

## 12 Drug Discovery and Development

---

**12.001 Anti-inflammatory effects of R-954 in an experimental model of endometriosis.** Carvalho PR, Paiva BJP, Sirois P, Fernandes PD UFRJ – Ciências Biomédicas

**Introduction:** Endometriosis is a gynecological condition characterized by the growth of endometrium-like tissues within and outside of the pelvic cavity [1]. Its clinical symptoms are often manifested as chronic pelvic pain and infertility [2]. Currently available drugs are only efficacious in treating endometriosis-related pain [3]. However, it's not a targeted treatment. Thus, the search for new drugs that could improve the quality of life of women with endometriosis continues to be a goal. In this sense, the aim of this work is to evaluate the effects of R-954 in a murine model of endometriosis. **Methods:** Female Swiss Webster mice (25-30g, n=7) were anesthetized with intraperitoneal injection of ketamine/xylazine. The abdomen was opened to expose the uterus. One uterine horn was ligated at both the uterotubal junction and the cervical end, and the intermediate segment was removed and split longitudinally, with 5-mm pieces were sectioned. These explants were then anchored onto the peritoneum on the right side in abdominal wall. After 35 days, the animals were anesthetized, and the abdomen was opened to assess the viability of the endometrial explants. With confirmation of the ectopic cysts, the animals were divided and treated with: R-954 (2 and 5 mg/kg, sc), progesterone (1 mg/kg, po) or vehicle for 15 consecutive days. At last day the animals were euthanized and blood or ectopic cyst were collected for quantification of pro-inflammatory cytokines. Analysis of the size of each ectopic cyst was also measured using the ImageJ program. The results are presented as mean±SD and statistical analysis were performed by ANOVA followed by Newman-Keuls post-test (\*p<0.05). Protocols for animal use received number #DFBCICB015-04/16 (COBEA/UFRJ/Brazil). **Results:** All doses reduced the levels of TNF- $\alpha$ , IL-6 and IL-1 $\beta$ , in both blood and ectopic cyst. In ectopic cyst, TNF- $\alpha$  reduced in 47.7% ( $6.3\pm1.6^*\text{pg/mL}$ ) and 79.0% ( $2.5\pm1.4^*\text{pg/mL}$ ), when compared to vehicle  $12\pm6.3\text{ pg/mL}$ . IL-1 $\beta$  reduced in 64.7% ( $7.8\pm1.9^*\text{pg/mL}$ ) and 54.5% ( $10\pm6.9^*\text{pg/mL}$ ), when compared to vehicle  $21.7\pm15\text{ pg/mL}$ , to 2 and 5 mg/kg, respectively. However, only 5 mg/kg dose significantly reduced IL-6 production: 74% ( $0.5\pm0.3^*\text{pg/mL}$ ) when compared to vehicle  $2\pm0.8\text{ pg/mL}$ . In the blood, the same inhibition profile was observed to TNF- $\alpha$  and IL-6. TNF- $\alpha$  reduced in 56.5% ( $320.0\pm136.9^*\text{pg/mL}$ ) and 58.4% ( $306.3\pm146.0^*\text{pg/mL}$ ) when compared to vehicle  $736.1\pm298.4\text{ pg/mL}$ . IL-6 significantly reduced about 83.2% ( $41.8\pm30.7^*\text{pg/mL}$ ) and 84.8% ( $37.8\pm19.4^*\text{pg/mL}$ ) when compared to vehicle  $248.7\pm106.7\text{ pg/mL}$  at doses 2 and 5 mg/kg, respectively. With regard to the size of the ectopic cyst, a significant reduction was observed only at the highest dose (5 mg/kg),  $0.72\pm0.35\text{mm}^*$  when compared to vehicle  $1.7\pm0.5\text{mm}$ . **Conclusions:** Our results suggest that R-954 presents significant effect after 15 days of treatment reducing production of cytokines and the size of endometriotic ectopic focus located in peritoneal cavity. **Technical Support:** Alan Minho **References:** [1]Liang, B. Reprod Biol Endocrin. 16, 1-13.2018; [2]Wang, X. M. Eur Rev for Med and Pharmacol Sci. 22,2513-2518.2018; [3]Barra, F. Expert Opin Investig Drugs. 27,445-458.2018 **License number of ethics committee:** #DFBCICB015-04/16 **Financial support:** CAPES, CNPq, FAPERJ and Institute Vital Brazil (animal donation)

**12.002 Security assessment of the LASSBio-788: A new antiatherogenic drug candidate.** Maia IC<sup>1</sup>, Moreira TJ<sup>1</sup>, Gontijo LS<sup>1</sup>, Motta NAV<sup>1</sup>, Ribas JAS<sup>1</sup>, Kümmerle AE<sup>2</sup>, Brito FCF<sup>1</sup>, Maróstica E<sup>1</sup> <sup>1</sup>UFF – Fisiologia e Farmacologia, <sup>2</sup>UFRRJ – Química

**Introduction:** LASSBio-788 is a thienylacylhydrazone derivative that has an antiatherogenic effect with antiplatelet, anti-inflammatory, vasodilatory, antioxidant and hypolipidemic properties well established (Motta *et al.*, J Pharmacol Sci 123: 47, 2013). Therefore, it is important to evaluate the toxic effects caused by this potential drug candidate for the treatment of atherosclerosis. Thus, the aim of this study is to evaluate the possible toxic effects of LASSBio-788 on the different tissues in rats, comparing to simvastatin. **Methods:** (CEUA 695/16) Male Wistar rats (60-day old) were randomized in 6 groups (n=6/group), receiving standard chow (CO) or hypercholesterolemic (HC) diet for 45 days. In the last 15 days, rats were treated with LASSBio-788 100 µmol/kg, i.p. (CO+788 or HC+788) or simvastatin 10mg/kg, gavage (CO+SIMVA or HC+SIMVA). Animals were anesthetized, and blood samples were collected for biochemical analyzes. Testes, liver, kidney and soleus muscle were removed, weighed and processed for morphological analysis. Spermatogenic evaluation (motility, sperm number, membrane integrity and hypo-osmotic swelling test) was performed using sperm from epididymis cauda. Values are mean±SEM (one-way ANOVA, Newman-Keuls;  $P<0.05$ ). **Results:** LASSBio-788 did not change body weight or relative weights of the liver, kidney and organs of the reproductive tract, except for testis which was also different for SIMVA when administrated with the HC diet (CO: 0.46±0.02, HC+788: 0.54±0.01, HC+SIMVA: 0.53±0.02 g). Morphometric analysis of the testis showed that LASSBio-788 partially prevented the deleterious effects of HC diet on the height of the seminiferous epithelium, while SIMVA aggravated this effect. In addition, LASSBio-788 decreased germ cells count only in the early stages, while SIMVA did so at all stages of spermatogenesis, as well as it decreased Sertoli cells number. In the spermatogenic evaluation, both drugs partially prevented HC diet effects on the male gamete. Morphological analysis of liver, kidney and skeletal muscle did not suggest toxic effects induced by LASSBio-788; however, SIMVA confirmed its myotoxicity. The function and integrity of these tissues were evaluated by biochemical parameters. LASSBio-788 did not alter hepatic transaminases, albumin, alkaline phosphatase, uric acid, urea, creatinine, calcium, magnesium and creatine phosphokinase. Nevertheless, it increased the plasma phosphorus (CO: 7.1±1.0; CO+788: 10.5±1.2 mg/dL) and total protein (CO: 6.1±0.13; CO+788: 6.7±0.16 g/dL). Regarding hematimetric parameters, LASSBio-788 alone slightly reduced the hematocrit (CO: 50.2±1.2; CO+788: 47.3±1.8 %) but ameliorated the acute inflammatory profile induced by HC diet, effects that were not observed with SIMVA. **Conclusion:** Our preliminary results showed that the new compound LASSBio-788 does not have significant toxic effects on the male and male gamete reproductive tract, as well as on the liver, kidney and skeletal muscle, being a potential candidate for the antiatherogenic drug, as safe as or better than statins considering the effects on the male reproductive tract and skeletal muscle. **License number of ethics committee:** CEUA 695/16 **Financial support:** FAPERJ, CNPq, CAPES, PROPPI/UFF

**12.003 Study of acute oral toxicity of lyophilized aqueous extract of Cord01.** Souza-Junior FJC<sup>1</sup>, Andrade DM<sup>1</sup>, Barros MA<sup>1</sup>, Gomes BAQ<sup>1</sup>, Monteiro MC<sup>1</sup>, Maia CSF<sup>1</sup>, Pereira WLA<sup>2</sup>, Fontes-Junior EA<sup>1</sup> - <sup>1</sup>UFPA – Pharmaceutical Sciences, <sup>2</sup>UFRA – Ciências Patológicas

**Introduction:** Plants inserted in popular medicine are common study objects in the scientific environment, with the aim of developing new therapeutic possibilities. Your application as innovative drug depends primarily on systematic toxicological tests. Cord01 is an Amazonian plant species and little is known about the safety of your administration orally, claim activity for the treatment of bruises, showing promising in the treatment of pain and inflammation. In this context, the study evaluated the toxicity of Cord01 administered in an acute oral pattern. **Methods:** the Cord01 was obtained through the freeze-drying of an infusion of dry leaves. The evaluation of your toxicity was conducted in agreement with the guideline 423 of the organization for economic co-operation and development (OECD). Therefore, were used females of the specie *Rattus norvegicus* (Wistar, n=12) distributed in two groups: Control (vehicle, oral via, 0,1mL/100g) e Treated (2000 mg/kg of Cord01, oral via). After the administration, the rats were submitted to the hippocratic and behavioral assessment in the first four hours and daily for fourteen days, also being verified the weight gain and the water and feed consumption. On the 15th day after treatment, the animals were euthanized, being collected samples of organs (kidneys, liver, stomach and lungs) to macro and microscopic evaluation. It was also collected samples of blood to evaluate of biochemical markers of function (aspartate aminotransferase -AST; alanine aminotransferase -ALT; Alkaline phosphatase -ALP and gamma glutamyltransferase -GGT) and oxidative stress (nitric oxide production - NO; lipid peroxidation; total antioxidant capacity - TEAC; and reduced glutathione - GSH). **Results:** the administration of Cord01 (2000 mg/kg) presented low toxicity profile, promoting neither behavioral and consumption water and feed changes, nor deaths during all the period of evaluation. There was also no change in the function markers (ALT, AST), except ALP and GGT, which were at reduced levels in relation to the control, which may indicate hepatoprotective effect and possible pharmacological actions on pathological processes such as inflammation. This hypothesis is reinforced by the reduction of serum levels of the NO. The levels of GSH and total antioxidant capacity were found to be equivalent of the control group. On the other side, the lipid peroxidation level, measured from the concentration of malondialdehyde (MDA), was reduced, indicating possible reduction of oxidative cellular damage. The relative weight of the evaluated organs, as well as its macroscopic characteristics did not change under treatment with Cord01. Similarly, the histological constitution and architecture of the kidneys, liver, stomach and heart, they were preserved in relation to the control. **Conclusion:** The set of findings characterizes Cor01 as a xenobiotic of low toxicity. It didn't promote behavioral and development alterations, or vital organs damage, wich preserve the macro and microscopic normality. Additionally, revealed evidence of hepatoprotection and possible antioxidant and anti-inflammatory effects. **License number of ethics committee:** CEUA nº6029300817 **Financial support:** UFPA, CNPq, CAPES and FAPESPA

**12.004 Evaluation of the anti-inflammatory profile of LASSBio-2060 and LASSBio-2061.** Paiva JPB, Carvalho PR, Freitas RHCN, Fraga CAM, Fernandes PD UFRJ – Ciências Biomédicas

**Introduction:** Inflammation is a benefice response of the immune system in view of an infectious agent or tissue damage. During an inflammatory process, there are several vascular alterations, leukocytes migration and activation and systemic responses [1]. Due to several side effects of non-steroidal anti-inflammatory drugs the continue search for new substances is still a goal for researchers. In this respect LASSBio-1829 was firstly described with significant anti-inflammatory property. Some structural changes were made on LASSBio-1829, resulted in LASSBio-2060 and LASSBio-2061. So, the aim of the present work was to evaluate the anti-inflammatory effects of LASSBio-2060 and LASSBio-2061 using traditional methods of inflammation. **Methods:** Male Swiss Webster mice (28-32g, n=6) were used in models of formalin-induced licking or carrageenan-induced cell migration into the subcutaneous air pouch (SAP) models. Mice were orally treated with LASSBio-2060 or LASSBio-2061 (10, 30 or 100 µmol/kg). After 1-hour mice received intraplantar injection of formalin (2.5%, 20 µL) and the time that mice spent licking the formalin-injected paw was recorded with a chronometer. In SAP model, after oral administration of substances, mice received carrageenan (1%, 1 mL) or saline injection into SAP and 24 hours later mice were euthanized and exudate collected for further measurements. Results are presented as media ± sd. Statistical analysis were performed by ANOVA followed by Bonferroni test (\*p<0.05). The protocol for use of animals was approved by CEUA/UFRJ and received the number DFBCICB015-04/16. **Results:** Although none of the substance inhibited the 1<sup>st</sup> phase of formalin-induced licking both had an effect in 2<sup>nd</sup> phase as shown: vehicle-treated group: 161.4±67.1sec; LASSBio-2060, 30µmol/Kg: 173.2±9.6sec; 100µmol/Kg: 92.3±37.2\*sec. LASSBio-2061 10µmol/Kg: 128.2±50.8sec; 30µmol/Kg: 34.0±10.6\*sec; 100µmol/Kg: 80.3±43.6\*sec. LASSBio-2061 also inhibited leukocyte migration into the SAP at higher doses: injected vehicle-treated group: 79.3±28.9 x10<sup>3</sup>cells/µL; 10µmol/Kg: 72.5±35x10<sup>3</sup>cells/µL; 30µmol/Kg: 30.2±12.7\*x10<sup>3</sup>cells/µL; 100µmol/Kg: 48.4±7.6\*x10<sup>3</sup>cells/µL. **Conclusion:** The results suggest that both substances (LASSBio-2060 and LASSBio-2061) present anti-inflammatory activity since both reduced the 2<sup>nd</sup> phase of formalin-induced licking and LASSBio-2061 reduced cell migration induced by carrageenan. **References:** Chen, GY. Nat Rev Immunol. 10, 826-37, 2010 [1]. **Technical Support:** Alan Minho **License number of ethics committee:** DFBCICB015-04/16 **Financial support:** CAPES, CNPq, FAPERJ and Institute Vital Brazil (donation of animal)

**12.005 New molecules derived from ibuprofen with analgesics and anti-inflammatory activities.** Silva ACS<sup>1</sup>, Silva RC<sup>2</sup>, Correa LJS<sup>2</sup>, Nascimento V<sup>2</sup>, Fernandes PD<sup>1</sup>, Cordeiro NM<sup>1</sup> <sup>1</sup>UFRJ – Ciências Biomédicas, <sup>2</sup>UFF – Química

**Introduction:** Ibuprofen is a non-steroidal anti-inflammatory drug (NSAIDs), one of the most used group in the world, but even with effective effects, they present a wide range of side effects, such as renal and gastrointestinal dysfunctions<sup>1</sup>. Because of that, the discovery of new substances or improvement of existing ones with lesser side effects continues to be a goal. The present study **Aims:** is to evaluate the effects of new analogues of Ibuprofen in acute models of inflammation. **Methods:** The activities of R-60 and R-65 were evaluated after oral pretreatment one hour before tests in a dose of 10 mg/kg, through intraplantar injection of formalin. Formalin-induced licking response was evaluated as previously described<sup>2</sup>, Cell migration induced by carrageenan was evaluated in the subcutaneous air pouch model<sup>3</sup>. Carrageenan (1%) was injected into the air pouch performed on the back of the animal 1 hour after treatment with the substances. Experimental groups were composed of 6-8 (25-35g) male Swiss *Webster* mice per group (protocols number DFBCICB15-0416). The results are presented as mean  $\pm$  SD. Statistical significance was calculated by one-way ANOVA followed by Bonferroni post-test (\* $p < 0.05$ ). **Results:** R-60 and R-65 reduced the time of licking formalin-injected paw: 1<sup>st</sup> phase: R-60: 24.1 $\pm$ 13.9\* sec and R-65: 18.2 $\pm$ 6\* sec, when compared with vehicle-treated group: 55 $\pm$ 12.1 sec and 2<sup>nd</sup> phase: R-60: 92.5 $\pm$ 14.1\* sec and R-65: 61.8 $\pm$ 25\* sec, when compared with vehicle-treated group: 190.6 $\pm$ 54.4 sec. Cell migration was also reduced after the treatment with substances in a dose of 10 mg/kg. Ibuprofen (39.9 $\pm$ 15.6\* cells  $\times 10^6$ ) reduced 43.4% of leukocyte migration and its derivatives, R-60 (37.7 $\pm$ 17.5\* cells  $\times 10^6$ ) 46.4% and R-65 (39.4 $\pm$ 6.3\* cells  $\times 10^6$ ) 44.1%, when compared with vehicle-carrageenan group (70.4 $\pm$ 15.2 cells  $\times 10^6$ ). Protein extravasation was also reduced, but of the two compounds only the R-60 (0.9 $\pm$ 0.3) demonstrated significant reduction of 43.7% of protein exudate to the air pouch, the others compounds not demonstrated significant reduction, R-65 (1.2 $\pm$ 0.3) had 25% reduction and Ibuprofen (1.2 $\pm$ 0.5) also had 25% reduction, when compared with vehicle-group (1.6 $\pm$ 0.4). **Conclusions:** Our results suggest that the new analogues of ibuprofen seem to present significant anti-inflammatory effect which justifies the continuity of the studies. **Acknowledgments:** Alan Minho (technical support), Instituto Vital Brazil (Animal donation) 1. Peesa, J. J *Acute Dis.* V.5(5): 364; 2016. 2. [Hunskaa S.](#) *Pain.* V.30, 103; 1987. 3. Raymundo, L. J *Ethnopharmacol.* V134(3): 725; 2011. **License number of ethics committee:** DFBCICB15-0416 **Financial support:** CNPq, CAPES, FAPERJ

## **12.006 New analogs of phenacetin with antinociceptive and anti-inflammatory activities.**

Almeida PG<sup>1</sup>, Nascimento V<sup>2</sup>, Silva RC<sup>2</sup>, Fernandes PD<sup>1</sup>, Cordeiro NM<sup>1</sup> <sup>1</sup>UFRJ – Ciências Biomédicas, <sup>2</sup>UFF – Química

**Introduction:** Phenacetin is a compound with pharmacological activity very used in the past with antipyretic and analgesic effects (1). Three new analogues were synthesized, named 4-ethoxyaniline, R-71 e R-72, which are being tested for its pharmacological potential. **Methods:** Nociception and inflammation have been assessed by the formalin-induced licking response and carrageenan-induced cell migration. Male Swiss Webstermice (22-30 g, n = 6-8) donated by the Animal Production Centre of Institute Vital Brazil were used for both tests. The protocol for use of animals was approved by CEUA/UFRJ and received the number DFBCICB015-04/16. Results are compared with vehicle-treated group and expressed as mean  $\pm$  SD and statistical analysis were performed by ANOVA followed by Bonferroni test (\* $p < 0.05$ ). Formalin-induced licking response is mainly characterized for a neurogenic (0-5 min) and inflammatory (15-30 min) phases. The inflammatory stimuli are given by the injection of 20  $\mu$ L of formalin 2.5% in the left hind paw of the mouse. The time the animal spent licking injected paw is recorded (2). The animals received oral treatment of 4-ethoxyaniline, R-71 or R-72 at 10 mg/kg, acetylsalicylic acid (200mg/kg) and morphine (2.5mg/kg) 60 minutes before formalin injection. To access cell migration, the subcutaneous air pouch (SAP) was produced with a sterile air injection (10 mL) into the intrascapular area of the mice. After three days, another injection of air (7 mL) was performed to maintain the pouches. Three days later, animals received the oral treatment with 4-ethoxyaniline, R71 or R72 (at 10 mg/kg) one hour before the injection of sterile carrageenan (1%) or saline into the SAP. After 24 h, all animals were euthanized, the SAP was washed with 1 mL of saline and exudate were collected (3). **Results:** Our data demonstrated that treatment with the compounds significantly reduced licking time in the neurogenic and inflammatory phases of the paw licking model. 1<sup>st</sup> phase: vehicle= 52.7 $\pm$ 11.3 sec; 4-ethoxyaniline: 24.9 $\pm$ 3.5\* sec; R-71: 10.0 $\pm$ 3.0\* sec; R-72: 10.1 $\pm$ 3.8\*sec. 2<sup>nd</sup> phase: vehicle= 212.2 $\pm$ 56.3 sec; 4-ethoxyaniline: 107.8 $\pm$ 32.2\*sec; R-71: 76.5 $\pm$ 32.4\*sec; R-72: 60.4 $\pm$ 16.6\*sec. Regarding cell migration, 4-ethoxyaniline reduced 48% (42.8 $\pm$ 16.9 $\times 10^6$  cells/mL), R-71 52% (38.9 $\pm$ 11.39 $\times 10^6$  cells/mL) and R-72 in 67% (26.8 $\pm$ 11.1 $\times 10^6$  cells/mL) when compared with vehicle (82.6 $\pm$ 39.29 $\times 10^6$  cells/mL) that also received carrageenan into the pouch. **Conclusions:** We can conclude that the analogues presented anti-inflammatory effects and should be considered to further assays. **References:** 1. Smith and Reynard, Textbook of Pharmacology, p. 431, 1991 2. Hunskaar, Sand Hole, K., Pain, v. 30, p. 3, 1987 3. Raymundo, L.J.R.P.; Guilhon, C.C.; Alviano, D.S.; et al., J Ethnopharmacol, v. 134, p. 3, 2011 **License number of ethics committee:** DFBCICB015-04/16 **Financial support:** CNPq, CAPES, FAPERJ

**12.007 Evaluation of embryotoxicity of new semicarbazone in Zebrafish (*Danio rerio*).** Souza GS<sup>1</sup>, Silva JMM<sup>1</sup>, Pinheiro GM<sup>1</sup>, Souto KS<sup>2</sup>, Braoios A<sup>1</sup>, Junior SS<sup>3</sup>, Rita RMS<sup>4</sup> <sup>1</sup>UFG-Regional Jataí – Biomedicina, <sup>2</sup>UFG-Regional Jataí – Fisioterapia, <sup>3</sup>UFG-Regional Jataí – Física, <sup>4</sup>UFG-Regional Jataí – Ciências Biológicas

**Introduction:** In the search for new drugs with therapeutic potential, researchers are arousing interest in several classes of chemical compounds due to the versatility of their molecular structures and their biological effects. Semicarbazones are prominent and have a wide pharmacological profile. In this context toxicity and embryotoxicity tests are required. *Danio rerio* known as Zebrafish, exhibits small size, ability to absorb water substances, rapid metabolism, gene sequenced and with a high degree of homology to the man (about 70%). It is a great study model also because it has low maintenance costs and simplified handling. **Methods:** The experiment used 96-well round bottom plates. In plate 01, 180µL of reconstituted water and 20µL of the compound were added to the first well, 100µL of reconstituted water was added to the wells, and a serial dilution of 1: 2 from well 01 to 12 was performed, the final 100µL was discarded. Afterwards, 100µL of reconstituted water was added to all wells. In plate 02, one *Danio rerio* embryo was added to each well. To avoid further dilution of the drug, the liquid next to the embryo was withdrawn and in sequence the corresponding well solution from plate 01 was transferred to plate 02. On plate 02 the control group was created, a bottom line a of the experiment using 12 embryos in 200µL of reconstituted water. A medium control group was also made to check if the medium had contaminants. The experiment was performed in triplicate using 48 embryos with the control group. The evaluation was made by light microscopy observing the presence of death or teratogenic alterations. **Results:** All control embryos hatched and grew according to expected standards. From 500 to 125 µg/mL in 24 hours, 100% mortality was obtained. In 48 and 72 hours the result was maintained. But with 96 hours in 62.5µg/mL, it was verified that, 33% died and 66% had malformations of the cardiovascular and skeletal system. Below 62.5µg/mL, 100% of the embryos were alive and did not show developmental deformations. **Conclusion:** This drug had concentration dependent activity, being lethal only at concentrations above 62.5 µg/mL and malformation in this. It is necessary to perform *in vitro* toxicity tests with cell lines to elucidate the mechanism of action of the drug. **Ethics committee:** Protocol nº 002/3017/CEUA-UFG **License number of ethics committee:** 002/3017/CEUA-UFG

**12.008 Computational studies for rational planning of new Kinase C-kit inhibitors.** Pereira MBM<sup>1</sup>, Antunes JE<sup>2</sup> <sup>1</sup>UFJF – Life Basic Sciences, <sup>2</sup>UFJF – Farmácia e Farmacologia

**Introduction:** Protein kinase c-Kit (v-kit Hardy-Zuckerman 4 feline Sarcoma Viral Oncogene) is involved in several types of cancer of high prevalence such as lung, carcinoma, melanomas, colon cancer, rectum and leukemias<sup>1</sup>. Thus, this kinase becomes a potentially therapeutic target for the treatment of these different types of cancer. It has already been seen in the literature<sup>2,3</sup> that the presence of mutations in the gene of this enzyme are related to resistance to treatment with inhibitors, such as Imatinib®. The overall objective of this work was to develop c-Kit mutation (L576P) inhibitors, *i.e.*, c-Kit with mutation of the lysine residue at position 576 by the proline residue. The inhibitors will be application in the treatment of cancers that do not yet have treatment. **Methodology:** Through the use of bioinformatics, the mutation of the desired amino acid residue was performed to test new molecules capable of inhibiting the c-Kit with the mutation. The quinazolines were chosen for the inhibition test because several quinazolin have already been developed as inhibitors of kinases<sup>4</sup>. The inhibition test was performed computationally by *molecular docking* studies. The most promising molecules were synthesized through known synthetic routes<sup>5</sup>. **Results:** Through this study, several molecules were tested, and two new molecules were proposed as probable inhibitors of the c-Kit with the mutation (L576P). The two molecules of quinazolines (1: Nc2nc(nc1cc(OC)c(cc12)OC)Nc4ccc(OCc3ccccc3)cc4, 2: COc1cc(cc(OC)c1OC)Nc3ncnc2c3cccc2Cl) were synthesized. **Conclusion:** Computational studies such as this project can contribute to the development of new drugs in an efficient way, reducing the time and cost of the project because it was possible to synthesize only the most promising molecules. Thus, through this work we can synthesize two quinazolines with potential inhibition c-Kit (L576P) mutation. **References:** 1. Lennartsson, J.; Rönnstrand, L. *Physiol Rev.* **2012**, 92, 1619. 2. Gorre ME, Mohammed M, Ellwood K, Hsu N, Paquette R, Rao PN, Sawyers CL. *Science* **2001**, 293, 876. 3. Tamborini E, Bonadiman L, Greco A, Albertini V, Negri T, Gronchi A, Bertulli R, Colecchia M, Casali PG, Pierotti MA, Pilotti S. **2004**, 127, 294. 4. Vema, A.; Panigrahi, S. K.; Rambabu, G.; Gopalakrishnan, B.; Sarma, J. A. R. P.; Desiraju, G. R. *Bioorg. Med. Chem.* **2003**, 11, 4643. 5. Rewcastle, G. W.; Denny, W. A.; Showalter, H. D. H. *Curr. Org. Chem.* **2000**, 4, 679. **Financial support:** 1. PROPESQ of Federal University of Juiz de Fora - UFJF 2. FAPEMIG.



**12.009 Preclinical and clinical evidence to support safety and efficacy of BZ371 in a topical formulation for treatment of erectile dysfunction.** Borges PV<sup>1</sup>, Lacativa P<sup>1</sup>, Gross G<sup>1</sup>, Nunes da Silva C<sup>2</sup>, de Lima ME<sup>3</sup> <sup>1</sup>Biozeus Biopharmaceutical – Desenvolvimento de Fármacos, <sup>2</sup>UFMG – Bioquímica Toxicológica, <sup>3</sup>UFMG – Ensino e Pesquisa da Santa Casa de Belo Horizonte

**Introduction:** BZ371 is a synthetic peptide, derived from the isolated toxin fraction PnTx2-6 from the venom of Brazilian spider *Phoneutria nigriventer*, which causes priapism in male individuals bitten by this spider. BZ371 was created by molecular modeling studies of PnTx2-6, in order to maintain the erection but without the undesired effects of the toxin (Silva CN, J Urol, 194: 1481, 2015). BZ371 acts on penile erection by stimulating nitric oxide (NO) production, enhancing NO synthase, via isoforms neuronal and inducible, being independent of endothelial, triggering the cGMP cascade, thus inducing SM (smooth muscle) relaxation, vasodilation and increased local blood flow in the genital area.

**Methods and Results:** BZ371 treatment ( $10^{-8}$ M), in corpus cavernosum (CC) tissues from male health Sprague-Dale (SD), spontaneous hypertensive rats (SHR) and STZ (streptozotocin)-diabetic mice, submitted to electric field stimulation (EFS: 8Hz), potentiates CC relaxation and erection function. BZ371 treatment also reached to the same relaxation levels as control in both models, as well as enhanced cGMP production in diabetic CC, corroborating a relaxation response mediated by NO. Accordingly, *in vivo* STZ-diabetic SD rats treated with BZ371 topically administered (400µg) in gel, 30 min after EFS, restores erectile function, as assessed by intracavernosal pressure/main arterial pressure (ICP/MAP), reaching similar levels of normoglycemic rats when compared to vehicle. BZ371 also showed an additive effect when co-administered with Sildenafil (25mg/kg,sc). In Biodistribution of Iodine<sup>123</sup>BZ371 topically (10µCi) or intravenous (20µCi) in mice was mostly found in the penis and testicles, demonstrating a kind of “chemotaxis” for this organ. In line of this, BZ371 (5mg/Kg,IP) did not exhibit any histopathological modifications in the analyzed tissues (kidney, heart, lungs, liver, brain) of mice, only the CC of the penis had a dose-dependent vasodilation. We also demonstrated *in vitro*, according to OECD guidelines that BZ371 caused no dermal or genital mucosa irritation, nor sensitization, genotoxicity, neither cardiotoxicity. The first exploratory clinical trial performed in 12 healthy subjects (6/6 men, women) after topical application of BZ371 (1, 2, 4mg) on genital area, in a gel formulation, confirmed its safety, with no local irritations, edema, headache, hypotension, electrocardiogram or blood hemogram changes, prolonged erection, priapism or penile pain, being well tolerated. As well, the first signals of efficacy assessed by doppler scan, to detect blood flow changes in the genital area from both genders, after 10 e 30 min BZ371 topical administration (1 and 2mg), revealed an increase in arterial inflow. In addition, pharmacokinetics human analysis of BZ371 demonstrated a limited systemic exposure in the blood (LLOQ<5ng/ml).

**Conclusion:** BZ371 may emerge as a promising drug for erectile dysfunction treatment, being topically administered and with a desirable safety profile. **License number of ethics committee:** For Animals: Israel Animal Welfare Act (# IL-17-4-167), UFMG (protocol 216/11) and For humans: Faculty of Jaguariúna (# 79372817.7.0000.5409). **Financial support:** BIOZEUS, UFMG, FAPEMIG AND FUNED

## 12.010 Critical assessment of binding assays for determination of drug-receptor residence time. Monteiro FM, Noël F UFRJ – Farmacologia

**Introduction:** Besides their affinity and efficacy, the residence time of drugs at their receptor has been shown to constitute an important element with respect to the clinical performance of some drugs. The objective of present work was to critically compare different assays that have been proposed for measuring the kinetics of unlabeled drugs binding and estimate their residence time. We used digoxin and digoxigenin since we previously showed that these two cardiac steroids inhibit the pig kidney  $\text{Na}^+/\text{K}^+$ -ATPase with similar potency but very different kinetics. **Methods:** binding assays were performed with a pig kidney preparation and 2 nM [ $^3\text{H}$ ]-ouabain in a 3 mM Mg-Pi medium at 37°C, following three different protocols: 1. Full competition association assay (FCAA): binding of [ $^3\text{H}$ ]-ouabain was measured at 9 different times in the absence (control) or presence of three different concentrations of the drug and fitting of the data was performed according to the model of Mahan & Motulsky (1983) to obtain  $k_{+1}$  and  $k_{-1}$  values. 2. Simplified competition association assay (SCAA): only one concentration of the drug was used (around its  $\text{IC}_{50}$ ) and two parameters were analysed: ratio of [ $^3\text{H}$ ]-ouabain bound at 30 min and 480 min (KRI) and half-time for [ $^3\text{H}$ ]-ouabain binding ( $T_{1/2}$ ). 3. Delayed association assay (DAA): here, the preparation was pre-incubated with a high concentration of the drug ( $7 \times \text{Ki}$ ) in order to occupy nearly all the binding sites before a jump dilution (50x) in a medium containing only the radioligand, for analyzing its binding time-course.  $T_{1/2}$  is here increased if the drug has a long residence time so that it delays the binding of the radioligand. **Results:** 1. FCAA revealed the following rate constants ( $n=3$ ): Digoxin:  $k_{+1} = 5.62 \times 10^5 \pm 1.56 \times 10^5 \text{ M}^{-1} \cdot \text{min}^{-1}$ ;  $k_{-1} = 0.0085 \pm 0.0007 \text{ min}^{-1}$ . Digoxigenin:  $k_{+1} = 19.6 \times 10^5 \pm 0.2 \times 10^5 \text{ M}^{-1} \cdot \text{min}^{-1}$ ;  $k_{-1} = 0.1465 \pm 0.0015 \text{ min}^{-1}$ . 2. SCAA: KRI values were significantly different between digoxin, digoxigenin and control (0.668, 0.222 and 0.438, respectively ( $p < 0.0001$ ), indicating that digoxigenin binds and dissociates more rapidly than digoxin. 3. DAA: in this protocol, the  $T_{1/2}$  for [ $^3\text{H}$ ]-ouabain binding was 143 min for digoxin and 61 min for digoxigenin, indicating that digoxigenin dissociates more rapidly than digoxin. **Conclusion:** 1. FCAA is theoretically the best assay since it allows obtaining the rate constants and calculation of residence times ( $1/k_{-1}$ , 118 min for digoxin and 6.8 min for digoxigenin). On the other hand, such assay is time- and material-consuming and frequently gives unprecise values, at least in our conditions. 2. SCAA is a rapid and cost-effective assay for screening a large number of drugs for discriminating binding kinetics profiles, but interpretation of differences in the KRI parameter is not straightforward since both  $k_{+1}$  and  $k_{-1}$  influence the time-course of radioligand binding. 3. DAA is an assay that addresses the dissociation kinetics of the drug, without interference of the association kinetics so that it is a good complementary assay for differentiating drugs on the basis of their residence time, at least qualitatively. All three assays indicate that digoxin and digoxigenin have very different kinetics profiles albeit their affinities are not too different ( $\text{Ki} = 53 \text{ nM}$  and  $246 \text{ nM}$ , respectively). **Financial support:** CNPq, FAPERJ

**12.011 Evaluation of isoniazid hydrazone derivatives as myeloperoxidase inhibitors.** Quimas JVF<sup>1</sup>, Santos DC<sup>2</sup>, Lopes Jr MAA<sup>3</sup>, Henriques RR<sup>3</sup>, Romeiro NC<sup>3</sup>, Souza ALF<sup>3</sup>, Silva LL<sup>1</sup> <sup>1</sup>UFRJ-Macaé – Farmácia,<sup>2</sup>UFRJ-Macaé – Química, <sup>3</sup>UFRJ-Macaé – Química

**Introduction:** The enzyme Myeloperoxidase (MPO) catalyzes the oxidation reactions of a series of endogenous compounds, from its reaction with H<sub>2</sub>O<sub>2</sub>, and can also produce HOCl, a highly reactive species that contributes strongly to oxidative stress. Physiologically, MPO, present mainly in neutrophils, acts in innate defense against infections [1]. However, the pro-oxidant effects of MPO and its products, as well as its presence in several pathological conditions, has an important pharmacological target for the treatment of several conditions, such as: cardiovascular diseases, neurodegenerative diseases, inflammatory diseases, renal diseases and immune-mediated diseases [2]. This work aims to investigate the profile of a series of N-acylhydrazonic derivatives (NAH) synthesized from isoniazid (ISO), an antimicrobial used for the treatment of tuberculosis, with known activity on MPO [3], in order to identify candidates for prototypes of MPO inhibitor drugs.

**Methodology:** The ability to sequester free radicals was verified by the method of DPPH (2,2-diphenyl-1-picrylhydrazyl) (300 µM) and derivatives was screened at 300 µM and the potency of de active compounds (CI<sub>50</sub> ± standard error) was determined (1.56 to 100 µM) reading at 517 nm. Quercetin was used as a positive standard. The effect on the peroxidase cycle of MPO was measured by the oxidation of TMB (tetramethylbenzidine) in the presence of H<sub>2</sub>O<sub>2</sub> in phosphate-citrate buffer pH 6, the reaction was quenched with H<sub>2</sub>SO<sub>4</sub> 1M and reading at 450 nm. The interference with the MPO chlorination cycle was measured by the production of HOCl, in the presence of H<sub>2</sub>O<sub>2</sub> and chloride ion, by the taurine/TNB (thionitrobenzoic acid) method, in phosphate buffer 0.05 M pH 7.5, the reaction was stopped with catalase (20 µg/ml) and reading at 412 nm. Rat bone marrow homogenate supernatant was used as the source of MPO. The derivatives were screened at 100 µM and the potency of the most active compounds was determined (0.01 to 300 µM) and the ISO was used as a positive standard. **Results:** The LCSO11, LCSO12 and LCSO67 derivatives were able to sequester the DPPH free radical, with the most potent LCSO67 (18.32 ± 1.11 µM), like the quercetin standard (15.17 ± 1.06 µM). The LCSO11, LCSO12, LCSO13 and LCSO67 derivatives showed inhibitory activity in peroxidase cycle, greater than 50%, with the LCSO11 derivative being the most active and with potency higher than ISO (1.6 ± 1.22 and 3.7 ± 1.34 µM, respectively). LCSO02, LCSO06, LCSO11 and LCSO13 were able to inhibit the chlorinating cycle by more than 80%, LCSO02 and LCSO13 being more potent than ISO (0.95 ± 1.24, 0.38 ± 1.25 and 4.1 ± 1.29 µM, respectively). **Conclusion:** NAH derivatives appear to act on MPO by different mechanisms, on the peroxidase cycle (LCSO67), on the chlorinating cycle (LCSO02) and on both (LCSO11), demonstrating that MPO-inhibitory activities of ISO was optimized in its derivatives, since we could identify the derivative LCSO13 that was 10 times more potent than prototype on the chlorinating cycle. References: [1] Klebanoff1, J Leukoc Biol, 77, 598, 2005. [2] Lazarević-Pasti et. al., Curr Drug Metab, 16, 168, 2015. [3] Soubhy et. al., ACS Med Chem Lett, 8, 206, 2017.

**12.012 *In silico* structural evaluation of the male contraceptive target eppin by homology modelling and molecular dynamics simulation.** Rosa LR<sup>1</sup>, Gomes AAS<sup>2</sup>, Borges RJ<sup>2</sup>, Fontes MRM<sup>2</sup>, Silva EJR<sup>1</sup> <sup>1</sup>IBB-Unesp – Farmacologia, <sup>2</sup>IBB-Unesp – Física e Biofísica

**Introduction:** New non-hormonal male contraceptive methods are under development. EPPIN (Epydidimal Protease Inhibitor) is a sperm-surface protein containing WAP-type four disulfide core (N-terminal) and Kunitz-type (C-terminal) protease inhibitor consensus sequences, which presents a crucial role in male fertility, since it regulates sperm motility acquisition after ejaculation. Thus, EPPIN is an interesting contraceptive drug target. Nevertheless, its structural properties are not elucidated yet, slowing down drug discovery process and further knowledge omits mechanism of action. Here, we constructed homology models of mouse EPPIN N- and C-terminal domains and then employed molecular dynamics simulations (MDS) to evaluate their spatial conformation and rearrangement.

**Methods:** 3D homology models for murine EPPIN (NP\_083601) N-terminal (P22-C78) and C-terminal (L70-T134) domains were built using Swiss Model Workspace. Four templates were initially chosen based on their QMEAN Z-scores, percentage of identity, coverage and WFDC- and Kunitz-type domains conservation. The best two models for each domain were submitted to 100 ns MDS in Gromacs Software under Gromos force field solvated with water and 100 mM NaCl. We evaluated Root Mean Square Deviation (RMSD), Root Mean Square Fluctuation (RMSF), Solvent Accessible Surface Area (SASA), Radius of Gyration (RG) and Free Energy Landscape (FEL). **Results:** For EPPIN N-terminus modelling, template identification revealed elafin (PDB ID: 1fle) and antileukoproteinasase (2z7f) as best templates since they showed highest percentage identity with EPPIN N-terminus (37 and 31%), resulting in models with 86% and 82% sequence coverage, QMEAN Z-scores=-2.56 and -1.15, and conservation of WFDC domain disulfide bonds. In MD simulations, 1fle- and 2z7f-EPPIN models showed similar RMSD (<0.5 nm), RMSF (0.06-0.60 nm), SASA (41-52 nm<sup>2</sup>), and FEL converging to a similar state in both models. RG analysis showed that 2z7f-EPPIN model (1.11-1.25 nm) presented a more compact structure than 1fle-EPPIN model (1.07-1.26 nm). As for EPPIN C-terminus, selected templates were tissue factor inhibitor (1adz) and bikunin (1bik), with 39% and 33% identity to EPPIN C-terminus, providing models with 95% and 97 % sequence coverage, QMEAN Z-scores=-3.28 and 0.68, and conservation of Kunitz domain disulfide bonds. In MD simulation, 1adz- and 1bik-EPPIN models showed similar RMSD (<0.5 nm) and RMSF (0.07-0.60 nm) values. SASA values were slightly higher and more stable for 1adz-EPPIN model (42-51 nm<sup>2</sup>) than 1bik-EPPIN model (35-47 nm<sup>2</sup>), which was consistent with a FEL with one minimum-energy state. RG analysis showed that 1bik-EPPIN model (1.03-1.18 nm) was more compact than 1adz-EPPIN model (1.15-1.25 nm). **Conclusions:** We were able to build optimized structural homology models for mouse EPPIN's WFDC and Kunitz domains, providing new insights into its structure. Collectively, our results provide a promising *in silico* platform for the understanding of EPPIN's structure and mechanism of action and for the rational design of potential EPPIN-binding contraceptive drugs. **Financial support:** FAPESP (2015/08227-0)

**12.014 Neuroprotective effect of arylnitrones in *in vitro* models of stroke.** Boni MS<sup>1</sup>, Dias AG<sup>2</sup>, Costa PRR<sup>3</sup>, Costa DSS<sup>1</sup>, Castro NG<sup>1</sup> <sup>1</sup>UFRJ – Farmacologia e Inflamação, <sup>2</sup>UFRJ – Química Orgânica, <sup>3</sup>UFRJ – Produtos Naturais

**Introduction:** Stroke is a neurologic disease that represents the third most frequent cause of deaths in the whole world and second in Brazil. Focal ischemia leads to excessive release of the excitatory neurotransmitter glutamate, triggering the excitotoxicity process, which contributes to neuronal injury in the ischemic core. Thrombolysis with alteplase and mechanical thrombectomy are the only therapies available for the acute treatment of ischemic stroke. However, blood reperfusion leads to a massive production of reactive oxygen (ROS) and nitrogen species (RNS), promoting neuronal death in the penumbra zone and lesion propagation. Arylnitrones related to the antioxidant alpha-phenyl-tert-butyl-nitron (PBN) synthesized by Laboratório de Química Bioorgânica from UFRJ showed a protective effect in a peripheral model of ischemia followed by reperfusion (Kim et al., Bioorg. Med. Chem., 15: 3572, 2007). **Aim:** Considering the redox imbalance in neural ischemia-reperfusion, our aim was to study the possible antioxidant and neuroprotective effects of the novel arylnitrones in *in vitro* models of stroke. **Methods:** A high-content live-dead fluorescence assay was used to evaluate the cytotoxicity of the compounds after 24-hour exposure of HT29 human colon epithelial cells. Using rat cortex cell cultures, we analyzed the effects on excitotoxicity induced by a brief exposure to 500  $\mu$ M of glutamate + 10  $\mu$ M of glycine through colorimetric quantification of lactate dehydrogenase (LDH) released. Using acute cortical slices (300  $\mu$ m) from adult rats, we studied the neuroprotective effects of nitrones in oxygen-glucose deprivation (OGD) followed by reperfusion to simulate ischemia. **Results:** None of the tested compounds was cytotoxic to HT29 cells in the 50-1000  $\mu$ M concentration range. Glutamate with glycine induced an increase of 4.8 times in LDH release (27.1% of total content) compared with the control group (cells not exposed to glutamate) (6.3% of total LDH). Initially we performed a screening of twenty arylnitrones at 500  $\mu$ M, and the compounds LQB 123, LQB 534, LQB 537, LQB 539 showed respectively 58%, 21%, 55% and 49% of protection compared with the glutamate group (N = 3 independent experiments in triplicates), while 100  $\mu$ M of LQB 536 showed 56% of protection in this assay. The cLog P of the compounds determined *in silico* (ChemDraw program) ranged between 0.241 and 4.855. The most potent substances in the glutamate assay showed the highest cLog Ps, which is promising regarding possible brain penetration. OGD followed by reperfusion of cortical slices increased 4.2 times the LDH release (7.2% of total content) compared with the control group (slices not exposed to OGD) (1.7% of total). None of the nitrones tested at 1 mM was protective in this assay. **Conclusion:** Thus, the arylnitrones were not toxic in a large range of concentration and four compounds showed an interesting protective effect in the excitotoxicity assay. On the next steps, it would be interesting to investigate whether neuroprotection is associated with an antioxidant effect of these compounds using other models and appropriate biochemical endpoints. **License number of ethics committee:** DFBICB029 **Financial support:** CNPq-MS-MCTI, CNPq and CAPES fellowships

**12.015 Antinociceptive effect and mechanism of action of LASSBio-1822.** Salomé DC, Cordeiro NM, Freitas RHCN, Fraga CAM, Fernandes PD UFRJ – Ciências Biológicas

**Introduction:** Pain is a major health problem as it affects the daily routine of individuals, once it influences their activities. Drugs such as morphine have long been used to limit various types of painful processes, however it has many undesirable effects such as dependence and tolerance [1]. For this reason, the continuous search for new analgesics with lesser or few undesirable effects is an important goal. So, in this work our aim was to evaluate the antinociceptive activity of LASSBio-1822, an n-acylhydrazone derived from LASSBio-1524. **Methods:** Female Swiss *webster* mice (22-25g, n=4-6) were pre-treated orally with LASSBio-1822 (0.003, 0.03 or 0.3 mg/kg) or morphine (2.5 mg/kg) or vehicle (polyssorbate 80) and the animals were placed on a hot plate (Insight Equipment, Brazil) set at  $55\pm 1^{\circ}\text{C}$ . At successive intervals of 30 min after oral administration, the reaction time was observed when the animals licked their hind paws and jumped. The antinociceptive effect was quantified as area under the curve (AUC) of responses measured between 30 and 180 minutes. To evaluate a possible mechanism of action, the animals were treated with the following antagonists: opioid (naloxone, 1 mg/kg, ip), muscarinic (atropine, 1 mg/kg, ip) and nitroergic (L-NAME, 3 mg/kg, i.p.). After 15 minutes, mice were orally treated with 0.3 mg/kg of LASSBio-1822 and 30 minutes later animals were placed on the hot plate to evaluate the antinociceptive effect as described previously. Capsaicin-induced licking time was performed to evaluate a possible mechanism of action. Animals were orally pre-treated with LASSBio-1822 (0.3 mg/kg) or vehicle 1h before capsaicin injection (0.0016 mg/paw) 20  $\mu\text{L}$  into the hind paw. The licking time was evaluated during 5 minutes. Results are mean  $\pm$  SD. The Software GraphPad Prism 5.0 (La Jolla, CA, USA) calculated the AUC. Statistical analysis was performed by ANOVA and Bonferroni's post-test ( $*p<0.05$ ). Protocols for animal use received number #DFBCICB015-04/16. **Results:** Pre-treatment with 0.003, 0.03 or 0.3 mg/kg of LASSBio-1822 increased the AUC when compared with the vehicle-group, and this effect can be comparable with morphine (vehicle group:  $786 \pm 112$ , 0.003 mg/kg:  $3,206 \pm 1,588^*$ , 0.03 mg/kg:  $5,371 \pm 2,012^*$ , 0.3 mg/kg:  $9,059 \pm 1,803^*$ , morphine:  $12,133 \pm 2,356^*$ ). Pretreatment with all the antagonists reversed the antinociceptive effect of LASSBio-1822 (at 0.3 mg/kg): LASSBio-1822:  $9,059 \pm 1,803$ , LASSBio-1822+ atropine:  $5,244 \pm 1,585^*$ , LASSBio-1822 + L-NAME:  $4,741 \pm 1,175^*$ , LASSBio-1822 + naloxone:  $1,734 \pm 408^*$ . In the capsaicin-induced licking model, when compared to the vehicle group, LASSBio-1822 (0.3 mg/kg) caused a significant reduction in the licking time (vehicle group:  $53.6 \pm 16$  seconds and treated group:  $15.2 \pm 6.5^*$  seconds). **Conclusion:** Our results indicate that LASSBio-1822 demonstrated significant peripheral and central antinociceptive activities and possible mechanism of action through muscarinic, opioid and nitroergic pathways. **Bibliographic References:** 1- GHOLAMI, M. et al. IJPR, 14-303, 2015. **License number of ethics committee:** DFBCICB015-04/16 **Financial support:** CAPES, CNPq, FAPERJ and Institute Vital Brazil (donation of animals).

**12.016 *Stevia serrata* essential oil produces antinociceptive and anti-hyperalgesic effects.** Cordeiro MS<sup>1</sup>, Simas DLR<sup>2</sup>, Silva AJR<sup>2</sup>, Fernandes PD<sup>3</sup>, Giorno TBS<sup>3</sup> <sup>1</sup>UFRJ – Farmacologia e Química Medicinal, <sup>2</sup>UFRJ – Química Orgânica, <sup>3</sup>UFRJ – Farmacologia e Inflamação

**Introduction:** *Stevia serrata* is a plant from Asteraceae family that grows in Central America and Mexico and in northern South America (1). The aim of this study was to evaluate the antinociceptive activity of the essential oil (EO) from *Stevia serrata*. **Methods:** EO was obtained by hydrodistillation using a Clevenger-type apparatus for 2 h. Swiss Webster mice (20-25g, n=6) were orally pretreated with 10, 30 or 100 mg/kg doses and evaluated in formalin-induced licking response, hot plate test and thermal hyperalgesia test. One hour after treatment, mice received intraplantar injection (20 µL) of formalin (2.5%). The time (in seconds, sec) that animal spent licking the injected paw was counted with a stopwatch during the 1<sup>st</sup> 5 minutes (min) (1<sup>st</sup> phase) and between 15 and 30 min (2<sup>nd</sup> phase). In hot plate test, animals were placed on a plate (Insight Equipment, Brazil) set at 55±1°C. At 30 min intervals, between 30 and 180 min after oral administration of EO or vehicle, the reaction time was recorded when the animals licked their fore- and hind-paws and jumped. Antinociception was calculated by the area under the curve (AUC) of responses between 30 and 180 min after drug administration. In the thermal hyperalgesia test, 1h before treatment, the animals received 1% carrageenan in the hind paw and were placed on a hot plate at the times of 1h, 2h, 4h and 6h after the injection of carrageenan and the time of reaction of the carrageenan-injected paw of the heated plate was recorded. **Results:** EO significantly reduced 1<sup>st</sup> and 2<sup>nd</sup> phases of formalin-induced licking, 1<sup>st</sup> phase: vehicle-treated group= 42.4±7.7 sec versus 23±10.4\* sec (45.7%); 18.1±3.6\* sec (57.3%) and 16.2±7.1\* sec (61.9%) to 10, 30 and 100 mg/kg, respectively; and 2<sup>nd</sup> phase: vehicle-treated group = 224.7 ± 25.7 sec versus 195.5±16.6(12.1%); 127.7±31.3\* sec (43.2%) and 77.6±26.3\* sec (65.5%) to 10, 30 and 100 mg/kg, respectively. EO significantly increased the AUC values when compared with vehicle-treated group in hot plate test. Vehicle-treated group=986.5±193.1; 10mg/kg=1,725.5±370 (74.9% increase); 30mg/kg=1,229.5±152.2(24.6% increase); 100 mg/kg=1,868.5±339.1 (89.4% increase). In Thermal hyperalgesia test, the dose of 100 mg/kg increased the latency time in e 100%, 128.6%, 123.1% and 266.6% at 1h, 2h, 4h and 6h after carrageenan injection, respectively. 30 mg/kg increased in 71.1% and 166.6% at times of 4h and 6h and 10 mg/kg increased in 133% at time of 4h. **Conclusions:** Our results are the first evidence that EO from *S. Serrata* produces peripheral and central antinociceptive and anti-hyperalgesic effects. (1)SIMAS,D.L.R., et al..Chemical Composition and Evaluation of Antinociceptive Activity of the Essential Oil of *Stevia serrata* Cav. from Guatemala. **Nat. Prod. Res.**, 1: 1-3, 2017. Acknowledgements: Alan Minho for technical assistance, Institute Vital Brazil (Niterói, Brazil) for donation of mice. **Financial support:** CAPES, CNPq and FAPERJ. Ethics committee of the CAUAP/UFRJ protocol number: DFBCICB015-04/16. **License number of ethics committee:** DFBCICB015-04/16 **Financial support:** CAPES, CNPq, FAPERJ

**12.017 Investigation of the antibacterial activity of cashew gum-based zinc oxide nanoparticles**, Souza JMT<sup>1</sup>, Araújo AR<sup>1</sup>, Silva DA<sup>1</sup>, Leite JRSA<sup>2</sup>, Eaton P<sup>3</sup> <sup>1</sup>Biotec-UFPI, <sup>2</sup>UnB – Morfologia, <sup>3</sup>ULisboa – IMM

**Introduction:** Cashew gum (CG) is a heteropolysaccharide, obtained from the exudate of cashew tree stem, which has been recently used in the synthesis and stabilization of nanoparticles. Zinc oxide nanoparticles (ZnO-NPs) are one of the most commercially important nanomaterials, due to their number of applications and low toxicity. This work aimed to synthesize ZnO-NPs, through a green synthesis route, using CG as a biotemplate and to investigate their potential against different species of bacteria. **Methods:** For the nanoparticles synthesis, a 1.5% (w/v) CG solution was prepared and left under heating and constant stirring for 2 hours. Subsequently, 2g of Zn(NO<sub>3</sub>)<sub>2</sub> were added to the CG solution and left under the same conditions for 12 hours. After this process, the zinc-gum solution was treated by calcination, at 500 °C, for 4 hours. UV-visible spectroscopy (UV-vis), Fourier-transform infrared spectroscopy (FTIR) and Atomic Force Microscopy (AFM) were used to characterize the nanoparticles. The antibacterial assay was carried out using a 96 well plate microdilution method, as described by CLSI, against *Staphylococcus aureus* ATCC 29213, *Staphylococcus epidermidis* ATCC 12228, *Salmonella enterica* Thyphimurim ATCC 1401 and *Escherichia coli* ATCC 25922. The species were exposed to different concentrations of nanoparticles, varying from 1000 µg/mL to 15.25 µg/mL. ZnO-NPs biocompatibility was assessed by a haemolysis assay, where erythrocytes were exposed to the same concentrations used for the antibacterial test. **Results:** Uv-vis results showed a sharp band at 376 nm, which confirmed the synthesis of the nanoparticles. A strong absorption band at 440 cm<sup>-1</sup> was observed through the FTIR spectrum, which corresponds to Zn-O stretching. AFM imaging enabled the observation of round-shaped nanoparticles, with a mean size of 129 nm. The ZnO-NPs synthesized in this work were able to inhibit all the bacteria tested, with the exception of *Escherichia coli* (MIC > 1000 µg/mL), showing MIC concentrations of 62.5 µg/mL, 31.25 µg/mL and 500 µg/mL to *S. aureus*, *S. epidermidis* and *S. enterica*, respectively. To observe the ZnO-NPs effects against the bacteria, the most susceptible strain (*S. epidermidis*) was chosen for AFM imaging. The images revealed no clear morphological differences between the tested and the control bacteria, as well as no significant differences in the mean surface roughness between the two groups. However, the surface profiles analysis showed that the surface of the bacteria exposed to ZnO-NPs exhibited some depressions, with dimensions varying from 150-250 nm of width and 15-30 nm of height, which also suggest that the bacteria may suffer a decrease in size. In the range of 1000 µg/mL to 15.25 µg/mL, no haemolytic activity was observed for the ZnO-NPs, what means that the material may be considered safe. **Conclusion:** In this work, ZnO-NPs were successfully green synthesized, using CG as a template, showing an interesting antibacterial potential. Moreover, the absence of haemolytic activity shows that the material is biocompatible. More detailed studies are required, however, to elucidate the mechanism of this material against the micro-organisms. **Financial support:** CNPq, CAPES



**12.018 Search of novel phenylpiperidine cholinesterase inhibitors for Alzheimer's disease with less cholinergic side effects.** Guimarães MJR<sup>1</sup>, Viegas Jr C<sup>2</sup>, Castro NG<sup>1</sup> <sup>1</sup>UFRJ – Farmacologia e Inflamação, <sup>2</sup>Unifal – Química

**Introduction:** Alzheimer's disease (AD) is a progressive neurodegenerative disease that initially affects cognitive abilities due to the dysfunction and death of entorhinal and hippocampal neurons. A cholinergic hypothesis proposes that neurodegeneration in the basal forebrain causes the levels of acetylcholine to fall in the cortex and hippocampus, possibly leading to their dysfunction. This contributes to the cognitive symptoms evident in those with the disease. In agreement with this hypothesis, AD is currently treated through the use of anticholinesterase substances, such as galantamine, donepezil and rivastigmine. However, the high cost and a high presence of side effects, mainly due to activation of peripheral muscarinic receptors, drive the demand for new drugs.

We have planned and synthesized novel AD multi-action drug candidates with anticholinesterase and anti-inflammatory properties, designed by hybridization and molecular modification from the phenylpiperidine framework of donepezil with an acylhydrazone spacer unit, which in addition to the anticholinesterase activity unit could also have other beneficial activities in AD, interacting with other targets. One of the goals is to add an inhibition of M3 receptors, which mediate adverse effects such as nausea and diarrhea related to increased gastrointestinal secretion and bowel motility. **Methods:** All samples were first analyzed for their inhibitory effect on cholinesterases by the Ellman method with purified acetylcholinesterase of *E. electricus* (AChE) and equine serum butyrylcholinesterase (BuChE). Calcium fluorimetry assays were performed with the ratiometric indicator fura-2 to determine a possible antagonistic action of the M3 muscarinic receptor on human intestinal epithelial cells (HT-29 strain). Cytotoxicity assays were performed using a Live / Dead methodology for calcein AM and propidium iodide fluorimetry. **Results:** Fourteen of 54 substances that were screened at 30  $\mu$ M presented an inhibitory action on AChE greater than 50% and were selected to obtain concentration-response curves. The IC<sub>50</sub> were between 3.3 and 22.8  $\mu$ M for AChE and between 12.8 and 18.9  $\mu$ M for BuChE. Thus, most of the analyzed substances demonstrated selectivity between cholinesterases. We obtained the concentration-response curve of carbachol in standardization tests of the calcium assay and began the evaluation of the antagonistic effect on M3 receptor, preincubating the cells with the phenylpiperidine derivatives before the addition of carbachol. None of the compounds (at 30  $\mu$ M) seemed to inhibit carbachol-induced calcium mobilization in HT-29 cells. None of samples evaluated was cytotoxic (at 30  $\mu$ M). **Conclusion:** The novel donepezil analogues were active as AChE inhibitors, but additional structural modifications in our current series of compounds may be required to incorporate a significant antimuscarinic activity. **Financial support:** CNPq, FAPEMIG and a CAPES fellowship

**12.019 Antimicrobial and antioxidant activities of silver nanoparticles *Terminalia fagifolia* Mart. extract-based.** Barros AB<sup>1</sup>, Araújo-Nobre AR<sup>1</sup>, Nogueira KM<sup>1</sup>, Andrades EO<sup>1</sup>, Brito MP<sup>1</sup>, Nunes PHM<sup>2</sup>, Marinho-Filho JDB<sup>1</sup>, Medeiros JVR<sup>1</sup>, Silva DA<sup>1</sup>, Leite JRSA<sup>3</sup> <sup>1</sup>Biotec-UFPI, <sup>2</sup>UFPI –Plantas Medicinais, <sup>3</sup>UnB – Medicina

**Introduction:** Silver nanoparticles (AgNPs) have been the subjects of researchers because it may have several applications in the medical field. The green synthesis of silver nanoparticles enables the formation of systems with different shapes and sizes, with low cost, safer, benign and ecological. *Terminalia fagifolia* Mart. is a specie rich in secondary metabolites with various ethnopharmacological uses. These secondary metabolites have potential for green synthesis of bioactive nanoparticles that may be alternatives for the treatment of infectious and non-infectious diseases. In this context, this work aimed to synthesize silver nanoparticles with aqueous extract of *T. fagifolia* and test its antibacterial and antioxidant potential. **Methods:** The AgNPs were synthesized with the plant aqueous extract (0,05% and 0,025%) and AgNO<sub>3</sub> (1mM) in a ratio of 1: 1 under constant stirring for 24 hours in the dark, adjusting at pH of synthesis at 7 and 9. The AgNPs characterization was performed by UV-vis spectroscopy, dynamic light scattering (DLS), Zeta potential determination, Fourier transform infrared spectroscopy (FT-IR). Antioxidant activity was assessed by DPPH and ABTS assays. The antibacterial activity was performed against *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922 bacteria, by the microdilution method according to M07-A10 (CLSI, 2015). AgNPs biocompatibility was assessed by a haemolysis assay. **Results:** The presence of color change and plasmonic band in the wavelength between 420 and 424 nm confirmed the AgNps synthesis. The mean size of AgNPs according to the DLS ranged from 23.09±0.71 to 50.23 ± 0.35 nm with polydispersity of 0.3 a 0.6 for both samples. The Zeta potential analyzed indicated that systems might be considered stable with negative surface charge. By the FT-IR analysis, it was possible to observe the reduction of the characteristic bands of the constituents of the aqueous extract. All nanoparticles showed sequestration capacity of the DPPH and ABTS. No haemolytic activity was observed in the concentrations tested and AgNPs tested were active against both strains used, however *E. coli* was more susceptible. **Conclusion:** Therefore, the green synthesis was performed efficiently, presenting nanoparticles with good physico-chemical characteristics, with antibacterial and antioxidant potential. **Financial support:** CNPQ

**12.020 Stathmin 1 as a target for anticancer therapy: Heterologous expression.** Silva CSMR<sup>1</sup>, Branco PC<sup>1</sup>, Barbosa GH<sup>2</sup>, Jimenez PC<sup>2</sup>, Costa-Lotufo LV<sup>1</sup>, Machado-Neto <sup>1</sup>ICB-USP – Farmacologia, <sup>2</sup>Unifesp – Bioprodutos e Bioprocessos

**Introduction:** Marine pharmacology is a branch of the pharmaceutical sciences that focuses on the search of substances with pharmacological properties in marine organisms. The marine environment is an exceptional warehouse for new bioactive natural products with unique structural and chemical characteristics, including those with anticancer properties. One target of these new anticancer-based marine bioactive products can be stathmin 1 (STMN1). STMN1, also known as oncoprotein 18 (OP18), is a phosphoprotein that regulates the microtubule dynamics due to its participation in the destabilization of these microtubules, allowing the promotion of microtubule catastrophe and/or sequestration of alpha/beta tubulin heterodimers. The interaction between STMN1 and microtubules accelerates cell cycle progression, and increases cell proliferation, migration, and survival. STMN1 overexpression is associated with a variety of cancers in humans, such as breast cancer, prostate cancer, colon carcinoma and hematological malignancies. Of note that specific inhibition of STMN1 using siRNA or shRNA reduces the proliferation and clonogenicity in multiple cancer models. Bioaffinity chromatography is a novel technique and can be used to find biological affinity between proteins of interest and extracts that exhibit cytotoxicity, since it implants the biological affinity as the matrix for the isolation of the compound. Thus, this innovative technique may find bioactive compounds that interact with key proteins that participate of important pathways in the maintenance of cancer phenotype. In this way, the aim of the present study was to standardize heterologous STMN1 expression to be used in bioaffinity chromatography assays. **Methods for heterologous expression of STMN1:** *E. coli* Rosetta bacterial strains already came transformed with the plasmid containing STMN1 (pQTEV-STMN1, Addgene, USA). The transformed bacteria were induced with different concentrations of IPTG, 0.25 mM, 0.5 mM, 1.0 mM and 1.5 mM. We also evaluated this expression in small and large scale and also in two different temperatures: 16 and 37°C; we also evaluated the expression with different times of induction. Validation of overexpression was evaluated on polyacrylamide gel by Western Blotting. Proteins were purified by immobilized metal ion affinity chromatography (IMAC) and subsequent dialysis. **Results:** In the tests for the heterologous expression of STMN1 protein, best results were obtained with the concentration of 1.0 mM of IPTG for induction. The growth of the bacteria was also better on the small and large scale at 16°C. **Conclusion:** STMN1's heterologous expression method was standardized, which will aid in future research with this protein, reducing cost and production time. STMN1 is an interesting therapeutic target and for this reason it was chosen for the performance of the functional chromatography with the different marine extracts. The studies involving STMN1 and marine extracts present potential for new anticancer therapies. **Financial support:** CAPES, CNPq and FAPESP (2018/06522-2; 2017/24993-0; 2015/17177-6)