroborated these findings, indicating that prolonged exposure to 4d, 4f, and vorinostat, but not to venetoclax or 5-FU, was capable of completely eliminating neoplastic cells. **Conclusion:** Among the compounds tested, 4f demonstrated the highest potency. The next steps of this study will involve evaluating the effects of combining HDAC inhibitors with venetoclax and 5-FU in the model of oral cavity cancer. **Financial support:** CNPq, CAPES, and FAPESP.

10.002 Multi-Target Inhibitor NT157 Exhibits Antineoplastic Activity in Pancreatic Cancer Cells.

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Introduction: Pancreatic cancer remains one of the most lethal malignancies worldwide, largely due to its late diagnosis and poor responsiveness to existing therapies. Signaling pathways mediated by insulin-like growth factor receptor (IGF1R), insulin receptor substrates (IRS), and AXL receptor tyrosine kinase are known to promote tumor progression, cell proliferation, and resistance to apoptosis. NT157 is a small molecule that targets IGF1R/IRS, STAT3, and AXL signaling, previously shown to exert antitumor activity in other cancer models. **Objectives:** To evaluate the expression of IGF1R- and AXL-related signaling components in pancreatic cancer cells and to characterize the molecular and cellular effects of NT157. **Methods:** Using pancreatic cancer cell lines, MIA PaCa-2, AsPC-1, and PANC-1, we performed Western blot analysis to assess basal expression and phosphorylation levels of IGF1R, IRS1/2, STAT3, and AXL. NT157 treatment was then tested at multiple concentrations and time points to determine its effect on cell viability (MTT assay) and clonogenicity. Statistical analyses included ANOVA and Bonferroni post hoc test, with significance set at $p < 0.05$. **Results:** NT157 reduces cell viability in a dose- and time-dependent manner across all three cell lines. MIA PaCa-2 cells exhibited the greatest sensitivity, with an IC_{50} of 1.4 μ M at 72 hours. Clonogenic assays confirmed a marked decrease in colony formation following NT157 treatment. Molecularly, NT157 induced inhibitory phosphorylation of IRS1/2 on serine residues in MIA PaCa-2 and AsPC-1, accompanied by increased levels of phosphorylated ERK1/2 and JNK. In contrast, PANC-1 cells displayed increased total levels of IRS1/2 without increased inhibitory phosphorylation, suggesting alternative regulatory mechanisms in this lineage. In all cell lines, NT157 led to a reduction in total AXL protein, with this decrease being more pronounced in PANC-1 cells. Regarding STAT3, PANC-1 cells showed a reduction in both total and phosphorylated forms. In MIA PaCa-2, only the phosphorylated (active) form was reduced, whereas AsPC-1 showed no significant changes. Cleaved PARP1 levels increased in all cell lines, indicating apoptosis, while γ H2AX, a marker of DNA damage, was elevated in MIA PaCa-2 and PANC-1. **Conclusion:** Taken together, these findings suggest that NT157 acts via multiple molecular pathways to exert antineoplastic effects in pancreatic cancer cells. The lineage-specific responses observed emphasize the complexity of signaling networks in pancreatic tumors. The dual targeting of IRS/IGF1R and AXL signaling by NT157 highlights its potential as a candidate for the development of novel therapeutic strategies against pancreatic cancer, a disease with urgent unmet medical needs. **Financial support:** CNPq and FAPESP.

10.003 **NT157 an IRS/IGF1R Inhibitor impacts on Nox4: a novel mechanism involved in anti-melanoma effects.** Rocha DS, Almeida BO, Veríssimo-Filho S, Machado-Neto JA, Lopes LR. ICB-USP, Dept Pharmacology, Brazil

Introduction: Melanoma, an aggressive and invasive skin cancer, is caused by ultraviolet light-induced mutations. Over 50% of cases feature BRAF mutations which activate pro-survival pathways. Despite the existing BRAF inhibitors, such as Vemurafenib, most of the patients develop resistance after six months of treatment. In addition, tumor cells resistant to BRAF inhibitors can also develop mechanisms that allow them to bypass BRAF inhibition through the overexpression of tyrosine kinase receptors, such as the insulin-like growth factor receptor (IGF1R), which leads to the activation of the PI3K-AKT-mTOR pathway, an alternative route for cell survival and growth. In this context, the synthetic compound NT157, a tyrosine phosphorylation inhibitor under preclinical study, shows promising effects against vemurafenib-resistant melanoma by inhibiting IGF1R signaling. NT157 binds allosterically to IGF1R, causing dissociation and inhibitory phosphorylation of IRS1/2, which are then degraded. This blocks IGF-1-mediated PI3K-AKT survival signaling, promoting apoptosis. Thus, NT157 may overcome resistance to BRAF inhibitors. Furthermore, oxidative stress plays an important role in the context of neoplasms, with Nox4 being implicated in the cellular survival of this type of cancer. Within this framework, this study aims to investigate the possible involvement of Nox4 in the death of melanoma cells under the action of NT157. **Methods:** The IC₅₀ and the minimum incubation time of the compound were determined for vemurafenib-sensitive and-resistant cells — A375 and A375R, respectively — using the MTT assay. These parameters were then used to carry out clonogenic assays, using pre-determined concentrations and exposure times. Additionally, Western blot analyses were performed using both cell lines to investigate NT157 induced phosphorylation and degradation of IRS1/2 and whether these effects included Nox4. Based on these results, further Western blots were conducted after treatment with NT157 during a time course of 2, 4, and 6 hours. **Results:** The MTT assay showed that the concentration required to reduce cell viability by 50% (IC₅₀) was 3.8 μ M for A375 and 4.5 μ M for A375R. Regarding incubation time, 24 hours was sufficient to decrease A375 cell viability by 50%, whereas A375R required 48 hours. In the clonogenic assays, NT157 treatment led to a 66% reduction in the number of colonies and a 96% reduction in colony area for A375 cells. In contrast, for A375R cells, the effect was even more pronounced: 92% reduction in colony number and 99% reduction in colony area (*p < 0.05 vs. control). Western blot analysis revealed that Nox4 was decreased proportionally to phosphorylated IRS (p-IRS) under NT157 treatment. These findings support the hypothesis that the compound's effects in melanoma involve not only modulation of the IGF1R pathway but also Nox4 degradation. **Conclusions:** Thus, our findings confirm that NT157 reduces melanoma cell viability and suggest that its effects on melanoma may be associated not only with the modulation of the IGF1R pathway, but also with Nox4. These data provide a solid foundation for further experiments aimed at deepening the understanding of Nox4's role in the response to NT157 and its potential to overcome vemurafenib resistance.

10.004 *In vitro* and *in vivo* Antitumor Properties of Casearin J on Sarcoma 180 Cells. Santos IKS¹, Sousa RWR¹, Oliveira RAB¹, Ramos CLS¹, Rodrigues DCN¹, Barbosa SLM¹, Dittz D², Zalewski S³, Camargo JGS³, Carvalho FA³, Santos AG³, Ferreira PMP¹ ¹UFPI, Lab Experimental Cancerology (LabCancer), Dept Biophysics and Physiology, Brazil; ²UFPI, Dept Biochemistry and Pharmacology, Brazil; ³UNESP, Faculty of Pharmaceutical Sciences, Brazil

Introduction: *Casearia sylvestris* (guaçatonga) is known for its (ethno)pharmacological properties, particularly in the treatment of inflammatory, infectious, and painful conditions. **Objectives:** To evaluate the antiproliferative activity of Casearin J (Cas J), a compound isolated from *Casearia sylvestris*. **Methods:** Leaves of *C. sylvestris* (SisGen #A33EA7A, SISBIO 33429-1) were subjected to ethanolic extraction, followed by partitioning with ethyl acetate. Compounds were identified by retention time (tR) and UV spectra, indicating a predominance of clerodane diterpenes with λ_{max} between 221–238 nm. Cas J was isolated and initially evaluated for *in vitro* cytotoxicity using murine cell lines [S-180 (sarcoma 180) and L-929 (non-transformed fibroblasts)] via Alamar Blue (resazurin) and MTT assays, respectively (1.5–100 $\mu\text{g/mL}$) after 72 hours of exposure. Dimethyl sulfoxide (DMSO, 0.5%) and doxorubicin (0.005–5 $\mu\text{g/mL}$) served as negative and positive controls, respectively. Subsequently, female Swiss mice were subcutaneously inoculated with S-180 cells (6×10^6 cells/0.5 mL) and treated intraperitoneally for 8 consecutive days with Cas J (10 or 25 mg/kg/day), DMSO 5% (negative control), or 5-fluorouracil (5-FU, 25 mg/kg/day, positive control). On day 9, behavioural assessments were conducted using the open field and elevated plus maze tests. After euthanasia, tumor mass and relative wet weights of key organs were measured. Statistical analysis was performed using one-way ANOVA followed by the Student-Newman-Keuls test ($p < 0.05$). **Results:** Cytotoxicity assays revealed CI_{50} values of 0.61 (0.57–0.66) $\mu\text{g/mL}$ for S-180 and 1.18 (0.82–1.70) $\mu\text{g/mL}$ for L-929, resulting in a selectivity index of 1.93 for S-180 cells ($p < 0.05$). Both doses of Cas J (10 and 25 mg/kg/day) significantly reduced tumor growth (34.9% and 55%, respectively) compared to the negative control group (1.1 ± 0.07 g, $p < 0.05$). Cas J at 25 mg/kg/day and 5-FU impaired body weight gain and increased relative heart weights. Additionally, 5-FU reduced spleen weight and increased stomach weight. Increased locomotor activity was observed at the lower dose of Cas J. No deaths were recorded. **Conclusion:** Cas J demonstrates intense cytotoxic activity against sarcoma cells and promising *in vivo* antitumor effects without lethal toxicity, supporting its potential as an anticancer candidate. **Financial support:** This study was partially supported by FAPEPI and CNPq. **Approved** by the Ethics Committee on Animal Use (CEUA/UFPI #0795/2023).

10.005 Cannabidiol Nanoformulations Inhibit Cell Proliferation and Cancer-Stem Cell Marker CD133 In *in vitro* Models of Burkitt Lymphoma. Santos HAF, Souza VB, Nicolosi JS, Vinha P, Perozzo BR, Schenka NGM, Schenka AA Unicamp, School of Medical Sciences, Dept Pharmacology, Brazil

Introduction: Burkitt lymphoma (BL) is an aggressive mature B-cell non-Hodgkin's lymphoma, prevalent among children and immunocompromised individuals, and associated to high mortality. BL lacks more effective and less toxic treatment protocols. Studies indicate that, despite being a mature lymphoma, BL can express cancer stem cell (CSC) markers (such as ALDH1 and CD133). Recent studies suggest that cannabidiol (CBD), a non-psychotropic component of *Cannabis sativa* L., has inhibited cell proliferation and cancer-stem cell markers in some cancers; however, there are no studies evaluating such effects in BL to date. Aim: to evaluate the potential anti-proliferation and anti-CSC effects of CBD in *in vitro* models of BL. **Methods:** cells from three commercial immortalized LB cell lines (Daudi, Raji, and Namalwa) were cultured and, upon reaching 80% confluence, were submitted to cytotoxicity assays (MTT). Each cell line was exposed to control treatment (no drugs) or to one of the following drugs, at concentrations of 10⁻⁴ to 100 µg/mL: (1) daunorubicin (reference chemotherapy drug), (2) broad CBD nanoformulation (0%THC; Bisaliv® Power Broad, from Thronus), and (3) full CBD nanoformulation (0.3%THC; Bisaliv® Power Full, from Thronus). After 72h, the plates were subjected to the MTT protocol to assess cell viability (cytotoxicity). Absorbance was measured in a SpectraMax 340PC-384 plate reader (Molecular Devices, USA) at 570 nm. Three independent experiments were performed, with technical triplicates per group (n=9). In a second stage, BL lines were cultured in 6-well plates until semi-confluent (80%), treated for 24h with each drug at its respective IC₅₀ concentration or without drugs (control), collected to obtain cell blocks and sent for immunocytochemical detection of cell proliferation (Ki67) and CSC markers (CD133 and ALDH1). **Results:** The concentration-response curves obtained with the MTT assays show that daunorubicin and both CBDs are cytotoxic to all cell lines, reducing cell viability in a concentration-dependent manner. Comparing the IC₅₀ of each drug, we found that daunorubicin (IC₅₀ = 0.008-0.021 µg/mL) is significantly more cytotoxic than CBDs (IC₅₀ = 0.55-1.184 µg/mL) in 2 cell lines - Raji and Daudi (p<0.05, ANOVA + Tukey's post hoc test). Regarding cell proliferation, daunorubicin and broad CBD are associated with a significant reduction in Ki67 (up to 30%; p<0.05) in the Raji and Namalwa cell lines. Full CBD also reduces Ki67 (~30%), but only in Namalwa cells (p<0.05). Results of CSC marker expression indicate that all pharmacological treatments significantly reduce the number of CD133+ cells in all cell lines (p<0.001). No treatment reduces ALDH1. In Daudi cells, daunorubicin significantly increases the number of ALDH1+ cells (p<0.01). **Conclusions:** The *in vitro* cytotoxic, anti-proliferative and anti-CSC effects demonstrated in BL lines when exposed to nanoformulations of CBD suggest the potential of these compounds for *in vivo* antineoplastic actions (particularly, with the use of broad nano-CBD). However, given the general limitations of *in vitro* research, such anti-cancer potential must be thoroughly scrutinized by *in vivo* and clinical studies. **Financial support:** FAPESP (No do Processo: 2024/03837-3); FAEPEX (No do Processo: 2021/13726-6).

006 Antiproliferative Activity at Lung Cancer Cells and Acute Toxicity of N-Acylhydrazone Derivatives. Santos YV¹, Souza SM¹, Silva VR², Bezerra DP², Dias RB³, Dos Santos Filho JM⁴, Militão GCG¹ ¹UFPE, Dept. of Physiology and Pharmacology, Biochemistry and Physiology Graduate Program, Brazil; ²Fiocruz, Gonçalo Moniz Institute (IGM), Lab Tissue Engineering and Immunopharmacology, Brazil; ³UEFS, Dept. of Biological Sciences, Brazil; ⁴UFPE, Dept. of Chemical Engineering, Lab Design and Synthesis Applied to Medicinal Chemistry-SintMed®, Brazil

Introduction: Lung cancer is one of the leading causes of cancer-related deaths worldwide, and treatments have limitations such as low bioavailability and the development of drug resistance. *N*-acyl hydrazone derivatives have attracted interest for their antiproliferative and anti-inflammatory effects, representing promising agents for the development of new anticancer drugs. Therefore, the aim of this study was to evaluate the antiproliferative effects of *N*-acyl hydrazone derivatives on a lung cancer cell line and to analyze their acute toxicity. **Methods:** The *N*-acyl hydrazone-derived compounds SintMed65 and SintMed71 were synthesized through the introduction of alkyl and alkene side chains into the *N*-acyl hydrazone chemical structure, aiming to improve their physicochemical and biological properties. The antiproliferative potential was assessed by clonogenic assay in A549 cells seeded at a density of 500 cells per well (6-well plate) in RPMI-1640 medium, and treated with the SintMed65 (1–32 μ M) and SintMed71 (1.22–36 μ M) for 24 hours. After 10 days, colonies were fixed with 10% formalin, stained with 0.5% crystal violet and quantified using ImageJ software. IC₅₀ values were calculated using GraphPad Prism software (v. 8.4). In addition, acute toxicity was evaluated in BALB/c mice via intraperitoneal administration (50 and 300 mg/kg, n = 3/group), according to the guidelines of the Organization for Economic Cooperation and Development (OECD/423). Animals were monitored for 14 days and then organs were subjected to histological analysis. All procedures were approved by the Ethics Committee on Animal Use (CEUA nº 16/2022, IGM/FIOCRUZ). **Results:** The compounds reduced colony formation in A549 cells in a concentration-dependent manner. SintMed65 showed higher potency, with an IC₅₀ of 3.08 μ M (95% CI: 2.44–3.89), while SintMed71 had an IC₅₀ of 27.89 μ M (95% CI: 22.76–34.27). No gross pathological abnormalities, mortality or signs of toxicity were observed during the 14-day observation period. Cardiac tissue remained largely preserved, except in the group treated with SintMed65 at 300 mg/kg, which showed necrosis and inflammation. The liver exhibited mild to moderate alterations, with more pronounced necrosis in the SintMed71 50 mg/kg group. Lung tissue damage was dose-dependent, with more significant structural and inflammatory changes at higher doses, especially SintMed71 at 300 mg/kg. Renal tissue was preserved in SintMed65 (50 mg/kg) and SintMed71 (300 mg/kg) groups, whereas tubular necrosis, glomerular hyalinization, and vascular hyperemia were observed in SintMed65 (300 mg/kg) and SintMed71 (50 mg/kg and 300 mg/kg) groups. **Conclusions:** The compounds demonstrated significant antiproliferative effects in lung cancer cells, with SintMed65 showing better results. We observed controlled acute toxicity following single dose injection, indicating therapeutic potential. **Financial support:** Propesqi UFPE, CAPES, CNPq.

10.007 **Ivermectin Shows *in vitro* and *in vivo* Activity in a Sarcoma Experimental Model.** Sousa RCL, Porto JCS, Santos IL, Freitas MM, Barbosa SLM, Rodrigues DCN, Pinheiro-Neto FR, Ferreira PMP UFPI, Lab Experimental Cancerology (LabCancer), Dept Biophysics and Physiology, Brazil

Introduction: Sarcoma is a heterogeneous group of rare tumors derived from mesenchymal tissue. These tumors have a high metastatic potential and a recurrence rate of over 20%, resulting in a 5-year survival rate of 50% for localized tumors and less than 10% for metastatic ones. In this context, pharmaceutical repositioning can generate incremental innovation, improvements, and optimizations in products, services, or processes available on the market, aiming to replace conventional medications. Ivermectin, an anthelmintic widely used in clinical practice, has demonstrated antiproliferative effects in different preclinical models of carcinomas, but its effects on sarcoma are still poorly explored. **Objective:** To investigate the potential of ivermectin alone or associated with doxorubicin (Dox) for treating sarcomatous tumors. **Methodology:** Firstly, the *in vitro* antitumor effect of IVM (0.009 – 25 µg/mL) was investigated in primary culture of sarcoma 180 (S-180) using the Alamar Blue (resazurin) assay after up to 72 h exposure and in combination with the positive control Dox (0.002–5 µg/mL) at 48 h. Next, healthy Swiss mice (25 – 35 g; CEUA/UFPI #0795/2023) received S-180 cells (6×10^6 cells/0.5 mL) in the axillary region (n = 8/group): negative control (vehicle), positive control (Dox 1 mg/kg/day every 3 days, i.p.), IVM 5 mg/kg/day oral, IVM 10 mg/kg/day oral, and combinations of IVM 5 or 10 mg/kg/day oral + Dox 1 mg/kg/day every 3 days, i.p. Food consumption (daily), body weight (every 2 days), and tumor volume were recorded. The animals were euthanized on the 10th day, and tumor, liver, kidneys, heart, and blood were collected. Statistical analysis was performed using one-way ANOVA followed by the Student-Newman-Keuls test ($p < 0.05$), using GraphPad Prism and CompuSyn. **Results:** IVM alone significantly reduced S-180 cell viability in a time-independent manner, with IC_{50} values of 2.42 (1.71–3.41), 3.59 (2.30 – 5.61), and 2.51 (1.60 – 3.69) µg/mL for 24, 48, and 72 h, respectively. Meanwhile, combination studies showed that IVM reduced the IC_{50} of Dox alone (from 1.09 to 0.13 µg/mL), demonstrating synergism. Both doses of IVM ($58.11 \pm 11.83\%$ and $85.26 \pm 10.26\%$ for IVM 5 and 10 mg/kg/day) were able to reduce tumor mass when compared to the negative control group (2.72 ± 0.25 g, $p < 0.05$). Synergistic *in vivo* effects were not observed for the combinations of Dox and IVM. Hematological analysis revealed no changes in the blood profile. On the other hand, all groups receiving IVM alone or with Dox showed a reduction in the wet weight of kidneys and decreased food intake (also observed in the Dox group). **Conclusion:** Together, these findings demonstrate the antitumor potential of ivermectin against sarcomas, a rare tumor type with high rates of metastasis, recurrence, and limited therapeutic options. However, safety issues deserve further specific investigation. **Financial Support:** This was partially supported by CNPq and CAPES (Financial code 001).

10.008 The Role of the Protein Tyrosine Phosphatase Receptor N2 In Inflammatory Breast Cancer: Differential Profiles and Association with Clinicopathological Features. Alves BES¹, Oliveira PRA¹, Maia IFVC¹, Arruda LM2, Carvalho, MAS¹, Silva, RL¹, Cunha MPSS², Leitão RFC³, Almeida PRC⁴, Rogatto SR^{5,6}, Pereira Lima Júnior RC¹, Wong DVT¹ ¹UFC, Dpt of Physiology and Pharmacology, Lab Inflammation and Cancer Pharmacology, Drug Research and Development Center, Brazil; ²Haroldo Juaçaba Hospital, Cancer Institute of Ceara, Brazil; ³UFC, Dpt of Morphology, Brazil; ⁴UFC, Dpt of Pathology and Forensic Medicine, Brazil; ⁵UNESP, Botucatu Medical School Hospital, Brazil; ⁶University Hospital of Southern Denmark, Denmark

Introduction: Inflammatory Breast Cancer (IBC) is a rare clinical presentation that affects 1 to 5% of patients with breast cancer, seeing fast evolution and poor prognosis. The Protein Tyrosine Phosphatase of Type Receptor N2 (PTPRN2) has been studied and associated with different types of cancer, such as prostate, colorectal, and breast cancer, stimulating malignant neoplastic cells. Therefore, the role of PTPRN2 in IBC remains unclear. This study evaluated the differential expression of PTPRN2 in subjects with IBC versus non-IBC and its association with the clinicopathological parameters. **Methods:** This is a retrospective study. Formalin-fixed paraffin-embedded (FFPE) tumor samples and clinicopathological data (age at diagnosis, family history, presence of hormone receptors and HER2 status, metastasis, and Ki-67, a proliferation cell marker) were prospectively from 18 IBC and 16 non-IBC subjects from 2015 to 2020. FFPE tissue samples were subjected to immunochemistry and immunofluorescence staining of PTPRN2 expression. The coloration and fluorescent intensity were quantified using FIJI ImageJ software. The transcriptomic data of IBC and non-IBC microarray data from the Gene Expression Omnibus repository (GEO) were used to investigate the expression of PTPRN2 genes. Statistical analysis was performed using the t-test and Two-Way ANOVA. $P < 0.05$ was accepted. Study approval number: 5.010.710. **Results:** The phenotype analysis showed a significantly higher PTPRN2 expression in tumor tissue versus peritumoral tissue in the IBC group among the two methods [$p = 0.0016$]. The IBC patients presented comparable patterns of obesity, lymph node involvement, metastasis, and expression levels of ER, PR, HER, and Ki-67 above 20% ($p > 0.05$ vs. non-IBC). In the intergroup analysis, there was no difference in PTPRN2 expression. External validation by analyzing external GEO datasets showed similar PTPRN2 expression levels between IBC and non-IBC. However, samples positive for the presence of hormone receptors and HER2 exhibited higher scores and expression. When patients were stratified according to the clinicopathological parameters, a trend to a higher percentage of PTPRN2 protein expression was associated with IBC subjects with negative metastasis (105.9 ± 48.1 vs. 49.8 ± 34.0) versus positive samples in the same group and non-IBC-group [$p = 0.0646$]. The same trend of PTPRN2 expression in the IBC group was observed in patients with lymph node involvement (40.8 ± 30.4 vs. 1.1 ± 0.0). The patients in stage III showed a higher PTPRN2 expression pattern in the non-IBC group [$p = 0.019$]. **Conclusions:** The presence of PTPRN2 in tumor tissue is higher than in peritumoral tissue. Luminal and amplified HER2 tumors have more predisposition to the presentation of higher PTPRN2 expression. **Financial support:** PPSUS/FUNCAP-SESA-Decit/MS-CNPq; CAPES.

10.009 Protein Disulfide Isomerase Inhibition Decreases Anoikis Evasion in Melanoma Cells Resistant to Chemotherapy Nascimento A, Pagotti H, Verissimo-Filho S, Agostinho-Neto J, Lopes LR ICB-USP, Pharmacology Dept, São Paulo, Brazil

Introduction: Melanoma is the most aggressive form of skin cancer and has the worst prognosis due to its great metastatic and invasive potential and resistance to current chemotherapy. Most of the patients present the BRAF V600E mutation and are treated with the BRAF inhibitor vemurafenib. Unfortunately, 70% of these patients become resistant after 6 months of treatment and the tumor relapses. Redox mechanisms involved in the resistance to BRAF inhibitors in melanoma are being investigated by our group. Protein disulfide isomerase A1 (PDI) is a redox endoplasmic reticulum (ER) chaperone involved in maintaining the correct folding of native proteins through thiol disulfide exchanges. PDI can leave the ER and traffic to the plasma membrane where it can regulate cell surface receptors. We observed that PDI is highly expressed metastatic as opposed to primary tumors in 472 melanoma patients (TCGA). Previous data from our group indicates that PDI silencing decreases focal adhesions and reduces vascular smooth muscle cell migration. *Anoikis* is a form of programmed cell death that occurs when the disruption of cell–cell or cell–extracellular matrix (ECM) adhesion leads detachment and apoptotic cell death. To metastasize cancer cells, need to evade *anoikis*. Recent studies by our group demonstrate that PDI inhibition decreases migration in a melanoma cell line resistant to vemurafenib. Thus, this study intended to investigate PDI role in the regulation of cell adhesion and *anoikis* evasion in melanoma cells resistant to chemotherapy.

Goal: To evaluate the role of PDI in the regulation of cellular adhesion and *anoikis* evasion in melanoma BRAF inhibitor sensitive and resistant cells. **Methods:** In this study, we present a new method for investigating anoikis in melanoma cells. Our approach enhances the traditional clonogenic assay by simulating a bloodstream-like environment using a shaker incubator. By adjusting the rotation speed, we expect to create a laminar flow—where blood flows steadily through the blood vessel—while avoiding turbulent flow (a disordered pattern). This setup allows for a more precise assessment of anoikis resistance. This methodology is particularly valuable as it provides a quantitative and reproducible approach to measuring anoikis, offering deeper insights into melanoma progression and potential therapeutic targets. Human primary melanoma cells A375 and the vemurafenib resistant lineage A375R were maintained in agitation to prevent cell adhesion for 1, 2, 4, 6 and 8 hours. Next the same protocol was repeated in the presence of Bepristat 2a (25 μ M for A375; 33 μ M for A375R) a reversible and specific PDI inhibitor, which prevents substrate binding in the b' domain of the protein inhibiting its catalytic activity. Next the cells were plated, and colony formation (number and area of the colonies) were analyzed after 10 days. Actin filaments, cytoskeleton organization and cell adhesion were analyzed by optic microscopy after labeling cells with phalloidin. Cell survival rate was also investigated over a period of 24 hours during treatment with Bepristat. **Results:** Anoikis evasion was decreased in both A375 and A375R after 4 hours of treatment with Bepristat. In addition, BRAF resistant cells were more sensitive to Bepristat treatment showing a progressive decrease of 71% in colony area after 2 hours of treatment. In contrast BRAF sensitive melanoma cells only showed a similar effect (also a 71% decrease in the area of the colonies) 6 hours after treatment with Bepristat. A375R cells detached more after PDI inhibition by Bepristat as compared to A375, suggesting that PDI impacted more *anoikis* evasion in cells resistant to BRAF inhibitors. Phalloidin labelling indicates that PDI inhibition impacts negatively on cytoskeleton organization and disturbs cell polarity and mitigates the ability of melanoma cells to migrate (transwell, single cell migration assay). **Conclusions:** PDI inhibition led to cytoskeleton disorganization and decreased migration and *anoikis* evasion leading to increased cell death which contributed to reduce the colonogenic potential of melanoma cells resistant to chemotherapy. Therefore, we propose that protein disulfide isomerase could represent a novel therapeutic target to treat melanoma patients resistant to chemotherapy. **Financial support:** Redoxoma FAPESP #2013/07937-8 and CNPq # 313492/2020-4

10.010 Therapeutic Combination of McLTP1 Protein and Oxaliplatin in Antitumor Activity and Peripheral Sensory Neuropathy in CT26.WT Tumor Bearing Mice. Abucater BLSM¹, Moura MNS¹, Nascimento RR¹, França JC¹, Pereira AF¹, Bezerra BS¹, Ribeiro MVS¹, Rocha LR¹, Freire DC¹, Oliveira HD², Souza PFN¹, Brito DMS¹, Vale, ML¹ ¹UFC, Lab Inflammation and Cancer Pharmacology, Drug Research and Development Center (NPDM), Dept Physiology and Pharmacology, Faculty of Medicine, Fortaleza, Brazil. ²UFC, Pharmacology and Biochemistry Lab, Drug Research and Development Center (NPDM), Department of Biochemistry and Molecular Biology, Fortaleza, Brazil

Introduction: Oxaliplatin (OXL) is a key antineoplastic agent in colorectal cancer (CRC) treatment. However, its clinical utility is limited by peripheral sensory neuropathy (PSN), a debilitating neurotoxic side effect. The development of PSN often leads to dose reduction or treatment discontinuation, adversely affecting patient survival outcomes. Studies show that a protein purified from the seed of *Morinda citrifolia* (McLTP1) has analgesic and anti-inflammatory properties and prevents OXL-induced PSN in a mice model. There is still no validated therapy for the prevention or treatment of PSN by OXL and it is of interest that therapeutic proposals have an antineuropathic effect and at the same time do not diminish the antitumor effect of the antineoplastic agent. Thus, this study aimed to evaluate the impact of McLTP1 on both the antitumor efficacy of OXL and OXL-induced peripheral neuropathy (PSN) in CT26.WT tumor bearing mice. **Methods:** For this purpose, a tumor was induced with the colorectal adenocarcinoma CT26.WT cell line in the flank of male BALB/c mice (CEUA no. 20090522-0). After 10 days of inoculation, the animals were divided into 4 groups: control (OXL vehicle), group treated with McLTP1, OXL group and McLTP1 + OXL group. Tumor growth was monitored until the 14th day, concomitantly with the evaluation of NSP by mechanical and thermal allodynia tests. Additionally, the *in vitro* cytotoxicity of McLTP1 alone and in combination with OXL was evaluated. Cell viability was assessed by the MTT assay, and the IC50 was determined. Immunofluorescence was performed for the markers Ki67, CD31 and HMGB1. **Results:** *In vitro*, McLTP1 and OXL demonstrate a significant cytotoxic effect, decreasing cell viability. The combination of McLTP1 with OXL demonstrated a potentiating effect on this parameter. *In vivo*, McLTP1 inhibited the development of OXL-induced PSN, decreasing mechanical allodynia by 96.8% ($p < 0.001$) and cold allodynia by 85.2% ($p < 0.001$). McLTP1 and OXL decreased tumor size by 51.1% and 81.3% ($p < 0.001$), respectively, and tumor weight by 76.1% ($p < 0.05$). The combination of McLTP1+OXL decreased tumor size and weight by 63% and 78% ($p < 0.001$). There was no difference between the OXL group and the group treated with the combination (OXL+McLTP1). All treatments reduced Ki67 and CD31 expression. In contrast, HMGB1 levels were elevated in all treated groups. Notably, McLTP1 enhanced the effect of OXL, particularly on HMGB1. **Conclusions:** The results are promising considering the potential of McLTP1 as an adjuvant in colorectal cancer therapy with OXL, both for its neuroprotective and antitumor effects. **Financial support:** UNIVERSAL/FUNCAP; CNPq; CAPES

10.011 Investigation of the Prognostic Impact of PIP4K2s and its Pharmacological Cytotoxicity in Hepatocellular Carcinoma. Carvalho MFL, Parducci NS, Lima K, Machado-Neto JA ICB-USP, Dept Pharmacology, São Paulo, Brazil

Introduction: Hepatocellular carcinoma is the fifth most common type of cancer and one of the leading causes of cancer-related death worldwide. The PIP4K2 family consists of three lipid kinases that play a significant role in cell proliferation, survival, glucose uptake, and cytoskeletal organization - all processes reported to be dysregulated in cancer. Therefore, the aim of this project is to investigate the impact of PIP4K2 expression on survival outcomes, clinical features, and biological functions in patients with hepatocellular carcinoma from the TCGA cohort, and to characterize the antineoplastic potential of its pharmacological inhibition in hepatocarcinoma-derived cell lines. **Methods:** RNA sequencing data, clinical characteristics, and outcomes of hepatocellular carcinoma patients available from the cBioPortal for Cancer Genomics were used. RNA transcripts from the TCGA cohorts were pre-classified based on the differential expression of PIP4K2 isoforms, comparing samples with high and low gene expression. Overall survival, disease-free survival, and progression-free survival were analyzed using the Cox proportional hazards regression model. Gene Set Enrichment Analysis (GSEA) was performed to identify signaling pathways associated with PIP4K2 expression, considering FDR < 0.25 and p-value < 0.05 as statistically significant. For *in vitro* experiments, hepatocellular carcinoma-derived cell lines (HepG2 and Huh-7) were cultured in media recommended by ATCC. The analyses included cell viability assays (MTT), clonogenicity (crystal violet-stained colony formation), protein expression (Western blotting), and evaluation of cell cycle and proliferation by flow cytometry. **Results:** The results indicated that PIP4K2s are more highly expressed in tumor tissue compared to adjacent normal tissue. High expression of PIP4K2s was associated with worse clinical outcomes in patients with hepatocellular carcinoma. In the GSEA analysis, several cellular and molecular events were positively enriched according to PIP4K2s expression. The high-expression phenotype showed enrichment in the PI3K/AKT/mTOR signaling pathway, epithelial-mesenchymal transition, inflammatory response, KRAS signaling, mitotic spindle, and TGF- β signaling in hepatocellular carcinoma. High expression of these genes was associated with downregulation of the P450 epoxygenase pathway. In hepatocellular carcinoma cell lines, low doses of the inhibitors led to increased cell death and DNA damage (increased cleaved PARP1 and γ H2AX), as well as reduced cell viability and colony formation. A decrease in p-YAP1/YAP and p-AKT/AKT expression was also observed. Flow cytometry showed that cells treated with the inhibitors had reduced proliferation. Cell cycle analysis revealed a progressive increase in the SubG1 population with a reduction in G1/S and accumulation in G2/M at higher doses, indicating that treatment with the inhibitors induced G2/M arrest and cell death (SubG1). **Conclusion:** The results obtained provide promising evidence that PIP4K2s may serve as potential prognostic markers and molecular targets for hepatocellular carcinoma. This identification paves the way for the development of future studies exploring the efficacy of this inhibitor in patients with this condition.

10.012 **Impact of the TP53 R248Q Mutation on the Interaction Between p53 and APR-246 in Acute Myeloid Leukemia.** Bertoline LMF¹, Lima K^{1,2}, Machado Neto JA¹ ¹ICB-USP, Dept Pharmacology, São Paulo, Brazil. ²FM-USP, Dept Internal Medicine, Hematology Division, São Paulo, SP, Brazil

Introduction: The TP53 gene encodes the tumor suppressor protein p53, a central regulator of cellular responses to stress and a key player in cancer biology. In acute myeloid leukemia (AML), TP53 mutations are observed in approximately 10–20% of cases and are strongly associated with poor prognosis and resistance to conventional chemotherapy. One promising small molecule, APR-246, is capable of reactivating mutant p53, thereby potentially restoring its tumor-suppressive functions. However, the diversity of TP53 mutations can differentially modulate treatment response, underscoring the importance of mutation-specific investigations. This study aims to explore the impact of TP53 mutations in AML patients, focusing on their association with clinical and laboratory features, therapeutic responses, and patient outcomes. Furthermore, it seeks to evaluate how specific mutations affect p53 protein structure and its interaction with APR-246.

Methods: To this end, two AML patient cohorts were analyzed for TP53 mutations. The first cohort comprises 942 samples from 805 patients, obtained from the Beat AML dataset (diagnosis-only samples; *Nature* 562:526–531, 2018; *Cancer Cell* 40:850–864, 2022). The second includes 200 samples from 200 patients provided by The Cancer Genome Atlas (TCGA) (*N Engl J Med* 368:2059–2074, 2013). Among these, 64 samples harbored TP53 alterations, in which a total of 59 unique mutations were identified: 35 missense, 10 frameshift, 6 nonsense (stop codon), and 8 splice-site mutations. **Results:** Notably, the R248Q mutation emerged as the most prevalent missense variant, detected in six independent samples. This mutation, recognized as a hotspot, is functionally characterized as dominant-negative and is predicted to be pathogenic and probably damaging by multiple bioinformatic tools. Although structural predictors suggest it may not cause damage to the protein structure, existing literature supports that R248Q induces conformational changes that compromise p53 function. Given its recurrence and clinical relevance, this mutation is a focus of deeper investigation. **Conclusion:** The analysis of two AML cohorts identified 59 distinct TP53 mutations, with a predominance of missense variants. Among these, the R248Q mutation emerged as the most frequent, detected in six independent samples. Although structural prediction tools did not indicate significant alterations in protein architecture, literature evidence suggests that R248Q induces conformational changes that impair p53 function. Its classification as a pathogenic, dominant-negative mutation further reinforces its disruptive role in the p53 pathway. These findings underscore the biological and clinical relevance of R248Q in AML and substantiate its selection for deeper investigation. By delineating the mutation spectrum and highlighting clinically impactful variants, this study enhances our understanding of TP53-related disease heterogeneity and strengthens the rationale for mutation-specific therapeutic strategies in AML. **Financial support:** FAPESP (2024/07906-0).

10.013 **Extracellular Vesicles from Obese-Educated Preadipocytes Enhance Migration and Alter Metabolic Profile in MDA-MB-231 Cells.** Botelho, LGX^{1,3}, Franco VA^{1,3}, Ramos IR², Barja-Fidalgo, TC³, Moraes JA⁴, Renovato-Martins, M^{1,3} ¹UFF, Lab Inflammation and Metabolism, Brazil, ²University of Miami, Dept Radiation Oncology, USA. ³ UERJ, Bioscience Post Graduation Program, Brazil ⁴UFRJ, Lab Redox Biology, Brazil

Introduction and Objective: Obesity is a well-known risk factor for breast cancer progression, yet the underlying mechanisms remain poorly understood. Preadipocytes, although often overlooked, may significantly contribute to this crosstalk. Among the mediators of this intercellular communication, extracellular vesicles (EVs) have emerged as key regulators capable of transferring bioactive molecules. This study aimed to: (i) develop an *in vitro* model to obtain EVs from obese (OB-PEVs) and eutrophic (EUT-PEVs) preadipocytes, and (ii) evaluate functional and metabolic alterations induced by these EVs in MDA-MB-231 breast cancer cells. **Methods:** Primary human preadipocytes were educated *in vitro* using the secretome from subcutaneous adipose tissue (AT) biopsies of obese or eutrophic individuals (CAAE: 36880914.0.0000.5259). After education, extracellular vesicles were isolated from the conditioned medium by centrifugation (20,000 × g), generating vesicles from obese-educated (OB-PEVs) and eutrophic-educated (EUT-PEVs) preadipocytes. MDA-MB-231 breast cancer cells were treated with OB-PEVs or EUT-PEVs, in the presence or absence of etomoxir (50 µM; ETO) or 2-deoxy-D-glucose (5mM; 2-DG), inhibitors of fatty acid oxidation and glycolysis, respectively. Migration was assessed using the wound healing assay. Metabolic parameters, including L-lactate and ATP levels, as well as glucose-6-phosphate dehydrogenase (G6PDH) and pyruvate kinase (PK) activity, were measured using colorimetric commercial kits. Statistical analysis was performed using one-way ANOVA with Sidak's post-hoc test ($p < 0.05$). **Results and Discussion:** The treatment with OB-PEVs increased MDA-MB-231 cell migration by approximately 3-fold and by 1.8-fold compared to untreated cells and cells treated with EUT-PEVs, respectively. In the presence of ETO, the migration induced by OB-PEVs was reduced by approximately 2.3-fold. Additionally, OB-PEV-treated cells displayed lower lactate levels relative to EUT-PEV-treated cells. This effect was reversed upon ETO exposure, which increased lactate levels and decreased G6PDH activity, indicating a shift towards glycolytic metabolism. **Conclusion:** Our findings reveal that OB-PEVs activate signaling pathways that drive the pro-migratory behavior of MDA-MB-231 cells, primarily through fatty acid oxidation. Inhibiting this pathway with Etomoxir effectively dampened OB-PEV-induced migration, triggering a metabolic shift toward glycolysis. Notably, this shift was marked by increased lactate production and a concurrent reduction in G6PDH activity. Altogether, these results underscore the role of obese-educated preadipocytes in the metabolic reprogramming of cancer cells, positioning extracellular vesicles as critical players in this intercellular dialogue. **Funding:** CAPES, CNPq, and FAPERJ.

10.014 Multifunctional Thermosensitive Nanocarriers for Local Treatment of Non-Invasive Breast Cancer: Tissue Localization, Cytotoxic and Molecular Effects. Passos JS^{1,2}, Machado-Neto JA¹, Panitch A¹, Lopes LB¹ ¹ICB-USP, Dept Pharmacology, Functional and Molecular Biology Program, Brazil; ²Georgia Institute of Technology, Dept Biomedical Engineering, USA

Breast cancer remains a major cause of mortality among women and a clinical challenge due to its heterogeneity and resistance to current therapies. Standard therapy typically includes surgery, radiotherapy, hormone therapy, and chemotherapy. However, less invasive approaches—such as intraductal drug delivery—offer promising alternatives for managing pre-tumor lesions and non-invasive forms of the disease, such as ductal carcinoma *in situ* (DCIS). This route enables localized drug delivery and reduces systemic toxicity, although technical limitations persist. In this scenario, nanosystems can help to achieve the full benefits of the route by improving tissue retention, selectivity and cytotoxicity. In this study, nanostructured lipid carriers (NLC) were developed and evaluated for paclitaxel (PTX) intraductal delivery. They presented size <600 nm, encapsulation efficiency >90% and stability. To improve tissue retention and add thermosensitive properties (i.e., responsiveness to biological temperature), two modifications were explored. In one, NLCs were dispersed in Poloxamer 407 to form *in situ* gels; in the other, they were coated with the thermosensitive polymer poly(N-isopropylacrylamide) (PNIPAM) to obtain hybrid nanoparticles (NP-H) functionalized with type I collagen-binding peptide (SILY), which is overexpressed in the mammary tumor microenvironment. Incorporating NLCs into the gel prolonged retention of hydrophilic (2.2–3.0-fold) and lipophilic (2.1–2.3-fold) fluorescent probes in mammary tissue *in vivo* compared to unmodified NLCs or probe solutions. PNIPAM coating increased NLC size by 140 nm and prolonged drug release for up to 120 hours in response to biological temperature. Functionalization with SILY promoted adhesion to collagen secreted by MCF-7 and T-47D breast cancer cells. Drug cytotoxicity in MCF-7 and T-47D 3D spheroids increased upon treatment with nanoparticles compared to the solution, although IC₅₀ values varied according to the type of nanoparticle: NLC (59.0–80.3 µM) > NP-H (90.6–116.5 µM). PTX-loaded NP-H functionalized with SILY showed selectivity evidenced by lower cytotoxicity towards non-tumor (MCF-10A) cells. PTX-loaded NLC and NP-H induced apoptosis and microtubule stabilization in MCF-7 and MDA-MB-231 cells, as evidenced by PARP-1, BAX, and acetylated α-tubulin expression, along with disrupted microtubule organization in the cytoskeleton. Notably, NP-H also increased γH2AX expression, indicating DNA damage and suggesting that nanoencapsulation alters PTX's mechanism of action. Unlike PTX solution, NP-H showed no vascular effects on the chorioallantoic membrane of fertilized eggs, yet effectively inhibited angiogenesis, reducing vessel growth by 6.1–7.8-fold. These results underscore the importance of thoughtful nanoparticle design to enhance therapeutic properties and enable novel targeted strategies for breast cancer treatment. **Financial support:** FAPESP 2018/13877-1, 2020/01208-8, 2021/12664-7, 2023/12246-6 and CAPES – Brazilian Federal Agency for Support and Evaluation of Graduate Education within the Ministry of Education of Brazil (finance code 001)

10.015 Proteomic profiling of *Bothrops jararaca* venom-induced alterations in cancer cells lines

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Introduction: Numerous studies have reported the potential antitumor properties of snake venom components, including those from *Bothrops jararaca*. Previous work from our group demonstrated that *B. jararaca* venom modulates proteins associated with cancer cell metabolism, immune response, and inflammation in breast cancer cell lines. However, its effects on other cell lines, including cervical cancer cells, remain underexplored. **Objectives:** We aim to analyze and characterize the effect of *B. jararaca* venom on different cancer cell lines, including cervical HeLa cell line and characterize the changes in the protein profile by mass spectrometry in order to determine specific proteins or pathways affected by the venom. **Methods:** HeLa cells were maintained at 37°C in 5% CO₂ in DMEM supplemented with 10% fetal bovine serum (FBS), ampicillin, and streptomycin. Upon reaching 80% confluence, cells were serum-starved for 24 h before treatment with sub-cytotoxic doses of *B. jararaca* venom (0.63 mg/mL and 2.5 mg/mL) for 24 h. Control cells were treated with PBS. Cell lysis was performed using lysis buffer supplemented with protease inhibitors. Proteins were reduced with TCEP, alkylated with CAA, and digested with trypsin following the SP3 protocol for desalting. Peptides were analyzed using an Orbitrap Exploris 480 mass spectrometer coupled to a Vanquish Neo UHPLC system. **Results and Discussion:** Morphological changes, including loss of adherence, were observed as early as six hours post-treatment. Proteomic analysis identified 6,241 proteins across all samples, with 3,645 proteins common to all conditions. Differential expression analysis revealed that at high-dose treatment (2.5 mg/mL), 12 proteins presented increased abundance (FC ≥ 1.5) and 83 presented decreased abundance (FC ≤ 0.5). Low-dose treatment (0.63 mg/mL) revealed 103 proteins with increased abundance (FC ≥ 1.5) and 12 proteins with decreased abundance (FC ≤ 0.5). Notably, several cancer-related proteins were modulated, including PARL, MET, NHSL3, WDR26, ALDH1A3, HEMH, and LPCAT1, which are implicated in proliferation, invasion, metastasis, and apoptosis. **Conclusion:** Our findings demonstrate that *B. jararaca* venom significantly alters the proteomic profile of HeLa cells, affecting key oncogenic pathways. Further functional and pathway enrichment analyses will provide deeper insights into the molecular mechanisms underlying these effects. This study supports the potential of venom-derived components as modulators of tumor-related pathways, warranting further investigation for therapeutic applications. **Financial Support:** This study was supported by Butantan Institute, São Paulo Research Foundation (FAPESP), National Council for Scientific and Technological Development (CNPq), and the Center of Toxins, Immune Response and Cell Signaling (CETICS). The authors gratefully acknowledge the financial support that made this research possible.

10.016 Characterization of Prednisolone Resistance in Acute Lymphoblastic Leukemia Cells.

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Introduction: Acute lymphoblastic leukemia (ALL) is the most common cancer in childhood. In this disease, an immature, genetically altered lymphocyte arises in the bone marrow due to a genetic error, giving rise to undifferentiated and non-functional leukemic blast cells. ALL can be classified into two subtypes: B and T, the B-cell subtype being more common in children (about 75% of cases). Current treatment for this neoplasm includes drugs such as vincristine, methotrexate, L-asparaginase, and prednisolone, among others. However, during treatment, some patients may develop resistance to these drugs. This resistance is characterized by the selection of progressively less sensitive cells through mechanisms that bypass the drugs' effects. Therefore, this study aimed to understand how leukemic cells acquire resistance and to identify relevant molecular targets involved in this process. **Methods:** To induce resistance, the B-cell ALL line NALM-6 was continuously exposed to prednisolone (1 and 10uM), with the concentration increased whenever the viability of treated cells (PRED) reached that of the untreated control cells (CTR). Once cells exposed to 10uM prednisolone reached 100% viability, their sensitivity to prednisolone (1, 3, 10, 30, 100, 300, and 1000uM) and Oncaspar® (0.3, 1, 3, 10, and 30 ug/mL) was assessed using the MTT assay at 24h, 48h, and 72h. At the same time points, a growth curve was generated for both CTR and PRED cells using the Trypan Blue exclusion assay. At each concentration change, RNA was extracted and stored for gene expression analysis. The expression of target genes was evaluated by real-time PCR. **Results and Discussion:** The MTT assay revealed a significant increase in the viability of resistant (PRED) cells exposed to prednisolone at concentrations ranging from 1 to 300uM, with higher viability compared to CTR cells. This effect persisted at 48h and 72h. Although a reduction in viability was observed at 100 and 300uM, PRED cells still exhibited approximately three times higher viability than CTR cells. No resistance was observed at the highest prednisolone concentration (1000uM) at any of the time points analyzed. The growth curve showed that PRED cells had a higher number of viable cells than CTR cells at all time points, indicating increased proliferative capacity. Regarding Oncaspar® exposure, PRED cells exhibited slight growth at 24h; however, at 48h and 72h, they were more sensitive to all concentrations of the drug compared to the CTR group. Finally, we evaluated the expression of *asparagine synthetase* (ASNS) and *galectin-3* (LGALS3), two molecular targets potentially involved in resistance in ALL. Both genes were upregulated in PRED cells compared to CTR cells, with a more pronounced increase observed in cells treated with 10uM versus 1uM prednisolone. **Conclusion:** These results indicate that prolonged exposure to prednisolone led to the development of resistance features in leukemic cells, including altered drug sensitivity, increased proliferation, and upregulation of key genes involved in survival and growth pathways. These resistant cells can be used in future assays to identify new therapeutic targets and mechanisms associated with treatment failure in ALL. **Supported by:** FAPERJ, FINEP, Fundação Maria Emília.

10.017 **Are Antidepressants a Reposition Option to Treat Mesenchymal Tumors?** Sousa RWR¹, Conceição MLP², Santos IL¹, Rodrigues DCN¹, Nascimento MLLB⁴, Barbosa SLM¹, Freitas MM¹, Pinheiro Neto FR⁶, Sales SLA⁷, Pereira EM⁵, Silva AS⁵, Monte SJH⁵, Pessoa C⁷, Castro e Sousa JM⁴, Ferreira PMP¹ ¹LabCancer-UFPI, Lab Experimental Cancerology, Brazil; ²Lid-USP, Lab Inflammation and Pain, Brazil; ³Lafac-UFPI, Lab Cardiovascular Pharmacology, Brazil. ⁴Lapgenic-UFPI, Lab Toxicological Genetics Research, Brazil. ⁵Lib-UFPI, Lab Immunogenetics and Molecular Biology, Brazil. ⁶UFPI, Lab Pain Pharmacology, Brazil. ⁷UFC, Lab Experimental Oncology, Brazil

Introduction: Sarcomas are highly heterogeneous malignant tumors from soft and bone tissues. Metastatic sarcomas respond poorly to radiation and chemotherapy. Furthermore, the 5-year survival rate for localized tumor is about 50% and < 10% for metastatic ones. Then, the development of novel agents remains an urgent need. **Objectives:** To evaluate *in vitro* and *in vivo* antiproliferative action of antidepressants and toxic effects. **Methods:** Firstly, different pharmacological classes of antidepressants were evaluated for cytotoxicity at 25 µg/mL by MTT or resazurin assays. The most active compounds [amitriptyline (AMI) and fluoxetine (FLU)] were further investigated for toxicity on *Artemia salina* nauplii (0.1-1000 µg/mL), cytotoxicity on a wide panel of normal and tumor cell lines (0.19-25 µg/mL), cell viability of Sarcoma 180 cells (S-180) by trypan blue and 7-aminoactinomycin D flow cytometry assays, morphological analyses by panoptic staining, and synergistic cytotoxic effects following combination with doxorubicin (Dox). For *in vivo* protocols (CEUA/UFPI #0712/2022), S-180 tumor-bearing mice were submitted to a 10-day treatment exposure by gavage with AMI and FLU (20 and 40 mg/kg/day), as well as their combination (10 mg/kg) with 5-fluorouracil (5-FU 10 mg/kg/day). Tumors and key organs were collected, as well as peripheral blood to measure oxidative stress (malondialdehyde) and nitrosative stress (nitrite) in tumors, and systemic toxicity through serum biochemical and hematological assessments. **Results:** AMI and FLU demonstrated > 50% of cell growth inhibition on different cell lines and toxicity upon *A. salina* [LC₅₀ values of 5.9 (4.4-8.0) and 9.3 (5.1-17.1) µg/mL, respectively]. Both of them display time- and concentration-dependent cytotoxic effects (24-72h, $p < 0.05$) and a cell death profile indicative of apoptosis, with loss of cell viability, pyknosis and onset of apoptotic bodies. *In vitro* association with Dox showed synergistic and additive effects. Treatment with AMI and FLU caused tumor growth inhibition of 65.5 ± 5.0 and $73.0 \pm 6.1\%$ and 59.7 ± 10.6 and $64.6 \pm 7.6\%$ for 20 and 40 mg/kg, respectively ($p < 0.05$). Even lower doses (10 mg/kg/day), both compounds reduced tumor mass during monotherapy (38.6 ± 13.9 and $47.5 \pm 15.3\%$) or in combination with 5-FU (56.3 ± 8.3 and $56.6 \pm 5.6\%$, respectively). AMI 20 mg/kg/day increased lipid peroxidation, whereas FLU elevated intratumoral nitrite levels. FLU 20 mg/kg/day induced leukopenia and AMI + 5-FU elevated serum GPT levels ($p < 0.05$). **Conclusion** AMI and FLU demonstrated apoptotic-related antitumor activities against S-180 cells and slight *in vivo* adverse effects, highlighting their promising potential for treating mesenchymal tumors. **Financial Support** This was partially supported by CNPq.

10.018 **Synergistic Effect of Ionizing Radiation And N-Hydrazinecarbothioamide B-Lapachone (BV3) Derivative Against Lung Cancer.** Andrade JKF¹, Goes AJF², Silva MSL², Silva TG², Oliveira APB³, Militao GCG⁴, ¹UFRPE, Dept Animal Morphology and Physiology, Brazil, ²UFPE, Dept. Antibiotics, Bioscience Center, Brazil, ³Institute of Radiotherapy Waldemir Miranda, Brazil, ⁴UFPE, Dept. Physiology and Pharmacology, Bioscience Center, Brazil

Introduction: β -lapachone undergoes redox cycles that increase intracellular ROS levels, most of which are catalyzed by the enzyme NAD(P)H:quinone oxidoreductase 1, causing tumor cell death. β -Lapachone and ionizing radiation kill cancer cells in a synergistic manner (Ahn *et al.*, 2013). The use of drugs acting as radiosensitizers is a strategy that has been discussed, since it can increase the effect of radiation, even at lower doses. We produced three thiosemicarbazone derivatives from β -lapachone, BV2, BV3 and BV5, all of them with cytotoxic potential toward cancer cells. Since lung cancer cells showed to be more sensitive to the new derivatives, we associated the β -Lapachone derivatives with γ -ray radiation. **Methods:** The NCI-H292 (lung cancer) cells were irradiated with 1 Gy gamma radiation (dose that cause 60 % survival) with two protocol: 24 h before treatment with compounds or immediately after treatment with compounds. After radiation, cells were trypsinized and seeded (10^3 /well) in six-well plates, 24 h later compounds in seven different concentrations were added to the plate, and incubated for 24 hours. After this period, the culture medium was removed and a clean medium was added. In another set of experiments, cells were treated with compounds at the IC₅₀ concentration during 24 h, then the drug was removed and radiation was applied. Growth was monitored during 11 days. Then, cells were fixed with 10% formaldehyde for 15 minutes. After washing with PBS, the cells were stained using 0.5% violet crystal for 5 minutes. A cluster of blue-staining cells (considered a colony if it comprises at least 50 cells) were counted in each well using an inverted microscope. **Results:** Among three compounds tested at the survival clonogenic assay on NCIH292 cell line, only BV3 presented a lower IC₅₀ (1.6 μ g/mL) when associated with 1 Gy pre-gamma radiation, compared to non irradiated BV3 treatment (2.8 μ g/mL). Pre-irradiation (1Gy) of NCIH292 did not improved the anticlonogenic effect of BV2 (IC₅₀ 2.6 μ g/mL for 1Gy + BV2 vs 2.1 μ g/mL for BV2 alone), nor BV5 (IC₅₀ 2.1 μ g/mL for 1Gy + BV5 vs 1.9 μ g/mL for BV5 alone), demonstrating no sensitization. The structural difference between BV3 and BV5 derivatives was the insertion of a nitro group at BV3, which enhanced the electron-withdrawing characteristics. This evidenced the role of the nitro group in the activity. When the NCIH292 cell line was first treated (24 h) with BV compounds at IC₅₀ concentration and then irradiated with 1 Gy, a significant reduction on colony survival was observed for all compounds tested, when BV2 alone at 2.1 μ g/mL, BV3 alone at 2.8 μ g/mL or BV5 alone at 1.9 μ g/mL resulted in 56 $\pm$ 2.51%, 50 $\pm$ 2.2% and 50 $\pm$ 2.7% of colony survival, respectively; BV2 + 1Gy, BV3 +1Gy or BV5 + 1Gy association treatments resulted in 42 $\pm$ 2.7%, 39 $\pm$ 2.28% and 41 $\pm$ 2.5% of colony survival, respectively. **Conclusion:** BV3 compound presented better radiosensitizer properties among compounds tested. **Financial Support:** Propesqi UFPE, CAPES, CNPq. **Ref.** Ahn *et al.* Enhancement of radiation effect using beta-lapachone and underlying mechanism. Radiat Oncol J. 2013 Jun;31(2):57-65.

10.019 **Cephalochromin Potentiates Clinically Used Drugs in Acute Lymphoblastic Leukemia Models.** Serra CSM¹, Lima GC¹, Vicari HP¹, Lima K², Nascimento MC², Rego EM², Ferreira MJP³, Costa-Lotufo LV¹, Machado-Neto JA¹ ¹Dept Pharmacology, ICB-USP, Brazil; ²Dept Internal Medicine, Hematology Division, FM-USP, Brazil; ³ Dept Botany, IB-USP, Brazil

Introduction: Acute lymphoblastic leukemia (ALL) is a malignant hematological neoplasm that arises from mutations in hematopoietic stem cells and affects precursors of the lymphoid lineage. In adult patients, rates of refractoriness and relapse to current treatments remain high, highlighting the need for new therapeutic options. In pharmacological therapy, natural products constitute the backbone of ALL treatment, with established examples including vinca alkaloids, anthracyclines, and cytarabine. Cephalochromin (CPCR), a compound derived from the fermentation of the fungus *Cosmopora vilior*, has demonstrated antiproliferative and cytotoxic activity by inducing cell cycle arrest in A549 (human lung cancer) cells. Our results confirmed that the reduced cell viability observed in ALL models treated with CPCR was associated with DNA damage, apoptosis, and autophagy. The upregulation of apoptosis-related genes, such as *BBC3* and *PMAIP1*, as well as cell cycle inhibitors including *CDKN1A*, *CDKN1B*, and *GADD45A*, suggests that these pathways are involved in the response to CPCR. Based on these findings, we investigated the effects of combining CPCR with vincristine, daunorubicin, and cytarabine, drugs well-established in ALL treatment.

Methods: ALL cell lines Jurkat, NALM6, and REH were treated with vehicle or increasing concentrations of cephalochromin (CPCR; 0.15 – 10 μ M), daunorubicin (1.9 – 32 nM), cytarabine (1 – 62 nM), and vincristine (0.12 – 20 nM), either as single agents or in combination, for 72 hours. Cell viability was assessed using the MTT assay. Drug interactions were analyzed using SynergyFinder, which calculates ZIP synergy scores interpreted as strong synergy (>10), moderate synergy (5 to 10), additive effects (–5 to 5), or antagonism (<–5). **Results:** In Jurkat cells (T-ALL), cephalochromin demonstrated strong synergistic effects with daunorubicin (ZIP score: 10.1) and vincristine (ZIP score: 12.4), and a moderate synergy with cytarabine (ZIP score: 6.4). In NALM6 cells (B-ALL), strong synergy was observed between cephalochromin and vincristine (ZIP score: 20.2), while combinations with cytarabine (ZIP score: 2.9) and daunorubicin (ZIP score: 5.1) showed additive effects or moderate synergy, respectively. Similarly, in REH cells (non-B/non-T-ALL), cephalochromin exhibited strong synergistic interaction with vincristine (ZIP score: 10.6) and additive effects with cytarabine (ZIP score: 3.7) and daunorubicin (ZIP score: –1.2). **Conclusion:** Our findings demonstrate that CPCR enhances the cytotoxic effects of clinically used chemotherapeutic agents in ALL cellular models. These results support the potential of CPCR as an adjuvant compound in ALL therapy and warrant further investigation in more complex models. **Financial support:** FAPESP, CNPq and CAPES.

10.020 **Investigation of Cytotoxic Potential of Melittin and Apitoxin in Bladder Cancer Cell T24.** Brayn GA¹, Poiato GEH¹, Oliveira AMB¹, Porto RM², Silva GN³, Almeida TC¹, Picolo G¹. ¹IB, Lab Pain and Signaling, São Paulo, Brazil; ²IB, Lab Development and Innovation, São Paulo, Brazil; ³UFOP, Toxicogenetic, Epidemiological and Clinical Study and Research Group, Ouro Preto, Brazil

Introduction: Bladder cancer is one of the most incident cancers around the world and the most common among the urinary tract. There are two forms of bladder cancer: muscle-invasive bladder cancer (MIBC) and non-muscle-invasive bladder cancer (NMIBC). MIBC can metastasize and accounts for 25% of cases. Treatment for MIBC consists of complete cystectomy or partial surgery alongside chemotherapy. The surgery presents long-term effects that impair quality of life, and chemotherapy is associated with resistance and high nephrotoxicity. Thus, adherence to treatment is often below the desired rate, stressing the need for new therapeutic options. Melittin, the main constituent of apitoxin, the honeybee (*Apis mellifera*) venom, has been studied as an antineoplastic compound in several types of cancer. Even though the specific mechanisms of action of melittin in bladder cancer have not yet been clarified. Then, it was our aim to investigate the cytotoxic effect of apitoxin and melittin on T24 bladder cancer cells. **Methods:** T24 bladder cancer cells were treated with apitoxin or melittin at concentrations 1.25-10 µg/mL. After 24, 48, and 72 hours, cell viability was measured by MTT assay. The same method was applied to MRC-5, a non-cancerous cell line, to analyze selectivity. Cell migration and morphological analysis were studied after incubation with melittin at concentrations of 1,25-7,5 µg/mL for 24 hours and photos were taken before and after treatment. Clonogenic survival was measured after treatment with melittin at concentrations of 3.75 and 7.5 µg/mL for 24 hours. Cell death mechanism was analyzed through flow cytometry. **Results:** Both melittin and apitoxin decreased cell viability at concentrations of 5.0, 7.5 and 10 µg/mL in all analyzed timeframes. Apitoxin also reduced cell viability at concentration of 1.25 µg/mL after 24 hours of treatment. Decrease in MRC-5 cell viability, evaluated as control normal cell, was observed for apitoxin only at concentration of 10 µg/mL on all timeframes, and at concentration of 5.0 µg/mL after 48 hours. Melittin decreased in MRC-5 cell viability was observed at concentrations of 5.0, 7.5 and 10 µg/mL after 48 and 72 hours of treatment, with no reduction after 24 hours. Melittin did not reduce clonogenicity. In addition, melittin at 5.0 µg/mL inhibited cell migration. Morphological analysis shows presence of apoptotic bodies and cell density reduction after treatment with melittin at 7.5 µg/mL. Cell death mechanisms analysis showed recent apoptosis at concentrations 5, 7.5, and 10 µg/mL, late apoptosis at concentrations 3.75, 7,5 and 10 µg/mL and necrosis at 3.75 and 10 µg/mL. **Conclusion:** Although more assays need to be performed in order to fully grasp the effect of melittin on signaling pathways and tumor cell death mechanisms, these results show the cytotoxic potential of melittin on bladder tumor cells.

Key words: apitoxin, bladder cancer, melittin.

10.021 **Evaluation of Hecpidin and Heparanase Expression in the Treatment of DU-145 and PC-3 Cell Lines with Heparin.** Kawamura JK, Santos PCJL, Vicente CM, Porto CS. Unifesp-EPM, Dept Pharmacology, Brazil

Introduction: Castration-resistant prostate cancer (CRPC), due to its resistance to androgen deprivation therapy, is more likely to progress to a metastatic stage, posing a greater threat to the patient's health and significantly increasing the risk of mortality. As a result, therapeutic strategies aimed at reducing cell proliferation – an essential step in the metastatic process – have become crucial to improving the patient's prognosis. Although primarily used as an anticoagulant, heparin has been explored as a potential adjuvant in cancer therapy due to its effects on signaling pathways involved in cell proliferation. Notably, the proteins hepcidin and heparanase, both implicated in oncogenic processes, may have their systemic levels altered after heparin administration, given their mechanistic role in modulating iron homeostasis and the tumor microenvironment. This study aims to investigate CRPC cell lines (DU-145 and PC-3, derived from brain and bone metastases, respectively) after treatment with porcine heparin. The main focus is to analyze the expression levels of hepcidin, a key regulator of iron metabolism, and heparanase, an enzyme implicated in extracellular matrix remodeling and tumor progression. This work was financially supported by FAPESP 2024/07284-9. **Methods:** To carry out this study, the cells will be treated with different concentrations of heparin and subsequently submitted to protein extraction for Western blot analysis, and RNA extraction for real-time PCR. They will also be used in cell viability assays with MTT and Trypan Blue to determine whether heparin treatment has any effect on metastatic cell proliferation. **Results:** The cells were treated with varying concentrations of heparin (50, 100 and 200 µg/mL) and analyzed by means of the MTT assay, which did not reveal statistically significant differences in cell viability. Currently, the Trypan Blue exclusion trial is being conducted to further evaluate cell viability. Our subsequent experiments will involve Western Blot and qPCR analysis to analyze hepcidin and heparanase expression after heparin treatment. **Conclusion:** The current results indicate that treatment with heparin at the tested concentrations has no significant impact on CRPC cell proliferation or death. The findings may contribute to the understanding of the pharmacodynamics of heparin in metastatic prostate cancer and support its potential repositioning as an adjuvant in oncology therapies. **Financial support:** FAPESP 2024/07284-9, Capes, CNPq.

10.022 PEGylation Reduces Nanoparticle Cytotoxicity in Non-Cancer Cells as a Function of PEG

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Introduction: The lack of selectivity and non-specific interactions of chemotherapeutic agents with healthy cells and tissues lead to a large number of adverse and toxic effects, which hinder cancer treatment. Targeting drugs and nanocarriers to tumors or hindering their interactions with non-tumor cells is a way to reduce side effects. Poly (lactic-co-glycolic acid) nanoparticles (PLGA-NPs) containing the new drug candidate seriniquinone (SQ) were previously developed by our group and have shown cytotoxicity against melanoma cells at 0.7-1.6 μ M. Nanoparticle PEGylation increases systemic circulation time as it reduces the uptake by cells of the mononuclear phagocyte system, enabling more time for the nanocarriers to reach and accumulate in the tumor tissue. However, how the PEGylation ratio affect interactions with cells is a matter of discussion. Here, we studied the influence of nanoparticle PEGylation on their cytotoxicity against tumor and non-tumor cells.

Methods: PLGA-PEG-NPs were prepared with two ratios of PLGA-PEG to PLGA (1:9 and 2:3 w/w), and dynamic and electrophoretic light scattering were used to characterize the size distribution and the zeta potential of the NPs. Encapsulation efficiency (EE) was quantified using high performance liquid chromatography, while cytotoxic effects against melanoma cells (SK-MEL-28 and SK-MEL-147) and THP-1 differentiated in macrophages (dTHP-1) were evaluated using a sulforhodamine B assay. **Results:** The diameter of PLGA-SQ-NPs and PEG-PLGA-SQ-NPs obtained with the 1:9 ratio were not statistically different (260.2 and 285.4 nm, respectively), particles obtained with the 2:3 ratio were significantly smaller (230.7 nm, $p < 0.05$), suggesting that an increase in the ratio of PEG-PLGA to PLGA resulted in smaller NPs. However, the polydispersity index increased by 2.39-fold. Adding PEG-PLGA reduced the EE from 83.1% to 69.7% (1:9, $p < 0.05$) and 61.2% (2:3). The lower half-maximal inhibitory concentration (IC_{50}) for SK-MEL-28, SK-MEL-147 cells and for macrophages were very similar for PEG-PLGA particles obtained with the 1:9 ratio compared to NPs without PEG. On the other hand, the 2:3 ratio nanoparticles displayed an increase in IC_{50} for melanoma cells by 1.52-fold (0.44 μ M) and 1.07-fold (1.05 μ M), respectively. In spite of this, IC_{50} for dTHP-1 had a 3.25-fold increase (4.47 μ M). **Conclusion:** The results suggest that a higher ratio of PEG-PLGA:PLGA could be a mechanism for inducing selectivity against melanoma cells, and thus, should be considered during formulation design to balance the long circulation/toxicity effects. Financial Support Information: FAPESP (2018/13877-1, 2022/15832-0 e 2024/01714-1)

10.023 **BR4Cl Inhibits Migration and Adhesion of SW-480 Colorectal Cancer Cells *in vitro***. Alves BS¹, Oliveira MP¹, Pereira FIA¹, de Sá RE¹, Pérez CN², Marinho-Filho JDB¹, Araújo AJ¹. ¹UFDPAR, Lab Cultura de Células do Delta, Brazil, ²UFG, Instituto de Química, Brazil

Introduction: Colorectal cancer (CRC) is among the most common types of neoplasms worldwide and is associated with a poor prognosis, mainly due to its high metastatic potential, which is the leading cause of morbidity and mortality in affected patients. In this context, there is increasing interest in identifying novel molecules capable of disrupting tumor cell dissemination. The synthesis of natural product-based compounds represents a strategic approach that enables the rational modification of bioactive structures to enhance their therapeutic efficacy and broaden their pharmacological applications. **Objectives:** In line with this perspective, the present study aimed to investigate the cytotoxic, anti-migratory, and anti-adhesive effects of a terpenoid-type chalcone (BR4Cl), a hybrid molecule derived from the structural combination of chalcone and ionone, on human colorectal adenocarcinoma cells (SW-480). **Methods:** The cytotoxic activity of BR4Cl was initially assessed using the MTT assay, and IC₅₀ values were determined at 24, 48, and 72 hours. Based on the 24-hour cell growth curve, subcytotoxic concentrations of 0.05 µM and 0.10 µM were selected for functional assays. Cell viability was confirmed using the Trypan blue exclusion method and morphological analysis through rapid panoptic staining. **Results:** BR4Cl exhibited IC₅₀ values of 12.44 µM, 4.62 µM, and 3.64 µM after 24, 48, and 72 hours of treatment, respectively. The selected subcytotoxic concentrations did not induce significant alterations in cell viability or morphology after 24 hours of exposure. In the clonogenic assay, BR4Cl did not significantly affect the number or size of colonies formed. However, in the cell migration assay, BR4Cl significantly inhibited SW-480 cell migration at both tested concentrations after 24 and 48 hours of treatment. Additionally, the cell adhesion assay demonstrated a marked reduction in cell adhesion to fibronectin, with inhibition rates of 32.68% and 44.16% at 0.05 µM and 0.10 µM, respectively, after 24 hours of pre-treatment. **Conclusion:** These findings suggest that BR4Cl exhibits cytotoxic activity against CRC and interferes with critical steps of the metastatic cascade, such as cell migration and adhesion, *in vitro*. Further studies are needed to elucidate the underlying molecular mechanisms and to advance the evaluation of its therapeutic potential. **Financial support:** This work was supported by CAPES (Coordination for the Improvement of Teaching Personnel); CNPQ (National Council for Scientific and Technological Improvement); INCT BioNat (National Institute of Science and Technology for Biodiversity and Natural Products); INCT Polysaccharides (National Institute of Technology-Science for Polysaccharides).

10.024 **Characterization of the Cytotoxicity of Seriniquinone in Glioma Cell Lines.** Leal AGB¹, Hirata AS¹, Fenical W², Costa-Lotufo LV¹ ¹ICB_USP, Dept Pharmacology Brazil; ²Scripps Institution of Oceanography University of California in San Diego USA

Introduction: Seriniquinone (SQ) was first isolated from the marine bacterium *Serinicoccus sp.*, showing promising antimelanoma activity regardless of the mutational status. The proposed molecular target for SQ is a protein called dermcidin (DCD), that has multiple functions in cancer cells, promoting survival, invasiveness and migration. DCD was also described to promote neuronal survival, being over expressed in neurons. Thus, the present study aims to initially characterize how glioma cell lineages respond to SQ treatment. **Methods:** Initially, SQ cytotoxicity was evaluated by MTT assays against HOG, T98G, U251MG and U87MG glioma cell lines. Subsequently, clonogenic assays were performed for HOG and T98G lineages using concentrations compatible with the IC₅₀ values obtained for each cell line. **Results:** MTT assays showed that SQ was active in three out of four tested cell lines, with IC₅₀ values of 0.26µM, 0.49µM and 0.23µM, for T98G, U251MG and U87MG respectively. It is important to mention that those cells are classified as glioblastomas, the most aggressive type of glioma, while HOG, an oligodendroglioma cell line tested, was not sensitive to SQ at tested concentrations (IC₅₀ greater than 5 µM). Clonogenic assays showed that SQ able to reduce clonogenic capacity of both T98G and HOG cells. Nonetheless, after 24h of treatment with SQ at 0.26 µM, almost no colonies were formed in T98G cells, while in HOG cells treated with SQ at 5 µM, the clonogenic capacity decreased approximately 50% when compared with control. **Conclusion:** SQ showed great cytotoxic activity for T98G, U251MG and U87MG cell lineages, but HOG showed itself more resistant towards SQ treatment. This difference instigates further studies on the mechanism of action involved in the unusual quinone activity.

10.025 **Combining Hypericin-Mediated Photodynamic Therapy and Low-Dose Cisplatin Boosts Cell Death and Necrosis of Cervical Cancer Cells.** Carobeli LR¹, Santos JJ², Martins LF², Caetano W², Consolaro MEL¹ ¹UEM, PGP Biosciences and Physiopathology, ²UEM, PGP Chemistry

Introduction: Cervical cancer is the fourth leading cause of cancer death among women. Chemotherapy treatment is often ineffective and associated with systemic adverse effects. In this context, combination therapies have been explored to reach multiple pathways and overcome chemotherapy resistance. Photodynamic therapy (PDT) is a minimally invasive treatment that targets cancer cells by generating reactive oxygen species upon light activation of a photosensitizer. Studies have shown that hypericin (HYP), a hydrophobic compound derived from *Hypericum perforatum*, loaded into Pluronic 123 (HYP-P123), is an effective photosensitizer for PDT. This study investigated the effect of combining cisplatin, the most common chemotherapy for cervical cancer, with HYP-P123 mediated PDT against human cervical squamous carcinoma cells. **Methods:** The SiHa cervical carcinoma cell line was treated with cisplatin (2.5-62.5 μ M, 24 h) or HYP-P123 (0.1-2.5 μ M, 90 min) with or without white light irradiation (450-750 nm, 30 min), or a combination of both (cisplatin 2.5-12.5 μ M for 24h, before or after HYP-P123 0.5 μ M for 90 min and light 30 min). Cell viability was assessed by MTT assay; the IC₅₀ values were calculated from dose-response curves using nonlinear regression. The combination index (CI) was determined according to the Chou–Talalay method to assess drug interaction. Long-term cell proliferation was evaluated by colony formation assay 14 days post-treatment. Cell death pathways were analyzed by flow cytometry using annexin-V and propidium iodide staining. All assays were performed in triplicates and cells only with culture medium were untreated controls. Data were analyzed using ANOVA with Tukey-Kramer multiple comparisons test, and Student's t-test for viability experiments. **Results:** Monotherapies had limited efficacy even at the highest concentrations. PDT with 2.5 μ M HYP-P123 led to 14% viability; cisplatin at 62.5 μ M reduced cell viability to 30%. The combination showed greater effects. Pretreating with cisplatin followed by HYP/PDT reduced viability to 21% while reversing the order reduced it to 9% ($p < 0.001$). The IC₅₀ values were 3.4 μ M for HYP/PDT, 40.7 μ M for cisplatin, and 1.8 μ M for cisplatin when combined with 0.5 μ M of HYP/PDT. This resulted in a CI of 0.19, indicating a synergistic effect. After 14 days, 84% of colonies remained post-cisplatin and 2% post-HYP/PDT. Combination treatment was better at reducing long-term cell proliferation, as it fully inhibited colony formation ($p < 0.0001$). HYP/PDT mainly induced late apoptosis (66%) in SiHa cells, cisplatin induced early apoptosis (49%), while the combination changed the death profile leading predominantly to necrosis (65%, $p < 0.0001$). **Conclusion:** The combined treatment enhanced cell death, mainly via necrosis, and reduced clonogenicity. Sequential HYP/PDT–cisplatin therapy was more effective, suggesting that HYP-P123 mediated PDT boosts the sensitization of SiHa cells to cisplatin and acts synergistically to induce cell death. This combination may allow the use of lower doses of chemotherapy, potentially reducing side effects. These findings support the potential of HYP/PDT and cisplatin combination as a promising strategy against cervical squamous cell carcinoma, and merit *in vivo* investigations.

10.026 **Melanoma and IAPs: Unraveling Therapeutic Opportunities through Integrated Bioinformatics and Experimental Approaches.** Silva CSMR, Machado-Neto JA, Costa-Lotufo LV ICB-USP

Introduction: Apoptosis is a tightly regulated process essential for development, immune function, and tissue homeostasis. In melanoma and other cancers, its dysregulation enables tumor cells to evade death and sustain proliferation. The inhibitor of apoptosis proteins (IAPs) are key regulators and attractive therapeutic targets. Although natural and synthetic IAP inhibitors have been explored to restore apoptosis in melanoma, important gaps remain in understanding their role, highlighting the need for further research and new treatment strategies. **Objective:** To investigate the role of IAPs in melanoma and assess the therapeutic potential of IAP inhibition, alone or combined with other agents, in preclinical models. **Methods:** Gene expression from RNA-seq data of melanoma patients and acquired common nevi were obtained from public databases and analyzed using bioinformatics tools for survival outcomes, clinical associations, and functional transcriptomics. Cell viability was evaluated by MTT assay in seven melanoma cell lines treated for 72 hours with drugs of diverse mechanisms: doxorubicin (topoisomerase inhibitor), PLX-4032 (BRAF inhibitor), cephalocromin (survivin inhibitor), YM155 (survivin/XIAP suppressant), prodigiosin (survivin inhibitor), and tolinapant (BIRC2/3 and XIAP inhibitor), across a concentration range. IC₅₀ values were calculated by nonlinear regression using GraphPad Prism 8. Combination treatments with PLX-4032 and tolinapant were performed in SK-MEL-28R cells and analyzed using SynergyFinder software. Gene expression was quantified by qPCR applying the $2^{-\Delta\Delta CT}$ method. A *p*-value < 0.05 was considered statistically significant. **Results:** Bioinformatic analysis revealed increased expression of *NAIP*, *BIRC3*, *XIAP*, and *BIRC5* in melanoma patients, with high *BIRC5* expression correlating with poorer clinical outcomes. Differential expression and gene enrichment analyses highlighted IAP-associated processes relevant to melanoma progression. Cell viability assays showed consistent IC₅₀ values across most cell lines, except SK-MEL-28, which was more sensitive to PLX-4032 (IC₅₀ < 0.7 μM). SK-MEL-28 and its resistant variant SK-MEL-28R were selected for further study. As expected, SK-MEL-28R was resistant to PLX-4032 and unaffected by tolinapant alone. However, combined treatment exhibited synergistic cytotoxicity (ZIP score: 4.17). qPCR analysis across cell lines showed no significant predominance in IAP family member expression. **Conclusion:** Although bioinformatics data suggested melanoma as a suitable model for studying IAPs, *in vitro* results revealed a more complex biology than anticipated. The observed drug synergy, especially at higher concentrations, suggests a therapeutic window. Despite differing from stronger effects seen for embelin (XIAP inhibitor) in leukemia models, these findings support further exploration of IAP-targeting strategies and combination therapies for treatment-resistant melanoma. **Financial support:** FAPESP (#2023/08735-1;2024/14964-6); CAPES (#88887.893747/2023-00); CNPq.

10.027 Antitumoral Effect of Lemon Gum (*Citrus X Latifolia*) In A Murine Colon Carcinoma Model (CT26.WT) *in vivo* and *in vitro*. Oliveira MMF¹, Alves GOM¹, Araújo DD¹, Araújo GS¹, Machado FS¹, Cajado AG², Lima RCP², Lopes ALF³, Medeiros JVR³, Ribeiro FOS⁴, Silva DA⁴, Souza LP⁵, Silva FBF⁵, Araújo AJ¹, Marinho JDB¹ ¹UFDFPAR, Lab Cultura de Células do Delta, Brasil, ²UFC, Lab Farmacologia da Inflamação e do Câncer, Brasil, ³UFDFPAR, Lab Inflamação e Gastroenterologia Translacional, Brasil, ⁴UFDFPAR, Núcleo de Pesquisa em Biodiversidade e Biotecnologia, Brasil, ⁵UFDFPAR, Brasil

Introduction The pharmacological importance of natural polysaccharides in cancer treatment is increasingly recognized due to their immunomodulatory and antitumor properties. Lemon gum (LG) is a biocompatible polymer, indicating potential for several future biomedical and biotechnological applications. No studies with *in vivo* investigation regarding its antitumor action have been verified. Thus, highlighting the importance of new studies that add information about LG from different perspectives. **Objectives** This study aimed to evaluate the effects of LG obtained from the polysaccharide extracted from the species *Citrus x latifolia* on cytotoxicity against tumor and non-tumor cells *in vitro* and its antitumor potential *in vivo* in BALB/c mice transplanted with murine colon tumor cells. **Methods** *In vitro*, the 3-4,5-dimethyl-thiazol-2yl-2,-diphenyltetrazolium bromide (MTT) test was performed to evaluate the cytotoxicity of LG and, for *in vivo* tests, CT26.WT cells, previously cultured *in vitro*, were inoculated subcutaneously in the left axillary region of the animals. Treatment began 24 hours after tumor induction and lasted 18 consecutive days, using two daily doses of LG, respectively, 50 and 100 mg/kg/day. Saline solution was administered as a negative control and the standard chemotherapy agent 5-Fluorouracil as a positive control. The percentage of tumor inhibition was performed based on the daily measurement of tumor growth, after the appearance of the palpable tumor. Hematological and biochemical assays were performed using blood and serum samples collected from the animals. The relative and histopathological weight of the dissected organs of the animals was performed. And qRT-PCR was used to analyze the expression of genes related to the inflammatory cytokines *Il4*, *Il6*, *Il1b*, *Tgfb* after treatment with LG. **Results** LG did not show cytotoxicity *in vitro*, but showed an antitumor effect *in vivo* with tumor inhibition of 16.46% and 33.16% at doses of 50 and 100 mg/kg/day, respectively. Regarding toxicological aspects, no evidence of toxicity was found in biochemical and hematological tests at both doses; however, a slight increase in the relative weight of the liver was observed at both doses of LG tested, which in the histopathological analysis of the liver demonstrated that the organ was slightly affected by the treatment, indicating reversible toxicity. In the analysis of cytokine gene expression by qRT-PCR, an increase in the expression of *Il1b* was identified, in contrast, *Il6*, *Il4* and *Tgfb* did not show significant differences. **Conclusion** LG was able to inhibit tumor growth of the CT26.WT cell line *in vivo*, despite not showing direct cytotoxicity against this cell line *in vitro*. The results found suggest that LG has some interesting anticancer activity that may be associated with its immunostimulatory properties. And it opens new perspectives for future investigations into its antitumor potential. **Financial Support** This work was supported by CAPES (Coordination for the Improvement of Teaching Personnel); CNPq (National Council for Scientific and Technological Improvement); FAPEPI (Piauí State Research Support Foundation); INCT BioNat (National Institute of Science and Technology for Biodiversity and Natural Products); INCT Polysaccharides (National Institute of Technology-Science for Polysaccharides).

10.028 **IL-33 Expression and M1 Macrophage Polarization in Triple-Negative Breast Cancer: A Translational Study.** Maia IFVC¹, Vidal DBD¹, Alves BES¹, Freitas GL¹, Maia CB¹, Barrozo AEO¹, Silva JMR¹, Almeida PRC², Cunha MPSS³, Júnior-Lima RCP¹, Wong DVT¹ ¹UFC, Dpt of Physiology and Pharmacology, Lab Inflammation and Cancer Pharmacology, Drug Research and Development Center; ²UFC, Dpt of Pathology and Forensic Medicine; ³ICC, Haroldo Juaçaba Hospital

Introduction: Triple-negative breast cancer (TNBC) is an aggressive subtype characterized by the absence of estrogen and progesterone receptors and little/absence of overexpression of the human epidermal growth factor 2 (HER2). Identifying new prognostic markers is essential for improving therapeutic outcomes. **Aim:** To evaluate the association of IL-33/ST2 expression with clinicopathologic parameters in TNBC, in addition to investigating *in vitro* the polarization of macrophages after exposure to tumor cells incubated with chemotherapy drugs. **Method:** The clinical study was a retrospective and cross-sectional design. Clinical-pathological data from 73 patients with TNBC treated at Hospital Haroldo Juaçaba were included. Sociodemographic data was obtained from the patient's electronic medical records. A Tissue Microarray (TMA) was performed on the samples to evaluate the expression of IL-33 and ST2 using indirect immunofluorescence (IIF) and Immunohistochemistry (IHC). *In vitro*, the IL-33/ST2 pathway was investigated in TNBC (MDA-MB-231) and luminal (MCF-7) tumor cell lines, which were incubated or not with doxorubicin (DOX) and/or paclitaxel (PTX) using IFI. To evaluate the macrophages, the RAW 264.7 strain was exposed to the supernatant of the tumor cells, incubated or not with the DOX/PTX. The supernatant was collected to dosage cytokines. IL-33 was also measured in the tumor cell lines. In addition, the expression of iNOS and Arginase-1 was assessed by IFI. Gene expression for the M1 (*Tnf-alpha*, *inos*, *Il-18*, and *Tgf-beta*) and M2 (*Mmp9*, *Cd206*, *Arg1*) phenotypes by qPCR, as well as nitrite dosage. Chi-Square, Fisher's Exact, and/or ANOVA tests with a statistical significance of $p < 0.05$ were used for statistical analysis (Ethics committee approval n. 5.614.936). **Results:** Most patients were 50 years old or older (63.01%), with a mean age of 53 years. A family history of breast cancer was reported in 41.10%. The Ki-67 index was predominantly greater than 20% (84.93%). Low IL-33 expression was observed in 56.45% ($p = 0.0068$), Ki-67 > 20% ($p = 0.0068$), and tumor recurrence ($p = 0.0440$) in TNBC. There was no significant difference in overall survival between patients with low and high IL-33 expression. There were no significant associations with ST2. *In vitro*, there was an increase in IL-33 expression, especially in the groups incubated with DOX or DOX/PTX ($p < 0.05$). In RAW 264.7 activated with LPS, nitric oxide (NO) production was elevated following incubation with tumor cell supernatants. In the other groups, even without LPS stimulation, NO production was also observed. There was an increase in TNF- α levels ($p < 0.05$) and the gene expression of *Tnf-alpha*, *Il-18*, and *inos*, suggesting polarization towards M1. **Conclusion:** Low IL-33 expression is associated with a high Ki-67 proliferation index and tumor recurrence. The conditioned medium from tumor cells incubated with chemotherapy induces the polarization of macrophages towards the M1 phenotype. **Financial support:** CNPQ, Ministério da Saúde, FUNCAP, CAPES. **Keywords:** IL-33; triple-negative breast cancer; macrophage

10.029 Aurelianolides Downregulate Macroautophagy and HSP90 Activity, with Mitochondria Disruption. Silva GWS¹, Marques AM², Fontão APGA¹, Figueiredo MR², Alves, SV³, Santos, LMG³, Sampaio ALF¹ ¹Farmanguinhos-FIOCRUZ, Lab Farmacologia Molecular; ²Farmanguinhos-FIOCRUZ, Lab Tecnologia para Biodiversidade em Saúde/TecBio; ³INCQS-FIOCRUZ Lab Alimentos

Introduction: Leukemia stands for around one third of child cancer, with more than 30 thousand annual deaths of children below 14 years old [1]. Historically, natural products represent a majority in antitumoral drug origin, acting as bioactive compounds or basis for specific synthesis [2]. Aurelianolides A and B are isolated from *Athenaea fasciculata*, and show great antitumoral potential against leukemia, with apoptosis induction and high selectivity [3,4]. Autophagy is an intracellular recycling process that maintains homeostasis [5]. Mitochondria is a key organelle, closely related to energy production, iron metabolism and apoptosis [6]. **Methods:** In this work, we investigated the mechanism of action of aurelianolides (SisGen AB5D582). After 24-hour treatments (1.5 to 5.0 μ M), western blotting was performed to evaluate autophagy related and heat shock proteins. Mitochondria polarization was assessed using JC-1 probe and CD71 membrane expression was evaluated, both by flow cytometry. Intracellular iron levels were assessed by atomic absorption. **Results:** The ATG5- 12-16L1 complex and Beclin-1 (macroautophagy-related proteins) are greatly reduced after 24-hour treatment with aurelianolides, reaching 70% expression decrease. However, Chaperone-Mediated Autophagy (CMA) proteins are upregulated, with HSPA8 increasing 3-fold. Also, HSP90 interaction is visible by upregulation of HSP70, one of its client proteins, which increases in a dose-dependent manner. Mitochondria membrane potential is also greatly reduced after 24-hour treatments, with almost 80% of depolarization in higher concentrations (5 μ M aurelianolide B). Iron uptake and intracellular levels are also reduced, with CD71 expression and intracellular iron levels greatly reduced ($p < 0.01$), indicating iron metabolism impairment. **Conclusion:** Aurelianolides can disrupt mitochondrial activity, macroautophagy and iron metabolism, probably by its interaction with HSP90. This work was supported by CNPq PROEPFAR II Proc 440018/2022-6 and CNPq PROEPFAR Proc 447630/2024-5. **References:** [1] World Health Organization (WHO). *Cancer Today*. Disponível em: . [2] Newman DJ, Cragg GM. Natural Products as Sources of New Drugs over the Nearly Four Decades from 01/1981 to 09/2019. *J Nat Prod*. 2020;83(3):770-803. [3] Silva GWS, Marques AM, Fontão APGA, et al. Aurelianolides from *Aureliana fasciculata* var. *fasciculata* Trigger Apoptosis With Caspase Activation in Human Leukemia Cells. *Anticancer Res*. 2023;43(3):1245-1253. [4] Marques AM, Brito LC, Mendonça SC, et al. An Integrated Strategy of UHPLC-ESI-MS/MS Combined with Bioactivity-Based Molecular Networking for Identification of Antitumoral Withanolides from *Athenaea fasciculata* (Vell.) I.M.C. Rodrigues & Stehmann. *Molecules*. 2024;29(18):4357. [5] Debnath J, Gammoh N, Ryan KM. Autophagy and autophagy-related pathways in cancer. *Nat Rev Mol Cell Biol*. 2023;24(8):560-575. [6] Vringer E, Tait SWG. Mitochondria and cell death-associated inflammation. *Cell Death Differ*. 2023;30(2):304-312.

10.030 Impact of LGALS3 Gene Silencing on the Sensitivity to Chemotherapy in Acute Lymphoblastic Leukemia Cells. Haus LLFA^{1,2}, Salgado MTSF¹, Almeida MS³, Carvalho RS¹ ¹ICB-UFRJ, Lab. of Pain and Inflammation Pharmacology, Brazil; ²UFRJ, PPG Biological Systems Engineering, Brazil, ³CENABIO-UFRJ, National Center for Structural Biology and Bioimaging, Brazil

Introduction: Acute lymphoblastic leukemia (ALL) is a malignant hematologic disorder marked by the abnormal proliferation of immature lymphoid cells, leading to bone marrow infiltration, suppression of normal hematopoiesis and potential metastasis. While T-cell ALL is more common in adults, B-cell ALL predominates in children, representing the leading cause of cancer-related mortality in this group. Standard treatment includes corticosteroids, vincristine, and L-asparaginase. Despite L-asparaginase's importance in therapy by depleting plasma asparagine, inhibiting protein synthesis, and inducing apoptosis, resistance remains a major challenge. Proposed mechanisms include drug inactivation by antibodies and asparagine synthetase overexpression. However, these do not fully explain all resistant cases. Galectin-3 (Gal-3), a protein involved in immune modulation, inflammation, and tissue remodeling, is overexpressed in several cancers and has been associated with cancer progression, emerging as a potential therapeutic target. It is related to ALL by promoting communication between B-cell precursors and bone marrow stromal cells, which contributes to relapse and is import for leukemia migration. This study investigates the role of Gal-3 in modulating the response of B-ALL cells to L-asparaginase and corticosteroid. **Methods:** Galectin-3 gene (*LGALS3*) silencing was performed in the B-ALL cell line NALM-6 through viral transduction, resulting in two cell groups: scramble control (shSCRB) and Galectin-3-silenced cells (shGAL3). Real-time PCR and immunoblotting were conducted to confirm gene knockdown. A proliferation curve was also performed at 24, 48 and 72 hours by trypan blue assay. Cell viability was assessed using the MTT assay with Prednisolone (1, 3, 10, 30, 100, 300, and 1000 μ M) and Oncaspar® (0.3, 1, 3, 10, and 30 μ g/mL) at the same time points. **Results and Discussion:** The gene silencing process generated two batches of shGAL3 cells, named L1 and L2. When compared to shSCRB cells, the L1 cells reduced *LGALS3* expression about 60% and the L2 cells showed a decrease of 70%, indicating that silencing was successful. Therefore, the L2 was chosen for the following experimentation. In addition, shGAL3 cells proliferation profile was investigated through the growth curve. At 24, 48, and 72 hours, the cell counts were, respectively, 134%, 163% and 180% of shSCRB cell counts. In the MTT assay with Prednisolone, all concentrations reduced cell viability compared to the control group, and the 1000 μ M concentration presented the highest effect at all times analyzed. Compared to shSCRB, shGAL3 L2 cells were more resistant to the treatment and had more cells at 48 hours. For Oncaspar, the MTT assay also decreased cell viability at all concentrations, but 10 and 30 μ g/mL showed no significant difference between them at 48 and 72 hours. In comparison, shGAL3 cells were more sensitive than shSCRB cells at 48 and 72 hours. **Conclusion:** These findings suggest that Galectin-3 may influence drug sensitivity and proliferation in B-ALL cells, highlighting its potential as a therapeutic target. **Supported by:** FAPERJ, FINEP, Fundação Maria Emília.

10.031 **Antiproliferative Activity of a Natural Clerodane Diterpene on Breast Cancer Cells.** Barbosa SLM¹, Almeida ML¹, Ramos RWS¹, Freitas MM¹, Feitoza LLS¹, Zalewski S², Camargo JGS², Carvalho FA², Santos AG², Castro e Sousa JM³, Dittz D³, Ferreira PMP¹ ¹UFPI, Dept Biophysics and Physiology, Lab Experimental Cancerology (LabCancer), PP Pharmaceutical Sciences Brazil, ²UNESP, Faculty of Pharmaceutical Sciences, Lab Pharmacognosy, Brazil, ³UFPI, Dept Biochemistry and Pharmacology, Brazil

Introduction Breast cancer is the most common type among women, and its etiology relates genetic, environmental and behavioral factors. Natural compounds, such as those derived from *Casearia* species, have gained prominence for the searching of new therapeutic options, based on their antioxidant, anti-inflammatory and antimicrobial pharmacological properties. **Objectives** To evaluate the antiproliferative action of Casearin J (Cas J), a diterpene from *Casearia sylvestris*, on tumor lines of breast cancer. **Methods** Leaves of *C. sylvestris* (SisGen #A33EA7A, SISBIO 33429-1) were submitted to ethanolic extractions, partitioned by ethyl acetate and identified by comparison of time of retention (tR) and UV spectra, suggesting the predominance of clerodane diterpenes with λ_{max} between 221-238 nm, mainly. Tumorigenic lines murine (4T1, triple negative), human (MCF-7, estradiol dependent; MDA-MB-231, triple negative) and a non-tumorigenic murine fibroblast (L-929) were plated at 5×10^3 cells and treated by Cas J (0.19 - 25 $\mu\text{g/mL}$) and the positive control doxorubicin (dox, 0.03 - 5 $\mu\text{g/mL}$) for 72h. Cell proliferation was analyzed by the Alamar blue (resazurin) assay. Morphological investigations included panoptic staining and trypan blue exclusion tests in which 10^4 4-T1 cells were treated with Cas J (0.025-1.0 $\mu\text{g/mL}$) and Dox (0.1 and 1.0 $\mu\text{g/mL}$). Next, cell migration by *in vitro* wound healing assay of 4-T1 cells was analyzed after exposure to Cas J (0.01-0.1 $\mu\text{g/mL}$) and Dox (0.05 $\mu\text{g/mL}$) for 48 h. Statistical analysis was performed using one-way ANOVA followed by the Student-Newman-Keuls test ($p < 0.05$) using GraphPad Prism. **Results** Casearin J non-selective cytotoxic action, with IC_{50} values of 1.19, 0.81, 0.75 and 6.19 $\mu\text{g/mL}$ for L929, 4T1, MCF-7 and MDA-MB-231 lines, respectively. Trypan blue staining indicated viability cell reduction up to 1 $\mu\text{g/mL}$ ($p < 0.05$), whose cellular changes presented apoptosis-related features, including shrinkage, karyorrhexis and pyknosis. Casearin J delay cell migration in a concentration-dependent manner, showing a significantly open area following 48 h of observation. **Conclusion** Casearin J displays strong cytotoxic action on breast cancer cells and potentiality for antimetastatic inhibition.

10.032 **Cytotoxic and Cell Death Mechanisms Induced by Cannabidiol in Colorectal Cancer: Role of Cannabinoid and Vanilloid Receptors.** Jesus MFIO, Pereira THR, Zamarioli LDS, Trindade CB Unifesp-EPM, Depto de Farmacologia

Introduction. Colorectal cancer is the third most diagnosed type of cancer worldwide and has one of the highest mortality rates. This high lethality is primarily due to challenges in early diagnosis and the limited availability of effective pharmacological treatments. Among the therapeutic strategies being investigated to improve outcomes in colorectal cancer treatment, the use of cannabinoids and/or extracts from *Cannabis sativa* has shown promising results in experimental studies.

Objective. To evaluate the therapeutic potential of *Cannabis sativa* constituents, specifically cannabidiol (CBD), in colorectal cancer cell lines and to determine whether this substance can modulate the cellular response to 5-fluorouracil. **Material and Methods.** The HT29 and SW480 cell lines, derived from primary human colorectal tumors, were exposed to cannabidiol (CBD - 1.5 to 100 μ M), and cell viability was assessed using the MTT assay (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide), which measures the reduction of MTT to formazan crystals. Additionally, the cannabinoid receptors CB1 and CB2, as well as the transient receptor potential vanilloid 1 (TRPV1), were characterized using immunofluorescence assays. The modalities of cell death, including ferroptosis, necrosis, and apoptosis, were investigated by pre-treatment with inhibitors of these pathways, followed by exposure to CBD. To analyze cell death, flow cytometry was performed using 7-AAD/Annexin-V-FITC staining in addition to the MTT assay. The involvement of cannabinoid receptors in CBD-induced effects was assessed using the MTT assay in conjunction with specific antagonists targeting these receptors. **Results:** CBD demonstrated cytotoxicity in both colorectal cancer cell lines analyzed, particularly at concentrations between 25 and 100 μ M. The mode of cell death induced by CBD varied between the studied cell lines, with apoptosis being predominant in SW480 cells and necroptosis in the HT29 cell line. Regarding receptor involvement, experiments using specific antagonists revealed that, although cannabinoid and vanilloid receptors were present in both cell lines, the effects of CBD were partially mediated exclusively through its interaction with CB2 receptors in both and TRPV1 in SW480. **Conclusion:** These results suggest that the mechanism of action of CBD may be influenced by the unique molecular profile of each cell line. Furthermore, the characterization of CB1, CB2, and TRPV1 receptors confirmed their presence in both colorectal cancer cells. The predominant role of CB2 in both cell lines, along with the exclusive contribution of TRPV1 in SW480, reinforces the hypothesis that CBD interaction with these receptors is crucial for modulating the observed cytotoxic effects. Understanding the mechanisms involved in CBD-induced cell death may contribute to the development of new therapeutic strategies for colorectal cancer. **Financial support:** CAPES, FAPESP and CNPq

10.033 Oxidative Stress Induction by Thiosemicarbazone DT-10 as a Therapeutic Strategy in Cholangiocarcinoma Cell Lines. Vasconcelos BV¹, Moraes ADTO², Da Costa VCM¹, Leite ACL³, Rêgo MJB¹ ¹UFPE, Programa de Pós-Graduação em Inovação Terapêutica ²UFPE, Programa de Pós-graduação RENORBIO ³UFPE, Programa de Pós-Graduação em Ciências Farmacêuticas

Cholangiocarcinoma (CCA) is an aggressive malignancy with a poor prognosis. The first-line therapy, based on gemcitabine, offers limited survival, highlighting the need for new therapeutic strategies. Thiosemicarbazones have shown remarkable antitumor activity, often associated with their ability to induce oxidative stress in cancer cells. Therefore, the objective of this study was to evaluate the therapeutic potential of the novel thiosemicarbazone DT-10, investigating its mechanism of action via the production of Reactive Oxygen Species (ROS) in the HuCCT-1 and RBE CCA cell lines. The cytotoxicity of DT-10 was initially accessed by MTT assay to establish the IC₂₅ and IC₅₀ concentrations. Subsequently, RBE and HuCCT-1 cell lines were treated for 72h with DT-10, gemcitabine (25 μ M), and their respective controls (untreated and vehicle DMSO 0.1%). Superoxide anion production was assessed by flow cytometry (FACSCalibur™) using the DHE probe. Results from three semi-independent experiments performed in duplicate were normalized for statistical analysis. Treatment with DT-10 induced a significant, dose-dependent increase in superoxide levels in both CCA cell lines, surpassing the effect of gemcitabine at equivalent concentrations. At the IC₂₅ dose (12.5 μ M), DT-10 consistently induced a pronounced increase in ROS while maintaining substantial cell viability. In the RBE cell line, this resulted in a mean 1.23-fold increase in fluorescence intensity, an effect greater than that observed with gemcitabine. The HuCCT-1 cell line exhibited even greater sensitivity, with the IC₂₅ dose of DT-10 inducing a 2.9-fold increase in ROS, compared to only a 1.1-fold increase caused by the highest dose of gemcitabine. At the IC₅₀ concentration, a pronounced cytotoxic effect was observed, evidenced by the loss of over 50% of cellular events. The few surviving cells displayed substantial ROS production, corresponding to a mean 3.2-fold increase in fluorescence intensity compared to the control. To confirm that cytotoxicity was indeed mediated by ROS, rescue experiments with the antioxidant N-acetylcysteine (NAC) were performed. Preliminary results, although technically challenging, indicated that pre-treatment with NAC could partially reverse the superoxide production induced by DT-10, especially in the HuCCT-1 cell line. These findings support the need for further studies to directly assess the correlation between the antioxidant effect and the rescue of cell viability. This study establishes that DT-10 is a potent agent against CCA, with superior efficacy in inducing oxidative stress when compared to the positive control, gemcitabine. The differential sensitivity observed between the cell lines suggests that DT-10 exploits the redox vulnerabilities of different CCA subtypes. Additional studies are necessary to fully validate the safety and efficacy profile of the compound, aiming for its future clinical development in the treatment of CCA.

10.034 **Assessment of Diaporthein B Cytotoxicity in Gastric Cancer Cells.** Domingos HV¹, Peña-Hidalgo M², Ferreira MJP², Miguel RA³ Lima K¹ Machado-Neto JA¹ Costa-Lotufo, LV¹ ¹ICB-USP Departament of Pharmacology, Brazil, ²IB-US,P Dept Botany, Brazil, ³UFDPAr, Delta Cell culture Lab, Brazil

Introduction: Many antineoplastic drugs used in clinical practice are derived from natural products, which drives the continuous search for new natural compounds that could impact cancer therapy. Diaporthein B (DTB) is a diterpene derived from an endophytic fungus cultivated from *Bignonia magnifica* W. Bull, an ornamental plant native to Colombia, with significant antifungal and cytotoxic activity in colon cancer cells. The aim of the present study was to assess the activity of this diterpene using gastric cancer (GC) cells as a model.

Methodology: Cytotoxicity was assessed using the MTT assay. DTB was tested at increasing concentrations (0.002–27 μ M, and 0.003–50 μ M, respectively) to determine its IC₅₀ values in AGS and HGC-27 gastric carcinoma cells after 72 hours of incubation. Doxorubicin (0.0004–5 μ M) was used as a positive control. To evaluate colony formation, the cell lines were treated with DTB for 24 hours at concentrations of 0.2, 0.4, and 0.8 IC₅₀. Cell viability was assessed using the trypan blue exclusion method, and the cell cycle was analyzed by flow cytometry after 48 hours of treatment with DTB at concentrations of 0.5 IC₅₀, IC₅₀, and 2x IC₅₀. Morphological changes, apoptosis induction, and potential mechanisms of action were further evaluated using Western blot (WB).

Results: Treatment with DTB exhibited cytotoxic activity in AGS and HGC-27 cells, with IC₅₀ values of 1.01 μ M and 1.59 μ M, respectively. Colony formation assays showed a reduction in colony area, intensity, and number in both GC cell types (AGS-1 and HGC-27) treated with DTB at all tested concentrations (0.2 IC₅₀, 0.4 IC₅₀, and 0.7 IC₅₀ for AGS, and 0.7 IC₅₀ for HGC-27), indicating inhibition of cell proliferation. Similarly, cell viability, as assessed by the trypan blue assay, decreased significantly in a dose-dependent manner in both cell lines. The HGC-27 and AGS cell lines showed a significant increase in the SubG1 population when treated with DTB at concentrations of 0.8 and 3.2 μ M (HGC-27) and 1.04 and 2.08 μ M (AGS). Morphologically, DTB-treated cells showed significant blebbing and alterations, indicative of cell death by apoptosis. Apoptosis, as assessed by annexin V staining, was significantly increased in the AGS cell line at concentrations of 1.04 μ M and 2.08 μ M. No significant changes were observed in the expression of proteins (PARP-1, P-Yap-1, Yap-1, and H2AX) analyzed by WB. **Conclusion:** DTB demonstrated strong cytotoxic activity against gastric cancer cells and significantly inhibited colony formation compared to the control group. The results showed that DTB induces cell death through apoptosis, but the underlying mechanisms of action are not yet clear and need to be further elucidated. **Financial support:** CNPq/MCTI/CT-BIOTEC-Projeto:440472 and FAPESP: 2014/50926-0; 2023/08735-1

10.035 Nanoemulsion Containing Limonene: A Promising Strategy for Topical Transdermal Approach to Chemoprevention Breast Cancer. Nunes JR, Teixeira MAS, Lopes LB ICB-USP

Nanoemulsion (NE) is a promising system for topical-transdermal drug delivery and for the incorporation of lipophilic compounds for local delivery. Given the high incidence of breast cancer and the limited pharmacological options to prevent progression or recurrence, it becomes evident the need to develop new therapeutic approaches. This study proposes the incorporation of the retinoid fenretinide into NE aiming promote topical-transdermal therapy as a novel approach for breast cancer chemoprevention, aiming to combine efficacy, safety, and patient comfort. **Methods:** The nanoemulsion was produced using probe sonication under an ice bath and characterized by dynamic light scattering. The concentration of limonene in the formulation varied between 1% and 2.5% (w/w). The irritation potential was assessed using the Hen's Egg Test on Chorioallantoic Membrane (HET-CAM assay), this procedure was approved by the Ethics Committee (protocol 70/2016) from Biomedical Science Institute. Skin permeation and the effect of the NE on skin barrier function were evaluated using Franz diffusion cells and porcine ear skin (Ethical Committee approval wasn't required for this experiment). Cytotoxicity was assessed against MDA-MB-231 and MCF-7 breast cancer cells, as well as HaCaT keratinocytes, using the MTT (3-(4,5-Dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium Bromide) assay over a 48-hour period. **Results:** All nanoemulsions exhibited droplet diameters below 200 nm at the time of production, with a monodisperse profile (Polydispersity index < 0.2). None of the formulations induced vascular responses compatible with irritation in the HET-CAM model, regardless of limonene concentration, indicating that limonene addition did not increase the irritation potential of the system. A relationship between droplet size, viscosity, and skin penetration of fenretinide was established: after 12 hours, smaller droplets and lower-viscosity systems—both observed in the nanoemulsion containing 2.5% limonene—resulted in significantly enhanced skin penetration and permeation (3.4-fold increase in penetration and 9-fold in permeation) compared with the NE with limonene 1%. As such, the 2.5% limonene formulation was selected for further studies. In the resistivity assay, used to evaluate skin barrier function, the NE promoted a 1.2-fold reduction in skin resistivity, highlighting its ability to modulate the stratum corneum and potentially enhance compound permeation. The NE containing both limonene and fenretinide significantly enhanced cytotoxicity in MCF-7 cells, evidenced by a 3-fold reduction in IC₅₀ compared with control. In MDA-MB-231 cells, however, no marked difference was observed. For HaCat cells, the IC₅₀ of the fenretinide and limonene-loaded NE was approximately 4-fold higher than for MCF-7, suggesting some degree of selectivity toward tumor cells. **Conclusion:** Despite the addition of limonene, none of the tested systems exhibited irritant properties, indicating their safety for future *in vivo* applications. The incorporation of 2.5% limonene enhanced both skin permeation and penetration, significantly increasing fenretinide levels in viable skin layers. The NE containing fenretinide and 2.5% limonene effectively induced cytotoxicity in both breast cancer cell lines, particularly MCF-7, presenting a promising alternative strategy for breast cancer treatment, especially considering its activity appears to be independent of hormone or retinoid receptor expression. **Financial support:** This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001 and Fundação de Amparo à Pesquisa de São Paulo (FAPESP) grants 2024/03868-6, 2021/06755-0 and 2018/13877-1.

10.036 Exploring Kinin Receptor Modulation as a Therapeutic Approach in Head and Neck Squamous Cell Carcinoma. Neculqueo GW^{1,2}, Farias ABS², Estrázulas M^{1,2}, Campos MM^{1,2} ¹PUCRS, Escola de Medicina, PPG Medicina e Ciências da Saúde, Brazil. ²PUCRS, Escola de Ciências da Saúde e da Vida, INTOX, Brazil

Head and neck squamous cell carcinoma (HNSCC) is an aggressive tumor linked to alcohol and tobacco (Feng Y. *Oncol Rep*, vol.51, p.79, 2024). Kinins and their receptors B₁ and B₂ are present in various tumors and involved in cancer progression (Deepak K. *Biochim Biophys Acta-Rev Cancer*, vol. 1877, p.188807, 2022). We assessed the impacts of B₁ (R954) and B₂ (HOE140) receptor antagonists, as well as B₁ (des-Arg⁹-BK) and B₂ (BK) agonists (1-100 mM) vs. cisplatin (20 µM), on

HNSCC CAL27 and HN30 cell viability and invasion, using MTT and wound healing assays. C57Bl6 and BALB/c nude mice of both sexes were used with approval from the local Animal Ethics Committee (11350;11877). The effects of kinin ligands plus 5-fluorouracil (5-FU) were tested *in vivo*, in a CAL27 xenograft model. CAL27 cells were injected into the posterior flank. Drugs were administered intratumorally from day 21 to day 27 as follows: negative control; tumour control; 5-fluorouracil (5-FU, 2.5 mg/site); HOE140 (3 nmol/site); R954 (80 nmol/site); BK (3 and 10 nmol/site); des-Arg⁹-BK (30 and 100 nmol/site); HOE140 (3 nmol/site/5 days)+5-FU (2.5 mg/site/2 days) and R954 (80 nmol/site/5 days)+5-FU (2.5 mg/site/2 days). Animals were euthanized on day 28 for sample collection. We also used a chemical model of oral carcinogenesis with 4-nitroquinoline 1-oxide (4-NQO -50 µg/ml, in drinking water) for 16 weeks. From 20 weeks, C57BL/6 mice received daily intraperitoneal treatment as follows: negative control; tumor control (PBS); cisplatin (2 mg/kg); HOE140 (50 nmol/kg), and R954 (160 nmol/kg). Euthanasia was performed at week 21 for sample collection. *In vitro*, cisplatin significantly reduced CAL27 and HN30 cell viability ($p<0.01$). Neither kinin antagonist showed significant differences in CAL27 cells ($p>0.05$), but an increase in cell viability was seen in the HN30 cell line for R954 (1 and 3 µM) and HOE140 (1 µM) ($p<0.01$). Neither kinin agonist exhibited any significant effect in both cell lines, and no differences were observed in cell invasion across treatments ($p>0.05$). *In vivo*, HOE140 or R954 significantly reduced the tumour volume in xenografted mice on days 26 and 27 ($p<0.01$). A reduction in body weight and hematological counts was seen in the 5-FU group ($p<0.01$). 5-FU, HOE140, or R954 lessened the number of Ki-67-positive cells ($p<0.01$). BK (3 and 10 nmol), but not des-Arg⁹-BK (30 and 100 nmol), significantly increased the tumor volume from day 25 onwards. Kinin antagonists plus 5-FU triggered a significant reduction in tumor volume from day 23 onwards ($p<0.01$). In the 4-NQO model, body weight was significantly reduced by cisplatin ($p<0.01$), and cisplatin or both kinin antagonists significantly reduced tumor numbers ($p<0.01$), while HOE140 diminished tumor areas ($p<0.05$). Histopathologically, HOE140 significantly reduced carcinoma development compared to the control (OR=0.170; $p<0.05$), while R954 showed no significant effect (OR=0.909; $p>0.05$). We show new evidence for the role of kinins in HNSCC *in vivo*. Further studies are underway to determine which cells are involved in the beneficial effects of kinin antagonists *in vivo*.

10.037 Integrative Network Analysis Reveals Stage-Specific Molecular Modules in Glioblastoma Progression and Potential Intervention Points for Thiazolidinedione Derivatives. Silva Junior CLC¹, Pitta MGR², Rego MJB², Rosa MM² ¹UFPE, PPG Therapeutic Innovation, Brazil. ²NUPIT-SG-UFPE, Research Center for Therapeutic Innovation - Suely Galdino, Brazil

Glioblastoma (GBM) is an aggressive primary brain tumor characterized by a high recurrence rate despite multimodal therapy. Given the limitations of current treatment protocols, understanding the molecular alterations driving GBM progression is critical for developing novel therapeutic strategies. Thiazolidinedione (TZD) derivatives have emerged as promising candidates due to their anticancer effects and traceable ability to cross the blood-brain barrier. To investigate transcriptomic changes during GBM progression and identify signaling pathways modulated by TZD derivatives, we analyzed protein-coding mRNA data from The Cancer Genome Atlas Program using RStudio. Differential Expression Analysis (DEA) was performed for two key comparisons: Primary GBM vs. Normal Tissue (PN); Recurrent GBM vs. Primary GBM (RP). The resulting differentially expressed genes (DEGs) and expression matrices were further analyzed through: Over-Representation Analysis (ORA) to identify enriched pathways; Gene Set Enrichment Analysis (GSEA) to evaluate pathway-level expression changes (both using the Reactome database). Pathways consistently upregulated or downregulated in both enrichments were selected to construct a protein-protein interaction (PPI) network (STRINGdb, confidence score >700). Functional modules within the PPI network were then identified using the Louvain community detection method, applied separately to each GBM comparison (PN and RP). DEA-PN showed 3,830 upregulated mostly in signaling related to DNA homology and repair ($p < 1 \times 10^{-75}$) while 1,888 downregulated genes related to neuronal functions, particularly neurotransmitter receptor activity ($p < 1 \times 10^{-26}$). DEA-RP Comparison revealed 252 upregulated genes associated with GABAergic signaling ($p < 1 \times 10^{-25}$) and ECM/collagen pathways ($p < 1 \times 10^{-15}$), whereas 3,047 downregulated genes tight junction interactions ($p < 1 \times 10^{-27}$) and xenobiotic metabolism ($p < 1 \times 10^{-19}$). PPI disclosed 575 differentially expressed proteins in PN and 191 in RP. The Louvain Algorithm showed distinct modules. The “Synaptic Plasticity & Neuronal Adhesion” module (PN), presenting five proteins predicted as TZD derivative targets, showed downregulated GABA subunits (GABRA1: $\log_2FC \approx -8.4$, $p < 1 \times 10^{-11}$; GABRB2: $\log_2FC \approx -7.8$, $p < 1 \times 10^{-19}$) and the upregulated Serotonin Transporter (SLC6A4: $\log_2FC \approx +5.6$, $p < 1 \times 10^{-5}$). Separately, RP presents “Xenobiotic Metabolism (CYP450)” module with downregulation of cytochrome P450 (CYP2A6: $\log_2FC \approx -8.35$, $p < 1 \times 10^{-12}$) and upregulation of Cathepsin S (CTSS: $\log_2FC$ approximately +1.50, $p < 0.012$) within the “Collagen Biosynthesis & Modification” module. These findings highlight substantial transcriptomic reprogramming between PN and RP GBM, with pronounced regulation of GABAergic and Serotonergic TZD targets, suggests a primary disruption in synaptic signaling pathways for PN. In RP, TZD targets localize to ECM remodeling (CTSS) and xenobiotic metabolism (CYP2A6), indicating a shift toward microenvironment adaptation and treatment resistance mechanisms. This divergence implies that TZD-based therapeutic strategies may need stage-specific optimization—targeting neuronal dysfunction in early disease versus ECM/drug metabolism in recurrence. Funded by CAPES.

10.038 **ACEA, A Selective Cannabinoid Receptor 1 Agonist, enhances the antitumoral activity of Oxaliplatin and inhibits the Associated Peripheral Sensory Neuropathy in a Murine Colorectal Adenocarcinoma Model.** Nascimento RR^{1,3}, Carvalho VM¹, Pereira AF¹, França JC¹, Moura MNS¹, Alves BWF¹, Muniz PTG¹, Rodrigues EDD¹, Freire DC¹, Ribeiro MVS¹, Rocha LR¹, Abucater BLSM¹, Bezerra BS¹, Nicolau LAD², Vale ML¹ ¹UFC, Dpt of Physiology and Pharmacology, Brazil; ²UFDP, Biotechnology and Biodiversity Center Research, Brazil; ³Medical University of South Carolina, Charleston, Dpt of Regenerative Medicine and Cell Biology, USA

Background: Colorectal cancer is the third most commonly diagnosed cancer worldwide and a leading cause of cancer-related mortality. Chemotherapy remains a cornerstone of treatment for metastatic colorectal cancer, with oxaliplatin (OXL) being a key first-line chemotherapeutic agent. Despite this, OXL use is limited by its dose-dependent peripheral sensory neuropathy (NSP), a debilitating side effect that often necessitates dose reduction or treatment discontinuation. Cannabinoid agonists modulate the endocannabinoid system and have been shown to prevent the development of NSP. However, there are no studies that demonstrate whether ACEA (arachidonyl-2'-chloroethylamide), a selective cannabinoid receptor 1 (CB1) agonist, interferes or not with the antitumor and neuropathic effect of oxaliplatin (OXL). **Objective:** To evaluate whether ACEA interferes with the antitumor and antineuropathic effects of oxaliplatin (OXL) in both *in vivo* and *in vitro* models using murine colorectal adenocarcinoma (CT26) cells. **Methods:** Male Balb/c mice (CEUA No. 16110422-0) were subcutaneously inoculated with CT26 colorectal adenocarcinoma cells and treated with ACEA, either alone or in combination with oxaliplatin (OXL). Tumor growth was monitored until day 12, at which point tumors were collected for analysis. Neuropathic pain symptoms were evaluated using von Frey and acetone tests. For *in vitro* assays, CT26 cells were cultured in 96-well plates and treated with increasing concentrations of OXL and ACEA. After 72 hours, cell viability was assessed. **Results:** ACEA inhibited the development of NSP, significantly reducing mechanical (100%) and cold allodynia (67.09%), as well as ATF-3 immunoexpression in the dorsal root ganglion (81.72 %, $p < 0.05$). Kinetic analysis demonstrated that both OXL and the combination of ACEA+OXL exerted significant antitumor effects, with a tumor growth inhibition (44.15%; 79.06%, respectively). ACEA also significantly reduced CD31 (72%) and Ki-67 (36.9) immunostaining. *In vitro*, OXL and ACEA decreased CT26 cell viability, with IC_{50} values of 2.23 μ M and 49.46 μ M, respectively. Moreover, co-treatment with OXL and ACEA reduced the IC_{50} of OXL to 0.01 μ M, enhancing its antitumor effect. **Conclusion:** ACEA exhibited an antineuropathic effect without interfering with the antitumor activity of oxaliplatin (OXL). Although ACEA alone did not demonstrate antitumor efficacy, its combination with OXL reduced tumor growth *in vivo* and enhanced cytotoxicity *in vitro*. These findings are promising and support further investigation into the use of selective cannabinoid receptor 1 agonists as adjuvant agents in colorectal cancer therapy involving OXL.

10.039 Friedelin Induces Caspase-3-Dependent Apoptosis Without Causing Systemic Toxicity in Mice Bearing Ehrlich Ascitic Carcinoma. Silva ELES¹, Silva FA¹, Souza TPM¹, Silva JP¹, Santana JR¹, Flor JF¹, Figueiredo CG¹, Santos EC², Bassi JE², Leal DC³, Silva JC³, Reis MDS¹, Lins MP¹, Barreto EO¹, Ferro JNS¹ ¹UFAL, Cellular Biology Lab and ² Lab Virology and Immunology Research; ³UNCISAL, Brazil

Friedelin (FD) is a naturally occurring triterpene with well-documented *in vitro* antitumor potential (SUBASH-BABU, Exp. Toxicol. Pathol., v.8, p.630, 2017). Previous data presented at the 55th SBFTE demonstrated that FD reduces tumor progression and extends survival time by decreasing tumor cell viability, modulating inflammatory components within the tumor microenvironment, and attenuating angiogenesis in the murine Ehrlich ascitic carcinoma (EAC). Therefore, this study investigated the effect of FD on the modulation of apoptotic signaling pathways and to evaluate its possible systemic effects in healthy and EAC-bearing mice. For this purpose, female Swiss mice (10–14 weeks old, CEUA/UFAL15/2019) were inoculated with EAC cells (5×10^6 ; i.p.) and randomly assigned to the following experimental groups: tumor-bearing (TM), 5-fluorouracil (5-FU; 200 $\mu\text{mol/kg}$), vehicle (EtOH; 2%), and friedelin (FD; 50 $\mu\text{mol/kg}$). To assess the systemic effects in healthy animals, non-tumor-bearing groups were included and treated with vehicle (NT) or (NTFD). Treatments were administered intraperitoneally from the day to day 10 following tumor induction. Twenty-four hours after the last treatment, the animals were euthanized for the collection of tumor ascites, blood and tissues. Tumor cell viability was evaluated by dual fluorescent staining using acridine orange (AO) and propidium iodide (PI). Apoptosis, CXCR4 expression, and cleaved caspase-3 detection were analyzed by flow cytometry. Gene expression of Bcl-2 and Bax proteins was assessed by RT-qPCR. To investigate possible systemic effects of FD, parameters including water and food intake, hematological indices, bone marrow cellularity, and histological analysis of the liver and kidneys were monitored. FD treatment resulted in a 22% increase in AO/PI-positive cells, indicating a reduction in tumor cell viability, along with a significant increase in apoptotic cells, particularly in the late apoptotic stage. Gene expression analysis revealed an increase in the Bax/Bcl-2 ratio (4.03 in TM and 18.93 in FD), and flow cytometry showed a significant upregulation of activated caspase-3 expression ($p= 0.01$ vs TM), suggesting that FD induces caspase-3-dependent apoptosis. CXCR4, a chemokine receptor broadly associated in tumor progression, survival, proliferation, and metastasis, showed a 20% reduction in expression levels following FD treatment, indicating its potential role in modulation tumor progression mechanisms. Regarding systemic safety, FD administration did not alter water and food intake, leukocyte production and circulation remained preserved, and no hematological disturbances or histopathological alterations were observed in hepatic or renal tissues. In conclusion, FD exhibits significant antitumor potential by promoting caspase-3-dependent apoptosis in EAC model, without evidence of systemic toxicity. These results support FD as a promising candidate for the development of new anticancer therapies. However, further studies are needed to elucidate the molecular mechanisms involved in the FD response in the EAC model. **Keywords:** Natural product. Death cell. Toxicity. **Financial support:** CNPq and CAPES.

10.040 *In Vitro* Cytotoxic Action of Anthelmintics on Glioblastoma Cells. Santos IL¹, Feitoza LLS¹, Sousa RWR¹, Rodrigues DCN¹, Porto JCS¹, Machado FS², Marinho Filho JDB², Araújo AJ², Sales SLA³, Pessoa C³, Ferreira PMP¹ ¹UFPI, Lab Experimental Cancerology (LabCancer), Dept Biophysics and Physiology, Teresina, Brazil; ²UFDPAr, Lab Cell Culture from Delta (LCCDelta), Parnaíba, Brazil; ³NPDM-UFC, Dept Physiology and Pharmacology, Center for Drug Research and Development, Fortaleza, Brazil

Introduction: Pharmaceutical repositioning has been considered a strategy to develop drug options for the treatment of neoplasias, and anthelmintics have shown promising pharmacological activities. **Objective:** To evaluate the *in vitro* antiproliferative and cytotoxic activity of anthelmintics on different cell lines. **Methodology:** First, the cytotoxic activity (0.19–25 µg/mL) of ivermectin (IVM), mebendazole (MBZ), albendazole (ABZ), praziquantel (PZQ), piperazine (PIP), and niclosamide (NIC) on B16-F10, L-929, HCT-116, PC-3, HL-60, SNB-19, NCI-H460, and HaCaT cells was assessed using the MTT assay after 72 hours of exposure. The viability of human glioblastoma multiforme SNB-19 cells treated with NIC (0.05, 0.1, 0.5, and/or 1 µg/mL) was analyzed using the trypan blue exclusion assay (24–72 hours). Morphological changes were evaluated by panoptic staining, while membrane integrity, cell cycle distribution (propidium iodide), and mitochondrial depolarization (rhodamine 123) were assessed by flow cytometry after 72 hours of incubation. IC₅₀ values and their 95% confidence intervals were obtained by nonlinear regression using GraphPad software. Statistical analysis was performed using one-way ANOVA followed by the Student–Newman–Keuls test ($p < 0.05$). **Results:** NIC was the most cytotoxic compound for most cell lines, with IC₅₀ values ranging from 0.05 µg/mL (human leukemia HL-60 cells) to 1.05 µg/mL (SNB-19), followed by MBZ and ABZ. IVM showed moderate effects, with IC₅₀ values ranging from 5.5 µg/mL (HL-60) to 9.9 µg/mL (murine melanoma B16-F10). NIC significantly reduced cell viability at concentrations of 0.1, 0.5, and 1 µg/mL, caused membrane damage (0.1 and 1 µg/mL), induced mitochondrial depolarization, and promoted cell cycle arrest in the G1 phase at higher concentrations (0.1 and 1 µg/mL), with a reduction in the S and G2/M phases compared to the negative control ($p < 0.05$). No alterations in the sub-G0/G1 population were detected. Morphological analysis revealed a larger nucleus-to-cytoplasm ratio in NIC-treated SNB-19 cells, along with chromatin condensation, cell shrinkage, and cytoplasmic rarefaction. **Conclusion:** NIC demonstrated strong antiproliferative effects on SNB-19 cells in a concentration- and time-dependent manner. **Financial support:** This study was partially supported by CAPES (Financial Code 001).

10.041 Synthetic Hydroxylated Chalcone A3 Impairs Migration and Adhesion in Oral Cancer Cells.

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Introduction: Oral squamous cell carcinoma (OSCC) is the most common type of head and neck cancer, characterized by aggressive local invasion and a high propensity for metastasis, the primary cause of mortality among affected patients. Conventional chemotherapy, although widely used, is limited by severe side effects and poor tumor selectivity, ultimately compromising both treatment efficacy and patient quality of life. In this context, medicinal chemistry has emerged as a promising field for the discovery of new anticancer agents. Among the most studied classes, chalcones have attracted significant attention due to their well-documented antiproliferative and antimetastatic properties. **Objectives:** This study aimed to evaluate the cytotoxic potential of the synthetic hydroxylated chalcone A3 in a panel of human cancer cell lines and to investigate its effects on key steps of the metastatic process of oral squamous cell carcinoma using an *in vitro* model. **Methods:** The cytotoxicity of chalcone A3 was initially assessed in MEL-501, HCT-116, SCC4, CAL27, HL-60, and HSC3 cell lines. The HSC3 line, representative of OSCC, was selected for further investigation. Cytotoxicity assays were conducted at 24, 48, and 72 hours of exposure, and IC₅₀ values were determined. Based on these results, subcytotoxic concentrations (0.25, 0.5, and 1.0 µM) were used in subsequent functional assays, including trypan blue exclusion, morphological analysis, clonogenic assay, migration assay, and adhesion assay to fibronectin. **Results:** Chalcone A3 exhibited time-dependent cytotoxicity, with IC₅₀ values ranging from 47.29 to 8.77 µM. Subcytotoxic concentrations did not impair cell viability or clonogenic capacity. However, treatment with A3 significantly inhibited cell migration at both 24 and 48 hours and markedly reduced cell adhesion to fibronectin, indicating interference with critical events in the metastatic cascade. **Conclusion:** Chalcone A3 demonstrates promising antimetastatic activity by impairing migration and adhesion of OSCC cells, suggesting its potential as a therapeutic agent for controlling tumor dissemination. Further studies are warranted to elucidate the molecular mechanisms underlying their antimigratory and antiadhesive effects. **Financial support:** This work was supported by CAPES (Coordination for the Improvement of Teaching Personnel); CNPQ (National Council for Scientific and Technological Improvement); INCT BioNat (National Institute of Science and Technology for Biodiversity and Natural Products); INCT Polysaccharides (National Institute of Technology-Science for Polysaccharides).

10.042 **Mechanistic Insights into the Cytotoxic Action of a Withanolide from *Athenaea velutina*: DNA Damage and Autophagy in Colorectal Cancer Cells.** Oliveira MP¹, Sá RE¹, Machado FS¹, Almeida BO¹, Garnique ADMB², Costa-Lotufo LV², Machado-Neto JA², Rocha KAD³, Pessoa ODL³, Araujo AJ¹; Marinho-Filho JDB¹ ¹ UFDFar, Lab Cultura de Células do Delta, LCCDelta, Brasil, ²USP, Depto de Farmacologia, Instituto de Ciências Biomédicas, Brasil, ³UFC, Depto de Depto de Química Orgânica e Inorgânica, Brasil

Introduction: Colorectal cancer (CRC) is among the most prevalent and lethal malignancies worldwide, highlighting the need for therapeutic advances that ensure clinical efficacy while minimizing toxicity. This study investigated the cytotoxic potential of a withanolide isolated from the plant *Athenaea velutina* against various tumor cell lines. **Methods:** The molecules were extracted from the leaves with hexane/ethyl acetate 1:1 at room temperature. SisGen Registration Number: A3D4962. Phenotypic, morphological and molecular assays were performed in monoculture models, in addition to validation of the effects in a threedimensional spheroid culture. **Results:** The compound demonstrated a significant reduction in cell viability across a panel of colorectal cancer (CRC) cell lines, including HCT-116 and its TP53-deficient variant, with effects dependent on concentration and exposure time. Treatment for only six hours was sufficient to induce persistent phenotypic alterations, such as loss of cell adhesion, cytoskeletal reorganization, and cytoplasmic vacuolization. Additionally, cell cycle arrest at the G2/M phase was observed, accompanied by increased expression of DNA damage markers γ H2AX and p21. Regarding autophagy, treatment induced a transient increase in acidic vesicular organelles, followed by disruption of autophagic flux, suggesting interference with autophagic dynamics. Finally, cytotoxic activity was confirmed in three-dimensional cell culture models, highlighting the compound's robustness under more physiologically relevant conditions. **Conclusion:** The results reinforce the cytotoxic potential of compounds from *A. velutina* and highlight the compound as a promising candidate for the development of novel chemotherapeutic strategies for colorectal cancer treatment. **Financial support:** This work was supported by CAPES (Coordination for the Improvement of Teaching Personnel); CNPQ (National Council for Scientific and Technological Improvement); INCT BioNat (National Institute of Science and Technology for Biodiversity and Natural Products).

10.043 Analysis of the Antiangiogenic and Cytotoxic Effects of Dihydromyricetin in Preclinical Models. Gomes JGF¹, Barbosa BS¹, Acha BT², Leite NFB³, de Sousa AA⁴, do Lago JPAD⁵, Castro e Sousa JMC⁶, Carvalho GD⁷, Freitas GBL⁸ ¹UFPI, PPG Farmacologia, Brasil; ²UFPI, PPG Biotecnologia, Brasil; ³UFPI, PPG Química, Brasil; ⁴UFPI, PPG Ciências e Saúde, Brasil; ⁵UFPI, Biomedicina, Brasil; ⁶UFPI, PPG Ciências Farmacêuticas, Brasil; ⁷Universidade Federal do Maranhão, Brasil; ⁸UEM, PPG Farmacologia, Brasil

Introduction: Dihydromyricetin (DHM) is a flavonoid from *Ampelopsis grossedentata* and *Hovenia dulcis* with reported antioxidant, anti-inflammatory, pro-apoptotic, autophagy-inducing, and antiangiogenic effects. **Methods:** This study evaluated DHM's antiangiogenic and cytotoxic properties using *in silico*, *in vivo*, and *in vitro* preclinical models. **Results:** In the *in silico* phase, homology modeling was performed for eight tyrosine kinase receptors (FGFR1/2, HER2/3, PDGFRA/B, VEGFR1/2), validated by structural superposition (RMSD < 2 Å) and Ramachandran plots (>90% residues in favorable regions). Blind docking with DHM and reference inhibitors (Futibatinib, Lapatinib, Nintedanib, Regorafenib) showed favorable binding for all active sites, with energies comparable to or surpassing controls, notably for FGFR1/2 and VEGFR2. ADMET predictions indicated low general toxicity, no CYP450 inhibition, non-mutagenicity, and compliance with Lipinski's Rule of Five, but low CNS permeability due to high polarity/low lipophilicity and probable P-glycoprotein interaction, suggesting reduced oral bioavailability. The *in vivo* antiangiogenic assay used the HET-CAM model with *Gallus gallus domesticus* embryos (n=8/group), treated with 20 µL/disc DHM at 1.33, 2.66, 5.32, and 6.98 nM. Dexamethasone (2.56 nM) and distilled water served as positive and negative controls. IKOSA CAM Assay AI analysis revealed dose-dependent inhibition of vascular parameters. At 6.98 nM, DHM reduced vascular area by 57.43%, vessel length by 51.12%, thickness by 38.70%, and branching points by 49.21% ($p<0.05$). The *in vitro* cytotoxicity (MTT assay) was tested against 4T1 (murine breast carcinoma) and L929 (fibroblasts) at 0.78–100 µg/mL. DHM significantly reduced cell viability, with IC₅₀ values of 29.23 µg/mL (4T1) and 15.16 µg/mL (L929). Although cytotoxic to both, DHM showed greater tumor selectivity at intermediate doses. Doxorubicin (control) presented IC₅₀ < 1 µg/mL. **Conclusion:** DHM demonstrates potent antiangiogenic and cytotoxic activities with a favorable safety profile and multitarget action involving FGFR, PDGFR, HER, and VEGFR receptors. These findings support DHM as a promising candidate for antiangiogenic antineoplastic drug development. Further studies should optimize its structure to enhance tumor selectivity and assess *in vivo* efficacy in murine cancer models. **Ethical Approval:** Not required, as Brazilian legislation does not regulate the use of *Gallus gallus domesticus* embryos. **Financial support:** Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES). **References:** ANNESE, T.; TAMMA, R.; RIBATTI, D. IKOSA® CAM Assay Application to Quantify Blood Vessels on Chick Chorioallantoic Membrane (CAM). *Methods in molecular biology*, p. 129–139, 27 set. 2022. AS, M. N. *et al.* Establishment of an in ovo chick embryo yolk sac membrane (YSM) assay for pilot screening of potential angiogenic and anti-angiogenic agents. *Cell Biology International*, v. 42, n. 11, p. 1474–1483, 11 set. 2018. BABU, C. *et al.* Development of novel HER2 inhibitors against gastric cancer derived from flavonoid source of *Syzygium alternifolium* through molecular dynamics and pharmacophore-based screening. *Drug Design, Development and Therapy*, v. 10, p. 3611–3632, 1 nov. 2016. BABY, B.; ANTONY, P.; VIJAYAN, R. Interactions of quercetin with receptor tyrosine kinases associated with human lung carcinoma. *Natural Product Research*, v. 32, n. 24, p. 2928–2931, 12 out. 2017. BATISTA-DUHARTE, A. *et al.* The Hen's Egg Test on Chorioallantoic Membrane. *International Journal of Toxicology*, v. 35, n. 6, p. 627–633, 13 out. 2016. CARUGO, O.; PONGOR, S. A normalized root-mean-square distance for comparing protein three-dimensional structures. *Protein Science : A Publication of the Protein Society*, v. 10, n. 7, p. 1470–1473, 1 jul. 2001. KELLEY, L. A. *et al.* The Phyre2 Web Portal for Protein modeling, Prediction and Analysis. *Nature Protocols*, v. 10, n. 6, p. 845–858, 7 maio 2015. KENNY, P. W. Hydrogen-Bond Donors in Drug Design. *Journal of Medicinal Chemistry*, v. 65, n. 21, p. 14261–14275, 25 out. 2022. LASKOWSKI, R. A. *et al.* PROCHECK: a program to check the stereochemical quality of protein structures. *Journal of Applied*

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10.044 *In Vitro* Antiproliferative Activity of Oleoresin, Volatile and Resinous Fractions of *Copaifera reticulata* Ducke from the Tapajós National Forest – Belterra/Pará. Caetano-Frota JK^{1,2}, Oliveira JC¹, Pontes RS¹, Sartoratto A³, Marsaioli AJ⁴, Oliveira ASS³, Freitas AS¹, Silva MC⁵, Oliveira ECP⁶, Foglio MA¹, Ruiz ALTG¹, Carvalho JE¹ ¹UNICAMP, Lab Phytochemistry, Pharmacology, and Experimental Toxicology, Brazil, ²UFOPA, Institute of Water Science and Technology, Brazil, ³Unicamp, Multidisciplinary Center for Chemical, Biological, and Agricultural Research, Brazil, ⁴Unicamp, Lab Biocatalysis and Organic Synthesis, Brazil, ⁵ IFPA, Postgraduate Program in Environment and Development, Brazil, ⁶ UFOPA, Lab Biotechnology of Medicinal Plants, Brazil

Copaiba oleoresin is exuded from the trunk of trees of the *Copaifera* genus. Its use is widespread, especially in traditional Amazonian medicine, and it has anti-inflammatory, healing and antimicrobial properties. Cancer is one of the main causes of thousands of deaths around the world, with high incidence rates in recent years. Considering the pharmacological properties of this natural product, the investigation of its antiproliferative effect may be an alternative for obtaining new anticancer phytotherapeutics. Thus, the objective of the present study was to evaluate the *in vitro* antiproliferative activity of oleoresin and volatile and resinous fractions of *Copaifera reticulata* Ducke from the Amazon region. The oleoresin (OR) sample was collected in the Tapajós National Forest, Km 117, in the municipality of Belterra/Pará, SISBIO registration number 68739, SisGen number AC3C2CB and botanical identification number NID: 46/2022. The volatile (VF) and resinous (RF) fractions were obtained by hydrodistillation (simple distillation and with Clevenger apparatus), and fractionated by column chromatography. The chemical composition of the VF was analyzed by Gas Chromatography coupled to Mass Spectrometry (GC-MS). The *in vitro* antiproliferative activity was evaluated by the Sulforhodamine B method in tumor cell lines of glioblastoma (U251), breast adenocarcinoma (MCF-7), multidrug-resistant ovarian adenocarcinoma (NCI/ADR-RES), non-small cell lung adenocarcinoma (NCI-H460), colorectal adenocarcinoma (HT-29) and in the non-tumorous human keratinocyte cell line (immortalized) HaCat. In the VF, 16 compounds were identified, the major ones being α -bisabolene (30.02%), α -trans-bergamotene (26.39%) and α -selinene (10.85%). OR, VF, RF and fractions obtained by column chromatography showed cytostatic and cytotoxic effects on tumor cell lines, with emphasis on the subfractions from the volatile fraction chromatographic column, such as VFC10, VFC13, VFC18 and VFC20, which demonstrated cytotoxic effects on U251, MCF-7 and NCI-H460 cell lines at all concentrations tested. The results confirm the antiproliferative activity of *C. reticulata* Ducke oleoresin and its fractions, especially the subfractions obtained by VF column chromatography, which showed high potency and selectivity on the tumor cell lines evaluated, evidencing the pharmacological potential of this natural product in the development of new anticancer phytotherapeutics. **Keywords:** Natural products, copaiba oleoresin, antiproliferative, anticancer, phytotherapeutics. **Funding:** FAPESP (process 21/01280-3), CNPq (process 309264/2021-9), and CAPES/PROAP (process 106/2021).

11. Clinical Pharmacology, Pharmacokinetics, Pharmacogenomics and Toxicology

11.001 **Peritoneal Exposure of Anticancer Drugs in Hyperthermic Peritoneal Chemotherapy (HIPEC): Insights from Literature-Based Pharmacokinetic Modeling.** Toson N, Santos LG, Araújo BV
UFRGS PPG Medical Sciences Porto Alegre Brazil

Hyperthermic peritoneal chemotherapy (HIPEC) was a surgical strategy developed to increase the exposure of tumor cells with chemotherapeutic agents, aiming to eliminate residual cells after cytoreduction surgery (CRS). Consists in a perfusion system able to circulate the chemotherapy directly in peritoneal space. Despite the clinical benefits, there are few pharmacokinetic data correlating drug concentrations with clinical outcomes obtained for HIPEC. This study aimed to analyze the available literature data of concentration time profile of anticancer drugs and evaluate the pharmacokinetics changes in exposure of this application way. Data was extracted from literature using Webplotdigitizer. By extracted concentrations profiles from plasma and perfusate fluid in patients undergoing HIPEC, a non-compartmental analysis (NCA) was performed to evaluate PK parameters using PKAnalix (SimulationsPlus), obtaining the individual area under the curve (AUC), which was normalized by drug amount. These data were compared to I.V. concentration in plasma of cisplatin, mitomycin C and oxaliplatin – more frequent used drugs in HIPEC. To compare the drug exposure, we calculate $AUC_{Perfusate\_IP}/AUC_{Plasma\_IV}$, once perfusate data was not available after I.V administration. Statistical analysis was performed using multivariate linear regression, considering significance p value below or equal to 0.01. The results show that only route of administration was significant when compared to other factors like temperature, drug. The ratio was 26.5 for CIS, 17.4 for MMC and 4.5 for OXA demonstrating superior exposure in peritoneal space when compared to systemic treatment. These findings reinforce the pharmacokinetic advantage of intraperitoneal administration via HIPEC, particularly in achieving higher local drug concentrations compared to systemic delivery. The superior peritoneal exposure observed for cisplatin, mitomycin C, and oxaliplatin supports the rationale for using HIPEC in the treatment of peritoneal malignancies. Further studies integrating pharmacokinetics with clinical outcomes are warranted to optimize therapeutic strategies and individualize drug regimens in HIPEC protocols.

11.002 Linalool, Linalyl Acetate, and Lavender Essential Oil: Evaluation of Metabolism and Drug Interactions Using Canine Liver Microsomes and Recombinant Enzymes. Soares-Santos RR¹, Soto-Blanco B¹, Ferreira RCM², Zhu Z³, Court MH³ ¹UFMG, Veterinary School, Dept Veterinary Clinic and Surgery, Lab. of Toxicology, Brazil, ²FSCBH, Molecular Biology Lab., Brazil, ³Washington State University, College of Veterinary Medicine, Dept Veterinary Clinical Sciences, Program in Individualized Medicine, Comparative Pharmacogenomics Lab., USA

Interactions between natural compounds and hepatic cytochrome P450 (CYP) enzymes play an important role in the pharmacokinetics of many bioactive substances. Linalool (LL) and linalyl acetate (LA), the major components of lavender essential oil (LEO), have pharmacological relevance because of their therapeutic use in dogs. This study investigated the metabolism of LL, LA, and LEO by pooled canine liver microsomes (pDLMs) and recombinant CYP enzymes (rCYP), aiming to identify the metabolic mechanisms involved and potential for drug interactions. pDLMs with or without NADPH, boiled or unboiled pDLMs, and rCYP2B11, rCYP2C21, and rCYP3A12 were used. High-performance liquid chromatography–tandem mass spectrometry was used to evaluate the depletion rate of LL and LA from incubations. pDLMs from dogs treated with phenobarbital, clofibric acid, beta-naphthoflavone, and rifampicin were used to evaluate effects of enzyme induction. Chloramphenicol, ketoconazole, and fluconazole were used as CYP-selective inhibitors, and tramadol, cannabidiol, midazolam, methadone, and trazodone were used as CYP-selective substrates. Drug inhibition experiments were performed with and without NADPH pre-incubation. LA was metabolized to LL by pDLMs in the absence of NADPH, indicating involvement of hepatic esterases. The main enzymes involved in the metabolism of LL were CYP2B11 and CYP2C21, with depletion occurring only in the presence of NADPH. The rate of LL depletion increased proportionally with increasing pDLMs concentration, and saturated with increasing LL concentration with a Michaelis-Menten constant of $52 \pm 11 \mu\text{M}$. Phenobarbital induced LL depletion in pDLMs by more than 5-fold. LL and LA inhibited metabolism in pDLMs mediated by CYP2B11, but not by CYP2C21, CYP2D15, or CYP3A12. LL concentrations required to reduce CYP2B11 mediated metabolism in pDLMs by half (IC₅₀) were $7.1 \mu\text{M}$ for tramadol N-demethylation and $13.9 \mu\text{M}$ for methadone N-demethylation, while IC₅₀ values for LA were $2.7 \mu\text{M}$ and $8.0 \mu\text{M}$ (respectively) and IC₅₀ values for LEO were $2.5 \mu\text{M}$ and $8.6 \mu\text{M}$ (respectively). None of the terpenes studied directly inhibited rCYP2B11, rCYP2C21, or rCYP3A12, suggesting CYP2B11 activity in pDLMs may be indirectly inhibited by a terpene metabolite formed by another hepatic CYP enzyme. These data revealed significant details about the metabolism of LL and LA, highlighting CYP2B11 and CYP2C21 enzymes as key factors in their metabolic processing and implying important potential herb-drug interactions for clinical safety in the therapeutic use of essential oils in dogs. **Financial support:** Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and the William R. Jones endowment to Washington State University College of Veterinary Medicine.

11.003 Development of a PBPK Model to Predict Interactions of DMT and Harmine in Ayahuasca and Co-Administration with Synthetic Drugs Ribeiro GSG¹, Paranhos BAPB¹, Dörr F¹, dos Santos RG², Hallak JEC², Villanova B³, Guerra LTLG³, Raminelli AO³, Reis JAS³, de Paula CC³, Zacharias ABV³, Martins FS¹, Marcourakis T¹ ¹USP, São Paulo, Brazil ²National Institute of Translational Science and Technology in Medicine ³FMRP-USP

Introduction: Ayahuasca is a psychedelic brew traditionally used by indigenous groups in the Amazon, mainly composed of N,N-dimethyltryptamine (DMT) and β -carbolines such as harmine (HRM), a reversible monoamine oxidase A (MAO-A) inhibitor that enables the absorption of DMT and its distribution in the central nervous system (Riba, J. Drug Test. Anal., 7, 401, 2015). Fluoxetine (FL) and paroxetine (PR) are selective serotonin reuptake inhibitors (SSRIs) commonly prescribed for depression. FL is primarily metabolized by CYP2D6 and CYP2C9 into the active metabolite norfluoxetine (NFL) (Mandrioli, R. Curr. Drug Metab., 7, 127, 2006), while PR is metabolized by CYP2D6 and CYP3A4 (Bloomer, J. Clin. Pharmacol., 33, 521, 1992). Co-administration with ayahuasca may lead to serotonin syndrome, as both FL and PR strongly inhibit CYP2D6, the enzyme also responsible for the metabolism of HRM and DMT (Riba, J. Drug Test. Anal., 7, 401, 2015). This study aimed to develop PBPK models to predict interactions between HRM and DMT, as well as with SSRIs. Due to the scarcity of pharmacokinetic data on ayahuasca alkaloids, a clinical study was conducted in partnership with the Ribeirão Preto Medical School, University of São Paulo (FMRP-USP). **Method:** Models were built using PK-Sim® version 11, incorporating *in vitro* parameters (e.g., enzyme kinetics) and physicochemical properties (e.g., logP, MW), and qualified against clinical data from volunteers following oral administration of ayahuasca, FL, and PR. Enzyme kinetics and the inhibition constant (K_i) of HRM for MAO-A were included. The PBPK models for DMT and HRM were validated using clinical data from six healthy volunteers (internal data) who received ayahuasca and underwent sequential blood sampling, analyzed by LC-MS/MS. The models for synthetic drugs were validated using published clinical data. Validation involved visual inspection of plasma concentration–time curves and comparison of observed versus predicted PK parameters. **Results:** Plasma samples were successfully analyzed, enabling the construction of pharmacokinetic curves. The model validation criteria (MFE between 0.5 and 2) were met for DMT, HRM, and PR, with AUC ratios ranging from 0.73 to 1, 0.78 to 1, and 0.73 to 1.4, respectively. MFE values for FL and NFL were adequate, with AUC ratios ranging from 0.6 to 1.9 for FL and 0.7 to 1.7 for NFL. Interaction scenarios for FL-HRM, FL-DMT, PR-HRM, and PR-DMT are being simulated, and a relevant increase in exposure is expected due to potent CYP2D6 inhibition. **Conclusion:** Plasma analysis by LC-MS/MS provided essential data to validate the PBPK models of ayahuasca alkaloids. Simulations showed good agreement with observed data, with predicted parameters within accepted limits and satisfactory observed/predicted ratios. Notably, the input data, such as the inhibition constant (K_i) of HRM for MAO-A, allowed for a clear reproduction of the increase in DMT exposure, highlighting the effectiveness of PBPK modeling in kinetic characterization. This study was funded by the Coordination for the Improvement of Higher Education Personnel - Brazil (CAPES) - Finance Code 001. This work was supported by the São Paulo Research Foundation (FAPESP), grant number 2023/15903-8. The clinical study was approved by the Research Ethics Committee (CEP) of the Hospital das Clínicas of FMRP-USP on May 22, 2023 (CAAE: 69291023.5.0000.5440).

11.004 Behavioral Responses of Adult Zebrafish (*Danio rerio*) Exposed to Zein-Nanoencapsulated Cinnamon Essential Oil: An Investigation of Toxicity. Da Silva ACF¹, Barros, JM², Pimentel JRL¹, Chamberlain ALV¹, Stadnik MJ³, Müller LG⁴, da Costa C^{2,5}, Siebel AM^{1,6} ¹UFPR, Graduate Program in Pharmacology, Brazil ²UFSC, Graduate Program in Chemical and Food Engineering, Brazil ³UFSC, Graduate Program in Plant Sciences, Brazil ⁴IBET Institute of Experimental and Technological Biology, Portugal ⁵UFSC, Graduate Program in Textile Engineering, Brazil ⁶FURG, Graduate Program in Health Sciences, Brazil

Introduction: Synthetic fungicides are widely used in agriculture; however, their continuous use raises concerns about pathogen resistance and environmental contamination, which can often affect non-target organisms. Cinnamon (*Cinnamomum cassia*) essential oil, commonly used as a

spice, contains compounds with antifungal properties. However, its application is limited due to high volatility and low water solubility. To address these issues, a zein-based nanocarrier loaded with cinnamon essential oil (Z-CEO) was developed as an alternative strategy to manage bean anthracnose. Thus, investigating the toxicity of this nanostructured biopesticide is necessary. This study evaluated the potential toxicity of Z-CEO on the behavior of adult zebrafish (*Danio rerio*), an accepted model for non-target organisms. **Methods:** Z-CEO nanoparticles were developed through ultrasound-assisted nanoprecipitation and characterized via UV-Vis spectroscopy. Toxicity was evaluated according to the OECD 203 guideline, the internationally recognized standard for acute fish toxicity testing. Adult wild-type zebrafish (6 months old, both sexes, n = 20 per group; total of 120 animals) were acclimated for 15 days in laboratory conditions (2 animals per liter, temperature $26 \pm 2^\circ\text{C}$, pH 7 ± 0.3 , 14-10 h light-dark cycle) and fed twice daily with flaky fish food. Then, animals were randomly assigned to 96 h exposure in 6 experimental groups: negative control (NC, osmosis water reconstituted with salts); Z-NPs (empty zein nanoparticles); and Z-CEO – a colloidal dispersion of zein nanoparticles loaded with cinnamon essential oil at four concentrations (0.001, 0.01, 0.1, and 1 mg/L). After exposure, fish were transferred to washout conditions (in osmosis water with salts) for 24 h. Behavioral analysis was conducted using the novel tank test to evaluate the time spent in the upper zone, exploratory activity (traveled distance) in the upper zone, and line crossing. The Kolmogorov-Smirnov test verified data normality. Statistical analysis was performed using one-way ANOVA with Dunnett's post-hoc test or Kruskal-Wallis with Dunn's post-hoc test. **Results:** Behavioral parameters showed no statistically significant differences among Z-CEO, Z-NPs, and NC groups ($p > 0.05$). **Conclusion:** The lack of behavioral changes indicates that Z-CEO is safe for zebrafish under the tested conditions, supporting its biocompatibility. Nonetheless, further studies are needed to investigate its antifungal efficacy compared to conventional fungicides and its long-term ecological impacts. This study was supported by the CNPq (under grants 160666/2021-0, 402097/2023-8, 310989/2021-3) and CAPES (final code 001). Fish maintenance and experimental protocols of this study were approved by the institutional Ethics Committee (CEUA; UFPR; #004/2024).

11.005 Pharmacokinetic Profile of the JME-209 in Rats – A Novel Mexiletine Analogue with Potential for COPD Therapy. Santos GCM^{1,2}, Santos HBS², Rocha BM¹, Santos IB³, Vilela LT³, Elidio HSM³, Silva RCPF³, Costa JCS³, Silva PMR¹, Martins MA^{1,2} ¹IOC-Fiocruz, Lab Inflammation, ²Preclinical Pharmacokinetic Platform, ³NBA2 and NBA3 Animal Experimentation Platform, ⁴VPPIS-Fiocruz

Introduction: Local anaesthetics such as mexiletine and lidocaine have demonstrated efficacy in modulating bronchoconstriction and airway inflammation. In the search for new therapeutic strategies for controlling COPD pathological changes, attention has been directed to the development of local anesthetics analogues with improved potency and safety profile. Among these substances, 1-([1,1'-biphenyl]-4-aryloxy)-2-propanamine (JME-209), a structurally modified analogue of mexiletine, has emerged as a promising candidate. The present study aims to characterize the pharmacokinetic (PK) profile of JME-209 when orally administered to rats.

Methods: All procedures involving the use of experimental animals were approved by FIOCRUZ's ethics committee (L002/20 – A5). Wistar rats were treated with orally administered JME-209 at doses of 3, 15 and 30 mg/kg (3 rats/dose). Blood samples were collected via the gingival vein at 5, 15, 30, 60, 120, 240, and 480 minutes post-administration. Plasma concentrations of JME-209 were quantified using a validated in-house LC-MS/MS method. Pharmacokinetic parameters were calculated using the non-compartmental model for extravascular administration. Dose proportionality was evaluated through linear regression analysis. Additionally, to assess potential gingival lesions resulting from venipuncture, the mentolabial region of the animals was analyzed histologically. **Results:** We found that the maximal plasma concentrations (C_{max}) of JME-209 were influenced by the different doses administered (3, 15 and 30 mg/kg), with C_{max} 32.9 ± 17.2, 77.2 ± 5.1 and 341.2 ± 115.4 ng/mL, respectively. The time to reach maximum concentration (T_{max}) was 15.0 ± 0.0, 8.3 ± 5.8 and 5.0 ± 0.0 min for the respective doses, indicating rapid absorption at all tested dose levels. The absorption extension (AUC_{0-t}) was 2612.0 ± 546.3, 12669.9 ± 5407.5, and 26956.2 ± 7488.0 ng/mL · h for the 3, 15, and 30 mg/kg doses, respectively. The elimination half-life (T_{1/2}) was approximately 2.1 ± 1.5 h for the 3 and 30 mg/kg doses, and 4.2 ± 2.0 h for the 15 mg/kg dose. Significant differences were observed among doses for the C_{max}, AUC_{0-t} and AUC_{0-inf}. However, no significant differences were observed in T_{1/2} values. For T_{max}, a significant difference was observed only between the 3 and 30 mg/kg doses. Both C_{max} (β = 0.9429) and AUC_{0-t} (β = 0.9908) increase proportionally with the administered dose, suggesting linear pharmacokinetics. Histopathological analysis of the gingival tissue between the lower incisors predominantly revealed typical structures. **Conclusion:** The PK analysis of JME-209 demonstrated rapid absorption and dose-proportional increases in systemic exposure (C_{max} and AUC_{0-t}), supporting a linear PK profile within the tested dose range. Furthermore, the absence of histopathological changes in the gingival tissue following venipuncture validates the safety of the sampling method. These findings highlight JME-209 as a promising candidate for further investigation as a potential therapeutic agent in the treatment of chronic obstructive pulmonary disease, offering a favorable PK profile. **Financial support:** CNPq, FAPERJ

11.006 Fc Gamma Receptor Polymorphisms as Potential Biomarkers of Natalizumab Therapeutic Failure in Multiple Sclerosis. Pavani GK¹, Cardoso RC¹, Matos MD¹, Duarte LA^{2,3}, Guimenes BD¹, Gutman EG^{2,4}, Ferreira HFA², Pereira VCSR⁴, Domingues VB⁵, Farinhas JGD⁵, Fernandez KA⁵, Schmidt FR⁵, Montes GC¹, Alves-Leon SV^{2,4#}, Fontes-Dantas FL¹ ¹UERJ, NeuroPharmacoGenetics Lab, Dept. of Pharmacology and Psychobiology, Brazil; ²UNIRIO, Biomedical Institute, Translational Neuroscience Lab (LabNet), Brazil; ³UFRJ, PPG Clinical Medicine, Brazil; ⁴UFRJ, Dept. of Neurology, Hospital Universitário Clementino Fraga Filho, Brazil; ⁵UERJ, Pedro Ernesto University Hospital, Neuroimmunology Outpatient Clinic, Brazil

Therapeutic failure of monoclonal antibodies has been linked to genetic variants in genes encoding Fc gamma receptors (FcγRs). Natalizumab (NTZ), a humanized IgG4 monoclonal antibody, is used in relapsing-remitting multiple sclerosis (RRMS) to inhibit leukocyte migration into the CNS via α4-integrin blockade. Despite its high efficacy, some patients do not respond adequately to NTZ. This study investigated the association between *FCGR2A* (rs1801274) and *FCGR3A* (rs396991) polymorphisms and NTZ response in 116 Brazilian RRMS patients. Therapeutic response was determined based on modified Rio criteria, relapse rate, and MRI activity. Genotyping was performed via TaqMan-PCR, and cytokine levels (IL-6, IL-4, IL-18, IFN-γ) were quantified by Luminex. Thirteen patients were non-responders. The *FCGR2A* GG genotype was significantly enriched in non-responders ($p = 0.0010$), as was the *FCGR3A* AA genotype ($p < 0.0001$). ROC analysis yielded AUCs of 0.67 for *FCGR2A* and 0.55 for *FCGR3A*, suggesting moderate and weak predictive value, respectively. Interestingly, carriers of GG (*FCGR2A*) and AA (*FCGR3A*) genotypes had reduced plasma IL-18 and IFN-γ levels, potentially reflecting peripheral immune retention or altered effector function. Literature suggests these variants alter IgG binding and FcγR signaling, affecting immune complex clearance and inflammation. In endothelial cells, *FCGR2A* G alleles are linked to increased CRP binding and adhesion molecule expression, possibly impairing blood-brain barrier integrity. These findings highlight the complex immunogenetic landscape of NTZ response and underscore the potential utility of FCGR polymorphisms as biomarkers for personalized MS therapy. Further studies in larger, multiethnic cohorts are needed to validate these findings and integrate them into clinical decision-making. This work received financial support from Fundação Carlos Chagas Filho de Amparo à Pesquisa do Estado do Rio de Janeiro (FAPERJ).

11.007 **Exposure to Apresa Herbicide leads to Oxidative Stress in brain of Male and Female Wistar Rats.** Aguiar IS¹, Ferreira PYO¹, Santos EEO¹, Souza VKB¹, Campos HM¹, Ghedini PC¹, Reis DS², Rodrigues BM², Santiago ALG², Almeida LM², Amaral VCS², Mazzaro R¹, Damin V³, Leite JA¹ ¹ICB-UFG, Goiânia, Brazil; ²UEG, Anápolis, Brazil; ³UFG, School of Agronomy, Goiânia, Brazil

Introduction: Apresa® is a herbicide widely used for weed control in various agricultural crops. Its formulation combines two active compounds: flumioxazin and S-metolachlor. Toxicological studies have not identified significant adverse effects from flumioxazin exposure, and it shows low neurotoxic potential. In contrast, S-metolachlor has been associated with more concerning toxic effects, including hepatic, reproductive, and endocrine alterations in animal studies. These findings raise concerns about its continued use and the potential risks to human health and the environment. Pesticides can interfere with the nervous system and trigger processes such as oxidative stress, neuroinflammation, and neuronal death, which may contribute to the development of neurodegenerative diseases (NDs). **Aim:** To evaluate the effects of Apresa® treatment on oxidative stress parameters in the brain cortex, hippocampus, and striatum of male and female Wistar rats. **Methods:** Male and female Wistar rats (n = 6 per group) were divided into four groups: Control, Apresa® 250 mg/kg (low dose, LD), 500 mg/kg (intermediate dose, ID), and 1000 mg/kg (high dose, HD). The control group received corn oil, and the treated groups received Apresa® by oral gavage for 28 consecutive days. On day 29, the cortex, hippocampus, and striatum were collected to analyze lipid peroxidation (LPO), protein carbonylation (CP), and the activities of antioxidant enzymes: superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx). The study was approved by the Animal Ethics Committee (CEUA/UEG No. 003/23). **Results:** In both male and female cortex, Apresa® increased LPO and CP levels and decreased SOD, CAT, and GPx activities compared to the control group (p < 0.05). Similar effects were observed in the male striatum in the ID and HD groups. In the hippocampus, significant changes were found in the HD group, with increased oxidative damage and reduced antioxidant enzyme activities (p < 0.05). **Conclusion:** Apresa® treatment for 28 days induced oxidative stress in the cortex, hippocampus, and striatum of Wistar rats. This was evidenced by increased levels of LPO and CP and decreased activities of SOD, CAT, and GPx. These results suggest that Apresa® has neurotoxic potential. Further studies are necessary to better understand the mechanisms involved and the possible relationship with neurodegenerative processes. **Financial support:** FAPEG, CNPQ and CAPES.

11.008 Validation of a Liquid Chromatography Coupled with Mass Spectrometry (LC-MS/MS) Method to Quantify Artepillin C in Microdialysate Samples from Prostate Rats: Application to Preclinical Pharmacokinetic Study. Menin RH, Toson N, Olivo LB, Dias BB, Lemos JLS, Rodrigues VJ, Barnet L, Bastos JK, Araujo BV Menin RH¹, Toson N¹, Olivo LB¹, Dias BB¹, Lemos JLS¹, Rodrigues VJ¹, Barnet L¹, Bastos JK², Araujo BV¹ UFRGS, ²USP

Introduction: Artepillin C (ArtC), a phenolic compound from Brazilian green propolis, shows promising antitumor activity against prostate cancer. Despite its potential, pharmacokinetic data and tissue penetration studies are scarce. This study aimed to develop and validate an analytical method to evaluate ArtC penetration into the prostate of healthy male Wistar rats. **Methods:** The bioanalytical method was developed using high-performance liquid chromatography coupled with tandem mass spectrometry (HPLC-MS/MS) and validated according to both FDA and ANVISA guidelines. Male Wistar rats (n = 6, CEUA #42969), anesthetized with urethane (1.25 mg/kg, i.p.), received a single intravenous bolus dose of ArtC (15 mg/kg). Free concentrations of ArtC in plasma and prostate were collected over a 6-hour period using the microdialysis technique with a CMA®-20 2 mm probe. Quantification was performed using the validated HPLC-MS/MS method. The prostate penetration factor (fT) was determined using the following equation: $fT = (ASC_{0-\infty, free, prostate}) / (ASC_{0-\infty, free, plasma})$. **Results:** The method demonstrated adequate selectivity, linearity, accuracy, precision, carryover control and analyte stability. Calibration curves prepared in ringer's solution exhibited linearity over the concentration range of 5-200 ng/mL ($r^2 > 0.99$). The validated method was successfully applied to evaluate the penetration of Artepillin C into the prostate. The mean area under the curve was 4127.44 ng.h/mL for prostate and 6783.02 ng.h/mL for plasma and the fT obtained was 0.61. **Conclusion:** This study successfully validated an HPLC-MS/MS method to quantify ArtC in plasma and prostate microdialysate samples from male Wistar rats. For the first time, it was demonstrated that ArtC reaches prostate tissue with a penetration factor of approximately 0.61. **Acknowledgements:** Federal University of Rio Grande do Sul (UFRGS) and University of São Paulo (USP).

11.009 Agmatine Exerts Anti-Ferroptotic Effects in HEK-293 Cultured Cells. Cells Caversan S, Santer M, Ilha SH, Sperlich CL, Jacques MT, Farina M UFSC, Neuropathology Experimentation Lab, Dept Biochemistry

Iron (Fe) is an essential transition metal involved in several biochemical processes crucial for cell survival. However, excessive intracellular Fe accumulation can lead to toxicity primarily through ferroptosis, a distinct form of iron-dependent regulated cell death. This process is closely associated with the disruption of the cellular antioxidant defense system, which is regulated by key proteins, including the transcription factor NRF-2 (Nuclear Factor Erythroid 2–Related Factor 2). NRF-2 plays a critical role in reducing reactive oxygen species (ROS) levels and modulating the metabolism of glutathione (GSH), glutathione peroxidase 4 (GPx4), and iron homeostasis. Agmatine, an aminoguanidine derived from the decarboxylation of L-arginine, acts as a neurotransmitter and a precursor of polyamines and has been shown to positively modulate NRF-2 expression in mammalian cells. This study aimed to investigate whether agmatine exerts protective effects against ferroptosis in a cellular damage model induced by RSL3 in HEK-293 cells. The methodology was divided into three main phases: (i) assessment of toxicity (cell lethality) induced by RSL3 and agmatine; (ii) characterization of RSL3-induced ferroptosis; and (iii) evaluation of the potential protective effect of agmatine pretreatment via NRF-2 activation against RSL3-induced toxicity. HEK-293 cells were cultured in 96-well plates (4,000 cells/well) for cell viability assays and in 24-well plates (20,000 cells/well) for cell death and morphology analysis via fluorescence microscopy. Treatments included ML385 (a selective NRF-2 inhibitor; 0.1, 0.5, 1, 5, and 10 μ M), agmatine (0.5, 1, 2, and 3 mM), Ferrostatin-1 (a ferroptosis inhibitor; 10 μ M), and RSL3 (50, 100, 250, and 500 nM). Cell viability was assessed by MTT assay 48 hours after plating, while cell death and morphology were analyzed using Calcein-AM and Propidium Iodide (PI). Our data demonstrated that RSL3 induced a concentration-dependent decrease in viability and cell death, which was completely reversed by Ferrostatin-1, confirming the induction of ferroptosis in this model. Notably, pretreatment with agmatine (0.5 and 1 mM) for 6 h protected against RSL3-induced decrease of cell viability and mortality, in addition to preserving cell morphology, as assessed by Calcein-AM and PI assay. However, this protective effect was abolished in the presence of the NRF-2 inhibitor (ML385 (1 μ M)), indicating that the protective action of agmatine is NRF-2 dependent. In summary, these findings demonstrate that agmatine confers protection against ferroptosis-induced cell death in HEK-293 cells through NRF-2 activation, highlighting its potential as a therapeutic target for ferroptosis-associated conditions.

Keywords: Agmatine, Ferroptosis, NRF-2, HEK-293.

Financing: CAPES, CNPq and FAPESC

11.010 Comorbidities in People Living with HIV/Aids (PLWHA) on Antiretroviral Therapy (ARV) in a Brazilian Hospital. Lima-Franco I², Umanã-Rivas M¹, Velázquez D¹, Soares, LM¹, Noronha EF¹, Meiners MMA^{1,3}, Metzger IF^{1,2} ¹UnB, Graduate Program in Tropical Medicine, Center for Tropical Medicine, Faculty of Medicine, Brasília, Brazil, ²UnB, Dept Pharmacy, Faculty of Health Sciences, Brasília, Brazil ³UnB, Faculty of Health Sciences and Technologies, Brasília, Brazil

Introduction: The acquired immunodeficiency syndrome (Aids), caused by immunodeficiency virus (HIV), poses a significant global public health burden, affecting more than 39.9 million individuals living with HIV/Aids (PLWHA) worldwide. HIV infection induces progressive immunosuppression, thereby significantly elevating susceptibility to opportunistic infections. Advances in antiretroviral therapy (ART) have substantially increased the life expectancy of PLWHA, allowing the development of age-related comorbidities. Consequently, investigation into the prevalence of comorbidities is vital to enhance the overall quality of life and optimize healthcare management within this population. **Objective:** This study aims to characterize the sociodemographic, clinical profile and comorbidity burden among PLWHA receiving ART. **Methods:** This retrospective clinical study included PLWHA aged ≥ 18 years undergoing antiretroviral therapy at the University Hospital of University of Brasília (HUB-UnB) between January and December 2024. Exclusion criteria include pregnancy, participation in HIV Pre-exposure Prophylaxis programs, and incomplete medical records. Sociodemographic and clinical data were extracted from electronic medical records following signature of the Informed Consent Form. This study is part of a project approved by the Research Ethics Committee, with registration number CAEE 74241623.4.0000.5558. **Results:** A total of 151 research participants were enrolled in the study. This population was predominantly male (n=124, 82.1%) and single (n= 90, 60%). Participant age ranged from 20 to 74 years, with a mean age of 40.5 ± 12.2 years. Regarding self-declared race, the majority identified as mixed-race (pardos, n=85, 56.3%), followed by White (n=46, 30.5%) and Black (n=10, 6.6%). In terms of educational background, 41 (27.2%) had high school diploma, while 35 (23.2%) concluded university studies. Among the enrolled patients 88 (58.3%) exhibited at least one documented comorbidity and the five most prevalent comorbidities were cardiovascular diseases (17.9%), mental, behavioral, or neurodevelopmental disorders (16.6%), endocrine, nutritional, or metabolic diseases (12.6%), diseases of the digestive system (9.3%), and infectious or parasitic diseases (8.6%). **Conclusions:** The PLWHA receiving ART at HUB-UnB exhibited a predominance of male, mixed-race (*pardos*), had completed high school or higher education and most of the population have at least one comorbidity. Non-communicable diseases were the most prevalent comorbidities, despite the predisposition to opportunistic infections associated with HIV infection. This highlights the importance of clinical management of these patients, extending beyond the treatment of infectious diseases alone. **Keywords:** human immunodeficiency virus, acquired immunodeficiency syndrome, people living with HIV/AIDS, chronic diseases and comorbidity. **Funding:** Edital PIBIC/2024/2025 (CNPq/FAPDF/UnB) and EDITAL DPI/CDT/PCTEC Nº 01/2024

11.011 Impact of Non-Steroidal Anti-Inflammatory Drugs (Nsaids) During Gestation and Lactation on Hypothalamic Sexual Differentiation in Male Rats. Magalhães L¹, Castro RLM¹, Santos STC¹, Stein J¹, Nagaoka LT¹, Aquino JVF¹, Rosalem GF¹, Jorge BC¹, Arena AC^{1,2} ¹IBB-Unesp,, Brazil ²CIATOX-Unesp, Center for Toxicological Information and Assistance, Brazil

Nonsteroidal anti-inflammatory drugs (NSAIDs) are the most commonly used agents for treating pain, fever, and inflammation, including during pregnancy and lactation. NSAIDs mediate their effects primarily through the inhibition of cyclooxygenase enzymes (COX-1 and COX-2), resulting in decreased production of prostaglandin E2 (PGE2). In addition to their anti-inflammatory properties, NSAIDs have been identified as potential endocrine disruptors. Evidence highlights the critical role of PGE2 in the development of the nervous system, particularly in the process of hypothalamic sexual differentiation (HSD), as well as the detrimental effects of endocrine-disrupting compounds on this process. Given the fact that NSAIDs are capable of crossing the placental barrier and may negatively impact fetal development, their unregulated use during pregnancy and lactation raises significant concerns. For the present study, three medications were selected — acetaminophen (paracetamol), acetylsalicylic acid (ASA), and nimesulide — due to their broad therapeutic indications and distinct mechanisms of action. This selection allows for a comparative analysis of the effects, benefits, and risks associated with each compound. Therefore, the objective of this research is to assess the impact of perinatal NSAID exposure on the early development in male rat offspring. To this end, pregnant rats were assigned to four experimental groups and administered different oral treatments via gavage: 1. Control Group (1 mL/kg/day - water); 2. Paracetamol Group (125 mg/kg/day); 3. ASA Group (6 mg/kg/day) and 4. Nimesulide Group (11 mg/kg/day). The chosen doses were based on recommendation of use and converted to animal-equivalent doses according to the rats' body surface area. The treatment occurred from gestational day (GD) 15 until the end of the lactational period (lactational day – LD – 22), a critical period for HSD in males. The male offspring were evaluated for body weight and anogenital distance (AGD) on postnatal days (PND) 1, 13, and 22. On PND 22, the animals were euthanized for hematological analysis and assessment of organ weights. Additionally, the day of testicular descent and preputial separation was monitored as indicators of early sexual development. Preliminary data shows that paracetamol exposure reduced AGD on PNDs 13 and 22, anticipated the day of testicular descent and reduced body weight on this day, it also led to increased levels of erythrocytes and hematocrit on PND 22. ASA exposure led to increased seminal gland weight on PND 22. Nimesulide exposure caused higher body weight on PND 1 and increased AGD on PNDs 13 and 22. These alterations may indicate a possible androgenic alteration in the animals, further investigations will be conducted to elucidate the underlying mechanisms and confirm whether these effects are directly related to androgenic modulation derived from exposure to NSAIDs. **Financial support:** Fapesp (2025/02352-9). Ethics Committee: CEUA(7611070225).

11.012 *Crotalus durissus terrificus* Venom: Complexity of PLA2 Isoforms. Felipe AGC¹, Galizio NC¹, Carneiro IB¹, Sousa EP¹, Rodrigues MAF¹, Vidueiros J², Lima JAF³, Coelho GR⁴, Pimenta D³, Sant'anna S², Morais-Zani K^{1,2} ¹IB, Lab Fisiopatologia, ²IB, Lab Herpetologia, ³IB, Lab Ecologia e Evolução, ⁴IB, Lab Bioquímica, Instituto Butantan

Introduction: In Brazil, *Crotalus durissus* ssp. is a species of public health importance due to the severity of the envenomation and to its wide geographical distribution, being responsible for the most lethal snakebites events in this country. Due to the wide distribution of the species, *C. d. terrificus* populations may exhibit a considerable amount of geographic variation. In this context, considering the importance of analyzing individual samples in studies involving intraspecific venom variability, we proposed a comparative compositional characterization of individual *C. d. terrificus* venoms from four geographically distinct regions of three Brazilian States: São Paulo, Minas Gerais and Goiás. **Objectives:** This work proposes the evaluation of the geographic variability of PLA₂ from *C. d. terrificus* venom. **Methodology:** We analyzed 12 individual *C. d. terrificus* venom samples from different Brazilian regions. Venom protein profile was analyzed by 15% SDS-PAGE and HPLC using a C18 column. The immunorecognition of the venom samples by the anticrotalic antivenom produced by Butantan Institute was assessed by ELISA and Western blotting. **Results and Discussion:** The protein band composition evidenced by 15% SDS-PAGE and the pattern of chromatographic peaks obtained by HPLC of the individual venoms were consistent with *C. d. terrificus* protein profile described in previous works. HPLC analysis evidenced a geographic pattern regarding to PLA₂, in which it was evident that *C. d. terrificus* individuals from the state of São Paulo presented the highest number of peaks. Individual variability was also identified regarding the immunorecognition of the venoms by the anticrotalic antivenom assessed by ELISA, although the immunorecognition profile obtained by Western blotting was similar among samples where all the main protein bands, including those associated with PLA₂, were recognized by the crotalic antivenom in all individuals. The results highlighted the individual variability among the venoms of *C. d. terrificus* specimens. Importantly, our data point to a geographical variation of venom profile, as evidenced by PLA₂ isoforms complexity, which may explain the increasing in venom neurotoxicity from Northeastern through Southern Brazil reported for the species. This work is supported by FAPESP (2020/07268-2; 2021/07627-5) and CNPq (309995/2022-1).

11.013 Nanoencapsulation of Cinnamon Essential Oil in Zein: The Toxicity Evaluation in the Non-Target Organism Zebrafish (*Danio rerio*). Chamberlain ALV¹, Barros JMHF², Pimentel JRL¹, da Silva ACF¹, Stadinik MJ³, Müller LG⁴, da Costa C^{2,5}, Siebel AM^{1,6} ¹UFPR, Dept Pharmacology, Brazil, ²UFSC, Dept Chemical and Food Engineering, Brazil, ³UFSC, Dept Plant Sciences, Brazil, ⁴Institute of Experimental and Technological Biology (iBET), Portugal, ⁵UFSC, Dept Textile Engineering, Brazil, ⁶UFRGS, Dept Health Sciences, Brazil

Introduction: The prolonged use of synthetic fungicides has been linked to harmful effects, including biodiversity decline and the emergence of resistant pathogens. Interestingly, cinnamon essential oil (*Cinnamomum cassia*), widely recognized as a culinary spice, presents antifungal activity. However, its use is limited by its high volatility and poor solubility in water. To overcome these problems, a zein-based nanocarrier incorporating cinnamon essential oil (Z-CEO) was formulated as an alternative approach for controlling bean anthracnose, enhancing the stability, controlled release, and bioefficacy of essential oils. Therefore, evaluating the potential toxicity of this nanostructured biopesticide is crucial. Thus, the safety of Z-CEO was assessed through *in vivo* toxicity assays in adult zebrafish (*Danio rerio*). **Methods:** Z-CEO nanoparticles were produced using an ultrasound-assisted nanoprecipitation method and were characterized for hydrodynamic size, morphology, encapsulation efficiency, thermal stability, and durability. Toxicity testing followed the OECD 203 guideline, an internationally recognized standard for fish toxicity assessment. Adult wild-type zebrafish (6 months old, both sexes, n = 20 per group; total n = 120) were randomly distributed into the following groups: negative control (NC), empty zein nanoparticles (Z-NPs), and four Z-CEO concentrations (0.001, 0.01, 0.1, and 1 mg/L). Fish were exposed for 96 hours, with solutions renewed daily. After exposure, the novel tank test was conducted to assess freezing behavior. Subsequently, lipid peroxidation in the zebrafish brain was quantified by measuring thiobarbituric acid reactive substances (TBARS). Data normality was assessed using the Kruskal–Wallis test. Behavioral data were analyzed by one-way ANOVA with Dunnett’s post hoc test, and biochemical data by one-way ANOVA with Bonferroni’s post hoc test. **Results:** Z-CEO particles displayed a spherical shape, with a mean diameter of 208 nm and an encapsulation efficiency of 75%. Data from the novel tank test and TBARS assay showed no significant differences in freezing behavior or lipid peroxidation between zebrafish treated with Z-CEO and those in the negative control group. **Conclusion:** The results indicate that Z-CEO does not cause freezing behavior or oxidative stress in the brains of adult zebrafish. Nevertheless, additional studies are needed to evaluate potential impacts on non-target organisms, as well as the fate and degradation products of Z-CEO in aquatic ecosystems, given the possible environmental persistence of nanomaterials. This study was supported by CNPq (under grants 160666/2021-0, 402097/2023-8, 310989/2021-3; and through the Scientific Initiation and Technological Development and Innovation Program) and CAPES (final code 001). Fish maintenance and experimental protocols of this study were approved by the Ethics Committee for Animal Use (CEUA UFPR Protocol, #004/2024) and respected the National Council for Control of Animal Experimentation recommendations.

11.014 Effects of Non-Steroidal Anti-Inflammatory Drugs (NSAIDs) Exposure during Pregnancy and Lactation: Maternal Toxicological Assessment and Early Development of Female Wistar Rat Offspring. Castro RLM¹, Dos Santos STC¹, Magalhães L¹, Stein J^{1,2}, Nagaoka LT^{1,2}, Aquino JVF¹, Rosalem GF¹, Vitale OG¹, Jorge BC¹, Arena AC^{1,3} ¹IBB-Unesp-Botucatu, ²UNESP, PPG Biomolecular and Pharmacological Sciences, ³CIATOX-UNESP-Botucatu, Center for Toxicological Information and Assistance

The use of medications during pregnancy is common, although many substances lack specific safety and efficacy data for pregnant women. Among the various classes of medications, some of the most commonly administered during pregnancy are analgesics and non-steroidal anti-inflammatory drugs (NSAIDs). Due to the numerous physiological changes that occur during this period, perinatal exposure requires attention because of potential adverse effects on both the mother and the fetus. Previous studies have already demonstrated that NSAIDs can cross the placental barrier and act as potential endocrine disruptors, particularly during fetal development. Therefore, more information regarding their use by pregnant and lactating women is essential. This study aimed to investigate the toxicological effects of exposure to the NSAIDs paracetamol, acetylsalicylic acid (ASA), and nimesulide, during the gestational and lactational periods in Wistar rats, and their repercussions on the early development of female offspring. To this end, pregnant Wistar rats were divided into four experimental groups and exposed (via gavage) to water (control, 1 mL/kg), paracetamol (125 mg/kg), ASA (6 mg/kg), and nimesulide (11 mg/kg), from the gestational day (GD) 15 until the end of the lactational period (lactational day – LD – 22). The chosen doses were based on the therapeutic indications and converted to the animal dose concerning the rats' body surface area. After lactational day 22 (LD 22), the female progenitors were euthanized for hematological evaluation, as well as body and organ weight assessment. The female offspring were evaluated for body weight and anogenital distance (AGD) on postnatal days (PND) 1, 13, and 22. On PND 22, they were euthanized for hematological analysis and organ weight assessment. Regarding maternal parameters, hematological alterations were observed. Nimesulide caused a statistically significant reduction in red blood cells (RBC), as well as a tendency toward reduced hematocrit (HCT) ($p=0.05$), increased mean corpuscular hemoglobin (MCH) ($p=0.05$), and increased platelet count ($p=0.06$). Paracetamol significantly increased mean platelet volume (MPV), all compared to the control group. In female offspring, the evaluated parameters remained unchanged. The data suggest that, although no evident effects were observed in female offspring up to PND 22, maternal hematological toxicity indicates potential systemic compromise, with possible delayed repercussions that need to be further investigated.

11.015 **Precision Medicine in Pediatric Transplants: Developing a Population Pharmacokinetic Model for Busulfan.** Rodrigues VJ¹, Olivo LB¹, Wermann S¹, Dias BB¹, Pinhatti AV^{2,3}, Zuckerman J^{2,3}, Daudt LE⁴, Dalla-Costa T¹, Araújo BV^{1,2,1} UFRGS, Faculty of Pharmacy, Pharmacokinetics and PK/PD Modeling Lab, Brazil, ²UFRGS, PPG Medical Sciences, Porto Alegre, Brazil, ³HCPA-UFRGS, Pharmacy Service, Porto Alegre, Brazil, ⁴HCPA-UFRGS Hematology Service, Hospital de Clínicas de Porto Alegre -, Porto Alegre, Brazil

Introduction: Busulfan (BU), a cytotoxic drug, is used for conditioning regimens before hematopoietic stem cell transplantation (HSCT). Due to its high interindividual variability (IIV), especially in children, BU is susceptible to therapeutic drug monitoring. This work aimed to optimize dose individualization through a developed popPK model for BU in Brazilian pediatric patients.

Methods: The model was developed using NONMEM 7.4 (Icon®). Demographic and clinical exams were tested as covariates as different forms to explain interindividual variability (IIV). To confirm the predictive ability of the model, Mean error (ME) for population prediction, mean relative error (MRE) and relative root mean square error (RMSE) for individual prediction were calculated. The acceptance criteria are ME close to zero, MRE less than 20% and RMSE less or equal to 30%. Probability of target attainment (PTA) was estimated for the model-informed dose, regarding the area under the curve (AUC) range of 3,600 – 6,000 µM.min (14.7 – 24,7 mg/L/h). **Results:** A one-compartment-model with a first order elimination was built using data from 28 patients (20 – 111 months old) treated with BU. IIV was exponentially included in both PK parameters and were explained by adding body weight (BW) as a covariate in volume of distribution (Vd) and clearance (CL). The typical value of CL was 3.3 L/h with an IIV of 23.2% and Vd was 0.951 L with an IIV 15.7%. Data from 2 extra patients was used for model external validation, ME was -0.006 mg/L, MRE was 1.16% and the RMSE was 15.48%, both following the acceptance criteria. With the new dose predicted from the model 67.89% patients achieve the therapeutic window. Therefore, our model increased in 42.89% the probability of reaching the therapeutic range during conditioning regimen. **Conclusion:** A popPK model of BU was successfully built for Brazilian pediatric patients. The results showed that BW is a determining factor for this PK parameter. The model will be used for individualized BU therapy and ensure the successful conditioning of children undergoing HSCT. **Financial support:** This work was financed by the PPSUS/MS-FAPERGS, Rio Grande do Sul, Brazil [#17/2551-0001438-3 | 21/2551-0000065-1]

11.016 **Analysis of the Humoral Response Against SARS-CoV-2 of Immunocompromised Healthcare Professionals Participating in the SEVACOV-PRO Study.** Pereira, BM¹, Silva VEG¹, Silva DLM², Duarte, DB^{1,3} ¹UnB, Lab Pharmacological Assays, Dept Pharmacy, School of Health Sciences, University of Brasília, Brasília, Brazil, ²HUB, University of Brasília Hospital, Brasília, Brazil, ³UnB, Graduate Program in Tropical Medicine, School of Medicine, University of Brasília, Brasília, Brazil

Healthcare workers have been the most exposed group to the coronavirus disease-19 (COVID-19) since its beginning, and when those professionals are immunocompromised, they are considered a risk group. Thus, we investigated the immunological response before and after the COVID-19 vaccination in participants of the “Safety and Effectiveness of Vaccines Distributed by the National Vaccination Program, in Health Workers (SEVACOV-PRO)” study to evaluate whether immunosuppression negatively influences humoral response **Methods:** This is a national cohort study that included healthcare workers older than 18 years from 7 research centers in Brazil. The sociodemographic data and blood samples were obtained during visits in those centers, designated as V1 (first visit), V5, V6, V7 and V8 (12, 18, 24 and 30 months after first vaccine dose, respectively), and the extra visits (EVs) 1 and 2 (made after the first and second vaccine booster doses). The control group was randomly selected between participants without self-declared immunocompromise paired by sex, age and research center. The levels of antibody against Receptor Binding Domain to SARS-CoV-2 (anti-S RBD) were measured through the chemiluminescence microparticle immunoassay. Data was collected from September 2021 to December 2024. All the procedures are approved by the ethical committee (49561321.0.1001.5558). **Results:** The study sample had 101 immunocompromised participants with 204 paired controls. In our population, 52,4% are immunocompromised due to the use of immunosuppressant drugs for rheumatologic disease, followed by immunodeficiency (33,0%). We observed that 91,0% of the immunocompromised population was composed by the female gender, the average age was 47 years, 44,6% was self-declared white, 37,8% are specialists. The most related profession was nurse technician (38,8%), followed by nurses (25,2%) and physicians (7,7%). Clinically, the most common comorbidity was rheumatoid arthritis (29,1%), followed by 7,7% with Sjogren syndrome. Immunocompromised participants developed a humoral response that was not different from the controls across the study. From 101 immunocompromised participants, only one did not develop immunogenicity after vaccination. At the first visit, participants with or without immunosuppression with two booster doses had an increase of 110% and 148% in anti-S RBD levels when compared to those with only the primary regimen, respectively. Also, we demonstrated a positive relation between the doses and the levels of anti-S RBD antibody levels in all groups **Conclusion:** These results suggest that booster doses are effective in enhancing humoral responses even among immunocompromised participants, supporting current recommendations for additional COVID-19 vaccine doses in this population. Funding: Ministry of Health.

11.017 Dopaminergic Cell Model Comparison to Evaluate Harmine's Neuroprotective Potential Against Cocaine Toxicity. Terra LAG, Ribeiro JLF, Pereira ML, Dörr F, Marcourakis T University of Sao Paulo

Introduction: Chronic cocaine use is strongly associated with increased oxidative stress, mitochondrial dysfunction, and impaired neuroplasticity, leading to neuronal death and functional compromise of the central nervous system. Despite the clinical relevance and social impact of cocaine use disorder, no specific and effective pharmacological therapies are currently available. The antioxidant, anti-inflammatory, and pro-neurogenic properties of harmine, a β -carboline alkaloid found in ayahuasca, have been investigated in preclinical models. Considering the overlap between mechanisms of cocaine-induced neurotoxicity and the potential neuroprotective mechanisms of harmine, this β -carboline emerges as a promising candidate for innovative neuroprotective strategies. **Methods:** The human neuroblastoma cell line (SH-SY5Y), both undifferentiated and differentiated into dopaminergic neurons, was used. Differentiation was achieved through a sequential 5-day protocol with retinoic acid (10 μ M) followed by 5 days with brain-derived neurotrophic factor – BDNF (50 ng/ μ L). Culture characterization was confirmed by the expression of mature neuronal and dopaminergic markers (MAP2 and tyrosine hydroxylase) using immunofluorescence in confocal microscopy. To assess the toxicity profile, differentiated (d-SY5Y) and undifferentiated (und-SY5Y) cells were exposed to increasing concentrations of cocaine (1–10 mM) and harmine (1–2000 μ M), with determination of the lethal concentration 50% (LC50) and the no observed adverse effect level (NOAEL) by flow cytometry. **Results:** The cell differentiation protocol resulted in a homogeneous culture with evident and stable neuritic arborization, demonstrated by increased fluorescence intensity and the expression of dopaminergic neuronal markers. Cocaine exposure for 48 h significantly reduced the viability of d-SH-SY5Y cells starting at 4 mM, predominantly inducing necrotic cell death, with an LC50 between 4.8 and 6 mM. In und-SH-SY5Y cells, the reduction occurred from 2.5 mM, with an LC50 between 2.8 and 4 mM, with apoptosis as the main type of cell death. Regarding harmine, d-SH-SY5Y cells showed an LC50 of approximately 220 μ M (NOAEL: 50 μ M), while und-SH-SY5Y cells presented an LC50 of 180 μ M (NOAEL: 10 μ M). **Conclusion:** Our results suggest that cell differentiation alters the susceptibility to cocaine and harmine toxicity, indicating a maturation-dependent response. The greater resistance of d-SH-SY5Y cells may be associated with the activation of the BDNF/TrkB trophic pathway, promoting cell survival, as well as with metabolic and organellar maturation, contributing to an enhanced antioxidant capacity. These findings highlight differentiated cells as a valuable dopaminergic neuron model for investigating cocaine-induced neurotoxicity. Future studies will investigate key proteins and enzymes through Western blot assays, focusing on signaling pathways related to neurogenesis and cell survival, synaptic proteins and neuronal maturation markers, as well as enzymes involved in the kynurenine pathway. This work was supported by the São Paulo Research Foundation (FAPESP), process number 2023/09664-0 and number 2024/21822-3.

11.018 Exposure to Nitrosodimethylamine (NDMA): Toxicological and Reproductive Effects in Male Wistar Rats. Nagaoka LT¹, Stein J¹, Vitale OG¹, Marraschi GK¹, Aquino JVF¹, Castro RLM¹, Rosalem, GF¹, Santos STC¹, Magalhães L¹, Alves, JT¹, Jorge BC¹, Arena AC^{1,2} ¹Unesp, Dept Structural and Functional Biology, Embryology Sector, Lab Toxicology of Natural and Synthetic Products, PPG Biomolecular and Pharmacological Sciences, Brazil; ²CIATOX-Unesp, Toxicological Information and Assistance Center, Brazil

N-nitrosamines are environmental contaminants that arise as unintended byproducts of industrial production. Significant levels of these compounds have been identified in everyday products, drinking water sources, widely used drugs and others. Among these, Nitrosodimethylamine (NDMA) is one of the most abundant in the environment. Although the carcinogenic potential of N-nitrosamines is well established, few studies have investigated their adverse effects on reproduction, especially at low doses. Thus, this study evaluates the consequences of low-dose exposure to NDMA on toxicological and reproductive parameters of male Wistar rats. The animals (n=20/group) were daily exposed to distilled water (1 mL/kg) or 7.2 ng/kg of NDMA orally (gavage) during the preconceptional period, postnatal day (PND) 60 to 90, and mating period, PND 90 to 104, and subsequently euthanized to collect organs and blood. There was an increase in the absolute (p = 0.02) and relative weight (p = 0.01) of the prostate in the NDMA exposed group, as well as a significant increase in the platelet count (p = 0.04) in the hematological analysis and increase in the number of monocytes (p = 0.04) in the differential analysis of leukocytes. Regarding sperm parameters, there was no change in sperm morphology, however, the sperm count analysis showed significant differences both in the testes and epididymis. In the testis, there was a reduction of absolute (p = 0.0081) and relative (p = 0.0052) numbers of mature spermatids, as well as a reduction in the absolute (p = 0.0081) and relative (p = 0.0052) daily sperm production (DSP). In the epididymis, there was a delay in total transit time (p = 0.001) and sperm transit in the caput-corpus (p = 0.0013) and cauda (p = 0.0475) sections. Furthermore, motility parameters (%) showed reduced total motile sperm (p = 0.04), increased progressive motility (p = 0.04) and more slow-moving (p = 0.02) and static sperm (p = 0.04). Sperm kinetics also changed, with lower amplitude of lateral head displacement (ALH) (p = 0.008) and wobble (WOB) (p = 0.011). In the analysis of oxidative stress, the presence of the antioxidant enzymes glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) did not result in significant differences between the groups, however the NDMA group showed a significant reduction (p = 0.02) of malonaldehyde (MDA), a compound derived from the peroxidation of polyunsaturated fatty acids. Finally, in the histological analyses of the testes, the staging of the seminiferous tubules resulted in a significant increase (p = 0.02) in the number of seminiferous tubules in stages IX-XII, as well as a reduction in the number of tubules in stage XIV (p = 0.04) in the males exposed to NDMA. With the results obtained so far, we can hypothesize that, even at low doses, NDMA is capable of causing injury and negatively affect the reproductive capacity of male rats chronically exposed to this substance.

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11.019 Context-Dependent Modulation of Neuroinflammation and CD4+ T Cell Activation by Heated Tobacco and Cigarette Smoke. Scharf P¹, Sandri S¹, Borges P¹, Xavier LF¹, Silva T², Faria L², Olival GS³, Peron JPS², Farsky SHP¹ ¹USP, PPG in Pharmacy (Physiopathology and Toxicology), São Paulo, Brazil; ²USP, PPG in Immunology, São Paulo, Brazil; ³Hospital Santa Casa de São Paulo, Dept. of Neurology, São Paulo, Brazil

Multiple sclerosis (MS) is an autoimmune disorder of the central nervous system (CNS) that predominantly affects young adults. MS causes a wide spectrum of motor and cognitive impairments and is driven by overactivated CD4+ T cells and influenced by genetic and environmental factors. Cigarette smoke (CS) is a known risk factor for autoimmune diseases due to its combustion byproducts, including numerous immunotoxic compounds. Heated tobacco products (HTPs), which lack combustion and release fewer chemicals than CS, but similar nicotine levels, are increasingly used by young adults and often perceived as safer alternatives, once HTP is claimed as a harm-reduction tool for current smokers. However, this perception is based on limited data, and little is known about their long-term immunological effects, particularly in individuals with an autoimmune predisposition. Here, we investigated the impact of HTP exposure in the course of neuroinflammation and the biology of T CD4+ cells. C57Bl/6 mice (CEUA 5044220322) under experimental autoimmune encephalomyelitis (EAE) were exposed twice daily to air, HTP, or CS in a controlled puff-based system delivering nicotine concentrations similar to those found in heavy smokers. Two exposure protocols were used: 7 days before EAE induction (pre-exposure) or from day 7 to 13 post-immunization. On day 18, spinal cords and draining lymph nodes (DLNs) were collected for immunophenotyping and histological analyses. Sorted CD4+ cells from healthy donors and MS patients were exposed *in vitro* for 30 minutes to assess early and late activation markers, polarization, and proliferation by FACS (CAAE 44446221.5.1001.0067). HTP and CS pre-exposure worsened EAE clinical and increased frequencies of peripheral and CNS infiltrating Th1/Th17 cells, but only HTP enhanced T cell proliferation. Spinal cord analysis revealed that HTP, but not CS, increased microglial activation, suggesting a distinct mechanism of CNS damage. In the post-immunization group, only HTP significantly exacerbated disease severity. Moreover, HTP enhanced CD4+ T cell proliferation and the frequency of pathogenic Th17 cells. On the other hand, besides similar frequencies of Th1 and Th17, CS selectively promoted the activation of the immunoregulatory phenotype Th22 and aryl hydrocarbon receptor activation in both peripheral and CNS compartments. *In vitro*, HTP induced a hyporesponsive phenotype in CD4+ cells from healthy donors, marked by higher CTLA-4 expression, reduced early/late activation markers (CD69 and CD25), and proliferation but paradoxically increased cell activation and proliferation in MS-derived T cells, reinforcing the context-dependent effects of HTPs. CS enhanced activation in both groups but did not alter proliferation rates. These findings demonstrate that HTPs exacerbate neuroinflammation and differentially modulate immune responses according to the host's immune status, resulting in distinct, non-redundant immunotoxic effects. Our data challenge the assumption that non-combustible tobacco products are safe harm-reduction alternatives, particularly for individuals predisposed to immune-mediated diseases. Financial Support by FAPESP.

11.020 From Plasma to Effect: Integrating Interstitial Pharmacokinetics and MRSA Kill Dynamics with PBPK/PD Modeling. Olivo LB¹, Lemos JLS¹, Rodrigues VJ¹, Dias BB¹, Kretschmer DB², Cruz WA², Staudt KJ², Annaert P³, Araújo BV¹ ¹UFRGS, PPG Pharmaceutical Sciences, Brazil, ²URI-Santo Angelo, Brazil, ³KULeuven, Pharmaceutical and Pharmacological Sciences, Belgium

Introduction: Vancomycin (VAN) is a key antibiotic for treating MRSA infections, especially in patients with sepsis. But even today, dosing and monitoring are mostly based on free plasma concentrations. While these concentrations are commonly used as therapeutic targets, they may not accurately reflect drug levels at the site of infection, the interstitial space of tissues. In sepsis, physiological alterations such as increased vascular permeability and expanded interstitial volume can significantly affect drug distribution and tissue penetration. These changes can make it harder for VAN to reach its target but can be predicted by physiological mathematical models. This study aimed to build a physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) model to predict how VAN reaches the interstitial space of key tissues in sepsis to understand whether those concentrations are enough to treat MRSA infection. **Methods:** All data was extracted from literature using WebPlotDigitalizer® software. We developed the PBPK model using clinical data from healthy volunteers (validated with average fold error (AFE) criteria between 0.5-2), then adapted it for sepsis by including changes like altered protein binding, reduced clearance, and impaired endothelial function. The model was validated using real-world data from septic patients, including both plasma and interstitial fluid measurements. We also ran time-kill experiments using a clinical MRSA strain with a MIC of 2 µg/mL. To describe the effect, we built a PD model that accounts bacterial death and adaptive resistance (AR). We simulated VAN behavior in four key tissues affected by sepsis: kidney, liver, lung, and subcutaneous tissue, using both intermittent and continuous infusion regimens. **Results:** The PBPK model was validated with AFE values of 1.15, for plasma in healthy volunteers and, 1.17 in plasma and 1.15 in ISF of septic patients. The PD model was validated through diagnosis prediction plots and resampling of parameters. A modified Emax model was used to describe VAN effect with an EC50 of 1.05 µg/mL in the absence of AR. One important finding in simulations was the clear mismatch between plasma and ISF free tissue concentrations. In septic patients, tissue levels were lower and with less fluctuation, compared to healthy individuals. This means that even when plasma levels looked fine, the tissue concentrations were insufficient to reach effect. For MRSA with an MIC of 2 µg/mL, all tested regimens worked well. But when the MIC was 4 µg/mL, only continuous infusion showed partial improvement and even then, kidney and liver did not reach bactericidal concentrations. For MIC = 8 µg/mL, none of the regimens were effective in any compartment, including plasma. **Conclusion:** Tissue exposure can be lower, flatter, and not enough to kill the bacteria, even when plasma targets are met. By linking tissue-level drug exposure to bacterial kill curves, this PBPK/PD model gives us a clearer and more realistic view of antibiotic effectiveness. **Financial support:** CNPq Project number #423260/2021-9.

11.021 Parental and Postnatal NDMA Exposure Alters Reproductive and Toxicological Profiles in F1

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Environmental and lifestyle habits are among the factors that contribute significantly to infertility worldwide. The Developmental Origins of Health and Disease (DOHaD) and Paternal Origins of Health and Disease (POHaD) hypotheses explore how parental health influences offspring development. Nitrosodimethylamine (NDMA) is a harmful environmental contaminant found in water, food, and some drugs, with mutagenic, genotoxic, and carcinogenic effects, and it promotes the formation of reactive oxygen species. Data on reproductive and developmental studies is limited, especially at low doses. We aimed to assess the effects of parental (M-, P-, and MP-) and postnatal NDMA exposure on toxicological and reproductive outcomes in male and female F1 rats. The animals were exposed to control (distilled water, 1mL/kg/day) or to 7.2 ng/kg/day of NDMA. The F0 were divided into 4 groups: C- (control males X control females); M- (NDMA females X control males); P- (control females X NDMA males); and MP- (NDMA females X NDMA males), exposed from postnatal day (PND) 60 until mating, with continued exposure in females during gestation and lactation. Offspring were exposure from PND 22 until the end of sexual maturation, with 1 pup/sex/litter exposed to the vehicle (groups -) and the other to NDMA (groups +). The F1 offspring were evaluated as reproductive and toxicological parameters in adult life. In male offspring, there were a reduction in the first mount and first intromission latency in the C+ and M+ groups. No significant alterations were found in organ weight, hematological parameters, fertility test, sperm morphology/motility and Leydig cell count. In biochemistry analysis, elevated serum creatinine in the P- group, elevated urea in P-, C+, M+, P+ and MP+ groups and reduced total proteins in the MP- and M+ groups were founded. In the staging of seminiferous tubules, there was a decrease in the percentage of stages I-VI in the MP-, C+ and MP+ groups, and an increase in stages VII-VIII in the C+ group. Glutathione (GSH) and superoxide dismutase (SOD) were reduced in the testis from MP+, while the Malondialdehyde (MDA) showed high levels in the MP- group. In female offspring, no alterations were found in estrous cycle, sexual behavior, organ weight, hematological parameters or follicle count. However, we observed a decrease of alkaline phosphatase in the P- and P+ groups and increase in gamma glutamyl transferase in the M+ group. Furthermore, in the fertility test, females in the MP- group had a reduction in the weight of the uterus + fetuses, in the number of implantations and live fetuses. SOD was reduced in the M-, P-, MP-, C+ and M+ groups, while catalase (CAT) and GSH were reduced in the P+ group. Overall, NDMA exposure - both parental and postnatal - adversely affected adult offspring. **Financial support:** FAPESP (2022/15849-0; 2022/15364-7); CAPES (88887.809658/2023-00) Ethics Committee: 470426042

11.022 Using Microdialysis Free Concentrations to Model Tissue-Level Targets for Therapeutic Success in Glycemic Control of Type 2 Diabetes. Dias BB, Olivo LB, Braga A, Rodrigues VJ, Araujo BV UFRGS, Faculty of Pharmacy, PK/PD Modeling Lab, Pharmaceutical Sciences Graduate Program, Brazil

Introduction: Therapeutic effects of drugs are driven by the free drug concentration at the site of action, not plasma levels¹. Metformin (MET), the first-line treatment for type 2 diabetes mellitus (T2DM), lowers blood glucose by inhibiting hepatic gluconeogenesis and enhancing glucose uptake in muscle and gastrointestinal tract². This study evaluates the pharmacodynamic (PD) effect of MET on glucose reduction using a population PK/PD modeling approach focused on tissue-level exposure. **Methods:** Free MET concentrations in the main key tissues for effect, liver and muscle, were measured via microdialysis in healthy and streptozotocin-induced diabetic rats (n=5/group) after a 50 mg/kg oral dose (Ethics Committee in Animal Use UFRGS #25780) and quantified using HPLC/MS-MS. Human plasma MET concentrations and glucose responses during oral glucose tolerance test were digitized from published studies^{3,4} using WebplotDigitizer. Data were analyzed using NONMEM® with a sequential population approach and using allometric scaling to predict tissue exposure in T2DM patients. MET distribution was modeled using a multi-compartmental model, with central, liver, and muscle compartments, with intercompartmental clearances Q_{in} and Q_{out} . PD effect of glucose reduction was modeled using a sigmoidal indirect response, driven by free tissue MET concentrations. Simulations were conducted to evaluate glucose reduction following typical therapeutic regimens (500 mg or 850 mg every 8 or 12 hours), and responses were stratified by baseline glucose levels and estimated HbA1c. **Results:** In T2DM patients, MET PK parameters were: clearance 39.52 L/h, central volume 26.12 L, absorption rate 0.51 h⁻¹, bioavailability 55%, Q_{in} 79.38 L/h, Q_{out} 46.28 L/h, muscle volume 13.50 L, liver volume 113.08 L. The PD model showed higher sensitivity in muscle (E_{max} 0.137 h⁻¹, EC_{50} 0.201 µg/mL) than liver (E_{max} 1.27 h⁻¹, EC_{50} 20.7 µg/mL). Simulations indicated target glucose (≤ 180 mg/dL) was reached with at least 500 mg every 8 or 12 h for patients with HbA1c $\leq 10\%$, 850 mg every 12 h for HbA1c of 11%, and 850 mg every 8 h for HbA1c of 12%. **Conclusion:** This PK/PD modeling study demonstrates that the glucose-lowering effect of metformin is closely linked to its free concentrations in liver and muscle tissue, and muscle is more sensitive to metformin than liver, requiring lower concentrations to achieve significant glucose reduction. Simulations indicated that metformin monotherapy is effective in reaching glycemic targets (≤ 180 mg/dL) primarily in patients with mild to moderate hyperglycemia (HbA1c $\leq 12\%$), with dose requirements increasing alongside baseline HbA1c levels. For patients with more severe T2DM, combination therapy may be necessary. These findings emphasize the importance of tissue-specific drug exposure in guiding individualized therapy and aligning with current clinical recommendations for T2DM management. **Financial support:** CAPES. **References:** 1 - Gabrielsson & Green, J Pharmacol Exp Ther, 331(3):767-74, 2009. 2 - Davies et al. Diabetes Care. 45(11):2753–86, 2022. 3 - Sambol et al. J Clin Pharmacol. 36(11):1012-21, 1996. 4 - Lee & Kwon. Arch Pharm Res. 27(7):806-10, 2004.

11.023 Physiologically Based Pharmacokinetic (PBPK) Modeling of Empagliflozin in Pediatric Populations with Healthy and Obese Weights. Milhm, GP¹, Moreira, FL², Abraham-Vieira, BA¹ UFRJ, Lab Molecular Modeling & QSAR (ModMolQSAR), PPG Pharmaceutical Sciences, Brazil. ²UFRJ, Pharmacometrics Lab (LabFarma), Brazil

Introduction: Type 2 diabetes mellitus (T2DM) is multifactorial, and treating pediatric patients is challenging due to ethical and practical constraints on clinical trials in this population. Empagliflozin, an antidiabetic of the sodium–glucose cotransporter 2 (SGLT2) inhibitor class, reversibly inhibits glucose reabsorption in renal proximal tubules. Beyond glycemic control, it offers cardio- and nephroprotective effects, making it a strong candidate for pediatric T2DM. However, the optimal pediatric dose remains undefined. Physiologically based pharmacokinetic (PBPK) modeling, widely accepted by regulatory agencies, represents a promising strategy for prediction of safe, effective doses. This mechanistic tool partitions the body into physiological compartments linked by blood flow, enabling prediction of drug pharmacokinetics from physiological, physicochemical, and pharmacological parameters. This study aimed to build a PBPK model to simulate oral empagliflozin in children and adolescents with healthy weight and with obesity.

Methods: Physicochemical and pharmacokinetic data for empagliflozin were compiled alongside physiological information from selected studies for model building and validation using PK-Sim® v.12. First, an adult model was developed and validated by comparing simulated outputs with observed clinical data. The model used single- and multiple-dose studies with a dose range of 0.5–400 mg. Validation criteria required predicted/observed ratios between 0.5 and 2 for C_{max}, AUC, and T_{max}. Model calibration employed statistical tools, including sensitivity and parameter analyses, to qualitatively and quantitatively assess the influence of variables. Once validated, the model was scaled to pediatric populations with healthy body weight (EMPA-2) and with obesity (EMPA-3) by adjusting age-dependent physiological characteristics and verifying additional parameters available in the PK-Sim database. **Results:** During validation, intestinal permeability was refined, which yielded a more accurate pharmacokinetic profile as shown by graphical comparisons and observed/predicted ratios. Subsequent simulations used virtual populations physiologically aligned with the cohorts in the selected studies. The adult model demonstrated good predictive accuracy, with most predicted/observed ratios within the 0.5–2.0 acceptance range. Ratios were 0.70–1.31 for AUC, 0.66–1.72 for C_{max}, and 0.46–1.73 for T_{max}; the proximity of most values to 1 indicates high fidelity in reproducing observed data. After scaling to pediatric populations, the simulations indicated relevant changes in C_{max} and AUC, suggesting the need for dose adjustments, particularly in children aged 10–14 years, to ensure therapeutic efficacy and reduce potential adverse effects. **Conclusion:** A PBPK model of oral empagliflozin built in PK-Sim® v.12 was successfully developed, validated in adults, and scaled to pediatric populations with healthy weight and with obesity. The model supports rational pediatric dose selection—especially for ages 10–14—and can guide future pediatric clinical studies. These findings reinforce PBPK modeling as valuable allies in developing safer, more effective therapies for children and adolescents with T2DM. **Financial Support Information:** CAPES

11.024 Meropenem Dosing Regimens in Critically Ill Pediatric Patients Undergoing Continuous Renal Replacement Therapy. Vieira JW¹, Matsuno VK¹, Delgado AF², Benzi JRL¹ ¹Dept Pharmacy, School of Pharmaceutical Sciences, University of São Paulo, São Paulo, SP, Brazil. ²Pediatric Critical Care Unit, Dept Pediatrics, Faculty of Medicine, University of São Paulo, São Paulo, Brazil

Continuous renal replacement therapy (CRRT) is the preferred approach for critically ill pediatric patients with hemodynamic instability and acute kidney injury. Meropenem is frequently used in this population, but current pediatric dosing regimens recommendations and CRRT adjustments are based on limited studies and often extrapolated from adult data, which may not achieve optimal pharmacokinetic/pharmacodynamic (PK/PD) targets. Additionally, there is still a lack of outcome evaluation in pediatric population in CRRT and meropenem use. This retrospective observational cohort study aimed to evaluate the impact of meropenem dosing regimens on clinical outcomes in pediatric patients receiving CRRT. Fifty-six patients admitted to the pediatric intensive care unit at a

tertiary hospital between June 2018 and May 2021 were included. Patients were divided into two groups: those receiving standard CRRT meropenem regimen, 40mg/kg/dose every 12 hours (n=34) and optimized meropenem regimen, 40mg/kg/dose every 8 hours (n=22). The 12-hour group showed significantly higher mortality (76.5% vs. 31.8%, $p=0.001$), higher vasopressor use, and greater prevalence of hepatobiliary diseases. Statistically significant differences in body temperature and leukocyte count were also observed during the evaluation period. Propensity scores matching (PSM) by nearest neighbor analysis were conducted to evaluate covariates groups unbalance. After PSM, 12 patients were included in each dose regimen group. Logistic regression indicated a significant association between meropenem dosing every 8 hours and lower mortality (odds ratio and [95% confidence interval] of 0.14 [0.02-0.96], $p=0.045$). These findings underscore the potential inadequacy of standard CRRT meropenem dosing and the need for individualized antimicrobial therapy in critically ill pediatric patients on CRRT. Although it was not possible to measure meropenem serum concentrations to establish a PK/PD target correlation, mortality was significantly lower in the optimized meropenem regimen group (40mg/kg/dose every 8 hours). Further research is needed to optimize meropenem dosing in this population and prevent therapeutic failure due to underdosing. The study was approved by the Research Ethics Committee (CAAE No. 50570421.6.0000.0068) and received no financial support.

11.025 **Pharmacokinetic Evaluation and Marker Residue Determination of Topical Fipronil in Cattle.** Ferreira SM¹, Gotardo AT¹, Raspantini LER¹, Barreto F² ¹CEPTOX-FMVZ-USP Research Centre for Veterinary Toxicology, Dept Pathology, Pirassununga, Brazil, ²LFDA-RS, Federal Agricultural Defense Lab, Ministry of Agriculture and Livestock, Porto Alegre, Brazil

Introduction: One of the most widely used pesticides in Brazilian livestock farming is fipronil. Fipronil-sulfone is the main biological metabolite, while fipronil-desulfinyl is the main environmental metabolite, resulting from oxidation and photolysis, respectively. According to the VICH GL 46 guidelines, metabolism studies in food-producing animals should be conducted using the route of administration corresponding to the pharmaceutical form proposed for the product. However, the selection of the marker residue for tissue depletion studies was based on investigations conducted in rodents exposed to fipronil via the oral route. It is noteworthy that fipronil-based products currently registered with the Ministry of Agriculture and Livestock (MAPA) for use in cattle are formulated for topical administration, highlighting a discrepancy between the route of exposure used in the metabolism studies and the route intended for clinical use in the target species. Thus, in Brazil, ANVISA's Normative Instruction N^o. 162/2022 establishes the acceptable daily intake (ADI), the maximum residue limit (MRL), and the marker residue (fipronil + fipronil-sulfone) for bovine matrices. Given that fipronil photodegrades into fipronil-desulfinyl, is considered more toxic and lipophilic, important questions arise: following topical administration, could a portion of the fipronil undergo photodegradation? Furthermore, could this active metabolite be systemically absorbed and considered a potential marker residue? **Objective:** To generate knowledge on the pharmacokinetics and metabolite profile of topically administered fipronil in beef cattle. **Materials and Methods:** Seventeen Nelore cattle raised under extensive conditions were treated with Fipronil Topline® 1% (Boehringer Ingelheim) according to the product label. Blood samples were collected at various time points for plasma separation, followed by HPLC. **Results:** The following pharmacokinetic parameters were obtained: for fipronil, C_{max} of 42.27 ng/mL, T_{max} of 1.59 days, AUC of 333.12 ng/mL, and T_{1/2} of 4.51 days; for fipronil-sulfona, 60.23 ng/mL, 12.76 days, 2070.83 ng/mL, and 20.82 days, respectively; and for fipronil-desulfinyl, 0.17 ng/mL, 3.29 days, 2.07 ng/mL, and 0.06 days, respectively. **Conclusion:** The cattle showed lower systemic exposure to fipronil and a shorter half-life compared to subcutaneous administration (Cid, Thesis – UFRRJ/RJ, 2012). Fipronil-desulfinyl was detected in the plasma of cattle, indicating that photodegradation of fipronil on the skin surface may occur following topical application, with subsequent systemic absorption of this active metabolite. Further studies will be conducted to investigate the distribution and persistence of fipronil-desulfinyl in edible bovine tissues, aiming to assess the need for its inclusion as a marker residue in tissue depletion studies. Detection Identification of this metabolite in tissues could justify its consideration for the establishing of a MRL and the determining of appropriate withdrawal periods. **Funding agency:** CAPES. **CEUA Number:** 7756291123.

11.026 Effects of Low-Dose DEHP Exposure on Brain Function and Behavior: Gut Microbiota as a Potential Modulator of Its Toxicity. Silva LN¹, Carvalhinho-Lopes PS², Santiago MSA¹, Monteiro LA², Ribeiro AM², Perobelli JE¹ ¹UNIFESP, Lab Experimental Toxicology, Brazil, ²UNIFESP, Lab Neuroscience and Bioprospecting of Natural Products, Brazil

Introduction: Di(2-ethylhexyl) phthalate (DEHP) is a widely used plasticizer and a relevant environmental pollutant, commonly detected in soil, air, water, and food. Once inside the body, DEHP acts as an endocrine disruptor. The gut microbiota (GM) - a community of microorganisms inhabiting the gastrointestinal tract - plays a crucial role in modulating immune and endocrine functions and can influence the metabolism of xenobiotics, potentially altering DEHP toxicity. This study investigates the effects of sub-chronic, low-dose DEHP exposure on brain function and behavior in adult male rats, under normal or mildly altered GM conditions induced by the cesarean (C-section) birth pathway (BP). **Methods:** Male Wistar rats born via vaginal delivery (VD), vaginal delivery followed by cross-fostering (VDCF), or C-section followed by cross-fostering (CSCF) were assigned to either control or DEHP-exposed subgroups. DEHP-exposed groups received 48 mg/kg/day of DEHP in olive oil via oral gavage from postnatal day (PND) 25 to 90, while control groups received vehicle only. On PND 83, animals underwent behavioral tests to assess motricity/anxiety-like behavior and memory/learning, using the open-field test and object recognition test, respectively (n=10/group). On PND 90, animals were euthanized for brain biometric analyses (n=5/group), including weighing specific brain areas. Additionally, tyrosine hydroxylase (TH) immunostaining was performed to evaluate the density of dopaminergic neurons in selected brain regions (n=2-4/group). All procedures were approved by CEUA-UNIFESP (#2428081021). The study was supported by FAPESP (#2024/03315-7). Statistical analyses were conducted to compare: (i) only control groups, to assess the effects of BP and/or CF (One-way ANOVA); (ii) only DEHP-exposed groups, to evaluate whether BP and/or CF influenced responses to DEHP (One-way ANOVA); and (iii) control versus respective exposed groups, to assess DEHP toxicity (Independent T test). The significance level (α) adopted was 5% (statistically significant results when $p \leq 0.05$). **Results:** In the first analysis, CF resulted in reduced total distance traveled and average speed in the open-field test. BP and/or CF increased TH expression in the substantia nigra and olfactory tubercle, along with striatum weight. In the second analysis, restricted to DEHP-exposed groups, results indicated that BP and/or CF interfered with DEHP-induced changes in TH expression in the striatum. In the third analysis, DEHP exposure per se reduced both the time spent in the central zone of the open field and overall body weight, while increasing the relative weights of the hypothalamus and hippocampus. **Conclusion:** The findings indicate that CF was associated with mild locomotor impairment. Moreover, TH immunostaining was increased by BP followed by CF, and by DEHP only when co-occurring with these factors, suggesting that BP and CF enhanced the animal's sensitivity to DEHP effects. This indicates potential activation of catecholaminergic pathways. Further analyses, including RT-qPCR for markers of apoptosis, inflammation, and oxidative stress, are underway. **Keywords:** DEHP; toxicity; gut-brain axis; male reproduction; birth pathway

11.027 Locomotion Parameters as Indicators of Toxicity: Effects of Nanoencapsulated Cinnamon Essential Oil in Adult Zebrafish (*Danio rerio*). Pimentel JRL¹, Barros JM², da Silva ACF¹, Chamberlain ALV¹, Stadinik MJ³, Müller LG⁴, da Costa C^{2,5}, Siebel AM^{1,6} ¹UFPR, PPG Pharmacology, Brazil ²UFSC, PPG Chemical and Food Engineering, Brazil ³UFSC, PPG Plant Sciences, Brazil ⁴Institute of Experimental and Technological Biology, Portugal ⁵UFSC, PPG Textile Engineering, Brazil ⁶FURG, PPG Health Science, Brazil

Introduction: Synthetic fungicides are a standard approach for pest management in agriculture. However, their toxicity still poses substantial challenges. Cinnamaldehyde, compound derived from cinnamon (*Cinnamomum cassia*), displays potential antifungal effects. Although promising, certain limitations prevent its practical use, such as high volatility and limited water solubility. To overcome these issues, a zein-based nanocarrier loaded with cinnamon essential oil (Z-CEO) was developed to control bean anthracnose infestation without causing detrimental effects on non-target organisms. For this purpose, this study investigated the potential toxicity of a cinnamon-based pesticide on the behavior of adult zebrafish (*Danio rerio*). **Methods:** Nanoparticles were produced via ultrasound-assisted nanoprecipitation and characterized by UV-Vis spectroscopy. Toxicity was evaluated according to the 203 OECD protocol, a standard procedure for acute fish toxicity analysis. For that, adult zebrafish (*Danio rerio*) were randomly assigned (n=20) to 6 experimental groups: negative control (osmosis water reconstituted with salts), Z-NPs (empty zein nanoparticles) and Z-CEO colloidal dispersion at four concentrations (0.001, 0.01, 0.1, and 1 mg/L). Zebrafish were exposed for 96 hours and treatments were renewed every 24 hours. After exposure, the animals were maintained under washout conditions (in osmosis water reconstituted with salts) for 24 hours. Locomotion parameters were investigated using the novel tank test. Distance covered throughout all sections of the aquarium and average velocity were used as locomotion markers. Data normality was verified using the Kolmogorov-Smirnov test. As the data followed a normal distribution, groups were analyzed by one-way ANOVA followed by Dunnett's post hoc to assess differences between each experimental group in comparison to the control group. **Results:** Data indicated no significant differences among zebrafish exposed to Z-CEO and the negative control group ($p > 0.05$). **Conclusion:** These results indicate that Z- CEO is non-toxic at both low and high concentrations. Future research is encouraged to evaluate its potential efficacy compared to conventional pesticides. This study was supported by CNPq (under grants 160666/2021-0, 402097/2023-8, 310989/2021-3; and through the Scientific Initiation and Technological Development and Innovation Program) and CAPES (finance code 001). Additional support was also provided by CNPq through the Scientific Initiation and Technological Development and Innovation Program. Fish maintenance and experimental protocols of this study were approved by the institutional Ethics Committee for Animal Use (CEUA; UFPR; #004/2024).

11.028 A LC-MS/MS Method for the Simultaneous Determination of Nicotine and Cotinine in Mice's Plasma and Brain Samples after Exposure to Vaping Compared to Cigarette Smoking. Rocha, BM¹, Santos, GCM^{1,2}, Santos, HBS², Nobrega, MEG¹, Gomes, HS¹, Cotias, AC¹, Silva, PMR¹, Martins, MA^{1,2} ¹IOC-Fiocruz, Lab Inflammation, ²Fiocruz, Preclinical Pharmacokinetic Platform, Rio de Janeiro – Brazil

Introduction: The increasing use of electronic cigarettes (e-cigs) has led to amplified intake of nicotine, a rapidly absorbed psychoactive substance with several harmful properties. This work aimed to develop and apply bioanalytical methods using liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) for the quantification of nicotine in mice tissue samples.

Methods: LC-MS/MS methods were established for the simultaneous quantification of nicotine and its metabolite cotinine in plasma and brain tissues, following guidelines from ANVISA. Male and female A/J mice (n = 5/group) were exposed for 1 h/day over 4 days to air, nicotine-containing e-cig or conventional cigarettes. The e-liquid used was commercially obtained (Purple Panther - 12 mg/mL). Tissue samples were collected 24h after the last smoking session. All experimental procedures involving animals were approved by the FIOCRUZ Ethics Committee (L-002/2020-A5).

Results: The selected ion transitions were: *m/z* nicotine: 163→130; *m/z* cotinine: 176.9→98.1; *m/z* IS: 180→101 by MRM in positive ESI mode. Among the extraction methods evaluated, protein precipitation with methanol proved to be the most efficient for the tissues analyzed. Chromatographic separation was optimized using a reverse-phase C18 analytical column and a mobile phase consisting of methanol and 2 mM ammonium formate, operating in gradient mode. Sensitivity analysis revealed detection limits of 5 ng/mL for nicotine and 0.5 ng/mL for cotinine, as determined by a signal-to-noise ratio. The calibration curves for nicotine (10–500 ng/mL) and cotinine (1.0–500 ng/mL) demonstrated good linearity ($R^2 > 0.99$), as well as acceptable precision, accuracy, and selectivity, in accordance with ANVISA validation guidelines. In e-cig samples, plasma cotinine levels averaged 0.7 ± 0.1 ng/mL in females and 1.3 ± 0.3 ng/mL in males. Animals exposed to conventional cigarettes showed significantly higher cotinine concentrations (4.8 ± 1.6 ng/mL in females and 3.9 ± 0.7 ng/mL in males as compared to those exposed to e-cigs, $p < 0.0001$). In brain homogenates from animals subjected to conventional cigarettes, cotinine concentrations were 12.6 ± 3.1 ng/g in females and 9.2 ± 0.9 ng/g in males. Nicotine was undetectable in all tissue samples. **Conclusion:** The current LC-MS/MS method is robust, sensitive, and reproducible for the simultaneous quantification of nicotine and cotinine at low nanogram levels in plasma and brain tissues from A/J mice. Moreover, four daily one-hour exposures to e-cigarette or cigarette smoke allowed the detection of cotinine, but not nicotine, in plasma and brain 24 hours later, with no sex-related differences in these samples. **Financial support:** FIOCRUZ; CAPES; FAPERJ.

11.029 **Adverse Reaction to COVID-19 Vaccines in Healthcare Professionals in Brazil: An Analysis of the Associated Factors at the SEVACOV-PRO Study.** Rufino LG^{1,3}, Silva DLM^{1,2}, Duarte DB^{1,3} ¹UnB, Lab Pharmacological Assays, Dept Pharmacy, School of Health Sciences, Brasília, Brazil ²HUB-UnB, University of Brasília Hospital, Brasília, Brazil ³UnB, Graduate Program in Tropical Medicine, School of Medicine, Brasília, Brazil

Introduction: During the COVID-19 pandemic, diverse vaccine platforms were used to allow control the disease and its mortality. In Brazil, it was used inactivated virus, viral vector, and mRNA COVID-19 vaccines. Despite successfully controlling mortality, vaccine adherence was impacted by public hesitancy over the reactogenicity of the vaccines, besides other factors. The aim of this study is to investigate independent predictors of non-serious adverse events (AEs) among healthcare workers participants of the study SEVACOV-PRO cohort. **Methods:** This multicenter cohort study analyzed secondary data from the SEVACOVPRO project. Of 5,060 participants recruited, 5,031 were included after excluding unvaccinated individuals and those with missing key data. The outcome was the self-reported occurrence of any non-serious AE (binary variable). A multiple logistic regression model was used to identify independent predictors, with results presented as adjusted Odds Ratios (aOR) and 95% Confidence Intervals (CI). The study was approved by the National Research Ethics Committee (CONEP - CAAE: 49561321.0.1001.5558). **Results:** The final cohort (N=5,031) was predominantly female (81.0%), with a median age of 41.0 years (interquartile range [IQR]: 33-51). Overall, 83.1% reported at least one non-serious AE with local pain being the most frequent (76.7%). The multivariable analysis for the first dose revealed that, compared to the inactivated virus platform (CoronaVac), both the viral vector (AstraZeneca) (aOR = 3.98; 95% CI 3.42-4.64) and mRNA (Pfizer) (aOR = 2.48; 95% CI 1.60-3.95) platforms were associated with significantly higher odds of non-serious AEs. Additionally, female sex (aOR = 2.00; 95% CI 1.72-2.33) and younger age (aOR = 0.78 per decade; 95% CI 0.74-0.83) were also strong independent predictors of reporting an event. **Conclusion:** The reactogenicity of COVID-19 vaccines is a common, yet highly predictable phenomenon, with the vaccine platform being the main determinant, followed by host factors such as sex and age. The confirmation that mRNA and viral vector platforms are significantly more reactogenic than the inactivated virus platform provides crucial evidence for managing expectations in public health. This data allows for more precise clinical counseling and transparent communication, demystifying adverse events and reinforcing confidence in vaccination programs. Funding: Ministry of Health

12. Drug Discovery and Development

12.001 **Synergistic Anti-Schistosomal Efficacy of Curcumin-Anchored Silver Tungstate (AW-HRL-C).** Guimarães VH¹, Forde-Thomas J⁵, Anselmo MC¹, Duarte FO¹, Bianchi J¹, Fragelli BDL², Assis M³, Líbero LO², da Silva EL², Hoffmann KF⁵, Anibal FF¹ ¹UFSCar, Lab. of Inflammation and Infectious Disease, Dept. of Morphology and Pathology, Brazil, ²UFSCar, Center for Development of Functional Materials, Dept. of Chemistry, Brazil, ³Universitat Jaume I, Dept. of Physical and Analytical Chemistry, Spain, ⁴Unicamp, Inst. of Biology, Dept. of Animal Biology, Brazil, ⁵Aberystwyth University, IBERS, United Kingdom

Schistosomiasis remains a major public-health challenge in Brazil, where an estimated 6–8 million people are infected with *Schistosoma mansoni*. In response to the growing threat of praziquantel (PZQ) resistance, we evaluated ex vivo the anti-schistosomal performance of curcumin-anchored silver tungstate (AW-HRL-C) versus non-anchored silver tungstate (AW-HRL) and free curcumin against larval (schistosomula) and adult stages of *S. mansoni*. Schistosomula (NMRI strain) were obtained by mechanically transforming cercariae, shed by *Biomphalaria glabrata* snails, chilled on ice for 1h, drawn through needle to detach tails, then purified on a Percoll gradient and resuspended in wash media with concentration verified by counting. These schistosomula were exposed for 72h to serial dilutions (0.195–200µg/mL) of each compound in 384-well plates; viability and morphological alterations were quantified on an ImageXpress Micro 4 system and analyzed with MetaXpress software. Concurrently, adult worms of the NMRI (Puerto Rico) strain, recovered by hepatic-portal-vein perfusion from mice euthanized with sodium pentobarbital/heparin and perfused with DMEM/heparin, were treated at 12.5µg/mL and monitored at 24, 48, and 72h on a BioTek Cytation 7 Cell Imaging Multimode Reader using Gen5 software, with motility normalized to a DMSO vehicle control. Although literature EC₅₀ values for AW-HRL-C (13.28µg/mL), AW-HRL (20.68µg/mL) and curcumin (6.35–18.44µg/mL) have been previously reported, our results demonstrate that AW-HRL-C achieved the lowest active concentration (6.25µg/mL) required to induce pronounced phenotypic disruption and viability loss in schistosomula, compared to 12.5µg/mL for AW-HRL and 6.25µg/mL for free curcumin. In adult worms, AW-HRL-C reduced motility to –17 normalized units (± 1.2), statistically indistinguishable from PZQ and auranofin (AUR) controls (p > 0.05), outperforming AW-HRL (–12 ± 1.5) and matching curcumin (–16 ± 1.3). These findings underscore AW-HRL-C's central role as a broad-spectrum anti-schistosomal agent capable of impairing parasite viability and motility across life stages. The synergy between silver tungstate's oxidative properties and curcumin's anti-inflammatory actions merits further mechanistic investigation. AW-HRL-C is a compelling alternative to PZQ, exhibiting comparable ex vivo efficacy and laying the groundwork for novel therapeutic strategies against schistosomiasis.

Financial Support São Paulo Research Foundation - FAPESP (Process nº 2024/06545-3; CEPID Process: nº 2013/07296-2) **Keywords:** *Schistosoma mansoni*, schistosomiasis, drug discovery, semiconductor, anthelmintic

12.002 **Autophagy and Stress-Responsive Long Non-coding RNAs as New Putative Antischistosomal Targets in *Schistosoma mansoni*.** Fischer-Carvalho A, Poli PJ, Tahira AC, Verjovski-Almeida S, Amaral MS IBu, Cell Cycle Lab, São Paulo, Brazil

Introduction: Schistosomiasis is a neglected tropical disease for which praziquantel (PZQ) remains the only drug available for treatment. However, cases of parasite tolerance to PZQ and its inefficacy against schistosomula—the early larval stage—underscore the urgent need for alternative therapies. Autophagy is a conserved pathway involved in cellular homeostasis and stress response, and it also plays a developmental role across several organisms. In humans, many long non-coding RNAs (lncRNAs), transcripts longer than 200 nucleotides and highly species-specific, have been identified as pivotal regulators of the autophagy machinery. Previous data from our group indicated that genes associated with lysosomes and autophagy-related lytic vacuoles were downregulated in *S. mansoni* schistosomula under stress conditions, following incubation with plasma from self-cured rhesus macaques. Elucidating how autophagy is regulated in *S. mansoni*, particularly through the role of lncRNAs, may uncover novel molecular targets to overcome schistosomiasis.

Objectives: (i) To identify immune-stress-responsive lncRNAs and investigate their potential regulatory roles in the autophagy pathway. (ii) To evaluate the *in vitro* antischistosomal activity of autophagy modulators and determine their IC₅₀ values. **Methods:** We used RNA-Seq data to identify differentially expressed (DE) lncRNAs following incubation of schistosomula with plasma from self-cured rhesus macaques. Co-expression modules and hub genes were identified using Weighted Gene Co-expression Network Analysis (WGCNA). Two DE hub lncRNAs were validated by RT-qPCR and selected for RNAi-mediated knockdown using *in vitro* synthesized dsRNAs. Parasite viability post-silencing will be assessed by ATP quantification. Schistosomula were incubated *in vitro* for up to 120h with the autophagy inducer rapamycin and the selective autophagy repressors chloroquine, wortmannin, spautin-1, and bafilomycin A1. Viability was assessed every 24h using an ATP quantification assay. IC₅₀ values were calculated using five-parameter logistic (5PL) regression.

Results: WGCNA identified two lncRNAs (SmLNCA131832-IBu and SmLNCA163008-IBu) as DE and as hubs genes within the *brown2* module, which was functionally enriched for lysosomes and carbohydrate metabolism terms and composed of downregulated genes after plasma incubation. Both lncRNAs were validated by RT-qPCR as DE and are currently being silenced with dsRNAs to evaluate their roles in parasite viability. Rapamycin exhibited an IC₅₀ of 17.8 µM against *S. mansoni* schistosomula. Although the autophagy inhibitors induced visible phenotypic changes in schistosomula morphology and motility, they did not significantly reduce ATP levels. **Conclusion:** Our data suggests autophagy as a key stress-response mechanism in *S. mansoni* schistosomula. The lncRNAs identified may act as regulators of this pathway and represent promising molecular targets. Together, these findings support autophagy and its upstream regulators as potential therapeutic targets against schistosomiasis, as well as the repurposing of autophagy modulators as a promising pharmacological approach. **Funding** FAPESP and Fundação Butantan

12.003 Arylboronic Acids as Dual Inhibitors of Butyrylcholinesterase and β -Secretase 1 for Alzheimer's Disease Treatment. Bussinger ER¹, Hiller NJ², Faria RX³, Martins DL², Raimundo JM^{1,4}, Romeiro NC⁴, Silva LL¹ ¹UFRJ-Macaé, FarPBio, Brazil, ²UFF, LabCSI, Brazil, ³Fiocruz/RJ, LAPSA, Brazil, ⁴UFRJ-Macaé, PPG-ProdBio, Brazil

Cholinesterases (ChEs) catalyze the hydrolysis of acetylcholine and regulate cholinergic neurotransmission, being involved in the pathogenesis of Alzheimer's disease (AD). Acetylcholinesterase (AChE) is the main ChE in the healthy brain; however, in advanced AD, its activity is reduced by up to 90%, while butyrylcholinesterase (BChE) can increase by more than 120% and is also found in neuritic plaques. Arylboronic acids are described as reversible covalent inhibitors of hydrolases, as they react with the serine residue in the catalytic site of these enzymes. Therefore, they could act as ChE inhibitors (ChEIs), since these enzymes also have a serine essential for their activity. The enzyme β -secretase 1 (BACE1) also plays a role in AD pathophysiology, as it cleaves the amyloid precursor protein, promoting neurotoxic β -amyloid peptides that accumulate in senile plaques. In this sense, dual inhibition of ChEs and BACE1 represents a promising pharmacological strategy for AD. Thus, 30 derivatives—boronic and non-boronic—were evaluated as multi-target inhibitors for AD treatment. ChE activity was evaluated by Ellman's spectrophotometric method (412 nm) in phosphate buffer (50 mM, pH 7 or 8), 5,5'-dithiobis(2-nitrobenzoic acid) at 60 μ M, enzyme (0.25 U/mL) —AChE and BChE from *Homo sapiens* (*hsAChE* and *hsBChE*), derivatives (up to 300 μ M), and acetylthiocholine/butyrylthiocholine. Derivatives that inhibited ChE activity by more than 80% in the screening stage had their mean inhibitory concentrations (IC₅₀) determined and expressed as mean $\pm$ standard error of the mean (μ M). The reversibility of inhibition was assessed through a 100X dilution method by measuring enzyme activity before and after dilution. The inhibition mechanism was determined using the Lineweaver-Burk plot. BACE1 activity was evaluated by a fluorimetric method (ex. 320 nm /em. 405 nm) according to the manufacturer's instructions (Sigma-Aldrich); compounds concentration up to 300 μ M. The derivatives had their IC₅₀ values determined. For *hsAChE* NO-108 (46.9 $\pm$ 1.1 μ M) at pH 7, NO-09 (46.4 $\pm$ 1.1 μ M), NO-56 (38.7 $\pm$ 1.1 μ M), and NO-108 (50.1 $\pm$ 1.1 μ M) at pH 8. Against *hsBChE*, in ascending order of potency, NO-82 (0.1 $\pm$ 0.04 μ M), NO-13 (0.3 $\pm$ 0.1 μ M), NO-91 (4.4 $\pm$ 2.1 μ M), NO-03 (19.1 $\pm$ 1.6 μ M), NO-01 (24.8 $\pm$ 1 μ M), NO-79 (32.3 $\pm$ 4.91 μ M), NO-86 (46.7 $\pm$ 13.8 μ M), NO-09 (58.7 $\pm$ 1.6 μ M), NO-108 (59 $\pm$ 6.5 μ M) at pH 7; NO-82 (0.4 $\pm$ 0.02 μ M), NO-13 (0.7 $\pm$ 0.1 μ M), NO-91 (6.6 $\pm$ 1.9 μ M), NO-03 (34.2 $\pm$ 13.1 μ M), NO-56 (36.5 $\pm$ 2.3 μ M), NO-108 (42.2 $\pm$ 0.5 μ M), NO-09 (45.3 $\pm$ 3.7 μ M), NO-137 (83.5 $\pm$ 10.1 μ M), and NO-144 (85.5 $\pm$ 16.1 μ M) at pH 8. The more potent derivatives against *hsBChE* were evaluated in the BACE-1 assay; NO-13 and NO-56 were most active (IC₅₀: 65.6 $\pm$ 1.3 μ M and 46.5 $\pm$ 1.2 μ M, respectively). The inhibition of *hsBChE* by NO-13, 82, and 91 at pH 7 was reversible, with a competitive mechanism for NO-13 and 91, and mixed-type for NO-82. The boronic derivative NO-13 exhibited a multi-target profile by inhibiting BACE1 and being a potent reversible BChE inhibitor, highlighting the impact of the boron atom on the BChE selectivity.

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12.004 Neuroprotective Effect of Polymeric Resveratrol Nanoparticles in Parkinson's Disease.

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Parkinson's disease (PD) is a neurodegenerative syndrome that affects the central nervous system, impairing motor function and causing symptoms such as bradykinesia and tremors, as well as non-motor symptoms like urinary dysfunction. Current treatments are only symptomatic but do not halt disease progression. Natural compounds, such as resveratrol (RSV), have therapeutic potential due to their antioxidant, anti-inflammatory, and neuroprotective properties. Polymeric nanoparticles (NP) are delivery systems that carry active substances to specific sites, increasing their bioavailability and reducing side effects, making them a promising trend in pharmaceutical nanotechnology. This study aims to evaluate the effects of resveratrol-loaded polymeric nanoparticles (RNP) in a rotenone (ROT)-induced PD *in vitro* model. RNPs were obtained by nanoprecipitation, characterized in Zeta size (nanoparticle size - NS; polydispersity index - PDI,) and diluted in PBS (1.56 - 100 μ M) for experiments with PC12, and astrocyte (AST) dopaminergic neuron cell lines. Cytotoxicity of RNPs, dopamine, RSV, ROT, and the neuroprotective effect against ROT IC₅₀, were assessed by MTT assay. Experiments were performed in triplicate (n = 3), results expressed as mean $\pm$ standard error mean, and analyzed by One-way ANOVA (Dunnett's post-test). RNP and blank NP were obtained particle size 123.2 ± 73.16 nm (PDI = 0.379), and 169.4 ± 172.4 nm (PDI = 0.260), respectively. Nontoxic concentrations of dopamine (100 - 400 μ M), RNP (1.56 - 6.26 μ M for AST and 0.39 - 1.56 μ M for PC12), and RSV (3.12 - 12.5 μ M) were submitted to cytotoxicity induced by ROT after determining its IC₅₀ (33,78 μ M for AST and 19,58 μ M for PC12). In PC12 cells, RSV prevented ROT-induced damage in all tested concentrations. Similarly, at lower concentrations (0.39 - 1.56 μ M), RNP showed a similar or greater neuroprotective effect. In AST cells, RSV protection was enhanced in a concentration-dependent way, while for RNP, this effect was decreased in a dose-dependent way, although it was greater than that of dopamine and RSV at lower concentrations. The results of this study suggest that RNP possesses neuroprotective properties in the ROT-induced Parkinson's disease model at lower concentrations compared to RSV, being a potential therapeutic tool for neurodegenerative diseases. However, further studies are needed to better elucidate its potential, such as analysis of inflammatory markers, redox balance, and molecular docking studies. The authors thank to CAPES and CNPq for their financial support.

12.005 **Privileged Indole and N-Acylhydrazone Scaffolds in Multitarget Drug Discovery for Alzheimer's Therapy.** Leite BF¹, Romeiro NC², Farias AB², Silva LL¹, Raimundo JM^{1,2} 1UFRJ-Macaé, FarPBio, Brazil, ²UFRJ-Macaé, PPG-ProdBio, Brazil

Alzheimer's disease (AD) is a complex neurodegenerative disorder. Multiple mechanisms involved including oxidative stress, neuroinflammation and degeneration, biometals imbalance, and diminished synaptic transmission. They promote accentuate changes in the neural homeostasis, resulting in a process damage, leading to cognitive-memory injury and neuronal death. Privileged scaffolds such as indole and N-acylhydrazone can be used on development of multitarget-directed ligands (MTDL) directed targets enzymes like acetylcholinesterase (AChE), myeloperoxidase (MPO), and monoamine oxidase B (MAO-B) and chelator of metals redox as Fe²⁺ and Cu²⁺, involved in the pathophysiology of AD. Herein, the profile of six indole-N-acylhydrazone derivatives was evaluated as MTDLs for this targets for AD treatment. AChE inhibition was measured by Ellman's method (250 µL): phosphate buffer (0.05 M, pH 8), AChE (0.25 U/mL), DTNB (3 mM). Absorbance (412 nm) was read after adding acetylthiocholine (1.5 mM). MPO inhibition was assessed via HOCl generation (125 µL): NaSCN (50 µM), tyrosine (50 µM), urate (200 µM), BSA (1 mg/mL). Reaction was measured at 412 nm after H₂O₂ (50 µM) addition. MAO-A/B inhibition was analyzed fluorimetrically (ex. 320 / em. 415 nm) in 240 µL: enzyme preparation (0.5 mg/mL), phenylethylamine (20 µM) or phenylephrine (100 mM), HRP (0.65 U), tyrosine (250 µM), phosphate buffer (0.2 M, pH 7.6). Molecular docking validation was performed via redocking, with poses evaluated using multiple scoring functions and binding site radius (AChE: S293, 10 Å; BChE: W82, 20 Å; MPO: H95, 10 Å; MAO-A: Y407/F208/F352, 15 Å; MAO-B: I199/Y398/F343, 15 Å). ChemPLP yielded RMSD values of 0.43 Å (AChE), 0.4 Å (BChE), and 0.5 Å (MAO-B). ChemScore showed RMSD values of 0.52 Å (MPO) and 0.16 Å (MAO-A), confirming the co-crystallized ligand's binding pose. At 300 µM, the derivatives inhibited AChE catalytic activity. S5 was the least active (32.2±1% inhibition), while S2, S3, and S4 showed ~50% inhibition (45.5±4.9%, 54.2±1.6%, and 50.2±1.3%, respectively). S1 and S6 were the most actives (68.4±4.1% and 61.5±1.9%, respectively). In MPO assay the IC₅₀ of the most active derivatives was determined. Derivative S1 (37.9±8.4 µM) was less potent than S3, S5, and S6 (4.7±1.3 µM, 9.5±1.3 µM, and 13.6±2.1 µM, respectively). Concentration-response curves for MAO-B inhibition revealed derivative S4 was more potent (IC₅₀ = 0.35±0.1 µM) than S1 (6.2±0.8 µM) and S6 (3.7±0.4 µM; p<0.05). For MAO-A, S4 maintained superior potency (IC₅₀ = 1.8±0.2 µM) compared to S6 (13.8±3.9 µM) and S1 (14.3±3.5 µM). In biometal chelation at 150µM all formed complex (L+M/2:1) with Cu²⁺ and S4 complexed with Fe²⁺, Mg²⁺ and Zn²⁺ too. The derivatives acted as MTDL and S6 was the best candidate because was able to inhibit all enzymes and act like copper chelator. Molecular docking study validated the use of indol and NAH structures on drug development of MTDL and stablished a structure-activity relationship with biological assay results. **Financial support:** CAPES, CNPQ.

12.006 **Pharmacological Potential of Small Molecules from Marine Bacteria and Extremophiles: A Search for Antitrypanosomal Candidates.** Albuquerque V¹, Lago JHG², Santos C³, Pimenta DC⁴, Tempone AG¹ ¹IB, Lab. of Pathophysiology, PPG Toxinology, Brazil, ²UFABC, Center of Natural Sciences and Humanities, Brazil, ³Universidad de La Frontera, Dept Chemical Science and Natural Resources, Chile, ⁴IB, Lab. of Ecology & Evolution, PPG Toxinology, Brazil

Chagas disease, caused by the *Trypanosoma cruzi* protozoan, is a Neglected Tropical Disease, endemic in 21 countries in the Americas. It affects 7 million people worldwide, with only one toxic and ineffective drug available in Brazil. The search for new therapies is an urgent issue. Microbial natural products have been widely used as scaffolds for pharmaceutical development in several FDA-approved drugs. Marine and extremophilic microorganisms biosynthesize an array of metabolites, reflecting specialized cellular, molecular and biochemical adaptations, essential to survival under extreme conditions, as those found in the glaciers and hypersaline lakes. Considering the potential of these microbial metabolites, we explored the anti-*T. cruzi* activity of extremophiles from sediments of the Chilean Glacier Grey Lake, the hypersaline Bitter Lagoon (Patagonia), and marine bacterial metabolites from São Paulo coast. Sediments were plated in Marine agar and TSA agar medium, and the bacteria were isolated using conventional techniques. The identification of the bacteria was performed by MALDI-ToF/MS and 16S rRNA gene partial sequencing. The bacterial metabolites of nine Gram-negative and one Gram-positive bacteria were extracted with organic solvents, and the anti-*T. cruzi* (Y strain) activity was evaluated against trypomastigotes at 150 µg/mL for 24h incubation, using resazurin. Chemical analyses were performed using Nuclear Magnetic Resonance (¹H and ¹³C NMR) and Mass Spectrometry. The MALDI-ToF/MS analysis resulted in the identification of only one bacteria, the *Planococcus maritimus*. The 16S rRNA sequencing identified three isolates as *Pseudoalteromonas rubra*, *Pseudoalteromonas piscicida* and *Cyclobacterium plantarum*. Our results demonstrated that all organic extracts killed 100% of the parasites at the highest tested concentration, with EC₅₀ values between 1 and 58 µg/mL. Two potent extracts (PLASE1.1 and SMG37) were selected for NMR analysis and the results showed a mixture of compounds (< 1 kDa) containing aromatic rings and fatty acids. RP-HPLC bioguided fractionation resulted in one active peak, and by LC-MS/MS revealed a molecular weight of approximately 349 g/mol. Marine microorganisms and those living in extreme environments represent new sources of small molecules with potential antitrypanosomal activity. Supported by FAPESP 2024/16243-4 and CAPES. **Keywords:** neglected diseases, *Trypanosoma cruzi*, therapy, natural products

12.007 Development of a Poly(lactic-co-glycolic acid) Nanoparticle for the Delivery of Pradimicin-IRD for Colorectal Cancer Treatment. Gomes MAM, dos Anjos R, Costa-Lotufo LV, Lopes LB ICB-USP, Institute of Biomedical Sciences, Dept Pharmacology

Introduction: Colorectal cancer (CRC) is a serious global health problem, with 1.9 million new cases and over 900,000 deaths in 2022, accounting for 9.3% of all cancer-related deaths. There is also a concerning increase in incidence among younger populations. Conventional treatment with 5-fluorouracil (5-FU) is complicated by its low bioavailability and short plasma half-life, requiring intravenous administration at high doses and resulting in severe adverse effects. This scenario is further worsened by the development of resistance, highlighting the need for new pharmacological intervention strategies. Here, we propose a nanotechnological approach for the delivery of pradimicin-IRD (PRM-IRD), a cytotoxic molecule capable of inducing DNA damage and causing cell cycle arrest. More specifically, PLGA nanoparticles (PLGA-NPs) — a highly biodegradable copolymer of lactic acid and glycolic acid — were developed for the encapsulation of PRM-IRD. The synergistic effect of PRM-IRD and 5-FU was also evaluated to assess the possibility of coencapsulating both drugs. **Methods:** PLGA-NPs were developed using a single emulsion and solvent evaporation method. As the organic phase, a solution of ethyl acetate:dichloromethane in a 7:3 (v/v) ratio was selected, in which PLGA was dissolved (15–50 mg). The organic phase was then added dropwise into a surfactant solution (aqueous phase), forming an emulsion. Tween 80, Solutol, and Poloxamer 407 (0.5–1%) were evaluated as surfactants. The emulsion was then subjected to probe sonication and stirring (3 h). For PRM-IRD loading, 1 mg of the compound was dispersed in the organic phase. The particle diameter (d), polydispersity index (Pdl), and zeta potential (Z) of the produced nanoparticles were determined using dynamic light scattering (DLS). Encapsulation efficiency was determined using high-performance liquid chromatography (HPLC). Nanoparticle stability was evaluated over 28 days of storage at 4 °C. To assess the ratio of encapsulation of PRM-IRD and 5-FU, HCT-116 cells were grown at a density of 2×10^3 cells/well in 96 well plates and cell viability was evaluated after treatment with PRM-IRD, 5-FU and both drugs at 1:1 and 1:0.25 ratios for 72h by using MTT assay. The synergistic effect was determined by the Chou-Talalay method. **Results:** The type of surfactant influenced the characteristics of the nanoparticles. PLGA-NPs prepared with 0.5% (v/v) Tween 80 were selected, showing an average size of 155 nm, Pdl = 0.20, and Z = –34 mV. PRM-IRD encapsulation did not cause significant changes in these parameters. An encapsulation efficiency of 77% was observed, and the loaded PLGA-NPs remained stable for up to 28 days of storage at 4 °C. PRM-IRD and 5-FU showed a slightly additive effect at 1:1 ratio (CI = 1.14) and slightly synergistic effect at 1:0.25 ratio (CI = 0.83). **Conclusion:** The results demonstrate that the nanoparticles prepared using Tween 80 at 0.5% (v/v) had appropriate physical properties, and the encapsulation of PRM-IRD did not cause drastic changes in size, Pdl and zeta potential. The synergistic effect observed with PRM-IRD and 5-FU at 1:0.25 ratio further supports the coencapsulation of both compounds. **Financial support:** This work was financially supported by CAPES (001) and FAPESP (2018/13877-1).

12.008 Analysis of Cell Membrane Integrity of *Trypanosoma cruzi* Epimastigote Forms under Treatment With (2E,4E)-1-(2-hydroxy-3,4,6-trimethoxyphenyl)-5-phenylpenta-2,4-dien-1-one. Pinheiro KS¹, Duarte ALS¹, Fernandes MO¹, Ananias CF¹, Araújo JR¹, Ribeiro LR², Magalhães EP², Silva FRC², Gomes NDB², Maia MMG², Santos HS³, Martins AMC², Menezes RRPPB². ¹UFC, Faculty of Pharmacy, Dentistry and Nursing, Dept Clinical and Toxicological Analysis, Brazil; ²UFC, Faculty of Pharmacy, Dentistry and Nursing, Dept Clinical Analysis and Toxicological, Post Graduate Program of Pharmaceutical Sciences, Brazil; ³UVA, Center for Exact Sciences and Technology, Brazil

Chagas disease (CD) affects millions of people in Latin American countries and is caused by *Trypanosoma cruzi*. The currently available therapies, based on nitro-heterocyclic drugs such as benznidazole (Bzn), have limited efficacy in the chronic phase, high toxicity and risk of resistance. Chalcones, compounds with a broad bioactive spectrum, have shown promising trypanocidal action, including as inhibitors of the cruzain enzyme. This study investigated the effect of the chalcone (2E,4E)-1-(2-hydroxy-3,4,6-trimethoxyphenyl)-5-phenylpenta-2,4-dien-1-one (CPNC) on viability and cell membrane integrity in epimastigote forms of *T. cruzi*. *T. cruzi* strain Y epimastigote forms were grown in liver infusion tryptose (LIT) medium supplemented with SBF, penicillin and streptomycin, incubated at 28 °C. Parasites at exponential growth (10^6 parasites/mL) were treated with CPNC (31.2-1000 μ M) or 0.5% DMSO (vehicle group) in 96-well plates, and incubated at 28 °C. Untreated parasites were used as a control group. The viability was determined by counting on a Neubauer chamber under an optical microscope after 24, 48 and 72 hours of incubation, observing motility and typical morphology as criteria. These data were analysed by One-way ANOVA with Dunnet's post-test and nonlinear regression to estimate the concentration capable of inhibiting the growth of half the parasite population (IC₅₀). To analyse the death profiles, the parasites were treated with CPNC (250 and 500 μ M) for 24 h, stained with 7-AAD and annexin V-PE, and analysed by flow cytometry (FACSCalibur). 10,000 events were acquired per sample and the cells were classified according to their labelling as viable, necrotic, apoptotic or double-labelled. CPNC presented inhibitory effect at all the concentrations and times evaluated. The following IC₅₀ were obtained: IC₅₀/24h = 39.9 ± 4.2 μ M, IC₅₀/48h = 48.5 ± 3.7 μ M, and IC₅₀/72h = 37.8 ± 3.5 μ M for 72 hours. Regarding the flow cytometry analysis, the treatment of parasites with CNPC at both concentrations (250 and 500 μ M) demonstrated a reduction on the percentage of viable cells and an increase in 7-AAD-only labelled population in comparison to control group. No change was observed on annexin V-PE or double-labelled populations. CPNC showed an effect on epimastigote forms of *T. cruzi*, with an impact on the viability and cellular integrity of the parasites. This effect seems to be associated with membrane damage, suggesting a necrotic cell death mechanism. The findings show that the compound has antiparasitic potential, reinforcing its value as a promising candidate in the development of new therapeutic approaches for Chagas disease. **Financial support:** Capes, CNPq, FUNCAP.

12.009 Synthesis and Antiproliferative Activity of Triazol-Containing Derivatives Based on Minor Groove Binders and LSD1 Inhibitors. Peripolli JB¹, Theisen MC², Borba IAS², Castro AM¹, Maia BC², Librelotto DRN², Fumagalli F² ¹UFSM, Graduação em Farmácia, Brasil; ²UFSM, PPG Ciências Farmacêuticas, Brasil **12.009 Synthesis and**

Introduction: Cancer remains one of the leading causes of global mortality, with approximately 20 million new cases diagnosed annually. Despite therapeutic advances, cellular resistance and adverse effects still represent significant challenge. In this context, various molecular targets have been explored in the search for compounds with antitumor activity. Among them, the DNA minor groove stands out, offering greater selectivity and lower toxicity compared to traditional alkylating agents, as well as the histone lysine-specific demethylase 1 (LSD1), which plays a key role in epigenetic regulation and is frequently overexpressed in various types of cancer, with its inhibition associated with reduced tumor proliferation. This study aimed to synthesize a series of triazolic compounds derived from p-bromoacetophenone or acetovanillone, also known as apocynin. Apocynin is a natural metabolite with recognized antioxidant activity, isolated from the roots of *Apocynum cannabinum* and described as a NADPH oxidase inhibitor, although it remains underexplored in the antitumor context. **Methods:** The structural design was based on bioisosterism and scaffold hopping strategies, inspired by LSD1 inhibitors and DNA minor groove binders, focusing on evaluating antiproliferative activity against the MCF-7 breast cancer cell line. The triazole compounds were designed as bioisosteres of previously synthesized β -aminoalcohols and the amide derivative was planned with scaffold hopping of an LSD1 inhibitor. Structurally, the synthesized compounds feature an aromatic ring containing the guaiacol moiety from acetovanillone or a p-bromophenyl group, directly linked to the triazole core, followed by an aliphatic spacer (1 to 4 carbons), and a second aromatic ring p-substituted with bromine, an ethyl ester, N-((1R,2R)-2-(3,4-difluorophenyl)cyclopropyl)acetamide or hydrogen. **Results:** The triazoles were synthesized via a metal-free methodology using the aforementioned ketones, different amines, and p-nitrophenyl azide. The obtained yields were 30-93%. The amide derivative was synthesized from the ester derivative through a hydrolysis followed by acid activation with tetrachlorofluoroformamidinium hexafluorophosphate/N-methylimidazole (TCFH/NMI) obtaining 25% yield. The compounds were evaluated for cytotoxicity using the MTT cell viability assay on the MCF-7 cell line. The brominated derivatives exhibited relevant cytotoxic activity, although with high LogP values, suggesting potential limitations related to toxicity. The derivative containing an ester in the second aromatic portion presented a lower LogP and interesting activity at 60 μ M, and its amide derivative was the most active, with a GI₅₀ of 20.5 μ M and a cLogP below 4, indicating a more favorable lipophilic profile and potential activity. **Conclusion:** The results demonstrate that apocynin-derived triazolic compounds represent a promising approach for developing of new antitumor agents and more analogues of the amide derivative will be synthesized for a better understanding of the structure-activity relationship. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – Brasil (CAPES) – Finance Code 001 and Conselho Nacional do Desenvolvimento Científico e Tecnológico – CNPq.

12.010 Recombinant Expression of *Mycobacterium tuberculosis* Caseinolytic Protease (Clp) Components for Structural Analysis and Therapeutic Targeting. Ribeiro PHC, Silva CS, Rossini NO, Dias MVB USP, Lab. of applied structural biology, Brazil

Tuberculosis is an infectious disease caused by *Mycobacterium tuberculosis* and affects about 10 million people a year worldwide, making it one of the leading causes of death by infections. New therapy strategies against tuberculosis are needed, and the AAA+ degradation systems, such as the caseinolytic protease (Clp) system, are interesting targets for developing new antimicrobials. These are essential machines for the mycobacterium survival and should cause damage to the bacterial cell if this process is deregulated. The Clp system is constituted of three different subunits: ClpP1, ClpP2, and ClpC1. ClpC1 in *Mycobacterium tuberculosis* (MtbClpC) is a hexameric AAA+ unfoldase, while ClpP1 and ClpP2 are proteases. All the subunits of the Clp system are essential for protein degradation and, consequently, for *M. tuberculosis* survival. In this work, we aimed to produce these three subunits of the Clp system from *M. tuberculosis* recombinantly for structural and drug discovery application. that, synthetic fragment genes encoding ClpC1, ClpP1 and ClpP2 were subcloned into a pET28a plasmid. For the protein overexpression, these plasmids were transformed into BL21(DE3) cells and induced by IPTG. The proteins were purified using affinity and gel filtration columns. We were able to overexpress the three different subunits despite the low yield, fast degradation, and instability of the proteins. In the next step, we expect to optimize the protein purification protocols to obtain enough protein for structural, biochemical, and drug discovery studies. funding: Fapesp.

12.011 **Exploration of Cytotoxic Microorganisms from *Palythoa caribaeorum* collected at Brazilian Oceanic Islands.** Moura, MHS¹, Silva, CSMR², Sahm, BDB³, Costa-Lotufo, LV² ¹FCF-USP, Brazil, ²ICB-USP, Brazil, ³FCFRP-USP, Brazil

Introduction: Marine microorganisms are a remarkable source of molecules with cytotoxic activity, holding potential for cancer treatment. Marine sediments, in particular, harbor vast microbial biodiversity, including bacteria that produce secondary metabolites with pharmacological properties. **Objective:** To isolate bacteria from *Palythoa caribaeorum* samples collected in different oceanic regions of Brazil and to prospect the cytotoxic activity of extracts from their biological fermentations against human tumor cells. **Methodology:** The selection of bacteria begins with the dilution and plating of samples on Starch Casein Agar (SCA), Saltwater Agar (SWA), and Trace Minerals Agar (TMA) media, followed by incubation at 28°C for up to 90 days. *Palythoa caribaeorum* samples were collected in Fernando de Noronha Archipelago (FNA), PE, Saint Peter and Saint Paul Archipelago (SPSPA), PE, and Trindade Island (TI), ES. Strain isolation was based on phenotypic characteristics, and selected colonies were cultivated and purified using the stripping technique. After purity confirmation, bacteria were stored at -80°C. To obtain the organic extracts, pure strains were cultured in A1 medium under agitation (200 rpm, 28°C) for seven days. Subsequently, the fermentation broths were extracted with ethyl acetate, concentrated in the rotary evaporator, and resuspended in methanol for salt removal, yielding extracts for cytotoxicity tests. Antiproliferative activity was quantified by the MTT assay (50 µg/mL in triplicate) using HCT-116 tumor cells, measuring cell viability. **Results and Discussion:** So far, a total of 24 strains were isolated, being 10 from samples of *P. caribaeorum* collected at FNA, nine from SPSPA samples and five from TI samples. Half of the strains were obtained using Starch Casein Agar (SCA), six using Sea Water Agar (SWA), and six using Trace Minerals Agar (TMA). The obtained extracts will be tested for cytotoxicity. The variation in the number and distribution of isolates among the localities may reflect the nutrient richness in the SCA media. **Conclusion:** This study demonstrates the potential of *P. caribaeorum* from different Brazilian oceanic regions as a rich source of bacteria with biotechnological potential. The isolated bacterial strains represent a promising initial bank for prospecting secondary metabolites with cytotoxic activity. **Financial support:** FAPESP (2025/09280-3; 2023/08735-1); CNPQ (440472/2022-9; 441820/2024-7).

12.012 **Investigation of Bioavailability, Taste Masking Effect and Antimalarial Activity of Chloroquine-Ion Exchange Resin Complex.** Passos MXV¹, Mascarenhas ED¹, Sousa EGR², Guimarães TF², Rosas EC¹, Pádua TA¹ ¹Farmanguinhos-Fiocruz, Lab Applied Pharmacology, Rio de Janeiro, Brazil. ²Farmanguinhos-Fiocruz, Educação, Pesquisa e Inovação, Rio de Janeiro, Brazil

Introduction: Malaria is a parasitic disease that killed 597,000 people in 2023, with 76% of these deaths being children under five years of age. The available treatments, mostly in the form of oral tablets, require dose adjustment according to the weight of the pediatric patient, which often involves fragmenting the tablets intensifying the bitter medication taste, and contributing to treatment abandonment. Therefore, the complexation of bitter-tasting drugs, such as chloroquine diphosphate (CLQ) with ion-exchange resin represents a viable taste masking alternative to improve patient adherence to treatment. To determine whether the chloroquine resinate complex (CLQ-R) has the same bioavailability and antimalarial activity as the reference drug CLQ, but with a masking effect on the bitter taste. **Methods:** CLQ-R was prepared in a 1:1 (w/w) drug-to-resin (R) ratio using polacrilin potassium (PP) (Amberlite IRP88). Plasma concentration of CLQ or CLQ-R were studied in mice that received oral administration (p.o.) of CLQ or CLQ-R (15.6 mg/kg of chloroquine base). At scheduled times (0-6h) after dosing, mice (n=4/time-point) were euthanized, and plasma samples were collected and stored at -80°C until analysis. The toxic effect of antimalarial substances was evaluated after 24h and 48h by biochemical analysis. Mouse taste sensitivity of CLQ, CLQ-R or R was assessed by 48-hour two-bottle preference test. Mice had simultaneous access to bottles containing water and CLQ (10^{-5} ; 10^{-2} or 10 mM of chloroquine base), CLQ-R, or R solution (10 mM of chloroquine base) for 48h. The preference ratio was calculated as $PI = [\text{tastant intake}/(\text{tastant intake} + \text{water intake})] \times 100$. CLQ, CLQ-R or R antimalarial efficacy was evaluated in C57BL/6 mice (1×10^6 *Plasmodium berghei* ANKA-pRBC). Four-day suppressive was performed in mice daily treated up to day 4 after infection with CLQ, CLQ-R, or R (30 mg/kg of chloroquine diphosphate). For the recrudescence test, mice were treated on day 3 post-infection with CLQ, CLQ-R, or R (100 mg/kg of chloroquine diphosphate). Parasitemia determination was performed by flow cytometry in different days. The results are expressed as drug activity and were determined by the difference between the mean value of the control and experimental groups expressed as percent. **Results:** The conjugation of R to CLQ did not alter the profile of their plasma concentration and biochemical parameters. Regarding the bitter taste perception, the mice showed an increase (15.5%) in preference for CLQ-R compared to CLQ. Both CLQ and CLQ-R exhibited antimalarial activity with no statistically significant difference. The compounds CLQ and CLQ-R were able to reduce mice parasitemia by more than 95%. **Conclusion:** These results indicate that the CLQ-R presented similar biodistribution and antiparasitic activity profiles to CLQ. In addition, the CLQ-R formulation showed a significant improvement in palatability, making it a potential candidate for future pediatric treatments. CEUA-FIOCRUZ-license: LW-33/21 and LW-37/22 **Financial support:** FAPERJ, INOVA Fiocruz program. **Keywords:** Chloroquine, Ion exchange resin, Antimalarial activity, Taste masking

12.013 Pharmacology in the Shadows: Extracts of Bacteria Recovered from Brazilian Caves show Anticancer Potential. Ferraro ET^{1,3}, Lopes NP², Kato N², Costa-Lotufo LV¹, Salm BDB^{1,2} ¹ICB-USP, Brasil; ²FCFRP-USP, Brasil; ³USP, Escola de Artes, Ciências e Humanidades, Brasil

Cancer is a group of multifactorial diseases characterized by abnormal and uncontrolled cell growth that can affect various tissues and organs. Due to its high global incidence and mortality, there remains an urgent need for new therapies that are more effective, selective, and with milder side effects. In this scenario, natural products, especially those derived from microorganisms from extreme environments, have emerged as promising sources of bioactive compounds with relevant pharmacological potential. This study aims to investigate the cytotoxic potential of crude extracts obtained from bacteria isolated from caves in the region of the Alto Ribeira Tourist State Park (PETAR), an environment characterized by adverse conditions such as absence of light and nutrient scarcity, which favor the production of underexplored secondary metabolites. Bacterial strains were previously cultured and stored in the MicroCave-BR strain bank (ICB-USP), submitted to fermentation followed by organic solvent extraction. The resulting crude extracts were divided into two parts: one for biological activity assays and the other for chemical composition analysis via mass spectrometry. A total of 113 bacterial extracts were evaluated for their ability to inhibit cell growth using the MTT viability assay against three human tumor cell lines of different origins: 501mel (metastatic melanoma), HCT-116 (colorectal carcinoma), and MCF-7 (breast adenocarcinoma). Assays were conducted in three independent experiments at concentrations of 5 and 50 µg/mL under incubation of 72h. Results showed that 29 extracts (25,6%) exhibited over 90% inhibition at the highest concentration, whereas 6 extracts (5.3%) also maintained significant activity (≥70% inhibition) at the lower concentration, indicating high potency. The extracts BRC-016, BRC-066, BRC-071, BRC-090, BRC-096, and BRC-238 were selected for further IC₅₀ determination. Preliminary results showed that IC₅₀ values ranged from 0.04 µg/mL (BRC-096 in 501mel) to 19.43 µg/mL (BRC-090 in MCF-7). Additionally, a pattern of selectivity between extracts was observed depending on the tested cell line, suggesting the presence of bioactive substances acting on specific molecular targets. The bacterial strains producing the active extracts are currently undergoing molecular identification and the extracts will be submitted to preliminary chemical composition analysis by liquid chromatography coupled to mass spectrometry (LC-MS). These findings highlight the innovative potential of cave microbiota as a source of new antitumor agents, contributing to the advancement of natural product pharmacology in the context of human health.

12.014 Mechanistic Modeling of Biopharmaceutics Data to Enhance the Bioavailability of a Weakly Basic Drug: Ketoconazole–Succinic Acid Cocrystal Mitigates pH-dependent Absorption. Goes AKS^{1,2}, Soliman A^{1,3}, Olivera PR², de Moraes NV¹ ¹University of Florida, College of Pharmacy, Dept. of Pharmaceutics, Center for Pharmacometrics and Systems Pharmacology, USA ²Unicentro, Dept Pharmacy, Guarapuava, PR, Brazil. ³Helwan University, School of Pharmacy, Dept Pharmacy Practice, Egypt

Pharmaceutical cocrystals have emerged as a promising strategy to enhance absorption of poorly soluble drugs, particularly those classified as Biopharmaceutics Classification System (BCS) Class II. Ketoconazole (KTZ), a weakly basic antifungal agent, exhibits dissolution-limited absorption and variable oral bioavailability, both of which are highly sensitive to gastric pH. Cocrystallization of KTZ with succinic acid (SA) has been shown to improve its *in vitro* solubility and dissolution profile. In this study, we employed mechanistic modeling of *in vitro* biopharmaceutics data and physiologically based pharmacokinetic (PBPK) modeling to predict the *in vivo* performance of KTZ-SA cocrystal under varying gastric pH conditions, and in the presence of a drug–drug interaction (DDI) with omeprazole. The PBPK model was developed in SimCYP™ V24, incorporating *in vitro* solubility and dissolution data simulated and biorelevant media. Key parameters, including the bile micelle:water partition coefficients and particle size distribution (p-PSD) were estimated using the SIVA Toolkit. KTZ's nonlinear clearance was modeled by adjusting intrinsic clearance values across oral doses ranging from 100 to 800 mg. The model was verified using clinical pharmacokinetic data from healthy volunteers. For the KTZ-SA cocrystal, experimentally derived solubility parameters and p-PSD were integrated into the model. Simulations in healthy virtual individuals demonstrated accurate prediction of plasma concentration-time profiles, with area under the concentration-time curve from 0 to 24 h (AUC) ratios within the accepted 2-fold range. Under simulated achlorhydric conditions, KTZ exposure was markedly reduced (AUC: 1.11 µg·h/mL; fraction absorbed [Fa] < 0.2) compared to healthy subjects (AUC: 12.68 µg·h/mL; Fa = 0.8), reflecting impaired solubility and absorption. In contrast, KTZ-SA maintained high systemic exposure regardless of gastric pH, with similar AUC in achlorhydria (16.9 µg·h/mL) and healthy individuals (17 µg·h/mL). The gastric pH interaction module was used to simulate the interaction between omeprazole and ketoconazole. Our simulations showed reduced KTZ exposure when coadministered with omeprazole (AUC: 3.1 µg·h/mL), as compared to healthy subjects (AUC: 12.2 µg·h/mL), following a 200 mg dose. In contrast, KTZ-SA cocrystal achieved consistently similar exposure in healthy subjects either alone (AUC: 17.2 µg·h/mL), or when coadministered with omeprazole (AUC: 16.9 µg·h/mL). In conclusion, PBPK modeling successfully captured the influence of gastric pH on KTZ absorption. KTZ-SA demonstrated robust performance across physiological and pharmacological elevations of gastric pH, supporting its potential use to ensure consistent bioavailability in patients with altered gastric acidity.

12.015 Beyond Solubility: How Nanoencapsulation Boosts Seriniquinone Pharmacological Activity.

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Introduction: Seriniquinone (SQ) is a promising drug candidate for antifungal and antimelanoma therapy, having dermcidin as a putative, novel, and unique pharmacological target. However, efforts to enable its *in vivo* administration by improving its poor water solubility have not succeeded. In this work, we aimed to encapsulate SQ into poly(lactic-co-glycolic) acid (PLGA) nanoparticles (NPs), and to characterize and evaluate the biological activity of the nanocarriers. **Methods:** NPs were prepared by single emulsion-solvent evaporation and characterized via light scattering, electron microscopy, thermal analysis, and FTIR. SQ antifungal activity was tested by broth microdilution. Antimelanoma effects were evaluated in SK-MEL-28 and -147 monolayers using SRB, clonogenic assays, flow cytometry, and confocal microscopy. Homotypic and heterotypic (with THP-1 cells) spheroids were used to assess viability and NP-mediated drug penetration with fluorescent probes. PLGA-SQ-NPs were injected into *G. mellonella* at doses up to 80 mg/kg. **Results:** Spherical NPs (260–280 nm) with ~83% encapsulation efficiency were obtained. No drug precipitates were observed by microscopy, suggesting effective incorporation. Strong SQ–PLGA interactions were inferred, correlating with ~16% *in vitro* release after 96 h. SQ antifungal activity was preserved against *Cryptococcus* spp. and *S. brasiliensis*, but MICs increased ≥4-fold in *Candida* spp. Encapsulated SQ retained antimelanoma activity in monolayers and spheroids. In monolayers, NPs showed depot-like behavior, enhancing long-term cytotoxicity and SQ uptake via clathrin-mediated endocytosis. IC₅₀ values increased 3.7–14.7-fold in homotypic spheroids, reflecting model complexity. Yet, in SK-MEL-28 spheroids, encapsulated SQ showed a 2-fold lower IC₅₀ than free SQ. Fluorescent probes confirmed NP penetration and broader drug distribution. In SK-MEL-147 spheroids, NPs provoked an extra ~25% reduction in viability, likely due to solubility improvement. In spheroids co-cultured with THP-1 cells, IC₅₀ values increased by 2.2–2.7-fold, but NPs still reduced IC₅₀ by 14–50% across models. Encapsulation enabled a ~53-fold increase in the dose of injectable SQ in *G. mellonella*, without toxicity. **Conclusion:** In a nutshell, the PLGA-based platform goes beyond improving SQ solubility: it enhances pharmacological performance and enables its administration *in vivo*.

12.016 **Overcoming Drug Resistance in Osteosarcoma: Investigating Palladium-oxime Complexes as Potential Antitumor Agents.** Pereira THR¹, Guarache GC¹, Moura TR², Godoy Netto AV², Bincoletto C¹ ¹Unifesp-EPM, PPG Pharmacology, Brazil. ²Unesp, Institute of Chemistry, Brazil

Introduction. Drug resistance represents a significant challenge in tumor eradication. One of the main mechanisms driving this resistance is the increased expression of membrane transport proteins known as ABC proteins. These proteins play a crucial role in drug efflux to the extracellular space, thereby reducing the effectiveness of therapeutic agents. Among them, P-glycoprotein, encoded by ABCB1 (also known as MDR1, or Multidrug Resistance-1), is particularly noteworthy, as its overexpression is strongly associated with cellular resistance. In this context, Palladium-Oxime Complexes (POC) have been investigated as potential anti-tumor agents due to their ability to circumvent resistance mechanisms and induce cell death, even in highly resistant tumor cells. This study aimed to generate cell lines resistant to chemotherapeutics commonly used in osteosarcoma treatment, specifically methotrexate and doxorubicin. The research focuses on evaluating the metabolic and morphological changes acquired by these resistant cells while also investigating the antitumor potential of POC. **Methods.** Using the human osteosarcoma cell line MG-63, two resistant variants were generated: methotrexate-resistant (M63-MTX) and doxorubicin-resistant (M63-DOX). These resistant cell lines were assessed for viability via the MTT assay to determine both the degree of resistance and the cytotoxic potential of POC. Glycoprotein-P expression was evaluated through immunofluorescence assays and Western blotting. **Results.** Following successive exposures of MG-63 cells to methotrexate and doxorubicin, resistant cell lines M63-MTX and M63-DOX were successfully developed. Resistance was confirmed via MTT assays performed after 24 hours of treatment with the respective drug (12.5 μ M - 100 μ M). Furthermore, glycoprotein-P expression was significantly elevated in resistant cell lines compared to the parental MG-63 cells. Notably, MTT assays demonstrated the potent cytotoxic effects of POC, even against the resistant cell lines. **Conclusion.** The resistant cell lines showed a progressive increase in resistance with each successive drug exposure, accompanied by enhanced glycoprotein-P expression. This suggests that these cells developed distinct metabolic and morphological alterations compared to the parental MG-63 cell line, highlighting the remarkable adaptability of tumor cells. Importantly, POC exhibited strong cytotoxic activity, reinforcing their potential as chemotherapeutic agents, particularly against drug-resistant tumor cells. **Financial support:** CAPES, FAPESP and CNPq.

12.017 Drug Discovery: New PAD-4 Inhibitors HITs for The Treatment of Inflammatory Diseases.

Augusto PSA, Costa VF, Cunha TM, Cunha, FQ FMRP-USP

Pharmacotherapy options for inflammatory diseases are still limited. Neutrophil extracellular traps (NETs) triggered chronic inflammatory diseases, despite their physiological functions. Peptidyl-arginine deiminase 4 (PAD-4) is one of the key enzymes of NET formation by mediating histone citrullination. Therefore, discovering PAD-4 inhibitors would be a pharmacological approach to prevent NET formation and consequently reduce tissue damage in acute and chronic inflammatory diseases. The Center for Research in Inflammatory Diseases (CRID) labs have screened HITs for PAD-4 inhibitors for commercial chemical libraries which presents a broad chemical diversity, including isolated natural products, terpenes and alkaloids. The first aim of this study is to validate a high-throughput assay to measure NET release. Then, the main goal is evaluating *in vitro* PAD-4 inhibitory activity of HITs. Neutrophils of healthy volunteers were isolated by Percoll gradient, pre-treated with PAD-4 inhibitors HITs in a range from 3 nM to 1000 nM, or GSK-484, a standard PAD-4 inhibitor, and stimulated with ionomycin (CONEP – CAAE: 30248420.9.0000.5440). Free DNA levels were measured for 6 hours using nucleic acid stain. We confirmed PAD-4 dependent NET formation using Western Blot and ELISA for citrullinated histone (H3Cit) and immunofluorescence for H3Cit and myeloperoxidase (MPO). Ionomycin induced PAD-4 dependent NET release, measured as DNA-free cell and validated by H3Cit quantification measured in the confirmatory assays. HITs were effective in reducing NET induced by ionomycin in a concentration-dependent manner and were classified by IC₅₀. Also, 4 of them demonstrate activity in a post-treatment assay measuring cell-free DNA. HITs demonstrated no cytotoxicity measured by free-DNA and LDH levels. The inhibitors were submitted to confirmatory assays, which demonstrated effectively reduction of histone citrullination and, consequently, inhibition of PAD-4 activity. Free DNA levels measurement was characterized and validated as a high-throughput method to screening HITs for PAD-4 inhibitor drugs. Drugs presented the lower IC₅₀ will further be evaluated in *in vivo* models of rheumatoid arthritis, sepsis, COVID-19, and neuropathic pain. **Financial support:** The São Paulo Research Foundation (FAPESP).

12.018 AI-Driven Analysis Reveals Macrophage Reprogramming by TPPU for Anti-inflammatory Therapeutic Repurposing. Rodrigues WF¹, Miguel CB², Leite MS¹, Maraia ME³, Lazo-Chica JE⁴, Soares SC², Góes-Neto A¹, Santos MA¹ ¹UFMG, Postgraduate Program in Bioinformatics, Belo Horizonte, MG, Brazil ²UFTM, Postgraduate Program in Tropical Medicine and Infectious Diseases, Uberaba, MG, Brazil ³UFTM, Undergraduate Program in Biomedicine, Uberaba, MG, Brazil ⁴UFTM, Lab Cell Biology, Dept Structural Biology, Institute of Biological and Natural Sciences, Uberaba, MG, Brazil

Introduction: The pursuit of novel therapies for chronic inflammatory diseases, such as temporomandibular joint osteoarthritis (TMJOA), has accelerated AI-assisted drug repurposing. TPPU, a potent soluble epoxide hydrolase inhibitor with anti-inflammatory properties, is a promising candidate, yet its impact on macrophages remains underexplored. This study employed AI to decode the transcriptional reprogramming induced by TPPU in LPS-stimulated RAW264.7 macrophages, a cellular model mimicking the inflammatory environment of TMJOA. **Objective:** To identify the transcriptional signatures and molecular reprogramming patterns modulated by TPPU through AI approaches, thereby validating its potential for repurposing as a precision therapeutic agent for chronic inflammatory diseases. **Methods:** RNA-seq data (GSE285011), FPKM-normalized, were preprocessed and standardized using z-score transformation. Singular Value Decomposition (SVD) was employed to extract latent transcriptional components, followed by logistic regression to assess classification performance. Differential expression patterns were examined from the top-ranking genes, with visualizations via heatmaps, dendrograms, boxplots, and gene importance curves using Python pipelines with a reproducibility-focused workflow. **Results and Discussion:** AI-guided modeling revealed a robust stratification (AUC = 1.0) between TPPU-treated and control macrophages, indicating a distinct molecular response to the treatment. Analysis of the most influential genes suggests a functional reprogramming, characterized by the modulation of pathways associated with nuclear signaling, stress response, and biosynthetic activity. This transcriptional shift indicates a transition from a pro-inflammatory phenotype toward a more restorative or pro-resolving state, forming a coherent molecular fingerprint that reinforces TPPU's pharmacological relevance. **Conclusion:** This study demonstrates that TPPU triggers a coordinated transcriptional reprogramming in inflammatory macrophages, unveiled by AI tools. The identified molecular landscape provides a solid foundation for repurposing TPPU in chronic inflammatory contexts demanding targeted immunomodulation. These findings reinforce the synergy between AI and transcriptomics in accelerating therapeutic innovation and provide a reproducible computational framework for future biomarker discovery.

12.019 **The role of ERO1 α as a drug target for the inhibition of cellular proliferation.** Aljofan M, Khojayeveva K, Moldeshva A. Nazarbayev University, Dept Biomedical Sciences, School of Medicine, Kazakhstan

Background: Endoplasmic reticulum oxidoreductase 1 alpha (ERO1 α) is an endoplasmic reticulum-resident thiol oxidoreductase that catalyzes the formation of disulfide bonds for proper folding of proteins. ERO1 α facilitates oxidative protein folding by reoxidizing protein disulfide isomerase (PDI), thereby maintaining a redox homeostasis in the endoplasmic reticulum. Evidence shows that ERO1 α protein level is proportionally associated with poor prognosis in a range of malignancies including, fibrosarcomas, colon, lung, liver, pancreatic, cervical and breast cancer, including triple-negative breast cancer (TNBC). **Objective:** Given its critical role in tumor cell survival and poor prognosis, ERO1 α represents a promising molecular target for anticancer therapy. This study aims to investigate the level of ERO1 α in different breast cancer cells, and its effect on cancer cell survival. **Methods:** Protein expression level of ERO1 α assessed in different breast cancer cell lines including MCF-7, MDA-MB-468, T47D, and the TNBC model MDA-MB-231 cells. **Results:** Western blot analysis showed that MDA-MB-231 cells express a significantly higher ERO1 α protein levels compared to the other tested cell lines. A cellular treatment with 250 μ M of resveratrol, a naturally occurring polyphenol, significantly reduced ERO1 α protein levels, cancer cell viability in a dose-dependent manner, and Reactive Oxygen Species (ROS) levels as measured by the d-ROMs test. **Conclusion:** The current results warrant further study into the potential role of ERO1 α as a target for anticancer drug therapy.

13. Pharmacology Education and Technology

13.001 **Assessment of Healthcare Professionals in Public Hospital Knowledge on the Use of Pressurized Metered-Dose and Dry Powder Inhalers.** Moreira EM¹, Silva MCB¹, Oliveira HR², Nunes GM², Mendes GC², Araújo LCG², Borges AC², Nascimento AR¹, Fontes MLS¹, Silva GS¹, Gonçalves YS¹, Jacobina KF¹, Murcy, ACI, Castro PFS¹ ¹UEG, UnU Goiânia - ESEFFEGO, Brasil, ²UEG, UnU Itumbiara, Brasil

The treatment of obstructive respiratory diseases is based in administration of drugs through inhalation devices. The correct execution of the inhalation technique is essential for symptom relief, and prevention of complications. Therefore, healthcare professionals play a crucial role in the management of these devices. However, it has been observed that many of these professionals lack adequate knowledge and training, which may compromise the effectiveness of pharmacological therapy. This study aimed to assess, in a public hospital in the southern part of the state of Goiás, the level of knowledge of healthcare professionals regarding the correct use of dry powder inhaler (DPI) and pressurized metered-dose inhalers (MDI). This is a quantitative, descriptive, cross-sectional study carried out in a public hospital in southern Goiás, Brazil, from August 25 to 28, 2023. Pharmacists, nurses, and nursing technicians answered a validated and standardized questionnaire about the use of the MDI and DPI and handled them, simulating administration. In this scenario, researchers used a checklist to assess the inhalation techniques for two different devices. According to the Brazilian Society of Pulmonology and Tisiology Guidelines for Asthma Management 2012, the checklist details the steps involved in correctly using each device, and the researchers observed participants to determine if they followed the steps accurately. Professionals who were actively working during the data collection period, who agreed to participate and signed the consent form, were included. Research Ethics Committee: 6.140.301. Data were analyzed using Jamovi® software, applying the chi-square test, with a significance level of $p < 0.05$. A total of 41 professionals participated in the study, including 3 pharmacists, 14 nursing technicians, and 24 nurses. Overall, all groups demonstrated unsatisfactory performance in both theoretical and practical evaluations. The most common errors identified were: Failure to exhale before inhalation; Absence of an inspiratory pause of 5 to 10 seconds; Inadequate inhalation technique (fast and deep for DPI, slow and deep for MDI); Lack of mouth rinsing after corticosteroid use; and, for MDI, difficulty coordinating actuation with inhalation. Pharmacists performed better than the other professional groups, followed by nurses, and lastly, nursing technicians. A significant correlation was found between theoretical knowledge and practical performance, particularly in items such as correct mouthpiece positioning, proper inhalation, and breath-hold technique. The study revealed that healthcare professionals demonstrated insufficient theoretical and practical knowledge regarding the correct use of MDI and DPI. The most frequent errors included failing to exhale before inhalation, skipping the inspiratory pause, improper inhalation technique, and neglecting mouth rinsing. These findings highlight the need for continuous professional training and greater emphasis on this subject in academic curricula to improve therapeutic adherence and clinical outcomes for patients. **Financial support:** Universidade Estadual de Goiás (UEG).

13.002 Do Primary Health Care Professionals Know How to Use Inhaler Devices? A Study in Central-West Brazil. Oliveira HR¹, Borges TS¹, Vasconcelos CO¹, Fonseca FV¹, Silva MCB², Nascimento AR², Fontes MLS², Juliano FR¹, Castro PFS² UEG, UnU Itumbiara, Brasil, ²UEG, UnU Goiânia - ESEFFEGO, Brasil

Primary health care (PHC) provides vigilance for patients with respiratory diseases. Pharmacological treatment often involves the administration of drugs via inhaler devices. The correct execution of the inhaler technique by patients is a crucial practice for symptom relief. It has been observed that health care professionals in PHC often lack specific knowledge and training regarding the use of inhalers, and their educational background directly impacts the effectiveness of pharmacological therapy for patients. This study aimed to evaluate the level of theoretical and practical knowledge among PHC professionals in the South of Goiás, Brazil, regarding the correct techniques for using unit-dose dry powder inhalers (DPI) and pressurized metered-dose inhalers (MDI). Healthcare professionals were assessed through a validated, standardized, self-administered questionnaire comprising 22 objective multiple-choice questions related to the devices. According to the Brazilian Society of Pulmonology and Tisiology Guidelines for Asthma Management 2012, the practical demonstration of the technique with the devices in hand was also evaluated. Research Ethics Committee: 7.097.585. Data were analyzed using the Jamovi software, applying the chi-square test, with a significance level of $p < 0.05$. All 75 professionals from PHC were invited to participate between Dec 2024 and Mar 2025. Of these, 52 (69.3%) accepted the invitation, including 12 physicians (52.2%), 20 nurses (90.9%), 15 nursing technicians (62.5%), and 5 pharmacists (100%). The theoretical knowledge about MDI showed a significant difference ($p < 0.05$) in the question "How should you breathe before using the inhaler?" in the physician group. Regarding practical knowledge of MDI, for the item "Performing exhalation," a significant difference ($p < 0.05$) was also observed in the physician group. Similarly, the theoretical knowledge about DPIs showed a significant difference ($p < 0.05$) regarding the question "How should you breathe before using the inhaler?". For DPI use, the "Insert the capsule" item showed significance ($p < 0.05$) in the pharmacist group. This demonstrates a lower level of knowledge about how to prepare DPI for inhalation. Across all groups, there was a low percentage of correct answers (0.0%-26.7%) concerning a slight backward tilt of the head in both devices. Interestingly, the question "How should you inhale when using the medication?" showed a low percentage of correct answers for DPI (0.0%-33.3) and a high percentage for MDI (75%-100%) among the groups studied. The correlation between theoretical vs practical knowledge was significant ($p < 0.05$) for exhalation before inhalation and for 5-10 second apnea immediately after inhalation. The study concluded that the physicians' group presented a higher level of knowledge compared to nurses, nursing technicians, and pharmacists regarding the need for exhalation before inhalation. These results indicate differences in the academic training of the health professionals evaluated. Furthermore, the level of knowledge in MDI and DPI remains inconsistent across primary health care professionals. It indicates the need for pharmacology educational strategies to reduce this deficit.

Financial support: Universidade Estadual de Goiás (UEG).

13.003 Simulated Jury as an Active Learning Strategy for Teaching Medication Deprescribing to Medical Students. Rosa JG, Barros HMT UFCSPA, Porto Alegre, Brazil

Medication deprescribing is an essential yet underrepresented topic in medical education, especially in the context of increasing polypharmacy. This study describes the development and implementation of an innovative pedagogical strategy utilizing a simulated jury trial to teach deprescribing within a pharmacology curriculum. A total of 181 third- and fourth-year medical students participated in a structured four-phase educational model: Deprescribing Facts, D-Clinical Experience, Designation, and Debate. The intervention comprised theoretical instruction, a dedicated digital platform, real-patient data collection, and simulated jury sessions with role-based assignments. Clinical cases were analyzed using the Beers Criteria, STOP/START protocols, and the Medication Appropriateness Index. Educational outcomes were assessed through cognitive tests, Likert-scale perception surveys, and qualitative feedback. Mean cognitive scores ranged from 9.57 to 9.82. Perceived clinical relevance was rated between 4.24 and 4.71. Female students consistently outperformed their male peers. Thematic analysis highlighted high levels of student engagement, integration of theoretical and practical knowledge, and the development of clinical reasoning skills. The simulated jury model proved to be a high-impact educational approach for teaching deprescribing and is recommended for incorporation in intermediate or advanced stages of medical training. Keywords: Active learning; Deprescribing; Pharmacology education Funding: Teaching Initiation Project (PID) Scholarship – Federal University of Health Sciences of Porto Alegre (UFCSPA). Ethics approval: This study did not meet the criteria requiring submission to a Research Ethics Committee, in accordance with the Brazilian National Health Council Resolution No. 466/2012.

13.004 Pharmacology UNO and the Hypertension Card Deck: Gamified Strategies to Teach Lipid-Lowering and Antihypertensive Agents. Santa Clara-Amorim RC, Higashijima, AN, Lívero FADR UFPR, Lab. of Cardiometabolic Pharmacology, PPG Pharmacology, Brazil

Cardiometabolic diseases (CMDs) comprise a group of chronic conditions involving complex physiological processes and multiple treatment options. Dyslipidemia, for instance, plays a key role in the incidence and severity of these diseases, requiring a solid understanding of the mechanisms of action and potential adverse effects of lipid-lowering agents. Similarly, the treatment of hypertension demands clear knowledge of the major systems that regulate blood pressure, along with mastery of the main drug classes used in its management. These include diuretics, angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers, calcium channel blockers, and sympatholytics. This knowledge is essential for rational clinical practice, allowing for safer and more effective therapeutic decisions. However, the high density of content and the complexity of these mechanisms require approaches that go beyond simple memorization, promoting clinical reasoning as well. In this context, gamification emerges as a promising educational tool, capable of increasing student engagement and improving knowledge retention. This study presents two playful teaching tools: *Pharmacology UNO* (PharmUNO) and the *Hypertension Card Deck*. PharmUNO was adapted to review content on dyslipidemia, using colored cards to represent five agents—statins, ezetimibe, cholestyramine, niacin, and fibrates—and their respective mechanisms of action (HMG-CoA reductase inhibition, inhibition of intestinal cholesterol absorption, bile acid sequestration, inhibition of lipase activity in adipose tissue, and PPAR α activation with increased lipid oxidation, respectively). The game also includes standard UNO® booster cards (wild, reverse, skip, draw two, and draw four) to stimulate healthy competition and strategic thinking. Each player starts with seven cards, gameplay flows clockwise, and the goal is to discard all cards, shouting “UNO!” when only one remains. In PharmUNO, players must match a drug card with its corresponding mechanism to continue, reflecting the game’s pedagogical aim. The *Hypertension Card Deck*, on the other hand, is structured around forming logical sets of four related cards. Each player starts with four cards, and gameplay involves drawing a card from the pile or picking up the last discarded one. The objective is to match a medication—captopril, hydrochlorothiazide, amlodipine, propranolol, or spironolactone—with its mechanism of action and two characteristic side effects. The game ends when a player forms a complete combination—for example: captopril + ACE inhibitor + dry cough + angioedema. While the Hypertension Card Deck reinforces clinical reasoning and the connection between drug actions and adverse effects, PharmUNO emphasizes memorization and playful engagement. Together, these tools contribute to a portfolio of active learning strategies for innovative teaching in cardiometabolic pharmacology. By fostering engagement, reinforcing key pharmacological concepts, and encouraging critical thinking, PharmUNO and the Hypertension Card Deck exemplify how gamified resources can enhance the learning experience in complex subject areas. This project was supported by Universidade Paranaense (UNIPAR) and National Council for Scientific and Technological Development (CNPq; no. 306919/2021-4).

14. Pharmacology: Other

14.001 **Influence of Treatment Duration and Administration Routes on Therapeutic Approaches for Melasma:** A Systematic Review and Meta-analysis. Dalagrana H¹, Ribeiro MM², Galiciolli MEA², Oliveira CL², Garlet QI^{1,3} ¹UFPR, Curso de Farmácia, Brasil, ²IPPPP, Brasil, ³UFPR, PPG Farmacologia, Brasil

Introduction: Melasma is a chronic skin condition resulting from increased melanogenic activity, which causes a significant emotional impact on the patient's quality of life. The efficacy of melasma treatments depends on treatment duration, routes of administration, individual response, and the chosen therapeutic approach, which may include topical skin-lightening agents, oral drugs, and chemical peels. **Methods:** The aim of this study was to assess the impact of treatment duration and diverse routes of administration on the Melasma Area Severity Index (MASI). The protocol followed the PRISMA guidelines and was registered in PROSPERO (#CRD420202214886). The PICO acronym was used as follows: P = patients with melasma, I = available melasma treatments, C = with or without control group and O = primary: melasma control efficacy. A meta-analysis was performed using the MASI mean $\pm$ standard deviation (SD) with a 95% confidence interval. We analyzed continuous variables by the inverse variance method using a random-effects model and weighted means relative to the sample size of each study. The studies were grouped into short-duration treatments (SDT: 2-8 weeks) and long-duration treatments (LDT: 12-24 weeks). Within each time frame, we conducted a subgroup analysis considering the route of administration: oral, topical, chemical peel, and mixed administration routes (MAR). Heterogeneity among studies was assessed using the I^2 index, and methodological quality was analyzed using the ROBINS-I and RoB 2 tools. Additionally, funnel plots were used to evaluate publication bias. **Results:** Quantitative analysis included 34 studies. The meta-analysis by treatment duration detected a significant reduction in MASI for both short-term treatments (-1.32 [-1.65, -0.98]; $p < 0.00001$; $I^2 = 76\%$) and long-term treatments (-1.65 [-2.10, -1.21]; $p < 0.00001$; $I^2 = 94\%$). The overall effect was -1.52 [-1.82, -1.22]; $Z = 10.03$; $p < 0.00001$; $I^2 = 91\%$. Additionally, among SDT, stratified meta-analysis by route of administration showed a reduction in MASI after topical treatment (-1.68 [-2.28, -1.07]; $p < 0.00001$; $I^2 = 74\%$), chemical peeling (-0.72 [-1.08, -0.37]; $p < 0.0001$; $I^2 = 51\%$) and MAR (-1.53 [-2.08, -0.99]; $p < 0.00001$; $I^2 = 64\%$). On the other hand, among LDT, stratified meta-analysis by route of administration showed a significant reduction in MASI after topical treatment (-1.22 [-1.99, -0.46]; $p < 0.00001$; $I^2 = 96\%$), peeling (-2.08 [-3.31, -0.85]; $p < 0.00001$; $I^2 = 97\%$), and oral treatment (-1.50 [-2.65, -0.36]; $p < 0.00001$; $I^2 = 95\%$). Moreover, we detected an improvement in patients' quality of life after treatment (-1.26 [-1.49, -1.04]; Overall effect: $Z = 11.05$; $p < 0.00001$; $I^2 = 82\%$). **Conclusion:** Treatment of melasma, regardless of the route of administration, results in a significant improvement on MASI, which appears alongside treatment duration. Analysis by route of administration revealed that topical interventions and combined procedures had a greater magnitude of effect. Despite heterogeneity among the included studies, the data reinforce the effectiveness of the therapeutic approaches analyzed and highlight the need for clinical studies with more consistent methodologies and robust experimental designs to consolidate current evidence. **Financial Support:** None. **Keywords:** Melasma, quality-of-life, MASI, hyperpigmentation

14.002 **Economic Evaluation of Pharmaceutical Care in Warfarin-Anticoagulated Patients.** Oliveira IA, Vasconcelos PENS, Marcatto L, Santos PCJL Unifesp-EPM, Depto de Farmacologia, Brasil

Introduction: Warfarin is widely used in the prevention of thromboembolic events, but its safe and effective use depends on the time in the therapeutic range (TTR), a quality parameter measured by the INR (International Normalized Ratio) values. Due to individual variability in treatment response, interventions that favor adequate control of anticoagulation are essential. The performance of the clinical pharmacist has shown promise in the optimization of the TTR and, in this context, pharmacoeconomic studies are extremely important to determine the real cost-effectiveness of these interventions. **Objective:** To evaluate economically, in a partial way, the cost of increased TTR in patients using warfarin in pharmaceutical care. **Methods:** This study was approved by the Research Ethics Committee of the Instituto do Coração (InCor) da Faculdade de Medicina FMUSP da Universidade de São Paulo (SDC 4033/14/013). Patients were followed up for 12 weeks, INR values were assessed (INR target: 2-3) and, when necessary, dose adjustments were performed. During the first four consultations, the patients' INR was measured every 7 days, evaluated by a pharmacist and, if necessary, a medical consultation was made. After 4 weeks, if the INR was within the therapeutic range, the patient would return in 30 days, whereas if the INR was out, the patient would return in 7 days. Adherence assessment was measured by means of questionnaires and by counting the pills taken. For the economic evaluation, the following were considered: Hourly rate based on the average salary of the professionals who work in the Sistema Único de Saúde (SUS), considering cardiologists and pharmacists, divided by the number of hours worked monthly. This approach was adopted due to the absence of specific values in the Sistema de Gerenciamento da Tabela de Procedimentos, Medicamentos e OPM do SUS (SIGTAP). After determining the hourly cost of the consultation, an estimate of the average number of consultations performed during the twelve-week period was made to calculate the total cost per patient with the pharmaceutical intervention. **Results:** We included 285 patients with atrial fibrillation (AF) and without a stable dose of warfarin. The mean initial TTR of 1 year before was lower than the TTR value after 12 weeks of treatment with pharmaceutical care (0.320 ± 0.015 ; 0.540 ± 0.015 , $p < 0.001$). There was a 22% increase in costs of approximately R\$ 250.00 per patient. **Conclusion:** This clinical benefit suggests that it has a relatively low cost, which reinforces the relevance of management to provide pharmaceutical care in primary care. Consequently, it is suggested to reduce the care and financial burden on the health system.

14.003 **Evaluation of the Incidence of Electrolyte Disturbances in Patients Undergoing Allogeneic Hematopoietic Cell Transplantation Treated with Calcineurin Inhibitors.** Camargo MCF¹, Costa-Junior LC^{2,3}, Leão AML⁴, Fernandez TS⁴, Santos PCJL^{1,2} ¹Unifesp-EPM, São Paulo, PPG Farmacologia, Brasil, ²FMUSP, PPG Ciências Médicas ³INCa, Bone Marrow Transplant Center, Instituto Nacional de Câncer, Rio de Janeiro, RJ, Brasil, ⁴INCa, Cytogenetic Lab, Cell and Gene Therapy Program, Rio de Janeiro, RJ, Brasil

Introduction: Hematopoietic Cell Transplantation (HCT) is a well-established therapy in the treatment of several malignant and benign hematological diseases. Despite the advances, complications such as Graft-versus-Host Disease (GVHD) still compromise the success of the treatment. To prevent it, calcineurin inhibitors are widely used, such as tacrolimus and cyclosporine, which, although effective in immunosuppression, are associated with electrolyte alterations that potentially aggravate the clinical condition of patients. Objective: To evaluate the influence of calcineurin inhibitors on the incidence of electrolyte disturbances in HCT recipients.

Methods: A retrospective, single-center study was conducted including patients who underwent allogeneic HCT from 2010 to 2023. The presence of electrolyte disturbances was assessed in the first 21 days after the start of immunosuppressants. The diagnosis and degree of each clinical condition were classified according to the 2017 CTCAE criteria. Univariate logistic regression was also performed to analyze the association between the presence and degree of disorders and the occurrence of Acute Kidney Injury (AKI) and moderate or severe AKI (grade 2 or 3 according to the KDIGO). This work was financially supported by FAPESP 2024/07284-9. **Results:** 374 patients were included, most of whom were adults (67.1%), males (58.8%) and treated with cyclosporine (81.3%). The main diagnoses observed were acute and chronic myeloid leukemia and acute lymphoblastic leukemia, representing 67.9% of the patients. The presence of grade 2-3 AKI was represented in 28.9% of the patients. Low serum concentrations were predominant for all electrolytes evaluated, with hypokalemia present in 62.6% of the patients, hypomagnesemia in 85.6%, hyponatremia in 75.7%, and hypocalcemia in 86.6%. In patients who developed hypocalcemia, 49.7% already had grade 2 of the disorder. Significant associations were observed between the categories presence of grade 2-3 AKI and the presence of hypokalemia ($p < 0.001$), hypermagnesemia ($p = 0.001$) and hypernatremia ($p < 0.001$) and, more specifically, between the presence of grade 2-3 AKI and the grades of each of these disorders. Patients with AKI (any grade) were significantly more likely to have hypokalemia compared to those without AKI, and patients with grade 2-3 AKI are nearly three times more likely to have hypokalemia compared to those without these stages. Patients with hypermagnesemia were 2.34 times more likely to develop grade 2-3 AKI and patients with hypernatremia were 3.22 times more likely. **Conclusion:** These findings indicate that hypokalemia, hypermagnesemia, and hypernatremia are significantly associated with the occurrence and severity of AKI. Further, early correction of electrolyte imbalances in patients at risk of AKI may contribute not only as markers but also possibly as mediators of renal injury progression.

14.004 **Alternative Models to Murine Lethality Tests: Snake Venoms and Antivenoms.** Luders SS¹, Silva GSA¹, Schiess MC¹, Hosch NG¹, Dallazen JL¹, Tasima LJ², Azevedo AMT², Zambelli VO¹ ¹IBU, Lab Pain and Signaling, PPGTox, Brazil, ²IBU, Lab Development and Innovation, PPGTox, Brazil

Introduction: Every year, snake envenomation cases are responsible for a significant number of deaths or severe and long lasting physical and psychological injuries, and as such it is considered a neglected tropical disease by the WHO. In 2023, Brazil registered 32,595 cases of snake envenomation, a significant number that increased when compared to 2022. In Brazil, snake species from the genus *Lachesis* had the highest mortality rates, occurring mainly in the Amazon Rainforest. The most well-distributed treatment involves administering antivenom, a solution of highly specific immunoglobulin fractions, to the patient as quickly as possible. Antivenom production involves the use of mice to determine the potency of both venom and antivenom via lethality tests, the LD50 (venom dose that causes 50% mortality) and the EC50 (antivenom dose capable of protecting 50% of the mice from a previously determined high venom concentration). These results are then used to calculate and standardize antivenom efficiency. In accordance with the 3Rs principle, ethical and suitable alternatives must be considered to replace the use of vertebrate animals. In this study, we aim to substitute lethality tests on murine animals with the neuroblastoma cell line SH-SY5Y, as well as other possible models, for venom toxicity and antivenom efficiency testing. **Methods:** *Lachesis muta* venom and *Lachesis*-specific antivenom were tested on SH-SY5Y cell cultures to determine cell lethality induced by different concentrations of either. SH-SY5Y cells were cultivated in DMEM-F12 media, then plated into 96-well plates and treated with either venom or antivenom for 24h. Venom concentrations ranged initially from 0.30 µg/mL to 40 µg/mL for *Lachesis muta*, followed by a more precise range of 1 µg/mL to 13 µg/mL. Cells were incubated with different concentrations of antivenom to determine whether the antivenom was cytotoxic *per se* in high concentrations. Cell viability was determined by 3-(4,5-dimethylthiazol-2-yl)2,5-diphenyltetrazolium bromide (MTT) assay. Data was obtained by reading absorbance at 570nm and then analyzed by one-way ANOVA with GraphPad Prism. This same program was used to calculate the IC50 by a nonlinear regression curve. **Results:** Results indicate that *Lachesis muta* venom induces a dose-dependent neurotoxicity in SH-SY5Y cell cultures. When treated with venom, a drop in cell viability was observed between concentrations 3.5 µg/mL and 11.85 µg/mL. *Lachesis* antivenom induced significant cytotoxicity in concentrations of 12.5% and higher. An IC50 was successfully calculated, determined to be 10.53 µg/mL. **Conclusion:** The results indicate that SH-SY5Y can accurately represent *Lachesis muta* venom and antivenom toxicity and may be a viable alternative to murine models. Next steps include repeating the same experiments with other snake venoms (such as *Crotalus sp.* and *Bothrops sp.*) testing antivenom potency, as well as testing the nematode *C. elegans* as a substitution model. **Financial Support Information:** SES/SP and CAPES

14.005 **Fluorescence Microplate Reading for Evaluation of Calcium Influx in Mice Cortical Synaptosomes and Study of PEP2.** Alvarenga CC, Pereira TL, Moraes TR, Castro CJ FSCBH, Programa de Pós-graduação Stricto sensu em Medicina e Biomedicina, Belo Horizonte, MG

Introduction: Synaptic signaling is essential for understanding neurological processes and various diseases of the nervous system. Measuring synaptic activity is crucial for studying neurotransmission, receptor functionality, and the effects of pharmacological agents. Traditional methods such as spectrofluorimetry have scalability limitations. This study introduces an innovative method using plate-based microfluorimetry, which allows for the simultaneous assessment of intracellular calcium dynamics in multiple wells, reducing sample and reagent volumes, toxins, analysis time, and costs. **Objective:** To standardize the experimental conditions for measuring intrasynaptosomal calcium using plate-based microfluorimetry in synaptosomes from the mouse cerebral cortex, and to test the activity of PEP2, a synthetic peptide derived from Pha1 β , a toxin from the venom of the spider *Phoneutria nigriventer* with antinociceptive effects. **Methods:** Synaptosomes were obtained from the cerebral cortex of Swiss mice (30g, 12–15 weeks old) and incubated with Fluo-4 to assess the activity of voltage-dependent calcium channels upon stimulation with KCl. Several experiments were conducted to determine the optimal conditions, varying the Fluo-4 concentration, synaptosome dilution, and final volume in each well. After optimization, tests were performed using inhibitors, including the PEP2 peptide, to investigate its ability to reduce calcium influx. Readings were performed on a plate reader, enabling simultaneous data collection. The study was approved by the ethics committee (004/2018). **Results:** The optimal Fluo-4 concentration was 6 μ M, with the best synaptosome dilution being 150:500 (v/v, synaptosomal suspension/KRH volume). The final well volume that produced the greatest response to KCl was 150 μ L. Under these conditions, PEP2 was tested at different concentrations and showed an inhibitory effect on calcium influx, especially at a concentration of 0.01 nM, suggesting potential antinociceptive activity. **Conclusion:** The standardization of this plate-based microfluorimetry protocol represents an efficient strategy for investigating the effects of peptides and pharmacological agents on neuronal processes, contributing to the elucidation of neurotransmission mechanisms and receptor functionality. The PEP2 peptide proved to be promising in inhibiting calcium influx, reinforcing its potential as an antinociceptive agent. **Keywords:** calcium influx, synaptic signaling, synaptosome, PEP2. **Ethics committee:** 004/2018 (valid until 2026) **Financial support:** - FAPEMIG (Fundação de Amparo à Pesquisa do Estado de Minas Gerais - APQ_ 03767/2023; - CNPq (Conselho Nacional de Desenvolvimento Científico e Tecnológico); - CAPES (Coordenação de Aperfeiçoamento de Pessoal de Nível Superior).

14.006 **Latex Agglutination Assay Conjugated with Dispersin: A New Diagnostic Approach for Enteroaggregative *E. coli*.** Florencio C, Moraes CTP, Silva MA, Pollato JM, Piazza RMF, Elias WP IB, Bacteriology Lab, Graduate Program in Toxinology, Brazil

Introduction: Enteroaggregative *Escherichia coli* (EAEC) is one of the leading causes of acute and persistent diarrhea, particularly in developing countries. This condition is of concern due to its association with impaired growth and cognitive development in children living in socioeconomically vulnerable settings. Persistent infection and chronic intestinal inflammation caused by EAEC exacerbate the health burden in affected children, contributing to the vicious cycle of malnutrition and developmental delay. Accurate laboratory identification of EAEC is essential to guide therapeutic decisions and implement effective control strategies. Traditionally, EAEC detection relies on its characteristic aggregative adherence (AA) pattern observed in epithelial cell assays. However, this method requires sophisticated infrastructure and specialized personnel, limiting its use to reference laboratories and impeding implementation in resource-limited endemic areas. With advances in genomics and molecular biology, specific genetic markers of EAEC have been identified, including the *aap* gene, which encodes dispersin—a protein involved in bacterial dispersion across the intestinal mucosa. Dispersin has emerged as a promising target for the specific diagnosis of EAEC. In this study, we developed and evaluated a latex agglutination assay based on dispersin detection, aiming to provide a rapid, simple, and accessible diagnostic tool.

Methods: Recombinant dispersin was produced via gene cloning and expression and used to generate specific polyclonal antibodies. These antibodies were purified and conjugated to latex particles, forming the basis of the assay. The methodology was optimized by evaluating different culture media, including Tryptic Soy Broth (TSB) and MacConkey agar. **Results:** TSB showed superior performance, enabling the detection of 83% of EAEC strains positive for the *aap* gene. The latex agglutination test was compared to an indirect ELISA, which served as the reference standard, and demonstrated high concordance. **Conclusion:** The test exhibited 90% sensitivity, 100% specificity, and 95% overall accuracy, with positive and negative predictive values of 100% and 92%, respectively, indicating its high diagnostic accuracy even in low-resource laboratory settings. Furthermore, the low production cost (US\$ 2.15, approximately R\$ 11.07) makes it a viable and accessible alternative, especially in low-income regions with a high burden of enteric diseases. In conclusion, the latex agglutination test based on dispersin detection is a promising diagnostic tool characterized by ease of use, low cost, and high specificity, with significant potential to support early EAEC diagnosis and epidemiological surveillance in endemic regions. This study was supported by the funding agencies CAPES and FAPESP. All animal procedures were previously approved by the Animal Ethics Committee of the Butantan Institute (CEUA).

14.007 **Characterization of Depression- and Anxiety-Like Behaviours in a Mouse Model of Relapsing-Remitting Multiple Sclerosis.** Peres DS¹, Viero FT¹, Rodrigues P¹, Ruviano NA², Machado AC¹, Frare JM², Lima IR³, Martins G³, Silveira PCL³, Ferreira MA⁴, Silva AM⁴, Ferreira J⁴, Santos GT^{1,2}, ¹UFSM, Graduate Program in Pharmacology; ²UFSM, Graduate Program in Biological Sciences: Toxicological Biochemistry; ³Unesc, Graduate Program in Health Science; ⁴UFSC Graduate Program in Pharmacology

Introduction: Relapsing-remitting multiple sclerosis (RRMS) is an autoimmune neurological disease and is the most common subtype of MS. In addition, it is associated with the development of depression and anxiety. To date, depressive- and anxiety-like behaviours were only studied using models of progressive MS, which causes severe motor alterations. **Methods:** Thus, we sought to standardise the depressive and anxiety-like behaviours in an RRMS model induced by experimental autoimmune encephalomyelitis (RR-EAE) in mice. The RR-EAE model was induced in C57BL/6 female mice using myelin oligodendrocyte glycoprotein (MOG35-55) antigen and Quillaja saponin (Quil A) as an adjuvant. **Results:** The immunisation of RR-EAE did not induce locomotor alteration but caused relapsing-remitting induction of clinical scores in mice until 35 post-immunization (p.i.). Also, increased levels of tumour necrosis factor alpha (TNF- α), astrocyte marker (GFAP), and microglial markers (IBA-1) were detected in the prefrontal cortex at 35 p.i. of RR-EAE. In the open field test, RR-EAE mice showed decreased time spent at the centre and sniffing behaviour (at days 21 and 34 p.i.). Also, on day 35 p.i. the RR-EAE group spent less time in the open arms and had decreased open-arm entries compared to control mice in the elevated plus maze (EPM) test, confirming the anxiety-like behaviour. At day 36^o p.i. in the tail suspension test, mice showed depression-like behaviour with decreased latency time and increased immobility time. **Conclusion:** Thus, the RR-EAE model mimics the neuroinflammatory and behavioural features of the RRMS, including depression- and anxiety-like symptoms. **Financial support:** FAPERGS, CNPq and Capes.

14.008 Zinc-alpha2-Glycoprotein as a Predictive Biomarker and Therapeutic Target in Late-Onset Preeclampsia: Results from Proteomic Analyses. Bertozzi-Matheus M¹, Kaihara JSN¹, Chaguri JL², Santos LD³, Barbosa PO⁴, Cardoso VC⁵, Cavalli RC⁴, Sandrim VC¹. ¹IBB-Unesp-Botucatu, Dept Biophysics and Pharmacology, ²IBB-Unesp-Botucatu, Center of Toxicological Assistance, ³IBB-Unesp-Botucatu, ⁴FMRP-USP, Dept Gynecology and Obstetrics ⁵FMRP-USP, Dept Pediatrics, Brazil

Introduction: Preeclampsia (PE) is a condition characterized by hypertension after the 20th week of pregnancy, accompanied by proteinuria and/or target organ dysfunction. If diagnosed before the 34th week, the disease is classified as early-onset PE (EO-PE). If diagnosed after this period, it is classified as late-onset PE (LO-PE). In addition to hypertension, the disease is also characterized by metabolic alterations, especially in LO-PE. PE has no cure or specific treatment, and the search for pharmacological targets is essential to ensure better maternal and fetal outcomes. Therefore, the aim of this study was to identify potential predictors and therapeutic targets for PE before the development of clinical signs. **Methods:** Plasma from women who later developed LO-PE (6), EO-PE (6) or remained healthy during pregnancy (HP) were collected in the second trimester of pregnancy and analyzed using a liquid nanochromatography system coupled to mass spectrometry. Data were processed using MetaboAnalyst (version 6.0) and subjected to chemometric and univariate analyses. Correlations were assessed using Pearson or Spearman tests. One-way ANOVA, Kruskal–Wallis, and chi-square tests were used to compare clinical features, with $p \leq 0.05$ considered statistically significant. Additionally, a ROC curve was constructed to evaluate the predictive potential of the protein Zinc-alpha2-glycoprotein (ZA2G). **Results:** ZA2G emerged as the most relevant differentially expressed protein, exhibiting an 80% reduction in the LO-PE group. Its circulating levels showed a negative correlation with maternal BMI and strong discriminatory power for LO-PE (AUC 0.976; 95% CI 0.857–1). **Conclusion:** Our findings suggest that ZA2G, a protein involved in lipid metabolism and metabolic disorders, may serve not only as a potential biomarker but also as a therapeutic target in LO-PE. Modulation of this protein and its associated pathways may offer a promising strategy to alleviate the metabolic disturbances in this condition. **Financial support:** This study was supported by the São Paulo Research Foundation (FAPESP) (grant numbers 2008/53593-0 and 2021/12010-7) and by National Council for Scientific and Technological Development (CNPq) (grant numbers 308504/2021-6, 44682/2024-4, 302614/2025-7 and 301293/2018-0). Also, by Coordination for the Improvement of Higher Education Personnel (CAPES) (grant number: 88887940239/2024-00)

14.009 Cinnamaldehyde Modulates 5-FU-Induced Mucositis in a Murine Model: Involvement of Cytokines and Inflammatory and Proliferative Markers Sousa IJO¹, Barbosa BS¹, Silva RLT¹, Pinheiro Neto FR¹, Nogueira KM², Gonçalves RLG¹, Reis ML¹, Oliveira FA¹, Oliveira RCM¹. ¹UFPI, PPG Farmacologia, Brasil; ²UFC, Brasil

Introduction: Most of the classical chemotherapy arsenal employs drugs that affect the cell cycle, such as 5-fluorouracil (5-FU), whose mechanism of action directly interferes with mitotic processes. Due to its high proliferative rate, the gastrointestinal tract mucosa becomes an important target of these drugs toxicity. In this context, 5-FU-induced intestinal mucositis is an inflammatory-degenerative process, with no available curative intervention and a significant negative impact on the efficacy of cancer treatment. Therefore, new therapeutic alternatives have been sought, especially from natural products. In this context, this study aimed to evaluate the cytoprotective potential of cinnamaldehyde (CA), the major phenylpropanoid found in the essential oil of species of the genus *Cinnamomum*, in a murine model of 5-FU-induced intestinal mucositis.

Methods: *Mus musculus* (Swiss) mice were subjected to a 5-FU administration protocol for six days to induce intestinal mucositis and co-treated with CA (1, 10, 25, 50, or 100 mg/kg/day, dissolved in Tween 80 + saline 0.05%). The following were evaluated: weight loss; epithelial injury (luminal release of lactate dehydrogenase (LDH); systemic inflammation (serum C-reactive protein); tissue levels of TNF- α and IL-1 β ; and immunohistochemistry for Ki-67, CD45, and myeloperoxidase (MPO). For the mechanistic design, the most effective dose in the morphometric tests was used. **Results:** 5-FU reduced animal weight by $14.99 \pm 2.7\%$, while CA attenuated the loss by $60.7 \pm 1.9\%$ and $69.7 \pm 2.3\%$ at doses of 25 and 50 mg/kg/day, respectively. The villus/crypt ratio, reduced by $64.14 \pm 1.32\%$ by 5-FU, was preserved at $22.08 \pm 2.9\%$ and $11.67 \pm 2.3\%$ for the same doses. The mean luminal LDH release was 84 ± 8.8 U/L in the control, increasing to 267.6 ± 4.3 U/L with 5-FU, while co-treatment with CA reduced it to 59.8 ± 2.2 ; 64.33 ± 6.3 ; 59.17 ± 4.3 and 84.29 ± 17.1 U/L at doses of 10, 25, 50 and 100 mg/kg/day, respectively. CA (25 mg/kg/day) attenuated the reduction of Ki-67 and the infiltration of CD45 and MPO positive cells. For IL-1 β , 92.75 ± 7.0 pg/mg were observed in the control group, 265.8 ± 58.6 pg/mg in the 5-FU group, and 109.9 ± 11.6 pg/mg in the 5-FU+CA group; for TNF- α , 87.86 ± 14.1 , 174.8 ± 19.8 , and 108.4 ± 11.3 pg/mg, respectively. **Conclusion:** Cinnamaldehyde demonstrated the ability to attenuate weight loss, preserve villous architecture, reduce luminal LDH release, and modulate inflammatory and proliferative mediators, demonstrating its potential as an adjuvant pharmacological candidate in the management of intestinal mucositis associated with 5-FU chemotherapy.

14.010 **A Systematic Review of Predictive Clinical and Imaging Factors in Pediatric Chronic Recurrent Multifocal Osteomyelitis** Lemes FS¹, Maraia ME², Forte GV¹, Rodrigues WF^{1,3}, Miguel CB^{1,3}

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Introduction: Chronic Recurrent Multifocal Osteomyelitis (CRMO) is a rare, noninfectious autoinflammatory bone disorder that typically affects children and adolescents. The disease presents with diverse clinical patterns and imaging findings. Key variables such as age at onset, lesion distribution, and spinal involvement may influence therapeutic response and long-term prognosis. Despite its impact, predictive markers and standardized assessment tools remain limited. **Methods:** A systematic review was conducted using MeSH-based search strategies across six major databases and gray literature. Observational cohort studies reporting on age at symptom onset, lesion site (especially vertebral involvement), number of affected foci, and radiologic follow-up, particularly with whole-body MRI, were prioritized. A narrative synthesis approach was employed. **Results/Discussion:** Patients aged 3–17 years predominated across studies, with outliers such as a 33-year-old case from India (Gamalero et al., 2020). Vertebral involvement, reported in 20–40% of patients across studies, was consistently linked with more severe disease and higher risk of sequelae (Kaut et al., 2022; Andreasen et al., 2022). The Danish cohort demonstrated significant reductions in radiologically active lesions (RALs) and mRINBO scores following pamidronate treatment (median RALmax size from 39 mm to 28 mm; $p < 0.01$). Whole-body MRI emerged as a valuable tool for identifying subclinical lesions and evaluating treatment response. Patients without spinal involvement (Belgian and Indian cohorts) generally had more favorable clinical trajectories. Lesion multiplicity and bilateral bone involvement were also associated with greater therapeutic complexity. **Conclusion:** Age at onset, vertebral involvement, and lesion burden are relevant predictors of disease severity and treatment outcomes in pediatric CRMO. The incorporation of advanced imaging tools, particularly whole-body MRI, and standardized indices such as mRINBO may enhance clinical decision-making and prognosis assessment. **Financial support:** This work was supported by a scholarship from the Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq).

Uso Terapêutico do Nitrito na Hipertensão Gestacional: Investigação em Modelos *in vitro* e Potencial na Aplicação Clínica. Gabriela Morelli Zampieri, Karoliny dos Santos Nunes, Priscila Oliveira Barbosa, Vinícius Aniceto; Ricardo de Carvalho Cavalli e Valéria Cristina Sandrim. Universidade Estadual Paulista, Instituto de Biociências de Botucatu

A hipertensão na gravidez pode ser classificada como hipertensão crônica, hipertensão gestacional e pré-eclâmpsia (PE), e constitui uma das principais causas de mortalidade materno-fetal em todo o mundo. O tratamento visa a prevenção de complicações maternas, limitando a toxicidade dos medicamentos para o feto. Uma parcela significativa das pacientes não responde às terapias anti-hipertensivas atuais, evidenciando a necessidade de novas abordagens terapêuticas. A via nitrato-nitrito-óxido nítrico, via alternativa de produção de óxido nítrico, apresenta propriedades anti-hipertensivas, vasodilatadoras, angiogênicas, e antioxidantes promissoras, já exploradas em doenças cardiovasculares. O projeto investiga o uso terapêutico do nitrito em modelo *in vitro* de PE (células endoteliais de veia umbilical humana incubadas com plasma de gestantes com PE) avaliando a produção de NO, marcadores inflamatórios e de disfunção endotelial, estresse oxidativo e angiogênese. Em paralelo, estamos conduzindo um ensaio clínico randomizado, placebo-controlado e triplo cego com suplementação oral de nitrato de sódio em gestantes hipertensas crônicas, buscando melhorar os resultados materno-fetais.

Público-alvo: Pacientes gestantes e centros de pesquisa voltados à saúde materno-fetal e cardiovascular. **Diferencial competitivo:** O nitrito é um componente típico da alimentação, seguro para consumo durante a gestação. Diferente de outras terapias anti-hipertensivas com segurança limitada na gestação, o nitrito trata-se de uma abordagem inovadora com potencial de alta tolerabilidade.

Equipe: Equipe multidisciplinar com expertise em fisiopatologia da gestação, farmacologia vascular e ensaios clínicos, composta por pesquisadores da UNESP e USP. TRL: 3.

Conclusão e impacto: A proposta visa gerar um produto terapêutico seguro e eficaz, com potencial para mitigar os impactos dos distúrbios hipertensivos da gravidez, reduzindo complicações obstétricas.

Comitê de Ética: Aprovado no Comitê de Ética em Pesquisa da FMB (nº 6747398), e no Comitê de Ética do Hospital das Clínicas da Faculdade de Medicina de Ribeirão Preto da USP (nº 739082, nº 6764288). Clinical trials: NCT06105775 e NCT06387784. **Suporte Financeiro:** Financiamento: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP), bolsas números 2021/12010-7, 2023/10669-7 e 2023/07589-1

FK-1000: Fração Natural com Potencial Antimicrobiano Multifuncional em Saúde e Agropecuária.

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O avanço da resistência bacteriana em saúde humana, produção animal e vegetal representa um dos maiores desafios globais, exigindo alternativas que não acelerem a disseminação de multirresistência. O Laboratório de Bacteriologia do Instituto Butantan desenvolveu a fração natural FK-1000, composta por glicopeptídeos com múltiplos sítios de ação, não antagonistas e sinérgicos a antibióticos convencionais. Essa combinação impede a disseminação de bactérias multirresistentes pois se a bactéria for resistente a um princípio ativo outro pode eliminá-la.

A FK-1000 apresenta atividade bactericida contra bactérias multirresistentes em concentrações muito inferiores às citotóxicas para células humanas. Mantém estabilidade após processamento e armazenamento, essencial para produção. Seus mecanismos incluem degradação do DNA bacteriano, inibição da peroxidase e bloqueio da fosfatase alcalina, sem comprometer a integridade da membrana, sugerindo que não induz inflamação excessiva. O público-alvo inclui a indústria farmacêutica e o agronegócio, com aplicações em saúde animal e vegetal. O diferencial competitivo está no potencial de reduzir falhas terapêuticas e conter a disseminação de bactérias resistentes sem induzir citotoxicidade. A equipe do Instituto Butantan reúne expertise em imunologia, virulência bacteriana, antimicrobianos e caracterização de bioativos. O projeto integra a UNICEN (Argentina) com expertise em bacteriófagos e transferência gênica; o Instituto Biológico de Campinas em fitopatologia; a APTA Regional em sanidade animal; e o Instituto Adolfo Lutz em microbiologia médica e epidemiologia bacteriana. Alunos e técnicos apoiam a execução experimental. Após mais de 10 anos de pesquisa, o Procedimento Operacional Padrão (POP) de Produção foi consolidado. A tecnologia está em TRL 3, com provas de conceito concluídas e ensaios em andamento sobre resposta imune, fagos λ e modelos *in vivo* em tomateiros e galinhas com colibacilose. O projeto é financiado pela FAPESP. **Comitê de Ética:** Não se aplica. **Suporte Financeiro:** FAPESP- 2023/05870-5

Prevenção Da Dor Antes Que Ela Exista: Uma Inovação Terapêutica Através Do Veneno De Abelha.

Ana Maria Boaventura de Oliveira, Leonor MGR, Silva DF, Brazil MS, Almeida TC, Assis da Silva GS, Marques-Porto R e Pico G. Instituto Butantan

A dor crônica afeta milhões de pessoas sendo um dos maiores desafios na clínica, dado o baixo desempenho a longo prazo e frequentes efeitos adversos de terapias disponíveis. Frente a isto, propomos uma solução inovadora com uso da melitina, o principal peptídeo do veneno de abelha, que demonstrou, em nossos estudos em modelos pré-clínicos, capacidade de modular a dor crônica e também impedir sua instalação, quando administrada precocemente, sem a toxicidade conhecida da molécula. Para tanto, associamos a melitina à técnica da acupuntura, já reconhecida pelo Ministério da Saúde. Com esta combinação, chamada farmacoacupuntura, foi possível a aplicação de baixas doses da melitina de forma preventiva, para evitar o desencadeamento de dor crônica, proposta esta validada em animais. O tratamento pretende impactar pacientes com risco conhecido de desenvolver dor crônica, para os quais ainda não existe intervenção ou controle eficaz. O diferencial do projeto está na convergência entre inovação terapêutica, segurança e simplicidade de aplicação, além da ausência de alternativas similares na clínica. A equipe é composta por uma farmacêutica, com experiência em dor crônica e modelos animais; por uma veterinária, com formação em acupuntura, e por uma bióloga com experiência em toxinas animais e desenvolvimento de novos produtos, todas atuantes no Instituto Butantan. O projeto encontra-se entre TRL 3 e 4, com validação pré-clínica e prova de conceito estabelecidas. O avanço para TRL 5 ocorrerá por ensaios translacionais. Essa abordagem representa um salto inovador na prevenção da dor crônica, combinando o potencial terapêutico de um produto natural com uma técnica já reconhecida e acessível, eliminando efeitos adversos. Ao antecipar a intervenção e oferecer alívio antes do surgimento da dor, o projeto redefine a lógica de cuidado, abre possibilidades para novos protocolos clínicos e posiciona-se como uma alternativa disruptiva e segura. **Comitê de Ética:** Nº 7835170522 e 9080280324 **Suporte Financeiro:** FAPESP (2013/07467-1, 2024/04901-7 e 2024/04664-5)

PolissAg Derm: Curativo dérmico biodegradável e sustentável à base de Galactomanana e Nanopartículas de Prata para cicatrização de Feridas. Fernanda Alexandre da Silva, Everlaine Leite Estevam dos Santos, Luana Cristina Pincho, Jonata Felix Flor, James Henrique Almeida, Emilly Cristina, Daniel Medeiro Nunes, Priscilla Barbosa Sales de Albuquerque, Luiza Rayanna Amorim de Lima, Natalie Emanuelle Ribeiro Rodrigues, Ana Carolina de Carvalho Correia, Jamylle Nunes de Souza Ferro. Universidade Federal de Alagoas

Feridas cutâneas de difícil cicatrização são um desafio crescente à saúde pública, gerando impactos clínicos, sociais e econômicos. A busca por terapias eficazes, sustentáveis e acessíveis se intensifica diante da crescente resistência microbiana, altos custos e da necessidade de trocas frequentes de curativos, que elevam o risco de infecções. Nesse contexto, propomos o PolissAg Derm, um curativo dérmico biodegradável, sustentável e de baixo custo, formulado à base de galactomanana, um polissacarídeo extraído das sementes da *Cassia grandis*, contendo nanopartículas de prata (AgNPs) obtidas por síntese verde com extrato aquoso de *Anadenanthera colubrina*. Essa abordagem eco-friendly evita o uso de reagentes agressivos e torna a produção mais segura e ambientalmente responsável. Diferente dos curativos convencionais, o PolissAg Derm atua ativamente na cicatrização, promovendo a liberação controlada de AgNPs, que previnem infecções e contribuem para acelerar o reparo tecidual. Estudos *in vitro* e *in vivo* confirmaram que a estrutura do curativo foi eficiente em manter a estabilidade, a compatibilidade e em acelerar a cicatrização, por aumentar a reepitelização e deposição de matriz extracelular. O público-alvo são pacientes com feridas crônicas ou de difícil cicatrização, especialmente na atenção primária à saúde, onde há alta demanda por soluções eficazes e acessíveis. O diferencial competitivo do PolissAg Derm está na integração entre biotecnologia sustentável, bioatividade e viabilidade econômica, ao contrário de curativos inertes e caros. A equipe é formada por pesquisadores com experiência em farmacologia celular e molecular, biomateriais e nanotecnologia verde, comprometidos com a inovação sustentável. O projeto está no TRL 4, com validações *in vitro* e em modelo murino concluídas. A produção do PolissAg Derm foi depositada nas patentes BR1020230279180 e BR1020250027852, e os demais relatórios técnicos estão em fase final de elaboração. **Comitê de Ética:** CEUA nº 12/2023 **Suporte Financeiro:** FAPEAL e FACEPE

Desenvolvimento pré-clínico de formulação veterinária à base de Silexan® para o tratamento da ansiedade canina. Raquel Rodrigues Soares Santos e Renata Cristina Mendes Ferreira. Universidade Federal de Minas Gerais

A ansiedade é um dos distúrbios comportamentais mais comuns em cães, com impactos diretos no bem-estar animal e na relação com os tutores. Episódios relacionados a separação, ruídos intensos ou mudanças no ambiente podem levar ao abandono ou eutanásia. As terapias convencionais envolvem ansiolíticos sintéticos, cujos efeitos adversos e necessidade de uso crônico impulsionam a busca por alternativas naturais. Apesar do crescente interesse, há escassez de fitoterápicos veterinários validados. O Silexan®, óleo essencial padronizado de lavanda aprovado para ansiedade humana (ex. FDA), atua modulando receptores serotoninérgicos, canais de cálcio e receptores NMDA, com robusto histórico clínico. Estudos *in silico* e *in vitro* com microsomas hepáticos caninos demonstraram que seus compostos ativos (linalol e acetato de linalila) são metabolizados principalmente pela CYP2B11, com baixo potencial de interação medicamentosa. Propõe-se avançar para testes *in vivo* para validar segurança e eficácia em cães. O público-alvo inclui tutores, médicos-veterinários e a indústria de fitoterápicos. O diferencial da proposta reside no uso de uma substância amplamente conhecida, com dados farmacológicos consolidados, em um mercado carente de formulações veterinárias reguladas. A equipe reúne experiência em farmacologia, toxicologia e produtos naturais, com colaborações no Brasil e no Canadá. A tecnologia encontra-se no TRL 3 (prova de conceito *in vitro* e *in silico*), com meta de avançar ao TRL 4 (validação *in vivo*). A proposta visa gerar um produto seguro, eficaz e acessível, com impacto positivo no bem-estar animal, incentivo à indústria nacional e uso responsável da biodiversidade. **Comitê de Ética:** Não fornecido. **Suporte Financeiro:** Não se aplica.

Plant SUS: rede de pesquisa em plantas medicinais. Ionara Rodrigues Siqueira e Valquiria Linck Bassani. Universidade Federal do Rio Grande do Sul

Problema: Há vasta literatura sobre etnobotânica e pré-clínicos sobre as plantas medicinais no Brasil, mas estudos clínicos (EC) são raros. Os ECs, que trazem evidências de segurança e eficácia em humanos, exigem a necessidade de equipes articuladas e o desenvolvimento de produtos padronizados e sua produção com BPF. Solução: A constituição da rede multidisciplinar, “Plant SUS”, que conta com pesquisadores cujas especialidades cobrem toda a cadeia: desde a matéria prima, desenvolvimento e produção de produtos e de estudos clínicos, visa aportar soluções para este entrave. Nosso grupo analisou a eficácia de cápsulas de extrato seco de *V. officinalis*, prescritas, manipuladas e disponibilizadas no SUS (Igrejinha, RS) (10.46998/IJCMCR.2025.49.001224). O uso popular de *Achyrocline satureioides* (“macela”) no manejo de sintomas de infecções respiratórias virais, com avaliação de qualidade, foi validado no contexto da COVID-19 (10.1002/ptr.7976 e 10.3390/ph18060861). Executamos o projeto guarda-chuva que resultou em desenvolvimento tecnológico de cápsulas padronizadas de cápsulas contendo extrato seco de *M. ilicifolia*; estudo de potenciais hepatotoxicidade e interação medicamentosa; ECR duplo cego e controlado na DRGE e busca de mecanismos de ação (10.3390/ph17111559). A experiência do escalonamento deste processo para produção de cápsulas com BPF está descrita em 10.20944/preprints202503.1351.v1 (TLR5). Estamos aptos a interagir com diferentes parceiros, atendendo a demandas em diferentes níveis (1) execução de ECR de fitoterápicos disponíveis no mercado para outras indicações (2) ECR com produtos prescritos e fornecidos no âmbito do SUS, (3) desenvolvimento tecnológico até avaliação clínica de plantas de interesse. Temos projetos sobre alimentos funcionais, nutracêuticos e extratos de PM em fases iniciais. Nossos ensaios clínicos embasam a fitoterapia baseada em evidências, fortalecendo a PNPMF, assim agregando valor e amplificando o mercado. **Comitê de ética:** CEP — Hospital de Clínicas de Porto Alegre—HCPA (36540720.1.000.5327); CEP Grupo Hospitalar Conceição (36540720.1.3002.5530) e CEP Prefeitura de Porto Alegre (36540720.1.3004.5338); AS: CEP Universidade Federal do Rio Grande do Sul (4.514.201); VO: CEP UFRGS (64721022.5.0000.5347). **Suporte financeiro:** CAPES/Epidemias; FAPERGS PG; Edital Fitoterápicos CNPq/MS

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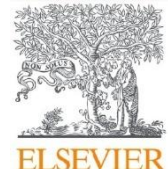
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