09. Natural Products and Toxinology

09.001 Rhamnogalacturonan as a potential therapeutic target for the treatment of ulcerative colitis. Maria-Ferreira D[1], Borato DG[1], da Silva LM, Corso CR[1], Nascimento AM[2], Cipriani TR[2], Watanabe PS[3], Santana DMG[3], van den Wijngaard RM, Werner MFP[1], Baggio CH[1] – [1]UFPR – Farmacologia, [2]UFPR – Bioquímica, [3]UEM

Introduction: Ulcerative colitis is a chronic disorder that affects the colon with unknown etiology. Despite several treatment options, not all patients are responsive to therapy and their use is related with side effects. Taking into account, the polysaccharides recently became the focus of studies exhibiting important pharmacological activities as immunomodulatory and antinociceptive. In addition, our group already showed that the polysaccharide rhamnogalacturonan (RGal) isolated from *Acmella oleracea* has potent antiulcerogenic and gastric ulcer healing activities (Maria-Ferreira *et al*., Plos One, v. 9, p. e84762, 2014). Therefore, in this study we investigate the protective effects of RGal in the dextran sulfate sodium (DSS)-induced colitis in mice and the underlying mechanisms in heterogeneous human epithelial colorectal adenocarcinoma cells (Caco-2). **Methods**: Colitis was induced by administration of DSS for 5 days followed by 2 days of water. The animals were orally treated with vehicle (water, 1 ml/kg) or RGal (10 mg/kg) daily. Colitis characteristics (body weight change, presence of blood in feces and fecal consistency) were monitored daily, and visceral allodynia was evaluated by von Frey filaments. After 7 days, the colons were collected and their weight and length were measured. The colonic tissues were used for the enumeration of total neurons of the myenteric plexus and histological evaluation. Caco-2 cells were challenged with IL-1β in a transwell epithelial cellular permeability assay and were used for analysis of IL-8 levels and also expression and distribution of tight junction proteins claudin-1, occludin and ZO-1 by immunoblot or immunofluorescence (CEUA/BIO-UFPR; approval number 721). **Results and Discussion**: The treatment with RGal reduced the colitis score from the day 5 (28%) to the day 8 (46%), protected mice from body weight loss (day 8, 51%) and reduced the macroscopic damage when compared to the DSS group. RGal also prevents the reduction of colon length (RGal: 9.1 ± 0.2 cm) when compared to DSS group (DSS: 7.1 ± 0.2 cm), reduced the withdrawal response to mechanical stimulation of the abdomen by von Frey hairs and protected the colonic ganglion cells when compared to the DSS group. In histological analysis, RGal increased mucosal and submucosal thickness in 22 and 54% and decreased the thickness of the muscular layer and total wall in 38 and 18% respectively when compared to the DSS group accompanied by the maintenance of mucosal enterocytes and goblet cells. In the transwell assay, RGal (100 and 1000 µg/ml, inner well) prevented the cellular permeability in 43 and 65%, respectively, and 77% at 1000 µg/ml (outer well), when compared to IL-1β group. We also observed a significantly reduction of IL-8 secretion (RGal: 807 ± 49.7 pg/ml) and a diminished claudin-1 expression (40%) when compared to IL-1β group. **Conclusion**: Collectively, we demonstrated that RGal reduces the severity of DSS colitis in mice through protecting colon epithelium and neurons of the myenteric plexus, reducing the abdominal allodynia, epithelial permeability and cytokine secretion and maintaining the integrity of junction complexes. Thus, RGal may represent a promising molecule for drug development to the treatment of ulcerative colitis. **Support**: CAPES, CNPq, Nuffic

09.002 The role of oxidative stress in indigo alkaloid protection against TNBS-induced colitis in rats. de Almeida ACA[1], de Faria FM[1], Manzo LPB[1], Dunder RJ[1], Socca EAR[1], Luiz-Ferreira A[2], Souza Brito ARM[1] [1]IB-Unicamp, [2]UFG – Ciências Biológicas

Introduction: Inflammatory Bowel Disease (IBD) is an exacerbated immune response in the intestinal tract and involves two conditions: Crohn Disease and Ulcerative Colitis. IBD incidence is increasing in the world and the treatment is rather ineffective, including various side effects and high costs. Thus, the research with active compounds may bring therapeutic alternatives for IBD. Indigo alkaloid is a secondary compound encountered in *Ingofera sp.* plants, with anti-inflammatory and antioxidant properties. **Aims:** The aim of this study was to investigate the effect of Indigo alkaloid in acute colitis induced by trinitrobenzenosulphonic acid (TNBS) in rats, an experimental model of Crohn Disease. **Methods**: Male rats HanUnib: WH (250 – 300 g) were divided in 7 groups (n = 7, per group) and treated orally with vehicle (NaCl 0.9 %, 10 ml/kg) or indigo (0.1, 1, 3, 10 and 30 mg/kg) at 72, 48, 24, 2 h before and 24 h after TNBS instillation. TNBS (30 mg) dissolved in ethanol (250 µl) was administered intra-rectally through a catheter 8 cm inside the anus. Rats were euthanized 48 h after colitis induction. The colon was removed, cleaned and photographed for macroscopic lesion analyses. The colon was homogenized for catalase (CAT), superoxide dismutase (SOD) and glutathione peroxidase (GPx) activity determination and lipid peroxidation (LPO), glutathione levels (GSH) assays. **Results**: TNBS induced an extensive lesion on colon, with edema, ulceration and necrosis (lesion area = 817 mm^2 and score = 9). Indigo treatment reduced significantly the lesion area, in all doses evaluated (reduction of 20 – 60 %), but just in rats treated with 3 mg/kg of indigo (IND_3 + TNBS) there was decrease of injury macroscopic score (lesion index = 6). We observed decrease in colonic CAT, GPx activity and GSH contents and increase in LPO levels and SOD activity in TNBS group, compared to Control (non-colitic). IND_3 + TNBS group presented basal LPO levels and increased SOD activity in colon, as while GPx and GSH were maintained as TNBS group levels. **Conclusions:** Oral treatment with indigo ameliorated TNBS injury, reducing inflammation area, but just 3 mg/kg doses decrease lesion score. This dose was further selected for biochemical assays. Indigo oral administration inhibited lipid peroxidation and increased SOD activity. Thus, antioxidant activity may contribute to protective effect of indigo alkaloid in TNBS colitis. **Financial Support**: FAPESP and CAPES. Approved by Ethical Committee of Animal Use (CEUA/Unicamp, protocol numbers 2399-1 and 3743-1).

09.003 Involvement of muscarinic and bradykinin receptors in the prolonged diuretic properties of *Echinodorus grandiflorus* and its relation to the prostaglandin and nitric oxide pathway. Tirloni ACS[1], Prando TBL[2], Barboza LN[3], Gasparotto FM[1], Lourenço ELB[2], Gasparotto Junior A[1] [1]UFGD – Farmacologia e Toxicologia de Produtos Naturais, [2]Unipar – Farmacologia e Toxicologia de Produtos Naturais, [3]UFPR – Farmacologia

Introduction: In Brazil, several medicinal species are used as diuretic drugs, but most of them lack pharmacological research that shows the molecular pathways that might be contributing to these effects. Among them, there is the *Echinodorus grandiflorus* (Cham. & Schltr.) Michel, native specie that occurs from southern Mexico to Brazil. In Brazil is popularly known as “chapéu de couro” and its leaves are habitually employed as diuretic, hypotensive and hypolipidemic drug (Bolson et al., 2015). **Aim**: Evaluate possible mechanisms involved in prolonged diuretic activity of the ethanol soluble fraction obtained from *Echinodorus grandiflorus* (ES-EG) and to assess its relationship with a hypotensive and antihypertensive activity using normotensive rats and with renovascular hypertension (2K1C). **Methods**: The diuretic effects of ES-EG (30-300 mg/kg; p.o.) were compared with hydrochlorothiazide in 7 days repeated-dose treatment. The urinary volume, sodium, potassium, chloride, bicarbonate, conductivity, pH and density were estimated in the sample collected for 24 hours by seven days. The plasmatic concentration of sodium, potassium, total protein, urea, creatinine, aldosterone, vasopressin, nitrite, acetylcholinesterase and angiotensin converting enzyme (ACE) activity were measured in samples collected at the end of the experiment (seventh day). Using pharmacological antagonists or inhibitors, we determine the involvement of bradykinin, prostaglandin, acetylcholine, and nitric oxide (NO) in the diuresis, hypotension and antihypertensive effects induced by ES-EG. In addition, activity of erythrocytary carbonic anhydrase and renal Na+/K+/ATPase were evaluated *in vitro*. All experimental procedures adopted in this study were previously approved by the Institutional Ethics Committee of Universidade Paranaense (UNIPAR, Brazil; protocol number 25454/2014). **Results**: ES-EG increased diuresis similarly to hydrochlorothiazide and also presented HCO_3^--sparing effects. Previous treatment with HOE-140, indomethacin and atropine fully avoided the diuretic effect of ES-EG, and including the L-NAME pre-administration, reduced the hipotensive and antihypertensive activity of ES-EG. In addition, the 7 days treatment with ES-EG resulted in increased plasmatic levels of nitrite. All other parameters were not affected by treatment with ES-EG. **Conclusion**: Our results suggest that the mechanisms through extracts of *Echinodorus grandiflorus* increase diuresis and reduces blood pressure in normotensive and 2K1C rats are mainly related to activation of muscarinic and bradykinin receptors with direct effects on the prostaglandins and nitric oxide pathways. **Acknowledgements**: We are grateful to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Diretoria Executiva de Gestão da Pesquisa e Pós-Graduação (DEGPP/UNIPAR) for financial support. **References**: Bolson, M. et al. Ethno-medicinal study of plants used for treatment of human ailments, with residents of the surrounding region of forest fregments of Paraná, Brazil. J Ethnopharmacol, v. 161, p. 1-10, 2015.

09.004 Effect of 2-Phenylquinoline in experimentally induced gastric ulcers: Pathways of gastroprotection. Breviglieri E[1], da Silva LM[1], Boeing T[1], Somensi LB[1], Gimenez A[2], Cechinel-Filho V[1], Andrade SF[1] – [1]Univali – Pharmaceutical Sciences, [2]Universidad Mayor de San Andrés

Introduction: The therapy for gastric ulcers is based in antisecretory drugs; however this treatment is linked to side effects and ulcer remission. In light of this, therapeutic alternatives are necessary and natural products are an important source for this. Previous studies in our laboratory revealed the gastroprotective potencial of 2-phenylquinoline (2-PQ) (Zanatta, F. Chem Biol Interact., 180: 312, 2009), but no mechanism involved in this effect was studied. **Aims**: The present study was carried out in order to elucidate the mechanisms involved in gastroprotective activity of 2-PQ, an alkaloid from bark of *Galipea longiflora* (Rutaceae). **Methods**: The gastroprotective activity of 2-PQ (10-100 mg/kg, p.o) was assessed by gastric ulcer induced by acidified ethanol (60% ethanol/0.03M HCl, p.o) in mice or indomethacin (80 mg/kg, p.o) in rats. Ulcerated tissue were processed for histological and biochemical analysis. In another series of trials, mice were pretreated with N^G-nitro-l-arginine methyl esters (l-NAME, 70 mg/kg, s.c), N-ethylmaleimide (NEM, 10 mg/kg, s.c), yoimbine (2 mg/kg, s.c) or indometacin (10 mg/kg, s.c) prior 2-PQ treatment to verify the involvement of nitric oxide, nonprotein sulfhydryl compounds, α 2-receptors and prostaglandin in the gastroprotection. Acid antisecretory effect was verified *in vivo* by pylorus ligation in rats. *In vitro* effects in gastric H^+,K^+-ATPase activity also was assessed. Carbenoxolone (200 mg/kg) or omeprazole (20 mg/kg) were used as positive controls. 2-PQ was identified by spectroscopic methods. **Results and Conclusion**: 2-PQ exerted gastroprotective activity against acidified ethanol reducing the lesion area up to 90% (vehicle ulcerated group: 84.4 ± 1.4 mm^2), and histological evaluation supported this effect. Furthermore, 2-PQ (30 mg/kg, p.o) also reduced in 85% the gastric ulcer induced by indomethacin (ulcerated vehicle group: 9.5 ± 3.9 mm^2). The gastroprotective action of PQ involved increasing in superoxide dismutase-, catalase- and glutathione-S-transferase- activity, as well as an increase in glutathione-reduced levels and an inhibition in neutrophyl infiltration [checked by the reduction in myeloperoxidase activity]. Additionally, NEM and Yoimbine, but not L-NAME or indometacin pre-treatment, reversed the gastroprotective effect of 2-PQ. Besides, 2-PQ (30 mg/kg, i.d) decrease the volume and total acidity of gastric juice in 56 and 71% (vehicle groups: 6.9 ± 0.7 ml and 0.056 ± 0.01 Eq H^+, respectively). In addition, 2-PQ (30 mg/kg, i.d) also reduced gastric acid prodution stimulated by histamine (10 mg/kg, s.c) or pentagastrin (400 µg/kg, s.c); but not by bethanechol (2.5 mg/kg, s.c). However, 2-PQ did not change the *in vitro* H^+/K^+- ATPase activity or the content of gastric adhered mucus in rats (vehicle groups: 1.45 ± 0.08 µM of Pi/min/mg of protein and 2600± 312 µg of Alcian Blue/g of tissue, respectively). Together, our results show that 2-PQ affords gastroprotection by different and complementary mechanisms, which include: 1) decrease in oxidative damage by a sparing effect on NP-SH reserve and increase in antioxidant enzymes activities; and 2) by inhibition in acid secretion acting mainly on the histaminergic and gastrinergic regulatory pathway, but not by H^+/K^+-ATPase inhibition. Thus, 2-PQ may be a suitable natural compound for the prevention and treatment of peptic acid diseases. **Financial support**: CNPQ, CAPES, FAPESC. Approval number CEUA: 38/14p.

09.005 Development of skin wound healing treatment: focus on *Passiflora mucronata* **plant extract.** Figueiredo J[1], Castro AB[1], Barreto A[1], Silva ICV[2], Calheiros AS[3], Ferreira AC[3], Frutuoso VS[3], Muzitano MF[1], Leal ICR[2], Bonavita AG[1] [1]UFRJ-Macaé, [2]UFRJ – Produtos Naturais e Alimentos, [3]Fiocruz – Imunofarmacologia

Introduction: Skin wound healing consists of a dynamic process involving tissues alterations to maintain the organism integrity. However this process could be affected by many conditions as diabetes mellitus, Cushing syndrome and old age. The development of bioactive molecules to enhance the healing process plays a key role in advancing wound-care management. The discovery of natural products that contain substances for skin regeneration has been showed promising. In this context, the Jurubatiba National Park (RJ) is a Restinga biome characterized by the presence of 821 plant species and is included in several research programs as the discovery of bioactive substances. **Aims**: In this work we performed the screening of plants extracts obtained from Jurubatiba National Park in animal model of skin wound healing and we evaluated the antioxidant and fibroblast migration effects of selected extract. We also tested the skin healing activity of plant extract included in gel. **Methods**: Female Wistar rats (180-200g) were anesthesiated and an excision of 1-2 cm^2 was made in dorsal skin. The wound was topically treated with crude hydroalcoholic extracts (3 µg/site/3 days) of *Passiflora mucronota (Pm), Peplonia asteria (Pa), Ocootea notata and Kielmeyera membranacea*. In another set of experiments, a gel formulation of plant extract was used to treat skin wounds. *In vitro* antioxidant activity was evaluated using the 2,2-diphenylpicrylhydrazyl (DPPH) assay where the scavenging activity of plant extracts was measured and compared with positive control *Ginko bilola*®. Fibroblast migration was evaluated by the scratch assay where $1x10^5$ cells (L929 cell line) were placed in 24 wells plate in presence of RPMI 1640 medium supplemented with BSF (10%) and maintained in 37ºC, 5% CO_2. Using a pipette tip a scratch was made in the middle of each well and then plant extracts (0.001 ng-0.1 mg/ml) was added and cell migration observed for 6, 24 and 48h. Cytotoxicity was measured by the MTT test in same conditions. **Results**: Screening of crude plant extracts showed that *Pm* and *Pa* extracts were active to promote wound healing with total healing time of 18 and 19 days respectively when compared with control group (28 days). *Pm* extract was selected for the next trials. The *Pm* extract showed antioxidant activity (EC_{50} of 133,3 µg/ml) and was able to induce fibroblast migration *in vitro* in concentration (0.001 mg/ml) were no cytotoxicity was observed. Since the use of phytotherapy is an approach used to treat wounds we tested *Pm* extract included in gel to treat rats wounds. The results using *Pm* extracted included in gel showed a better but not significant reduction of healing time compared with control group treated with gel alone. However when compared with not treated animals *Pm* extract-included gel was able to promote wound healing as well the crude extract. **Conclusion**: We identify *Passiflora mucronota* extract as a possible new phytotherapic to treat skin wound probably by it antioxidant activity and capacity to induce fibroblast migration. The challenge now is set an optimum dose of the *Pm* extract-gel for better skin healing. **Financial Support**: UFRJ, FAPERJ, CNPQ. CCS-UFRJ Animal Research Ethical Committee number protocol: MACAE003.

09.006 Evidences about gastric healing activity of *Maytenus robusta* Reissek: *in vitro* and *in vivo* studies. Costa P, da Silva LM, Boeing T, Somensi LB, Cury BJ, Steimbach VMB, Santin JR, Cechinel-Filho V, Andrade SF Univali – Pharmaceutical Sciences

Introduction: Maytenus robusta Reissek (Celastraceae) is a Brazilian folk medicine used to treat gastric ulcer in substitution to the M. ilicifolia, which is in extinction stage. The gastroprotective properties of M. robusta were demonstrated previously using preventive approaches (De Andrade, SF. J Ethnopharmacol., 113: 252, 2007). However, this study does not address its healing effects on installed gastric ulcer. **Aim**: This study was carried out to investigate the healing effects of hydroalcoholic extract (HEMR) from aerial parts of M. robusta in a chronic ulcer model and determine underling mechanism. **Methods**: To evaluate healing properties of HEMR in vivo, chronic gastric ulcer was induced in rats by 80% acid acetic. After ulcer induction animals were orally treated with vehicle (water, 1 ml/kg), omeprazole (20 mg/kg), or HEMR (1- 10 mg/kg) twice a day for 7 days. At the final of treatment the total ulcer area was measured and sample of ulcer tissue were processed for histological and histochemical analysis. Evaluation of glutathione reduced (GSH) and lipoperoxides (LOOH) levels; and glutathione–S-transferase (GST), superoxide dismutase (SOD), catalase (CAT) and myeloperoxidase (MPO) activity also was performed in site of ulcer. In parallel, radical scavenging activity, citoprotective effect, cell proliferative and anti-Helicobacter pylori action were determined *in vitro*. The antisecretory effect of HEMR was evaluated in pylorus ligated rats. Acute toxicity was evaluated by organ relative weight and biochemical parameters. In addition, evaluation of prokinetic properties was undertaken in mice. **Results**: Oral administration of HEMR (10 mg/kg) reduced the gastric ulcer area in 53% compared to vehicle group (120 ± 8.3 mm^2) and this effect was evidenced in histological analysis. Moreover, HEMR treatment increased gastric mucin content and reduced oxidative stress and inflammatory parameters in site of ulcer. In vitro, HEMR (100 µg/ml) was able to scavenge free radical DPPH and promotes citoprotection against H_2O_2 in fibroblasts (L929 cells). HEMR healing properties also were confirmed by enhancement of proliferation and coverage of scratched wounds in L929 monolayer. However, HEMR up to 1000 µg/ml not presented considerable *in vitro* activity against H. pylori and in vivo at 10 mg/kg also does not promote changes in volume, pH, total acidity or pepsin activity in pylorus ligature model. Regarding gastrointestinal motility, HEMR (10 mg/kg, p.o) does not provoke alterations. Besides, is important to mention that the oral administration of HEMR did not produce any sign of acute toxicity in the animals. **Conclusions**: The data here obtained show that M. robusta possesses an evident healing ulcer potential, mainly through the strengthening of protective factors of gastric mucosa, such as mucus layer, antioxidant defenses and cell proliferation. Taking into account the advantages in cultivation and harvesting of the M. robusta in relation to the M. ilicifolia and these evidences here presented, it is plausible to conclude that hydroalcoholic extract obtained from aerial parts of M. robusta is an interestingly source to development a phytotherapeutic formulation to treat the gastric ulcer. **Financial support**: CNPQ, CAPES, FAPESC. **Approval number CEUA**: 033/14p.

09.007 Anti-inflammatory effect of crude extract of S. hispidus's skin in allergic pleurisy murine model induced by ovalbumin. Muylaert FF[1], Chaves AS[1], Fernandes LDA[2], Ferraris FK[1], Amendoeria FC[1] – [1]Fiocruz – Farmacologia e Toxicologia, [2]IEAPM –Oceanografia

Indroduction: *Stephanolepis hispidus* is one of the most common filefish species in Brazil and is popularly known as "peixe-porco", "peroá" or "cangulo". Although some people living in the northern coast of Rio de Janeiro, Southeast of Brazil, usually consume *S. hispidus* and others filefishes as the main source of protein, meanwhile the *S. hispidus* skin is discarded. Its skin is traditionally used as a complementary treatment for inflammatory disorders. Many traditional fishermen in those areas consume water infusion of dried and powdered skin of filefishes as a complementary treatment for inflammatory disorders of the respiratory system. This study was undertaken in order to investigate the effect of aqueous crude extract of *S. hispidus* skin (SAE) in a model of allergic pleurisy. **Aim**: In the current study we investigated the antiallergic effects of SAE in a model of allergic pleurisy. **Methods**: The S. hispidus's samples were purchased from Porto do Forno fish market - Arraial do Cabo, Rio de Janeiro. Fish's skins were dried at room temperature and powdered. Male Balb/c mice (20 – 25g) obtained from the FIOCRUZ breeding colony, caged with free access to food and water and kept at 22°C (± 2), 12h light/dark cycle. Experimental procedures were performed according to Committee on Ethical Use of Laboratory Animals; license P-26/13-2. Mice were sensitized by subcutaneous injection of OVA (50µg) with $AlOH_3$ (5 mg). After 14 days, were challenged with intrapleural (i.pl.) OVA injection (12.5 µg/cavity) in 100 µl of sterile PBS.A pre-treatment 1 hour before the challenge, was done with the crude extract powder in different concentrations (62.5; 125; 250; 500 and 750 mg/kg) in saline (37ºC ± 2), orally administered by gavage. The stimuly controls and reference drug groups received, as treatment, saline and Dexamethasone (10 mg/kg) respectively. After 24 hours, mice were euthanized by urethane overdose and thoracic cavities washed with 1 ml of saline EDTA. Total cell counts were done by flow citometry (Cy-flow - Partec®). Diferential counts slides were prepared using a cytocentrifuge and giemsa stain. The data was reported as the mean ± standard error of the mean (SEM) and were statistically analyzed by analysis of variance (ANOVA), followed by the Student–Newman–Keuls test or Student's t-test. Values of $p \leq 0.05$ were significant. **Methods and Results**: The i.pl. injection of OVA induced an intense accumulation of total leukocytes (SAL = 3.6 ± 1.4 vs. OVA = 35.6 ± 12.3 x 10^5 cells/cavity, n=15, $p < 0.05$). The oral pretreatment with SAE (500 and 750 mg/kg) in previously sensitized BALBc mice impaired total leukocyte (SAE 500 = 18.5 ± 8.2 vs. OVA = 35.6 ± 12.3 x 10^5 cells/cavity, n=15, $p < 0.05$; and SAE 750 = 20.8 ± 6.2 vs. OVA = 35.6 ± 12.3 x 10^5 cells/cavity, n=15, $p < 0.05$) into pleural cavities triggered by the intra-pleural (i.pl.) challenge with ovalbumin. In accordance with such results, SAE induced antiallergic activity reducing eosinophil influx (SAE 500 = 2.24 ± 1.2 vs. OVA = 7.48 ± 3.6 x 10^5 eosinophils/cavity, n=15, $p < 0.05$; and SAE 750 = 2.83 ± 0.6 vs. OVA = 7.48 ± 3.6 x 10^5 eosinophil/cavity, n=15, $p < 0.05$) 24 h after OVA i.t. stimulation. **Conclusion**: Our results provide evidence that aqueous crude extract of *S. hispidus* skin can modulate allergic inflammatory response. **Financial support**: PAPES/CNPq.

09.008 Scorpion *Tityus apiacas*: identification of venom components with antimicrobial activity. Dal Mas C[1], Carvalho MA[2], da Silva Junior PI[3], Hayashi MAF[1]
[1]Unifesp – Farmacologia Celular, [2]UFMT – Biologia e Zoologia, [3]IBu – Toxicologia Aplicada

Introduction: The venom of scorpions is a complex mixture of bioactive molecules and their potential pharmacological and biotechnological applications has stimulated several studies. Many components with antimicrobial activity were identified in these venoms. Among the scorpion species of medical interest, the *Tityus apiacas*, which is found in Mato Grosso State, arises special interest due to the lack of biochemical studies and the limited information about the composition of its venom of this scorpion, which was only recently described by Lourenço (in 2002). **Aims** The purification and characterization of the components of the crude venom of the scorpion *Tityus apiacas* from Mato Grosso, aiming the identification of components with potential antimicrobial activity. **Methods**: This study was approved by the Research Ethics Committee UNIFESP / EPM No. 171905/13. The scorpions were collected in Apiacás in Mato Grosso (license for collection ICMBio/SISBIO 12764-3 (04/13)), and the crude venom was obtained from the animals (of both genders) by electrical stimulation of the telson. The crude venom was lyophilized for storage and then, it was fractionated by reverse-phase high performance liquid chromatography (RP-HPLC) using a Jupiter C18 semi-preparative column. The fractions obtained were also repurified using C18 analytical column (generating the subfractions). The fractions and subfractions were both evaluated in antimicrobial assays performed by dilution in microplates, and absorbance readings at 595 nm in a spectrophotometer. Fractions showing antimicrobial activity against Gram-positive bacteria *Staphylococcus aureus* (ATC 29213) or Gram-negative *Escherichia coli* (SBS 363) or yeast *Candida albicans* (MDM8) were evaluated again against these same strains of microorganisms. **Results**: 12 chromatography fractions obtained from the crude venom were chosen for this study. Gel electrophoresis analysis of crude venom and obtained fractions showed the predominance of components with molecular weights below 10 kDa. From the 12 analyzed fractions, five presented antimicrobial activity, among which: one inhibited the growth of *E. coli*, one showed activity against *S. aureus* and three fractions promoted the inhibition of growth of *C. albicans* yeast. Among them, only one showed activity against *E. coli* after repurification. **Conclusion**: Our results confirm the presence of compounds with antimicrobial activity in the venom of *Tityus apiacas* but they still need to be further purified for identification and characterization to allow possible identification of similarity with known compounds. The importance of studies of scorpions venoms relying on the search for new substances with antimicrobial properties is well recognized, taking into account the increasing drug resistance of microorganisms to the conventional antibiotics. On this point, this study has a clear potential to contribute to this area of medical interest, as the studies of natural peptides have increasingly contributed to the discovery of new drugs in the last few decades. **Financial Support**: FAPESP, CNPq and CAPES

09.009 Anti-diabetic, anti-inflammatory and antioxidant effects of *Euterpe oleracea* Mart. (Açaí) extract in Type 2 Diabetic Rats. The exercise training potentiates these effects? Bem GF, Costa CA, Santos IB, Cordeiro VSC, Carvalho LCRM, Souza MAV, Costa GF, Okinga A, Rocha APM, Ognibene DT, Resende AC, Moura RS UERJ – Farmacologia e Psicobiologia

Introduction: Type 2 diabetes (DM2) is characterized by metabolic defects, such as, insulin resistance. Polyphenols possess anti-inflammatory, antioxidant and vasodilator activities. Thus, the aim of this study was to evaluate the effect of treatment with açaí seed extract (ASE), rich in polyphenols, and exercise training, on metabolic disorders and hepatic morphological changes observed in an experimental model of DM2. **Methods**: The experiments were approved by the Ethics Committee of Animal Experiments of the UERJ (protocol: CEUA/058/2012). Two groups of Wistar rats were fed experimental diets: control (10% fat) and high fat (HF) diet (55% fat) for 5 weeks. In the third week, HF group received an intraperitoneal injection of streptozotocin (35 mg kg-1), that increased blood glucose levels to more than 250/100 ml. The animals received ASE (200 mg/kg^{-1}) by intragastric gavage and training on a treadmill for a period of four weeks, and were divided into eight groups: sedentary control and training (Sedentary C and Training C), sedentary control and training treated with ASE (ASE Sedentary C and ASE Training C), sedentary diabetic and training (Sedentary D and Training D) and sedentary diabetic and training treated with ASE (ASE Sedentary D and ASE Training D). Glycemia was measured with a glucometer. Insulin, TNF-α and IL-6 levels were determined by kit. Insulin resistance and beta cells function were calculated by HOMA index and HOMA β, respectively. Oxidative damage, nitrite levels and antioxidant enzymatic activity were measured in adipose tissue homogenates. The expression of IR, pIRS-1, pJNK, AKT, pAKT, GLUT-4 and adiponectin in adipose tissue homogenates were determined by western blotting. **Results**: The increased ($P<0.05$) glucose levels in diabetic animals were reduced ($P<0.05$) by exercise alone, and treatment with ASE alone or associated with exercise. Insulin and HOMA index were increased ($P<0.05$) and HOMA β was decreased ($P<0.05$) in Sedentary D group. Insulin and HOMA index were reduced ($P<0.05$) and HOMA β was increased ($P<0.05$) by treatment with ASE and exercise training. Sedentary D group showed increased ($P< 0.05$) TNF-α levels, which were reversed ($P<0.05$) by ASE associated with exercise. IL-6 levels were not different among groups. Malondialdehyde and protein carbonyl levels were increased ($P<0.05$) in Sedentary D group and reduced ($P<0.05$) by ASE and exercise. Antioxidant enzyme activities and nitrite levels were decreased ($P < 0.05$) in Sedentary D group and recovered ($P<0.05$) by ASE and exercise. In Sedentary D group, the reduced ($P<0.05$) expression of pIRS-1, pAKT, GLUT-4 and adponectin, and increased ($P<0.05$) expression of IR and pJNK were reversed ($P<0.05$) by treatment with ASE and exercise. AKT levels were not different among groups. **Discussion**: Treatment with ASE promotes antihyperglicemic, anti-inflammatory and antioxidant effects. These properties improve insulin sensibility and contribute to the antidiabetic effect. This study also demonstrated that the physical activity potentiated the effect of ASE by reducing glucose levels in the first week. These preclinical studies open a possibility for oral administration of ASE in the treatment of DM2. **Financial Support**: CNPq and FAPERJ.

09.010 Yerba mate extract increases bone markers expression on *in vitro* osteogenic differentiation of bone marrow-derived mesenchymal stromal cells from Wistar rats. Brito VGB, Chaves-Neto AH, Landim-Barros T, Oliveira SHP FOA-Unesp – Ciências Básicas

Introduction: Yerba mate (YM) consumption has been associated with higher bone mineral density. Recently we demonstrated that YM was able to increase matrix mineralization (SBFTE 2013), however studies that explain the related mechanisms to these effects are absent. **Aims**: Evaluate the mechanism involved on the stimulatory effect of the YM on the *in vitro* osteogenic differentiation of bone marrow-derived mesenchymal stromal cells (BMMSC) of Wistar rats. **Methods**: BMMSC culture was obtained from Wistar male rats (4 weeks old) with experimental protocol approved by Institutional Animal Research Ethical Committee (Process 00716-2012). BMMSC were harvested by flushing out bone marrow from femurs with culture medium (MEM plus 10% fetal bovine serum). Culture flasks were maintained at 37 °C in humidified atmosphere of 5% CO_2 and culture medium was replaced every 4 days. When reach confluence cells, were seeded in 24-well plate at 40000 cells/cm², which were used for all subsequent assays. Cultures were treated with osteogenic medium (culture medium plus 50 µg/mL ascorbic acid, 10 mM β-glicerophosphate and 10 nM dexamethasone) and 10, 20 and 50 µg/mL YM extract and bone markers expression and matrix metalloproteinase 2 (MMP-2) activities were analyzed at 7^{th} day by real time RT-PCR and zymography, respectively. **Results**: In the SBFTE 2013 we demonstrated that during osteogenic differentiation only the concentration 50 µg/mL YM showed significant cytotoxic effect at 10^{th} day (36% proliferation reduction). ALP presented an activity that peaked at 7^{th} day, indicating the osteogenic differentiation process, however there were no great changes related to YM treatment, but a slight increase in the concentration 10 µg/mL of YM at 7^{th} and 10^{th} days. On the other hand, the concentration 50 µg/mL caused a significant decrease in the ALP activity. Our current results show that YM did not affect the MMP-2 activity in any period of treatment or concentration. Bone markers expression were found increased at 7th day when treated with 10 µg/mL, including transcription factors RUNX2 (50%), Osterix (79%) and β-catenin (65%), as well as matrix protein osteopontina (74%), bone sialoprotein (88%), osteocalcina (260%) and bone morphogenetic protein (260%), justifying at least in part, the higher mineralization at 10^{th} day (290%). **Conclusion**: Our results suggest a direct promoter effect of YM extract *in vitro* osteogenic differentiation, explained in parts, by increased expression of osteogenic transcription factors and matrix mineralization-related proteins. **Financial support**: FAPESP (Grant# 2012/20547-1 and 2011/19458-1)

09.011 Antinociceptive, anti-inflammatory and gastroprotective effects of polysaccharides of *Croton cajucara* **Benth. in rodents.** Souza EFJ[1], Werner MFP[1], Nascimento AM[2], Cipriani TR[2] – [1]UFPR – Farmacologia, [2]UFPR – Farmacologia Bioquímica e Molecular

Introduction: Croton cajucara Benth., popularly known as sacaca, is a shrub native of Amazon region, which is widely used for stomachache, fever, liver injury and malária (Di Stasi, L. C. et al. Plantas Med. Amazonia, 1989). Phytochemical studies already demonstrated the presence of compounds such as terpenoids, steroids, flavonoids and essential oils (Maciel, M. A. M. et al. J Ethnopharmacol. v.70, p.41, 2000). However, there are no studies regarding the presence and biological activities of polysaccharides from Croton cajucara leaves (CCP). **Aims**: This study was designed to investigate the antinociceptive, anti-inflammatory and gastric antiulcer activities of CCP in rodents. **Methods**: Nociceptive behavior was evaluated in female Swiss mice (~20 g) treated intraperitoneally with saline (1 ml/kg) or CCP (0.01; 0.1; 0.3; 1; 3; 10 mg/kg) 30 min before formalin solution injection (2.5%, 20 µL/paw). Thermal hyperalgesia was evaluated in hot plate (52 ± 1 ºC) 1 and 3 h after carrageenan injection (20 µL, 300 µg/paw) and the paw edema was measured using a digital micrometer. Gastroprotection was evaluated in female Wistar rats (~200 g) after 18 h fast by ethanol (1 ml/rat, v.o.)- and diclofenac sodium (80 mg/kg, v.o.)-induced gastric ulcer models. Animals were orally treated with water (1 mL/kg), prostaglandin E2 (20 µg/kg), omeprazole (40 mg/kg) or CCP (1, 3, 10, 30 mg/kg), and 1 or 5 h thereafter, rats were euthanized and stomachs were collected to measure the lesion extension, mucus and GSH levels (CEUA/BIO-UFPR; approval numbers 837 and 657). **Results**: CCP 0.3 and 1 mg/kg attenuated second phase of formalin-induced nociceptive response behaviors in 45 ± 8 and 40 ± 7%, respectively. Furthermore, CCP 1 and 10 mg/kg increased the latency of carrageenan-induced thermal hyperalgesia (at 1 h: 113 ± 21 and 106 ± 35%; at 3 h: 126 ± 30 and 149 ± 36%, respectively), and only CCP 10 mg/kg decreased paw edema in 53 ± 4% when compared to the control. Moreover, CCP (3, 10 and 30 mg/kg) demonstrated gastroprotective effect on ethanol-induced lesions reducing lesions area in 56 ± 6; 80 ± 3 and 42 ± 5%, respectively (omeprazole 81 ± 5%) when compared to the control and increased mucus levels in 88 ± 13; 92 ± 19 and 58 ± 6%, respectively (omeprazole 91 ± 15%). However, no gastroprotective effect was observed diclofenac sodium-induced ulcers. **Conclusion**: Altogether, the present data indicate the therapeutic role of sacaca polysaccharides against formalin and carrageenan-induced inflammatory pain and paw edema, as well as gastroprotection by increasing mucus levels in ethanol induced gastric lesion. **Financial support**: CAPES

09.012 Reproductive characteristics of male Wistar rats supplemented with extract and fractions of fruits of *Tribulus terrestris* **L.** Oliveira NNPM[1], Félix MAR[2], Pereira TCS[2], Rocha LGP[2], Miranda JR[2], Zangeronimo MG[2], Pinto JEBP[1], Bertolucci SKV[1], Sousa RV[2] – [1]UFLA – Plantas Medicinais, [2]UFLA – Medicina Veterinária

Introduction: *Tribulus terrestris* L. (Zygophyllaceae), commonly known as tribulus, thorn, puncture vine, among other names, is originary from India, but it is widespread in warm regions around the world. By the Chinese and Indian traditional medicine, this plant has been used to treat infertility, sexual impotence, erectile dysfunction and low libido. Extracts obtained from this plant species contain steroidal saponins, alkaloids and flavonoids. Considering its popular application and incipient studies of the reproductive area, the aim of the present study was to assess the reproductive characteristics of male Wistar rats supplemented with extract and fractions of fruits of *Tribulus terrestris* L. **Methods**: Ethanolic extract was obtained by means of dynamic maceration of sprayed dried fruit. This extract was fractionated by liquid-liquid partition, using increasing polarity solvents. Twenty male rats were separated to four groups, 5 rats each. The control was supplemented with distilled water, while the others were daily given the ethanolic extract, hexanic or aqueous fraction soluble in methanol in a single dose of 42 $mg.kg^{-1}.day^{-1}$ for 70 days. At the end of the experiment, animals were anesthetized, euthanized and submitted to the wide opening of the abdominal cavity to the exposure of the reproductive organs that were collected and weighed. Statistical analysis was performed using an ANOVA test. **Results**: The mean testicular weight of groups supplemented with ethanolic extract (2.09±0.13g) and aqueous fraction soluble in methanol (1.89±0.36g) showed a significant increase when compared to the control (1.39±0.28g). The gonadosomatic index (1.18±0.18%), epithelial height of seminiferous tubule (80.2±7.4µm), tubular volume (1.69±0.08mL) and the total length of tubules (26.57±3.86m) were increased in the group supplemented with ethanolic extract when compared to the control (0.74±0.12%; 69.0±4.5µm; 1.16±0.23mL; 18.18±4.31m). The nuclear, cytoplasmic and individual volume of Leydig cells increased in animals supplemented with hexanic (155.1±31.0μm^3; 568.1±218.4μm^3; 723.2±237.3μm^3) and aqueous fractions soluble in methanol (155.8±30.9μm^3; 463.7±139.2μm^3; 619.4±165.2μm^3) when compared to the control (85.0±16.3μm^3; 214.1±58.8μm^3; 299.1±71.2μm^3), respectively. Conclusions: It is suggested that the ethanol extract of *Tribulus terrestris* L. influence on spermatogenesis by the changes evident in the tubular compartment of the testes such as an increase in the total tube length, tubular volume and height of the seminiferous epithelium; while the hexanic and aqueous soluble in methanol fractions promote changes in the intertubular compartment because they increase the nuclear, cytoplasmic and individual volume of Leydig cells. Financial Support: CAPES and CNPq. Experimental protocols were approved by the Institutional Animal Care and Use Committee for Experimentation (Standing Committees/PRP-UFLA) (nº 027/2014).

09.013 Investigation of gastroprotector potential of *Vernonia condensata* Baker, a Brazilian medicinal plant used in the treatment of gastric ulcer. Boeing T, da Silva LM, Somensi LB, Petreanu M, Niero R, Santin JR, Andrade SF – Univali – Ciências Farmacêuticas

Introduction: The leaves from Vernonia condensata Baker are so broadly used in the tradition folk as antiulcerogenic agent and in treatment of dyspepsia that have a potential to generate products of interest to Brazilian Public Health System (SUS). Thus, studies about pharmacological action of this species are important to its safe use. **Aim**: Our proposal is to contribute for scientific validation of popular use of V. condensata, at least pre clinically, by evaluation of gastroprotective activity of the crude ethanolic extract of the leaves of V. condensata (CEEV) in different ulcer models. **Materials and Methods**: First, the phytochemical screening of CEEV was perfomed. The evaluation of the CEEV antiulcer activity was assessed by ethanol- or indomethacin- induced gastric ulcer in rats and the acid anti secretory action was verified in ligature pylorus rats model, where also was evaluated the effect of CEEV in mucus content. Moreover, CEEV effects in gastric emptying and intestinal transit were evaluated in mice, as well as the *in vitro* antioxidant activity through the DPPH assay. Carbenoxolone (200 mg/kg, p.o) and Omeprazole (20 mg/kg, i.d) were used as positive controls in gastric models and ligature pyloric method, respectively. **Results**: The treatment with 30 and 300 mg/kg (p.o) of the extract reduced the lesion area induced by absolute ethanol (5 ml/kg, p.o) or indomethacin (80 mg/kg, p.o) in 55% and 87%; 80% and 71% respectively, compared to negative controls (67 ± 8.9 mm^2 and 9 ± 1.7 mm^2 respectively). In addition, the extract also decrease the volume of acid secretion, acidity and increase the pH in the major dose tested (300 mg/kg, i.d). However, the content of the adhered gastric mucus not was altered by CEEV administration. Besides, CEEV at a dose of 300 mg/kg (p.o) reduced the gastric emptying and intestinal transit in mice and showed a great ability of scavenge the DPPH radical at 1 µg/ml. In parallel, phytochemical trials confirmed the presence of triterpene and phenolic compounds in the extract. **CONCLUSION**: Herein, our results confirm the gastroprotective activity popularly attributed to Vernonia condensata and point out that its activity is related to the anti-secretory and antioxidant potential of constituents of this plant. Moreover, we have found that the extract present inhibitory action in gastrointestinal motility. At this time, studies are being conducted to elucidate the mechanisms involved in the experimentally observed effects and to verify active compound in the extract. **Financial support**: CNPQ, CAPES, FAPESC. **Approval number CEUA**: 020/13p.

09.015 The influence of calcium channels on vasorelaxant effect of (-)-borneol in superior mesenteric artery of l-name hipertensive rats. Souza FM, Silva-Filho JC, Azevedo PSS, Campelo RT, Rocha MS, Santos MEP, Lima GS, Snatos MRV, Oliveira AP NPPM-UFPI

Introduction: Monoterpenes have significant effects on the cardiovascular system, promoting, among other actions, vasorelaxation, decreased heart rate and hypotension (PEIXOTO-NEVES et al, Fundamental & Clinical Pharmacology: 24, 341, 2010). (-)-borneol is a bicyclic monoterpene present in essential oils of numerous medicinal plants, including species from Lamiaceae (*Rosmarinus officinalis* L. *e Salvia officinalis* L.) and Valerianaceae families (*Valeriana officinalis* L.) (HORVÁTHOVÁ et al, Food and Chemical Toxicology: 47, 1318, 2009). **Aim**: Evaluate the involvement of calcium channels on vasorelaxant effect of the (-)-borneol. **Methods**: Healthy adult male Wistar rats (230–250 g, n=5) received L-NAME (0,5 mg/mL, in drinking water) for 7 days. After euthanasia (Resolution N^{o}. 1000, 2012 – CFMV), rings of mesenteric artery (1-2 mm) with and without functional endothelium were kept in Tyrode at 37° C aerated with (95% O_2 and 5% CO_2) suspended by cotton thread and attached to force transducers coupled to a data acquisition system (AVS Projects/SP) for registration of isometric tension. After 1h of stabilization (tension of 0.75 g), the rings were pre-contracted with phenylephrine (10^{-5} M), α_1-adrenergic receptor agonist (EHRLICH, Nature, 336, 583, 1988), or 80 mM KCl acts by depolarizing the plasmatic membrane, activating the voltage-dependent calcium channel (KARAKI et al, Life Science, 62, 1629, 1998) or S-(-)-Bay K 8644, a Ca_vL activator (BARRÚS et al, Journal of Autonomic Pharmacology, 16, 161, 1996) different preparations. After equilibration, steady tension, (-)-borneol was added cumulatively (10^{-9} - 10^{-3} M). **Results**: In mesenteric artery rings of L-NAME hypertensive rats, (-)-borneol induced a vasorelaxant response on phenylephrine (10^{-5} M) induced pre-contractions in a concentration dependent manner, in endothelium-intact (pD_2= 5.08 ± 0.09) and endothelium-denuded (pD_2 = 4.94 ± 0.12), and the vasorelaxant effect was independent of vascular endothelium. In rings pre-contracted with KCl 80 mM (pD2 = 4,46 ± 0,10, *p<0,05), the concentration-response curve was moved to the right compared with the vasorelaxation after pre-contraction with phenylephrine in the endothelium-denuded (pD_2 = 4,94 ± 0,12), showing that in this tissue the (-)-borneol has a bigger capacity of relaxation in contractions induced by phenylephrine. In rings pre-contracted with Bay K 8644 (0.1 μM), the (-)-borneol promoted vasorelaxation in denuded rings of mesenteric artery (pD_2= 5,49 ± 0,17, *p<0,05). As it can be observed, (-)-borneol promoted a vasorelaxant effect in a manner concentration dependent, being enhanced in the rings pre-contracted with Bay K 8644 (0.1□M). **Conclusion**: In conclusion, the vasorelaxant effect induced by (-)-borneol might involve the Influx blockage of extracellular calcium, interfering in both channels operated by voltage and receptor. Support: UFPI/UFS/CAPES/FAPEPI/CNPq. All experimental protocols and procedures were approved by CEEA/UFPI nº 008/12.

09.016 Antimicrobial activity of (+)- Dehydrofukinone isolated from *Nectandra grandiflora* **essential oil.** Garlet QI[1], Pires LC[2], Spall S[2], Gressler LT[3,4], Bandeira Jr G[4], Vargas APC[4], Heinzmann BM[1] – [1]UFSM – Fisiologia e Farmacologia, [2]UFSM – Farmácia Industrial, [3]UFSM, [4]UFSM – Medicina Veterinária

Introduction: Microbiological control in aquaculture proceeding is a relevant concern, since bacterial contamination of fish and fish products goes to humans through feeding. *Plesiomonas shigelloides, Citrobacter freundii, Acinetobacter calcoaceticus* and *Aeromonas hydrophila* are Gram-negative bacteria linked with fish infection (JOH, S.J.Vet Microbiol, 163: 190, 2013). These microorganisms can also lead to infections and debilitating conditions in humans, such as gastroenteritis, peritonitis, pneumonia, skin and soft tissue infections (NEMEC, A. Int J Syst Evolut Microbiol, 65: 934, 2015). Nowadays, natural products, mainly essential oils (EO) and its components, are target for studies of new drugs with antibiotic properties that can bypass the microbial resistance observed with classical antibiotics. (+)-Dehydrofukinone (DHF) is a sesquiterpenoid found in *Nectandra grandiflora* (Lauraceae) EO and its antimicrobial potential against fish pathogenic bacteria was verified in this work. **Methods**: Leaves of *N. grandiflora* were colleted in Jaguari, RS, Brazil. EO extraction was performed by hydrodistillation in Clevenger type apparatus for 3h (BRAZILIAN PHARMACOPOEIA, 5th. ed., 2010). DHF was isolated by column chromatography procedure. Isolated DHF was characterized by GC-MS and GC-FID. The antimicrobial assay were performed by microdilution method according to *Clinical Laboratory Standards Institute* guidelines (2008), using strains of *P. shigelloides*, *A. calcoaceticus*, *C. freundii, A. hydrophila* from naturally infected silver catfish (*Rhamdia quelen)* and a standard strain of *A. hydrophila ATCC7966*. The applied concentrations ranged from 6400 to 3.12 µg/mL for determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC). **Results**: DHF amounted 24.7% of the EO and was isolated with 99.99% purity. The yield of the isolation process was 46.35%. MIC and MBC values observed, respectively, were: *P. shigelloides* (200 and 400 µg/mL), *A. calcoaceticus* (800 and >3200 µg/mL), *C. freundii* (3200 and >3200 µg/mL)*, A. hydrophila* (200 and 200 µg/mL) and *A. hydrophila ATCC7966* (1600 and >3200 µg/mL). **Discussion**: The antimicrobial properties of (±)-DHF were previously reported against *Bacillus cereus* strains (MIC: 256 µg/ mL) (BOLZAN, 2007, UFSM MSc. Dissertation), however there are no reports about the performance of the dextrorotatory isomer on antimicrobial activity. For natural compound derivatives, MIC values between 600 and 1500 µg/mL are considered as moderated inhibition and values below 600 µg/mL are strong inhibition. Following this thought, (+)-DHF inhibited moderately *A. calcoaceticus* strain and strongly *P. shigelloides* and *A. hydrophila* strains. Consequently (+)-DHF enclosures antimicrobial potential that could be better studied for aquaculture microbial control. **Financial Support**: FAPERGS/PRONEX; Ministério da Pesca e Aquicultura/MCT/FINEP; INCT ADAPTA; CNPq and FAPEAM.

09.017 Antidiarrheal activity of a sulfated polysaccharide extracted from seaweed *Gracilaria caudata* in rodents. Costa DS[1], Sousa NA[2], Souza LKM[2], Araújo TSL[2], Sousa FBM[2], Carvalho NS[1], Nogueira KM[3], Araújo S[3], Oliveira AP[3], Medeiros JVR[1,2]
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Introduction: Diarrheal diseases nowadays comprise a public health problem that affects developing countries, with distinct etiologic agents (AWE, J ETHNOPHARM, 137, 148, 2011). Gastrointestinal disorders are common in northeastern Brazil and local population is making use red algae species regionals, as *Gracilaria caudata* to treat these diseases, such as diarrhea, it lacks scientific proof. **Aim**: To investigate the antidiarrheal activity of a sulfated polysaccharide extracted from seaweed *G. caudata* in rodents. **Methods**: *G. caudata* specimen was collected, washed with distilled water, dried at 40° C and macerated with liquid nitrogen. In result, there were the filtration and extraction stages in distilled water and acetone twice and polysaccharide has been designated as "PLS" after characterization by NMR. Study was approved by local Ethics Committee Research (no. 11/2013). Antidiarrheal activity of PLS was evaluated for castor oil-induced diarrhea and *enteropooling*. Wistar rats (150-200g) were pretreated with PLS (10, 30, and 90 mg/kg, *p.o*.) or loperamide (5 mg/kg, *p.o*.), and after 1 h, was administered castor oil (10 ml/kg, *p.o*.) Animals were placed in cages lined with filter paper and observed for 3 h for the presence of diarrhea defined as watery (wet), unformed stool. Besides, were sacrificed and small intestine was isolated and volume of intestinal contents was measured by graduated tube. Intestinal motility was evaluated using activated charcoal. Mice (Swiss strain, 25-30g) received castor oil and 30 minutes later they were treated with PLS (90 mg/kg, *p.o*). After 1 h, all animals were received 0,2 mL of charcoal activated (10% charcoal suspension in 5% gum acacia *p.o*). 20 minutes later, animals were sacrificed, and the distance covered by the activated charcoal in the small intestine was measured. To evaluate the secretory diarrhea, it was used method of isolation of intestinal loops, evaluating the parameters: fluid levels, chloride ions and absorption, as described (TRADTRANTIP, PLOS NTD, 8, 1, 2014) and to evaluate the interaction between PLS, cholera toxin and GM1 receptor was performed ELISA test as described (SAHA, AAC, 57, 4373, 2013). **Results**: PLS (10, 30, and 90 mg/kg) was reduced significantly ($P<0.05$) the diarrheal severity (28.38, 26.13 and 49.41%, respectively), also decreased the frequency of defecation (20.65, 23.75 and 48.79% respectively) and total number of wet feces produced upon administration of castor oil as in enteropooling, where in all PLS doses significantly reduced ($P<0,05$) intestinal content (33.57, 35.71, 45.71% respectively). PLS reduced significantly the gastrointestinal transit ($P <0.05$) compared to control, however it didn't provide opioid action because naloxone didn't reverse the PLS effect. In choleric diarrhea, PLS decreased by presence of fluid (0.05 ± 0.007g/cm) significantly ($P <0.05$) when compared with group that received only cholera toxin (0.13 ± 0.017g/cm) and reduced in about 35% loss of chloride ions and didn't alter the pattern of intestinal absortion. ELISA test showed that PLS can bind to cholera toxin and the receptor GM1, reducing cholera diarrhea. **Conclusions**: PLS from *G. caudata* can act by interaction with GM1, and cholera toxin receptor, as seen in the model studied. However, studies are needed to understand mechanism's action completely. **Financial support**: CNPq/FAPEPI.

09.018 Role of species reactive oxygen mitochondrial and intracytoplasmic in the anti-inflammatory effects of hydroethanolic extract of *Dilodendron bipinnatum* Radlk. Oliveira RG[1], Miyajima F[2], Castilho GRC[1], Luz TE[1], Batista MS[1], Martins DTO[1]
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Introduction: *Dilodendron bipinnatum*, Sapindaceae, popularly known as "mulher-pobre", stem bark is used in the Mato Grosso Pantanal in the form of decoction and maceration in the treatment of inflammatory conditions. Recently, the inner stem bark of HEDb was shown to attenuate increases in the concentrations of the pro-inflammatory cytokines (IL-1β and TNF-α) and increased level of anti-inflammatory cytokine (IL-10) in peritonitis induced by lipopolysaccharide (LPS) and it inhibited paw edema induced by carrageenan (Oliveira et al., 2014). The phagocytic response of the immune system involves production of reactive oxygen species (ROS) which are essential components of the inflammatory immune response against infection in macrophages. **Aims**: To investigate the role of ROS in the anti-inflammatory effects of HEDb. **Methods**: The cell viability, alterations of ROS and mitochondrial membrane potential (ΔΨm) was evaluated using flow cytometry analysis. In brief, RAW 264.7 cells, of density $3x10^5$ cells, were plated overnight on a 12-well microplate, pre-treated with HEDb (1, 5 and 20 µg/mL) or N-acetyl-cysteine (NAC-10 µg/mL) for 1 h and stimulated with LPS for 24 h. The cells were harvested and washed twice with cold PBS and incubated with annexin V and 7-amino-actinomycin D (7-AAD), 2′,7′-dichlorofluorescein diacetate (DCFH-DA, 3 µM) or JC-1 (MitoScreen, 2 µM) for 30 min at 37°C in the dark for the evaluation of cell viability, ROS and ΔΨm, respectively. Fluorescence was captured using BD Accuri™ C6 Cytometer. **Results and Conclusions**: HEDb, LPS and NAC demonstrated to be non-cytotoxic with average viability being 80%. The level of ROS in response to LPS was significantly higher (776 ± 71.04; $p<0.001$) than that of unstimulated cells (222.8 ± 66.53). After pretreatment with 1, 5 and 20 µg/mL HEDb, ROS levels were reduced by 49.2, 55.8 and 67.5% ($p<0.001$), respectively, compared to the LPS group. NAC (10 µg/mL), the standard used in the assay, inhibited ROS production by 66.2% ($p<0.001$), compared to the LPS group. LPS treatment for 24 h caused a marked increase in the ΔΨm (19.2%, $p<0.001$) compared to unstimulated cells (3.1%). The three concentrations of HEDb significantly reduced by 7.3, 6.9 and 7.7% ($p<0.001$), respectively, the depolarization induzed by LPS (1 µg/mL). These results demonstrated that HEDb blocks apoptotic cell death of RAW 264.7 macrophage. HEDb significantly reduced the ROS synthesis and mitochondrial membrane depolarization induced by LPS. Furthermore, our data show that the underlying anti-inflammatory mechanism of HEDb is due, at least in part, to the inhibition of ROS. Financial support and acknowledgments: INAU, FAPEMAT, CAPES and UFMT. **Reference**: Oliveira, RG. et al. Evaluation of anti-inflammatory activity of hydroethanolic extract of *Dilodendron bipinnatum* Radlk. J Ethnopharmacol 155, 387-95, 2014.

09.019 Gastroprotective effect of ethanolic extract of *Samanea tubulosa* on naproxen-induced gastric damage in mice. Nogueira KM[1], Souza LKM[2], Pacífico DM[1], Araújo TSL[3], Costa DS[4], Sousa NA[3], Sousa FBM[3], Medeiros JVR[2,3], Sales PAB[1], Costa APR[1], Nicolau LAD[5] [1]UFPI, [2]UFPI – Ciências Biomédicas, [3]UFPI – Biotecnologia, [4]UFPI – Farmacologia, [5]UFC – Farmacologia

Introduction: Naproxen (NAP), a family representative of anti-inflammatory drugs non-steroidal drugs (NSAIDs) is often used in the treatment of chronic inflammatory diseases. However, this drug causes various problems in the gastrointestinal tract for carrying a decrease of prostaglandin synthesis and increased lipid peroxidation (Yoshikawa, T.; Gut, v.34, p.732, 1993). Natural products are a source of development of new alternative therapies for diseases of the gastrointestinal tract followed by a generally low toxicity. One example is the *Samanea tubulosa*, Fabaceae family member and popularly known as "old Bourdon", used widely to treat skin infections and parasitic infestations with little scientific evidence.. **Aim**: Thus, the aim of the study was to investigate the gastroprotective effect of the ethanolic extract of *Samanea tubulosa* (EEST) on NAP-induced gastric damage in mice. **Methods**: Swiss mice were pretreated with 0.5% carboxymethylcellulose (vehicle) or EEST (25, 50 and 100 mg/kg, *p.o.*). After 1 h, the animals received NAP (300 mg/kg) by gavage. Six hours later, the mice were euthanized and stomachs were removed and lesion measured using digital caliper (Santana, A. Nitric Oxide, v. 45, p. 35, 2015). Stomach samples were used for histological evaluation and malonyldialdehyde (MDA) concentration and myeloperoxidase (MPO) activity (Mihara M.; *Anal Biochem*, v. 86, p. 271, 1978). **Results**: NAP (300 mg/kg, *p.o.*) administration induced mucosal gastric damage (10.1 ± 0.83 lesion mm^2). However, pretreated with ESST (25, 50 and 100 mg/kg, *p.o.*) prevents NAP-induced lesions in a dose-dependent manner, with maximal inhibitory effects observed at dose 50 mg/kg EEST (1.36 ± 0.11 lesion mm^2), significantly ($P < 0.0005$). Histological analysis revealed that NAP increased hemorrhagic damage, edema, epithelial cell loss and inflammatory cell infiltration. The other hand, pretreatment with EEST (50 mg/kg, *p.o.*) decreased the infiltration of inflammatory cells, the formation of edema and the loss of epithelial cells. NAP increased MPO and MDA levels (26.7 ± 4.5 U/mg and 125.8 ± 18.7 U/mg, the gastric tissue, respectively). However, pretreatment ESST shows changed gastric biochemical parameters, reduced MPO (7.5 ± 1.3 U/mg of tissue), MDA (45.0 ± 7.6 U/mg of tissue). **Conclusion**: Our results suggest that EEST plays mainly a gastroprotective role against NAP-induced damage by inhibiting neutrophil infiltration, reducing oxidative stress and improving the preservation of gastric mucosal microscopic analysis. **Financial Support**: CNPq and FAPEPI. **CEP-UFPI**: Protocol Nº 33/2009

09.020 Intestinal anti-inflammatory activity of a standardized aqueous extract and butanolic fraction of *C. glaziovii* Sneth in acute DSS-induced colitis in mice.
Nogueira FM[1], Tanae MM[1], Landman G[2], Lima-Landman MTR[1], Lapa AJ[1,3], Souccar C[1] [1]Unifesp-EPM – Pharmacology, [2]Unifesp-EPM – Pathology, [3]Amazon Biotechnology Center – Pharmacology and Toxicology

Introduction: Ulcerative colitis (UC) and Crohn's disease (CD) are inflammatory bowel diseases characterized by a chronic and recurrent intestinal inflammation that results from genetic, immune and environmental conditions. These diseases also involve neurophysiological, neurochemical and morphological changes of the enteric nervous system (ENS), but their etiology is still unclear. Despite the available treatments, new safe therapies able to provide long-term remission are still required. Previous studies by our group have shown that a standardized aqueous extract (AE) and butanolic fraction (BuF) of *Cecropia glaziovii* Sneth exhibited hypotensive/antihypertensive, bronchodilator, anxiolytic and antidepressant-like properties. Both AE and BuF also presented antacid/antiulcer activity related to inhibition of the gastric proton pump (Souccar et al., Phytomedicine 15: 462, 2008). **Aims**: To assess the effects of AE and BuF of *C. glaziovii* on acute dextran sulfate sodium (DSS)-induced colitis in mice and its consequence on the myogenic response of colon preparations to cholinergic agonist. **Methods**: The plant AE and BuF were obtained according to Tanae et al. (Phytomedicine, 14: 309, 2007). Male C57Bl/6 mice (3-months old) were given tap water (control, C), or 2.5% DSS in their drinking water for 5 days and plain water for 48 h (DSS-mice). Additional control and DSS-mice were treated orally with AE (1.0 and 2.0 g/kg) or BuF (0.1 and 0.3 g/kg). Intestinal inflammation in all groups was assessed by clinical symptoms, histological analysis of colon sections and determination of myeloperoxidase (MPO) activity. Isometric tension recordings of colon segments in response to carbamylcholine (CCh) were analyzed in the presence of hexamethonium (0.3 mM). **Results**: Mice treated with DSS (5-7 days) presented bloody diarrhea and decrease in the body weights by 16% (C: 29.6 ± 0.4 g, n=10). The colon weight and colon length were decreased by 20% and 26% of the respective control value, while the tissue MPO activity was increased by 3.2-fold (C: 2.21 ± 0.13 mUn/g tissue). In control colon preparations, concentration-responses to CCh (1 nM - 1 mM) showed a greater muscle tension in distal (3.82 ± 0.06 g) than in proximal (1.67 ± 0.19 g) segments, with no significant difference between the agonist EC_{50} values. These results did not change in DSS-mice. Treatment of DSS groups with AE or BuF attenuated the intestinal inflammation in a dose-related manner, with the BuF being more effective. At 0.3 g/kg BuF reduced the effects of DSS-induced colitis on the body and colon weights, and prevented the increase of MPO activity. The responses to CCh in colon preparations were not affected. Similar results were obtained in positive control mice treated with prednisolone (3 mg/kg). **Conclusions**: The results show that at concentrations presenting antacid/antiulcer activity, both the AE and BuF attenuated the intestinal inflammation. BuF was the most effective indicating that the effect is probably related to its content in cathechins, procyanidins and flavonoids. **Financial support**: FAPESP, CAPES and CNPq Animal Investigation Ethics Committee Protocol Nº 6469270514

09.021 Lipid-lowering and antiatherogenic effects of *Cuphea carthagenensis* (JACQ.) J.F. Macbr. in rabbits. Barboza LN[1], Dalsenter PR[1], Prando TBL[2], Ribeiro RCL[2], Lourenço ELB[2], Gasparotto Junior A[3] [1]UFPR – Farmacologia, [2]Unipar – Farmacologia, [3]UFGD – Farmacologia

Introduction: Dyslipidemia and atherosclerosis are the leading causes of death and disability in Western countries. The process consists of chronic and progressive alterations in arterial wall characterized by inflammatory and fibroproliferative response (Buckley et al., 2015). The atherosclerotic lesion may affect several important arterial territories, accounting for 95% of coronary heart disease, 85% of lower limb intermittent lameness and 75% of strokes. Considering the impact of this disease to humans, in recent decades there was a great interest in the research of medicinal plants and their extracts in medication therapy. Recent researches have shown that extracts of *Cuphea carthagenensis* features cardiovascular benefits especially by its popular use in the treatment of hypertension and dyslipidemia (Bolson et al., 2015). **Aim**: Evaluate the hypolipemiant and antiatherogenic effects of ethanol soluble fraction obtained from *Cuphea carthagenensis* (ES-CC) in New Zealand rabbits submitted to high fat diet (HFD). **Methods**: Dyslipidemia and atherogenesis were induced by the administration of HFD (1% cholesterol) for 8 weeks. The ES-CC was administered orally at doses of 10, 30 and 100 mg/kg, once a day, for four weeks, starting from the 5nd week of HFD. The gain in body weight were measured weekly over the eight week study. Blood was collected and samples were analyzed at time zero and at the end of each month to measure the levels of triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), and very low-density lipoprotein cholesterol (VLDL-C). TBARS, alkaline phosphatase, creatinine and urea were also measured at the end of experimental period (8th week). At the end of the experiments it was withdrawn the aorta and its direct branches to perform pathological study. All experimental procedures were previously approved by the Institutional Ethics Committee of the Universidade Paranaense (authorization 25451/2014). **Results**: The HFD induced dyslipidemia and major structural changes in the aortic wall, including raising of the oxidative stress. The treatment with ES-CC was able to prevent the increase of TC, LDL-C, VLDL-C, triglycerides, creatinine and urea levels and increase HDL-C New Zealand rabbits. These effects were accompanied by a significant reduction in oxidative stress. Moreover, macroscopic lesions were significantly reduced in ES-CC-treated rabbits. **Conclusion**: This study demonstrated that ES-CC reduces the serum lipids and oxidative stress when orally administered to New Zealand rabbits. In addition, it was able to prevent arterial thickening induced by HFD. **Acknowledgements**: We are grateful to Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Diretoria Executiva de Gestão da Pesquisa e Pós-Graduação (DEGPP/UNIPAR) for financial support.

09.022 Acute toxicity and gastroprotective activity of *Wissadula periplocifolia* L. (Malvaceae) in mice. Silva AKM, Barros MEFX, Sales IRP, Formiga RO, Teles YCF, Souza MFV, Batista LM UFPB

Introduction: *Wissadula periplocifolia* (L.) C. Presl is a species from Malvaceae family, which is rich in terpenes, fatty acids and flavonoids, compounds referenced in literature with many biological activities (VENKATESH, S. J Ethnopharmacol, 67, 1999.). This species was selected for this study based on chemotaxonomic criteria, and, besides, some species of this family have already been referenced in literature as antiulcerogenic (LIMA, IO; Rev. Bras Pharmacog, 19, 2009). **Aims**: To assess the acute toxicity (behavioral testing and Lethal Dose 50%) and the gastroprotective activity of the ethanol extract obtained from the aerial parts of *Wissadula periplocifolia* (EEtOH-Wp) in gastric ulcer model induced by ethanol/HCl. **Methods**: In the acute toxicity essay (ALMEIDA RN, Rev. Bras Farm, 80, 72, 1999) Swiss (Mus musculus) mice (males and females) weighing between 25-35 g (n = 7-8) were divided into 4 groups (2 male and 2 female groups) and treated orally with vehicle (0.9% saline solution - negative control) or EEtOH-Wp (2000 mg/kg). Then, a behavioral evaluation of the animals was performed for 14 days, whose parameters such as death, consumption of water and food were quantified. For the gastroprotective activity evaluation, male Swiss mice were pre-treated orally with the vehicle (saline 0.9% - negative control), lansoprazole 30 mg/kg (positive control) and EEtOH-Wp (62.5, 125, 250 and 500 mg/kg). Sixty minutes after, it was given the harmful agent, ethanol/HCl to induce gastric lesions (Mizui, T., J. Pharmacol Jap, 33, 934, 1983 - With modifications). The results were expressed as mean ± standard desviation and analyzed by ANOVA followed by Dunnett test or T-test. **Results**: The EEtOH-Wp administered orally at a single dose (2000 mg/kg) induced no apparent changes in the central and autonomic nervous system when compared to the respective negative control group (NaCl 0.9%). Concerning with water and food intake, it was shown that the extract showed no significant changes to these parameters. There was no death during the experiment and it was not observed any significant change in the animals's weight or their organs (heart, kidney and spleen). However, there was a change in the weight of liver from male animals treated with EEtOH-Wp when compared to respective control group, which is an isolated data, inferring no signs of high toxicity. On gastric ulcers induced by ethanol/HCl protocol, the oral doses of 62.5, 125, 250 and 500 mg/kg showed gastroprotective effect with Ulcerative Lesion Index of 115.2 ± 11.9, with 19% lesion inhibition ($p < 0\ 01$), 110.5 ± 15.9 and 23% ($p < 0.01$), 105.0 ± 11.5 and 26% ($p < 0.001$), 102.3 ± 8.7 and 28% ($p < 0.001$) respectively when compared to the negative control group (143.2 ± 22.3). **Conclusions**: Thus, the results demonstrate that EEtOH-Wp showed low toxicity, and its LD50 is greater than 2000 mg/kg. Also, it possess gastroprotective activity due to the significant inhibition of lesions formation in the mucosa. However, future studies are needed to assess the gastroprotective activity in other models of induced gastric ulcers in animals. **Acknowledgements**: CNPq /CAPES/UFPB. Research approval by the Animal Research Ethical Committee (UFPB): 0105/14

09.023 Effect of the hydroalcoholic extract of *Croton antisyphiliticus* oxidative stress in mice with pre-hypertension induced by L-Name. Deus FA[1], Melo DS[2], Costa KB[2], Gregório LE[3], Rocha EV[2], Santos CFF[1] [1]UFVJM – Fisiologia e Farmacologia, [2]UFVJM, [3]Unifesp

Subject: The pre-hypertension term represents a blood pressure range between 120-139/80-89mmhg. This condition can double the risk for cardiovascular events, even in the absence of progression to hypertension. The *Croton antisyphiliticus*, a Brazilian cerrado native, has shown several pharmacological properties, however, no studies have evaluated these effects on the cardiovascular system. **Objectives**: Evaluate the effect of hydroalcoholic extract *Croton antisyphiliticus (EHCA)* on oxidative stress in the arterial pre-hypertension model induced by nitric oxide synthase inhibition in mice and evaluate the antihypertensive potential EHCA. **Methods**: Were included 32 mice Swiss, with initial weight of 30 ± 5g, divided into 4 groups: control (C, n=8); treated with extract (EX, n = 9); pre-hypertensive (PHA, n=7), pre-hypertensive treated with extract (PHAEX, n = 8). Pre-hypertension was induced by oral Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME) treatment in a dose of 60mg/kg/day. The effect of treatment on blood pressure (BP) was confirmed by non-invasive measurement of blood pressure, by tail plethysmography, which was performed before starting the approaches (control), after 4 days of treatment with L-NAME and after 7 days of oral treatment with EHCA in a dose of 250mg/kg/day. After the experimental protocol was performed humanitarian completion by cervical sprain, and blood, samples of heart, aorta and liver, was collected, weighed, immersed in phosphate buffered saline (pH 7.2), extensively washed to remove blood and stored at -80 until the time of the test. For determination of lipid peroxidation (TBARS) was used 0.1 ml tissue homogenate, it has been added to sodium dodecyl sulphate 8.1%, acetic acid 2.5 M (pH 3.4) and the thiobarbituric acid 0, 8%, the mixture was incubated for 60 minutes at 95 °C and reading of the samples was performed on spectrophotometer at 532nm. The project was approved by CEUA / UFVJM under no 001/2014. **Results**: Treatment with L-NAME increased animals BP (C PHA 98 ± 2 vs 121 ± 2 mmHg) and EHCA reversed these BP effects without reducing BP in normotensive animals (EX 102 ± 2; 120 ± 3 PHA; PAHEX 115 ± 4 mmHg). Dosages of TBARS showed that L-NAME increased significantly lipid peroxidation in the heart that was completely reversed by treatment with EHCA (C 6.6 ± 0.7, 19.2 ± 2.4 PHA; 5.1 ± 0 EX 6; PHAEX 5.6 ± 0.3 nmol MDA / mg protein). The same effect was observed in the liver (0.9 ± 0.2 C; PHA 2,0,2 ± 0.3, 1.1 ± 0.004 EX; PHAEX 1.1 ± 0.06 MDA nmol / mg protein). However, aortic lipid peroxidation induced by L-NAME was not reversed by EHCA (C 2.1 ± 0.1; PHA 2,8,2 ± 0.2, 2.2 ± 0.1 EX; PHAEX 3, 2 ± 0.3 nmol MDA / mg protein). **Conclusions**: The EHCA have therapeutic potential in the treatment of prehypertension and a significant protective effect, by reducing the lipid peroxidation in the heart and liver, thus reversing the factors involved in the pathophysiology of disorders related to the progression of hypertension. **Thanks**: CNPq, Capes and FAPEMIG

09.024 Hepatoprotective effect of *Cymbopogon citratus* **essential oil against acetaminophen-induced liver toxicity in mice.** Uchida NS, Rafael PA, Silva-Filho SE, Rodrigues PJ, Cardia GFE, Wiirzler LAM, Bersani-Amado CA, Cuman RKN UEM – Farmacologia e Terapêutica

Introduction: Acetaminophen (APAP; N-acetyl-p-aminophenol) is an analgesic and antipyretic agent commonly used. At therapeutic doses, it is usually safe and well tolerated, however, after an overdose, it can cause acute liver injury. Research aiming to propose new strategies of therapeutic intervention with minor adverse reaction includes the use of natural products as essential oils obtained from medicinal plants. *Cymbopogon citratus*, commonly known as lemon grass, is largely used in folk medicine, where antibacterial, antifungal, antimalarial and anti-inflammatory activities were reported. **Aims**: This study aims to evaluate the protective effects of *Cymbopogon citratus* essential oil (CCEO) against APAP-induced hepatotoxicity experimental mice model. **Methods**: The essential oil was obtained from dried leaves of *C. citratus* by hydrodistillation using a Clevenger apparatus. Experiments were conducted in male Swiss mice (30–40g) obtained from Central Animal House of the State University Maringá. The mice were divided into six groups of five animals each and were daily treated orally during seven days with CCOE (125, 250 or 500 mg/kg) and standard drug silymarin (SLM) (200 mg/kg). APAP (250 mg/kg) was administered on the seventh day to induce hepatic damage in mice. The hepatoprotective activity of CCEO was determined by assessing on biomarkers of hepatic damage aminotransferase (AST) and alanine aminotransferase (ALT). The data were expressed as mean ± SEM for each group. The results were statistically analyzed by using one-way variance analysis (ANOVA) followed by Tukey's test and were considered significant when $P < 0.05$*. **Results**: The AST (8754 ± 142.0 IU/L) and ALT (14332 ± 732.0 IU/L) levels were increased in animals receiving APAP when compared with mice did not receive any treatment. Pretreatment with 125, 250 and 500 mg/kg CCEO showed a significant reduction in the levels of serum markers AST (345.3 ± 168.7 IU/L; 408.2 ± 331.4 IU/L and 187.4 ± 105.6 IU/L, respectively) and ALT (1277 ± 823.4 IU/L ; 150.0 ± 106.6 IU/L and 916.6 ± 680.7 IU/L, respectively) when compared to animals APAP-induced hepatotoxicity. Pretreatment with SLM also showed a decrease in the levels of AST (983.0 ± 532.2 IU/L) and ALT (675.5 ± 348.5 IU/L) compared to animals that received APAP. **Conclusion**: Our data results suggest a hepatoprotective activity of CCEO in acute liver injury caused by acetaminophen. **Financial Support**: Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenadoria de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) and Fundação Araucária, Brazil. The experimental protocols were approved by the Ethical Committee in Animal Experimentation of the State University of Maringá (CEAE/UEM 045/2014).

09.025 Involvement of phospholipase A2 (PLA2) and cyclooxygenase metabolites in the contraction of rat isolated ileum and stomach by *Lachesis muta muta* **(South American Bushmaster) venom.** Stroka A[1], Dias L[1], Sousa NC[1], Melgarejo A[2], Hyslop S[1] [1]Unicamp – Farmacologia Básica e Clinica, [2]Instituto Vital Brazil – Zoologia Médica

Introduction: Envenoming by the South American bushmaster (*L. m. muta*) can result in coagulopathy, bradycardia, hypotension and manifestations of parasympathetic autonomic activation (sweating, nausea, abdominal cholic, diarrhea and vomiting). In this work, we examined the role of venom phospholipase A_2 (PLA_2) and endogenous arachidonic acid metabolites in the contractile activity of Peruvian *L. m. muta* venom in rat ileum and stomach. **Methods**: Male Wistar rats (300-400 g) were anesthetized with isoflurane (2% in 98% air), exsanguinated and segments of the ileum and stomach were removed and mounted under 1 g of tension in organ baths containing modified Krebs-Henseleit solution (KHS; composition, in mM: NaCl 118, KCl 4.7, $CaCl_2$ 2.5, $MgSO_4$ 0.45, $NaHCO_3$ 25, KH_2PO_4 1.03, D-glucose 11.1 and ascorbic acid 0.14, pH 7.4, at 37 °C) aerated continuously with 95%O_2-5%CO_2. Contractile responses were monitored continuously with PowerLab software (ADInstruments). After stabilization for 30 min, venom that had or had not been preincubated with *p*-bromophenacyl bromide (*p*BPB, a phospholipase A_2 inhibitor, 0.6 mM, 24 h, 37 ^oC) was added to the organ bath. In some cases, venom was preincubated with phenanthroline (a metal chelator; 5 mM, 1 h at room temperature) or tissues were preincubated (20 min, 37 ^oC) with indomethacin (a non-selective inhibitor of cyclooxygenase; 2 µg/ml). The results (mean±SEM) were compared statistically using ANOVA followed by the Tukey-Kramer test, with $p<0.05$ indicating significance. This work was approved by an institutional Committee for Ethics in Animal Use (CEUA/UNICAMP, protocol no. 2696-1). **Results**: Venom caused ileal contraction, with 5, 50 and 500 µg of venom/ml increasing the tension by 0.2±0.1 g, 2.1±0.6 g and 2.0±0.3 g, respectively ($p<0.05$ vs. basal; n=5). Venom also contracted rat stomach strips (increase in tension: 2.5±0.3 g, 3.8±0.3 g and 3.6±0.4 g for 50, 150 and 500 µg of venom/ml, respectively). Pretreatment with *p*BPB significantly attenuated the ileal responses to venom (50 µg/ml) (decrease in tension from 2.1±0.6 g to 0.3±0.1 g) ($p<0.05$, n=5). The contractile activity of the venom (50 µg/ml) was completely abolished by indomethacin and phenanthroline ($p<0.05$, n=5). Indomethacin also reduced the contractions in stomach strips (from 3.6±0.3 g to 1.0±0.1 g; $p<0.05$, n=5). **Conclusion**: These results show that venom PLA_2 has an important role in venom-induced contractions of rat ileum and stomach. The ability of indomethacin to abolish the responses to venom indicates that endogenous arachidonic acid metabolites, possibly derived from the action of venom PLA_2, are important mediators of this phenomenon. **Financial support**: CAPES, CNPq, FAPESP.

09.026 Topical anti-inflammatory effect of lavender essential oil. Cardia GFE, Aguiar RP, Rocha BR, Wiirzler LAM, Silva-Fillho SE, Uchida NS, Rodrigues PJ, Bersani-Amado CA, Cuman RKN UEM – Farmacologia e Terapêutica

Introduction: Lavender oil, chiefly composed of 1,8-cineole, camphor and endo-borneol is considered to be one of the mildest of known plant essential oils and has a history in wound healing. In recent years, considerable attention has been paid to screening new drugs with anti-inflammatory activity from natural sources, with fewer adverse effects than allopathic drugs. **Aims**: The main objective of this study was to investigate the effect essential oil of lavender on topical inflammatory response in mice. **Methods**: Male Swiss mice (weighing 25±5 g) were provided by the Central Animal House of the State University of Maringá. The animals were housed at 22±2°C under a 12 h/12 h light/dark cycle with water provided ad libitum. The effect of lavender in acute inflammatory response evaluated by ear edema induced by the topical application of 20 µL of an acetone solution that contained the irritant agent croton oil (200 µg) on the inner surface of the left ear of each mice (n = 6-8). The right ear was used as a negative control, which received an equal volume of vehicle (acetone). Sixty minutes before the application of the phlogistic agent, the animals were topically pretreated with vehicle (left ear), lavender essential oil (LEO) (0.125, 0.25, 0.5, 1, 2.5, and 5 mg/ear) or the antiinflammatory reference drug dexamethasone (0.1 mg/ear). Six hours after inflammatory stimulation, the mice were euthanized. Both ears were sectioned, and 6 mm plugs were removed. The plugs obtained from the right and left ears were used to analyze myeloperoxidase (MPO) activity and nitric oxide (NO) production. The protocol was approved by the Committee on the Ethics of Animal Experiments of the State University of Maringá (CEUA Nº 3024210315). **Results**: Topical pretreatment with LEO (0.25, 0.5, and 1mg/ear) inhibited croton oil-induced ear edema by 60, 35 and 30%, respectively. Topical pretreatment with the reference drug dexamethasone (0.1 mg/ear) inhibited ear edema by 79%. The activity of MPO was decreased by 55% and 58% in the group treated topically with 0.25 and 0.5 mg LEO per ear compared with the control group. Dexamethasone reduced MPO activity by 82%. The LEO-induced inhibition of MPO activity at doses of 1.0 mg/ear significantly reduced ear edema. but did not inhibit neutrophil migration. Topical pretreatment with LEO (0.125, 0.25, 0.5, 1, 2.5, and 5 mg/ear) significantly reduced nitric oxide production by 58, 62, 65, 73, 77 and 78%, respectively. Topical pretreatment with the reference drug dexamethasone (0.1 mg/ear) reduced nitric oxide production by 64%. **Conclusion**: These data show that LEO has significant anti-inflammatory effects in acute inflammation models. Treatment with LEO inhibited ear edema formation, neutrophil migration and reduced nitric oxide production. Our results support the possible use of LEO in the development of new anti-inflammatory drugs. However, further studies are needed to confirm this possibility **Supported by**: Fundação Araucária, CNPq and CAPES

09.027 Effect of Patchouli Essential Oil (*Pogostemon cablin*) on chemotaxis of leukocytes *in vitro.* Silva-Filho SE[1], Aguiar RP[1], Uchida NS[1], Wiirzler LAM[1], Rodrigues PJ[1], Cardia GFE[1], Cavalcante HAO[2], Bersani-Amado CA[1], Cuman RKN[1] [1]UEM – Farmacologia e Terapêutica, [2]FITL – Farmácia

Introduction: The Patchouli essential oil (PEO) is obtained from leaves of Pogostemon cablin, Lamiaceae. It is widely used in products such as perfumes, soaps and cosmetics. Researches have shown many biological activities of PEO, including: anti-emetic, trypanocide, antibacterial and antifungal. From PEO wereisolated as major compounds: patchouli-alcohol, α-bulnesene and α-guaiene. Analgesic and anti-inflammatory activities were demonstrated for the methanol extract of Pogostemon cablin, and also for their isolated constituents. **Aims**: In this work it was tested the effect of PEO on cell viability of neutrophils through MTT assay and also on neutrophils migration *in vitro*. **Methods**: PEO was purchased from Sigma-Aldrich (St. Louis, MO, USA). To MTT assay, neutrophils were obtained from the peritoneal cavity of mice 4 h after zymosan injection (1 mg/cavity, i.p.). Briefly, the cells were plated at a density of $5x10^5$ cells/well in a volume of 100 µl RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS) and 100 U/ml penicillin + 100 µg/ml streptomycin in 96-well plates. After 90 min exposure to PEO (3, 10, 30, and 90 µg/ml), 10 µl MTT (5 mg/ml) stock solution was added to each well. After 2 h of incubation at 37°C, 150 µl of the supernatant was removed, and 100 µl dimethyl sulfoxide (DMSO) was added to each well. The cells were incubated at 25°C for a further 10 min, and absorbance was measured using a Biochrom Asys Expert plus microplate reader at a wavelength of 540 nm. To evaluation of chemotaxis *in vitro*, neutrophils were isolated from mice peritoneal cavity, 4 hours after zymosan injection (1mg/cavity, i.p). The cell ($1x10^6$ cells/ml) in RPMI/BSA 0.1% were pretreated with PEO at concentrations of 1, 3, 10, 30, 60 or 90 µg/µl during 30 min, and placed in a chemotaxis Boyden chamber (48 wells). The neutrophils were allowed to migrate toward fMLP (10^{-6} M) or medium alone and the chamber was incubated at 37°C with 5% CO2 for 1 h. The membrane was removed, washed and stained using the Instant Prov. Neutrophils that migrated through the membrane were counted by optical microscopy (1000X). Data were expressed as the mean ± SEM of 3 separate experiments. Results were statistically analyzed by using one-way variance analysis (ANOVA) followed by Tukey's test ($p < 0.05$*). **Results**: In the cell viability assay, PEO was tested at concentrations of 3, 10, 30, and 90 µg/ml, and presented cell viability of 87.5, 97.2, 85.7, and 96.9%, respectively, indicating that it did not induce cell death, in the tested cells. Our data also showed that PEO pretreatment in concentrations of 1, 3, 10, 30, 60 and 90µg/µl inhibited *in vitro* leukocytes migration in 25, 35.3, 48.8, 46, 24.5 and 18%, respectively, showing an inhibitory effect on leukocytes chemotaxis. **Conclusion**: Our data suggested that PEO has inhibitory effect on *in vitro* leukocytes chemotaxis. **Financial Support**: CNPq; CAPES; Fundação Araucária. The experimental protocols were approved by the Ethical Committee on Animal Experimentation of the State University of Maringá (CEAE/UEM 009/2015).

09.028 Evaluation of topical anti-inflammatory activity of cinnamic acid in experimental model. Rodrigues PJ, Aguiar RP, Rocha BA, Silva-Filho SE, Cardia GFE, Wiirzler LAM, Uchida NS, Bersani-Amado CA, Cuman RKN UEM – Farmacologia e Terapêutica

Introduction: The cinnamic acid is a phenol can be found in plants, especially in products containing fragrances and essential oil of cinnamon. It has been widely used in medicine, pesticides, food preservatives, sweeteners, photosensitive resins, local anesthetics and fungicides. Despite being studied for its biological activities, there is still little research related to anti-inflammatory activity mainly topical. The topical anti-inflammatory activity of cinnamic acid (CA) was investigated using the experimental model of ear edema induced by croton oil in mice. It was evaluated myeloperoxidase activity (MPO) and nitric oxide concentration (NO) in sections of the mice ears.. **Methods**: The animals were divided into six treatment groups of eight mice each: Group I - control (vehicle acetone); Group II - CA 0.5 mg/ear; Group III - CA 1 mg/ear; Group IV - CA 2.5 mg/ear; Group V - CA 5 mg/ear; Group VI - dexamethasone (DEX) 0.1 mg/ear (anti-inflammatory drug reference). All drugs were administered topically in a volume of 20µL, 1 hour before the induction of the inflammatory process by phlogistic agent (croton oil). Drugs were administered in the left ears and the right were controls. Edema was induced by application of 20 uL of croton oil (200 µg) dissolved in acetone/water on the inner surface of the left ear (E) of the mice in each group. On the right ear (D) was applied to the vehicle only (acetone) (20 uL). After 6 hours, the animals were euthanized with isoflurane overdose by inhalation. The ears were cut into circular discs of 6.0 mm diameter and weight (mg) in an analytical balance. The sesctions ears were processed and the supernatants of the sections of the ears were collected for measurements of myeloperoxidase (MPO) and nitric oxide (NO). The results were statistically analyzed using ANOVA followed by Tukey's test. Differences were considered significant at $P<0,05$. The experimental protocol were approved by the Ethics Committee on Animal Experimentation of the State University of Maringa (CEUA/UEM 3657210315). **Results**: Topical administration of CA 0.5 and 5.0 mg/ear and DEX significantly reduced ear edema formation (24%, 63.5% and 82%, respectively). Addtionally, the groups treated with CA 0.5, 1.0 and 5.0 mg/ear and DEX significantly reduced cell infiltration – MPO activity (44.6%, 35.2%, 63.5% and 63%, respectively). Moreover, the CA did not influence the production of NO. **Conclusion**: The results suggest that the CA has a topical anti-inflammatory activity and that the efficacy is due at least in part, by the reduction of cell migration. **Sources of research support**: CAPES/CnpQ; Fundação Araucária-PR.

09.029 Cardiovascular responses to Bothropstoxins I and II, Phospholipases A2 from *Bothrops jararacussu* (Jararacuçu) snake venom. Rodrigues MAP, Dias L, Smaal A, Rennó AL, Lorenzetti R, Sousa NC, Panunto PC, Inoue BR, Hyslop S Unicamp – Farmacologia

Introduction: Systemic envenoming by Bothrops jararacussu (jararacuçu) in humans is characterized by coagulopathy, internal hemorrhage, hypotension, circulatory shock and renal failure. In rats, B. jararacussu venom causes dose-dependent hypotension. This venom is rich in phospholipases A2, the two best characterized of which are the basic myotoxins bothropstoxins I (BthTX-I, a catalytically inactive Lys49 PLA2) and II (BthTX-II, a catalytically active Asp49 PLA2). In this work, we examined the possible contribution of these two enzymes to venom-induced cardiovascular and histological alterations in rats. **Methods**: BthTX-I and -II were purified by a combination of gel filtration on Superdex 75 and ion exchange on SP-Sepharose. Toxin purity and identity were confirmed by SDS-PAGE, RP-HPLC and mass spectrometry. Male Wistar rats (300-400 g) were anesthetized with isoflurane (2% in 98% air); the left carotid artery was cannulated for blood pressure measurement (PowerLab, ADInstruments) and a femoral vein was cannulated for venom, BthTX I or BthTX II injection (all at a fixed dose of 0.5 mg/kg); only one agent was tested per rat. Heart rate and electrocardiogram (ECG) were monitored electronically and respiratory rate was determined manually. Changes in blood pressure were monitored for 120 min after which the rats were killed with an overdose of anesthetic. Selected organs were removed and processed for histological analysis after staining with hematoxylin-eosin. The results (mean±SEM) were analyzed using ANOVA followed by the Tukey-Kramer test, with $p<0.05$ indicating significance. The experiments were approved by an institutional Committee for Ethics in Animal Use (CEUA/UNICAMP, protocol nos. 1433-1 and 1739-1). **Results**: Venom caused immediate hypotension that was maximal within 1 min (decrease, from 92±7 to 54±10 mmHg, n=6), followed by gradual incomplete recovery. There were no significant changes in heart rate, respiratory rate or ECG. BthTX I had no significant effect on arterial blood pressure or other parameters compared to rats injected with 0.9% saline alone (n=6). In contrast, BthTX II produced abrupt hypotension (similar to venom) that was maximal at 5 min (decrease, from 102±4 to 66±6 mmHg, n=6). Neither of the toxins significantly affected heart rate, respiratory rate or ECG. Post-mortem examination revealed extensive pulmonary hemorrhage with venom that was confirmed histologically; there was no cardiac myonecrosis, edema or inflammatory infiltrate, but the kidneys showed an inflammatory response inflammation in the renal cortex. Neither of the toxins produced any macroscopic or microscopic alterations in the organs examined. **Conclusion**: Only BthTX II produced hypotension similar to that caused by venom, suggesting that this toxin (but not BthTX-I) may contribute to the hemodynamic alterations seen with venom. Neither toxin contributed to the systemic organ damage caused by venom. **Financial support**: CAPES, CNPq, FAPESP.

09.030 Doxycycline attenuates the hypotension caused by *Bothrops alternatus* (Urutu) snake venom: a role for venom metalloproteinases. Inoue BR, Dias L, Rodrigues MAP, da Silva IRF, Panunto PC, Hyslop S Unicamp – Farmacologia

Introduction: *Bothrops alternatus* (urutu) snake venom causes hypotension in experimental animals (rats and dogs). Venom components possibly involved in this phenomenon include metalloproteinases (MPs), serine proteinases (SPs), including kallikrein-like enzymes, and phospholipases A_2 (PLA_2). In this work, we used a combination of gelatin zymography and treatment with doxycycline (DOX), an inhibitor of metalloproteinases, to examine the role of matrix metalloproteinases (MMP-2 and MMP-9) and venom metalloproteinases on the hypotension caused by *B. alternatus* venom in anesthetized rats. **Methods**: Male Wistar rats (300-400 g) were anesthetized with isoflurane (2%/L air); the left carotid artery was cannulated for blood pressure measurement and a femoral vein was cannulated for injection of venom (0.4 and 0.5 mg/kg; FUNED, Belo Horizonte, MG) or DOX (30 mg/kg, 30 min before venom) and arterial blood sampling. Plasma MMP-2 and MMP-9 activities were determined by gelatin zymography after SDS-PAGE in 2.5% gels and densitometric quantification. In some cases, venom was preincubated with 10 mM DOX (1 h, room temperature) prior to injection. Venom proteolytic (caseinolytic) activity was assayed in the absence and presence of DOX. The results (mean±SD) were analyzed using ANOVA followed by the Tukey-Kramer test, with $p<0.05$ indicating significance. The experiments were approved by an institutional Committee for Ethics in Animal Use (CEUA/UNICAMP, protocol no. 3150-1). **Results**: Venom (0.4 mg/kg, i.v.) caused immediate hypotension that was maximal after 5 min and 15 min (mean arterial blood pressure (MAP) fell from 110±6 to 56±8 and 51±5 mmHg, respectively; n=4) but gradually returned to baseline over 60 min. In contrast, a dose of 0.5 mg/kg resulted in irreversible hypotension and death within 5 min (n=5). Pretreatment with DOX did not affect MAP in saline-treated (control) rats, but attenuated the hypotension with 0.4 mg of venom/kg (decrease in MAP in the absence of DOX 57±8 (50%) and 62±5 (55%) mmHg vs. decreases of 47±4 (42%) and 31±13 (27%) in the presence of DOX at 5 min and 15 min, respectively; n=4-5; $p<0.05$) and abolished the hypotension and lethality of 0.5 mg of venom/kg (n=5). Pre-incubation of venom with 10 mM DOX inhibited venom proteolytic (caseinolytic) activity (244±0.4 U/mg vs. 44±0.1 U/mg, before and after DOX, respectively; n=6) and attenuated the hypotension caused by 0.4 mg/kg to a similar extent to that seen in rats pretreated with DOX. There were no significant changes in heart rate and respiratory rate in any of the experimental groups. Zymography showed no significant changes in plasma pro-MMP-2 and MMP-2 activities or in MMP-9 activity in saline- or venom-treated rats in the first 30 min post-venom relative to pre-venom and control (saline) values. **Conclusion**: These results indicate that MMP-2 and MMP-9 do not have a major role in venom-induced hypotension since their activities were unaltered by venom during the hypotensive phase. In contrast, the attenuation seen in pretreated rats with DOX or after inhibition of venom proteolytic activity suggests a role for venom MPs in this hypotension. **Financial support**: CAPES, CNPq.

09.031 Evaluation *in vivo* **of the antioxidant activity of red wine and its residue from Vale do São Francisco in normotensive rats treated during 30 days by gavage.** Marques VFP[1], Santos IM[2], Oliveria WP[3], Biasoto ACT[4], Lima KM[2], Negro-Dellacqua M[2] [1]Univasf – Acadêmico, [2]Univasf, [3]UFBA, [4]Embrapa

Introduction: Wine is an alcoholic beverage produced by fermentation of the grape. In the composition of the wines are found phenolic compounds, including resveratrol which is one of the most famous compounds of the wine, since it is responsible for triggering cardioprotective mechanisms, for example, benefits in the regulation of lipoprotein metabolism. **Aims**: To evaluate the antioxidant activity of red wine and its residue when administered by gavage in normotensive Wistar rats for 30 days by checking the following biochemical parameters: total cholesterol, cholesterol fractions (LDL, VLDL and HDL), triglycerides and glucose. **Methods**: During 30 days of treatment by gavage, the groups (wine and residue) received 100 mg/kg/day of the lyophilized of red wine alcohol-free or the residue dissolved in water. The control group received water. The animals received food and water *ad libitum* throughout the treatment. At the end of the experiment, the animals were subjected to 8 hours fasting prior and anesthetized with ketamine solution (75 mg/kg, I.P) and xylazine (10 mg/kg, I.P) and subsequently euthanized for proceed the blood collection via cardiac puncture from the animal. The following biochemical parameters were analyzed: plasma glucose, total cholesterol (LDL fractions, HDL and VLDL) and triglycerides. After collecting blood, the biochemical analyzes were performed by automated equipment COBAS INTEGRA 400 Plus® using the enzymatic colorimetric technique with reagents kits from Roche Diagnostics®. **Results**: The results considered significant (represented by *) after thirty days of treatment were: control group, group wine and residue group presented respectively (mean values and standard deviation in mg/dL): triglycerides (105.10 ± 15.43; 80.73 ± 29.79; 61.94 ± 15.69*); HDL-cholesterol (23.95 ± 2.93; 22.16 ± 4.98; 30.68 ± 4.07*); VLDL-cholesterol (20.91 ± 3.30; 16.16 ± 5.96; 12.40 ± 3.13*). There was not statistical difference between the control group and the wine group or between the control group and residue group in parameters related to levels for total cholesterol, LDL-cholesterol and glucose. **Conclusion**: This study corroborates the results found in other studies cited in the literature, showing that the polyphenols can trigger benefits on human health, however, further studies with polyphenols from Vale do São Francisco products are required to support this hypothesis. **Financial Support**: UNIVASF/CNPq/CAPES/EMBRAPA Semiárido. Research approval by the Animal Research Ethical Committee (CEDEP-UNIVASF) - 0008/100614.

09.032 Effects of polyanions on some activities of *Bothrops leucurus* **venom.** Cons BL[1], Tomaz MA[1], Strauch MA[2], Monteiro-Machado M[1], Tavares-Henriques MS[1], Cruz JMT[1], Saturnino-Oliveira J[3], Melo PA[1] [1]UFRJ – Farmacologia e Química Medicinal, [2]Instituto Vital Brasil – Diretoria Científica, [3]UFS – Departamento de Morfologia

Snakebites accidents from genus *Bothrops* are common in Brazil, specifically among plantations of cacau in the Northeast, Bahia, where is high the incidence of accidents with *B. leucurus*, which is well adapted to such plantations. In this snake bites are observed edema, hemorrhage and myonecrosis. Preliminary studies show that polyanions such as, Heparin, Suramin, Sulfated Dextrans were able to neutralize toxins from *Bothrops* venoms. It is ascribe as the interctions between positive charges from toxins and the negative charges of these polyanions. We investigated the effect of polyanions abilities *in vitro* activities as well as phospholipase, proteolytic, hyaluronidase, myotoxic and in vivo activities as well as myotoxicity, hemorrhagic, oedema and tail bleeding. Phospholipase activity was assessed using chicken eggining azocoll with substrate. Hemorrhagic lesions were induced by an intradermic injection of the venom or the venom yolk incubated with 10 µg/mL of *B. leucurus* crude venom. Proteolytic assay was assessed in a solution containing azocasein incubated at concentration of 10 µg/mL of *B. leucurus* venom. Hyaluronidase activity was assessed using solution containing hyaluronic acid with substrate. Collagenase assay was assessed in a solution containcubated with the compounds. The *in vivo,* using swiss mices*,* myotoxicity were performed by i.m. injection of 1 mg/Kg of *B. leucurus* crude venom and the plasma CK activity analyzed 2 hours after the i.m. injection. Myotoxic experiments were performed *in vitro,* on mouse *extensor digitorum longus* muscle (EDL) and assessed by the increase of creatine kinase (CK) release following the exposure of muscle to the venom (25 µg/mL). Suramin 30 µM inhibited circa of 100% ± 0,5 of the *in vitro* myotoxic, phospholipase and hyluronidase activities and proteolytic activity only circa 30% ± 2,3. Fucosylated chondroitin sulfate inhibited circa of 40% ± 1,9; 70% ± 3,1 and 100% ± 2,4, the phospholipase, and proteolytic and hyaluronidase activities, respectively. Dextran sulfate inhibited about 60% ± 1,2 phospholipase activity. The proteolytic activity was inhibited by Suramin (30 µM) on circa only 30% ± 0,9. On the *in vivo* experiments, on the pre-incubated protocols, Suramin (30 mg/Kg) produced a protection, circa of 85% ± 1,5 of the myotoxic, 65% ± 1,1 of the oedema, 90% ± 2,2 of the hemorrhagic effects of *B. leucurus* crude venom. Suramin was not able to protect significantly the tail bleeding. Our results are shown polyanions inhibit some important activities of *B. leucurus* crude venom. All experiments we used the average calculation and standard error. CEUA Protocol: DFBCICB072-04/16
Financial support: CAPES, CNPq, PRONEX and FAPERJ

09.033 Gastroprotective effect of rosmarinic acid against NSAIDs and cold restrain stress induced ulcers in mice. Nascimento RF, Machado FDF, Sales IRP, Barbosa-Filho JM, Batista LM UFPB – Ciências Farmacêuticas

Introduction: Rosmarinic acid (RA) is a secondary metabolite of various plant species; it is chemically characterized as an ester of caffeic acid and lactic acid 3.4 dihydroxyphenyl. Its name is derived from *Rosmarinus officinalis*, the first plant from which it was isolated (Peterson, Phytochem, v. 62, p. 121, 2003). Several biological effects have been described for RA, among which we highlight: neuroprotective, antioxidant, anti-mutagenic, antibacterial, and antiviral (Peterson, Phytochem., v. 62, p. 121, 2003), anxiolytic (Pereira, Pharmacol Res, v. 52, p. 199, 2005), and antitumor activities (Lee, Biochem Pharmacol, v. 74, p. 960, 2007). **Aim**: The objective of this study was evaluate the gastroprotective activity of RA in two models of induced acute ulcer: stress (imobilizations and cold) and non-steroidal anti-inflammatory drugs (NSAIDs). **Methods**: For the cold restrain stress and NSAIDs induced ulcer protocol were used male mice *Mus musculus* (n= 5-7), weighing 25-35 g, fasted for 24 hours and pre-treated with vehicle (NaCl 0.9% p.o. - negative control), cimetidine 100 mg/kg (positive control) or RA (25, 50, 100 and 200 mg/kg p.o.). Posteriorly, they were performed the induction of ulcers by immobilization and cold stress (SENAY, R., Munksg., P.S.E.M., v. 92, 1121, 1971 – with modifications) or administration of piroxicam (30 mg/kg) subcutaneously (PUSCAS et al., Arzneim.-Forsch./Drug Res, v. 47, p. 568, 1997). The results were analyzed using ANOVA, followed by Dunnett's test.

Results and Discussion: In NSAIDs-induced gastric ulcer the results show a reduction in the ulcer index (UI) at all doses evaluated of RA (25, 50, 100 and 200 mg/kg) at 124.3 ± 19.67, 119.2 ± 11.86, 100.3 ± 18.90 and 63.83 ± 19.57 ($p < 0.001$), respectively, when compared to the negative control (195.40 ± 11.62). For the model of stress (immobilization and cold) the doses evaluated of RA (25, 50, 100 and 200 mg/kg), decreased the UI by 151.70 ± 32.17, 148.80 ± 28.09, 78.00 ± 14.46 and 72.33 ± 9.73 ($p < 0.001$), respectively, than compared with the negative control group (250.00 ± 47.34).Thus, the results of the present study demonstrate that RA has gastroprotective activity, as demonstrated by the significant inhibition of ulcer formation in both models, being possibly related to the presence of the bioactive molecules evidenced in phytochemical tests. However, future studies are needed to evaluate the gastroprotective activity in other ulcer models and to elucidate the mechanisms of this protective activity of the gastric mucosa. **Acknowlegments**: CNPq/UFPB. The experimental protocols were approved by the Ethics Committee on Animal Use (CEUA/UFPB) with number 0072015.

09.034 Inhibition of rat renal neutral endopeptidase 24.11 (NEP 24.11) activity by Bothrops snake venoms. Fernandes PCL, Torres-Huaco FD Unicamp – Farmacologia

Introduction: Snake venoms contain peptides that inhibit enzymes in venoms, e.g., snake venom metalloproteinases (SVMPs), and in the host, e.g., peptidases such as angiotensin-converting enzyme and dipeptidylpeptidase IV (DPP-IV). In this work, we examined the ability of *Bothrops* venoms (*B. alternatus*, *B. jararaca*, *B. jararacussu*, *B. moojeni*) to inhibit rat renal neutral endopeptidase 24.11 (NEP 24.11). **Methods**: Male Wistar (250-300 g) were anesthetized with 2% isoflurane, perfused with 0.9% NaCl via the aorta, and the kidneys removed and stored at -80 °C until used. Kidneys were homogenized in 0.1 M Tris-sucrose, pH 7.4, at 4 °C, centrifuged (3.000 *g*, 4 °C, 10 min) and the precipitate discarded. The supernatant was centrifuged again (20.000 *g*, 4 °C, 25 min) and the precipitate washed twice with homogenization buffer and resuspended in assay buffer. Protein concentrations were determined by the Lowry method. NEP 24.11 activity was assayed using N-dansyl-D-alanyl-glycyl *p*-nitrophenyl-alanylglycine as substrate. The reaction mixture (in black 96-well plates) contained 125-150 µl of 0.1 M Hepes, pH 6.4, 50 µl of kidney extract (9.6 mg/ml) and 25 µl of venom fraction that was incubated for 15 min at 37 °C prior to addition of substrate (25 µl, final conc. 40 mM). The fluorescence emission was monitored at 562 nm (excitation: 342 nm) for 30 min at 37 °C, with activity expressed in arbitrary fluorescence units (AFU). Venom samples (~75 mg) obtained from CETA (Morungaba, SP) were dissolved in 0.05 M ammonium bicarbonate buffer and fractionated on a Superdex-75 column (1.6x75 cm) equilibrated with the same buffer. The column was eluted at 0.5 ml/min and the elution profile was monitored at 280 nm; fractions of 1.5 ml were collected and screened for their ability to inhibit NEP 24.11. The results (mean±SEM) were analyzed by ANOVA followed by the Tukey-Kramer test, with $p<0.05$ indicating significance. The experimental protocols involving animals were approved by an institutional Committee for Ethics in Animal Use (CEUA/UNICAMP, protocol no. 3044-1). **Results**: Basal renal NEP 24.11 activity ranged from 603-645 AFU/mg (n=3). Gel filtration of *B. jararaca* venom yielded 10 major peaks (PI-PX). Peak VI showed the greatest inhibition of NEP 24.11 activity (81±18%), followed by peaks VII (69±28%), V (51±13%), IV (50±23%) and III (49±14%) (n=3). Gel filtration of *B. moojeni* venom yielded 8 major peaks (PI-PVIII). Peak III showed the greatest inhibition of NEP 24.11 (76±23%), followed by peaks IIIa (76±8%), II (59±16%), IIa (58±24%) and IV (30±14%) (n=3). Gel filtration of *B. alternatus* yielded 6 major peaks (PI-PVI). Peak IV showed the greatest inhibition of NEP 24.11 activity (84±18%), followed by peaks II (66±13%) and III (65±8%) (n=3). Gel filtration of *B. jararacussu* venom yielded 8 major peaks (PI-PVIII), but none of them inhibited NEP 24.11. **Conclusion**: These findings indicate that *Bothrops* venoms contain components capable of inhibiting renal NEP 24.11 activity, with at least one peak in three of the four venoms causing >70% inhibition. For *B. jararaca*, the component appears to be of low molecular mass (based on its elution profile), but its identity remains to be determined. **Financial support**: CAPES, CNPq, FAPESP.

09.035 Friedelin enhances angiogenesis and accelerate wound healing in diabetic mice. Correia ACC[1], Carmo JOS[1], Lima DJ[1], Aquino FLT[1], Ferro JNS[1], Broetto L[1], Conserva LM[1], Martins MA[2], Silva PMR[2], Barreto E[1] [1]UFAL, [2]Fiocruz

Introduction: Healing of diabetic wounds still remains a critical medical problem. Inadequate angiogenesis has been proposed as an important component in diabetic wound complications. Thus, treatments that improve angiogenesis could have important clinical applications. Previous studies have shown that triterpenes enhanced wound closure. Friedelin, a pentacyclic triterpene, has already been documented by its biological functions including antioxidant and anti-inflammatory activities. However, little attention has been paid to its potential effects on wounds associated with diabetes mellitus. **Aim**: In this study, we evaluated the healing activity of the friedelin in alloxan-induced diabetic mice. **Methods**: Diabetes was induced by alloxan at a dose of 65 mg/kg of weight by intravenous route in male Swiss mice. Three weeks after diabetes induction, the full-thickness skin was removed (1 cm diameter) from the dorsomedial back. The friedelin (0.1%) or saline solution (NaCl, 0.9%) were topically applied once daily for 11 days. Percentage of wound closure was calculated using macroscopic data. Wounds were harvested at 7 and 12 days for histopathology by H&E and Masson's staining, and immunohistochemical for CD31. **Results**: At 7 and 12 days post-wound, compared with the non-diabetic mice, diabetic mice exhibited delayed wound closure (38% and 27%) that was characterized by a marked increase in numbers of neutrophils (52% and 53%) and macrophages (29% and 43%), a lower number of fibroblast (77% and 31%), a decrease in collagen deposition (73% and 57%), and a drastic reduction in the capillary density only at 7 day pos-injury (49%). Interestingly, diabetic mice that receive topic treatment with friedelin (0.01%, 0.1% and 1%) had an accelerated healing process of their wounds ($p < 0.001$). The friedelin (0.1%) treatment also decreased the numbers of inflammatory cells ($p < 0.01$), restored the counting of fibroblast ($p < 0.001$), increased the levels of collagen deposition ($p < 0.05$), recovered the capillary density ($p < 0.001$) in all time points, and restored completely the vascular density ($p < 0.01$). **Conclusions**: Topical administration of friedelin affects the wound microenvironment leading to an improvement of healing in diabetic condition, suggesting a therapeutical potential to treat non-healing wounds. **Financial Support and Acknowledgments**: CNPq, CAPES. CEUA/UFAL (License 016/2014).

09.036 Adenosine receptor antagonism and 5´-Nucleotidase inhibition protect against lethal hypotension caused by *Bothrops alternatus* (Urutu) snake venom. Pereira-Marcelino E, Tamascia ML, Hyslop S FCM-Unicamp – Bioquímica e Farmacologia

Introduction: Purines present in snake venoms or released endogenously by venom enzymes such as 5'-nucleotidase (5´-Nuc) may play a role in venom-induced hemodynamic alterations. We have previously shown that adenosine contributes to edema and non-lethal hypotension caused by *Bothrops alternatus* snake venom. In this work, we examined the ability of adenosine receptor blockade and inhibition of 5´-Nuc to protect against lethal hypotension caused by this venom. **Methods**: Male Wistar rats (300-400 g) were anesthetized with urethane (2 g/kg, i.p.) and a carotid artery was cannulated for continuous blood pressure measurement (PowerLab, ADInstruments); a femoral vein was cannulated for administration of venom (0.75 mg/kg; CETA, Morungaba, SP) and antagonists. When required, rats were pretreated (30 min before venom, intravenously) with 1,3 -dipropyl-8-cyclopentylxanthine (DPCPX, adenosine A_1 receptor antagonist; 6 mg/kg; n=4), 8-(3-chlorostyryl) caffeine (CSC, adenosine A_{2A} receptor antagonist, 6 mg/kg; n=4), 3,7-dimethyl-1-proparglyxanthine (DMPX, adenosine A_{2B} receptor, 6 mg/kg; n=4), 3-propyl-6-ethyl-5-[(ethylthio)carbonyl]-2-phenyl-4-propyl-3-pyridine carboxylate (MRS1523, adenosine A_3 receptor antagonist, 6 mg/kg; n=4) or adenosine 5´-(a,b-methylene)diphosphate (AMP-CP, a selective 5´-Nuc inhibitor; 0.125 mg/kg, n=4). Only one venom dose was tested per rat. All agents were injected in a volume of 100 µl and washed in with a further 100 µl of 0.9% NaCl. The changes in mean arterial blood pressure (MAP) were monitored until death or for a maximum of 120 min. The results (mean±SEM) were analyzed statistically using ANOVA followed by the Tukey-Kramer test, with $p<0.05$ indicating significance. This work was approved by an institutional Committee for Ethics in Animal Use (CEUA/UNICAMP, protocol no. 1318-1). **Results**: A lethal dose of venom (0.75 mg kg) caused immediate hypotension (MAP fell from 117±4 to 20±2 mmHg; 83% decrease; n=4; $p<0.05$) followed by death within 5±1 min. DPCPX significantly attenuated the venom-induced hypotension by 57±3% (MAP decrease in the absence and presence of DPCPX: 97±2 and 52±2 mmHg, respectively; $p<0.05$). CSC also attenuated the hypotension (MAP decrease in the absence and presence of CSC: 97±2 and 36±2 mmHg, respectively; 62±2% reduction; $p<0.05$). Likewise, DMPX attenuated the hypotension by 45±3% (MAP decrease in the absence and presence of DMPX: 97±2 and 60±1 mmHg, respectively; $p<0.05$). In contrast, MRS1523 did not attenuate venom-induced hypotension since the decrease in MAP (from 114±6 to 26±2 mmHg; 77% decrease) was similar to that for venom alone. The 5´-Nuc inhibitor AMP-CP attenuated the hypotension by 74±2%. **Conclusion**: Adenosine generated by venom 5´-Nuc or endogenous ecto-5´-Nuc contributes to the hemodynamic alterations caused by *B. alternatus* venom since blockade of adenosine receptors or inhibition of 5´-Nuc protects against venom -induced hypotension and lethality.Because in contrast to the rapid death caused by venom alone, all of the rats treated with A_1, A_{2A},A_{2B} antagonists and AMP-CP showed recovery in blood pressure and survived until 120 min post-venom. **Financial support**: CAPES, CNPq, FAEPEX-UNICAMP, FAPESP.

09.037 Therapeutic potential of sulfated polysaccharide fraction extracted from seaweed *Hypnea musciformis* on acute and secretory diarrhea in rodents. Sousa NA[1], Souza LKM[2], Araújo TSL[2], Costa DS[2], Carvalho NS[2], Nogueira KM[2], Sousa FBM[2], Leódido ACM[2], Araújo S[2], Campos MS[2], Medeiros JVR[1] [1]UFPI – Biotecnologia, [2]UFPI

Introduction: Diarrheal diseases constitute one of the main problems that affect the population's quality of life in developing countries. In this sense, natural products derived from seaweed is the source of several bioactive compounds such as sulfated polysaccharides, which are involved in protective and modulating gastrointestinal function (DAMASCENO, B. J. P. 23, 320, 2013). However, the study of these compounds for the treatment of diarrheal disease is still sparse. **Aim**: Investigate the antidiarrheal activity of the fraction of Sulfated Polysaccharide (PLS) extracted from seaweed *Hypnea musciformis* on acute and secretory diarrhea. **Methods**: Initially the antidiarrheal activity of the PLS was evaluated by castor oil-induced acute diarrhea. Rats (120-160 g) were pretreated orally with saline, or loperamide (5 mg/kg), or PLS (10, 30 and 90 mg/kg). One hour later castor oil was administred (10 ml/Kg). After 3 h was recorded the total number of feces, total number of diarrheal feces, severity of diarrhea and enteropooling. The antidiarrheal effect of the PLS (90 mg/kg), was also evaluated by PGE_2-induced enteropooling (100µg/Kg, *p. o*.). To evaluate the involvement of the opioid system in the PLS activity on the gastrointestinal transit was administered a opiod antagonist, naloxone (2 mg/Kg, *s. c*) and the distance traveled by marker (charcoal preparation; p. o.) was measured. The effect inhibitory of the PLS on intestinal fluid secretion was induced by cholera toxin (1 µg/loop) inoculated into intestinal loops. Besides was analyzed chloride ion concentration of the intestinal contents. Fluid absorption in intestinal loops was performed separately. The interaction between PLS, cholera toxin and GM1 receptor was performed GM1- ELISA. **Results**: Pretreatment with PLS (10, 30 e 90 mg/Kg) reduced the total number of stools (46.68%; 35.46%; and 59.02%, respectively), the total number of diarrheal stools (50.82%; 46.83%; and 63.16%, respectively) ($P<0.001$) and also produced a reduction in the severity of diarrhea ($P<0.001$). The dose of 90 mg/kg significantly decreased the castor oil- and PGE_2-induced enteropooling with inhibition 43.31% and 50.68%, respectively. The PLS produced inhibition of intestinal transit (38.08 ± 1.75 %) ($P<0.01$). However, the naloxone was not able to antagonize significantly the effect exerted by the PLS. Thus, pretreatment with the PLS reduced cholera toxin-induced intestinal secretion (0.14 ± 0.016 g/cm) ($P< 0.001$), as well as decreased chloride levels (70.63 ± 17.82 mEq/l) in intestinal contents. Therefore, had no effect on intestinal fluid absorption. The results showed that PLS reduced cholera toxin detection by GM1 ELISA ($P<0.001$). **Conclusions**: The results of the study shown that PLS has antidiarrheal effect in acute and secretory diarrhea. The antidiarrheal activity of PLS probably stems from its ability to inhibit gastrointestinal motility, prevent the accumulation of intestinal fluid and reduce the secretion of water and chlorides in the intestinal lumen. Thus, this sulfated polysaccharide could be explored as a new therapeutic alternative for the treatment of diarrhea diseases. **Financial Support**: CNPq. **CEP**: Protocol Nº 11/2013.

09.038 Inhibition of angiotensin-converting enzyme activity by *Bothrops spp*. and *Lachesis muta muta* snake venoms. Brunieri LVP, Dias L, Rodrigues MAP, Lorenzetti R, Hyslop S Unicamp – Farmacologia

Introduction: *Bothrops* and *Lachesis* spp. snake venoms contain bradykinin (BK)-potentiating peptides that enhance BK-induced hypotension by inhibiting angiotensin-converting enzyme (ACE) responsible for the formation of angiotensin II (a vasoconstrictor) and the degradation of BK. In this work, we compared the ability of *Bothrops* and *Lachesis muta muta* (bushmaster) snake venoms to inhibit lung ACE. **Methods**: *Bothrops* and *Lachesis* venoms were obtained from CETA (Morungaba, SP) or FUNED (Belo Horizonte, MG). Male Wistar rats (300-400g) killed with an overdose of isoflurane were perfused intracardially with 0.9% saline prior to removal of organs that were stored at -80^{o}C until used. Tissues were homogenized (Polytron) in 50 mM sodium borate buffer, pH 7.4, at 4^{o}C, centrifuged (3,000 *g*, 30 min, 4^{o}C) and the supernatant then incubated overnight with 0.05% Triton X-100, followed by further centrifugation (10,000 *g*, 5 min, 4ºC). Protein was quantified by the method of Bradford. Tissue ACE activity was assayed fluorometrically (E_m=382 nm) using 25 mM hippuryl-histidyl-leucine and a standard curve of hippuric acid (reaction product). In some experiments, rats were anesthetized with isoflurane (2%/L air), prepared for mean arterial blood pressure (MAP) measurement and venom (0.4 mg/kg) was injected via a femoral vein. At various intervals post-venom, the rats were killed and lung tissue was removed for ACE quantification. The results (mean±SEM) were analyzed using ANOVA followed by the Tukey-Kramer test, with $p<0.05$ indicating significance. The experiments were approved by an institutional Committee for Ethics in Animal Use (CEUA/UNICAMP, protocol no. 2697-1). **Results**: ACE activity (□mol/min/mg protein) of different tissues was: lung 142±21, aorta 3.6±0.5, kidney 2.6±0.3, plasma 2.4±1.3, heart 0.5±0.2 and liver 0.07±0.0 (n=3). Incubation of lung extract with venoms (0.06, 0.6 and 2 mg/mL) resulted in significant ACE inhibition that, at 2 mg/mL, was: *B. alternatus* 55%(67±12), *B. atrox* 70%(34±4), *B. jararaca* 73%(26±11), *B. jararacussu* 88%(10±7) and *B. moojeni* 80%(31±17) (n=4; $p<0.001$ vs. basal activity). *Lachesis m. muta* venom was more potent than *Bothrops* venoms, with inhibition at 0.002-2.0 mg/mL at 91%(6±5) at 2 mg/mL. In kidney, *B. alternatus* venom (0.06, 0.6 and 2 mg/mL) enhanced ACE activity from 2.3±0.3 (basal) to 2.5±0.3 (8%), 4.6±0.9 (100%) and 8.1±1.4 (255%), respectively. In contrast, *B. jararaca* venom inhibited ACE activity by 74% (0.6±0.1), 94% (0.2±0.1) and 89% (0.3±0.2), and *B. jararacussu* venom by 22% (1.8±0.4), 61% (0.9±0.2) and 64% (0.8±0.2) (n=3). *B. alternatus* venom showed endogenous substrate cleavage (1.3±0.1) that was not inhibited by 110 nM enalaprilat. *B. alternatus* venom caused hypotension (maximal at 5 min; MAP fell from 109±4 to 57±5 mmHg; n=6; $p<0.05$) that gradually returned to baseline. Lung ACE was inhibited *in vivo* [from 3.0±0.8 (control) to 1.6±0.03 (venom), n=3, $p<0.05$] at 5 min. **Conclusion**: Lung ACE was inhibited to varying degrees by *Bothrops* and *Lachesis* venoms, with the latter being most potent. The inhibition of lung ACE *in vivo* at 5 min (maximal hypotension) suggests involvement of this enzyme in venom-induced hypotension. **Financial support**: CAPES, CNPq.

09.039 Sulfated polysaccharide fraction from marine algae *Gracilaria caudata* reduces mechanical hypernociception and inflammation during experimental arthritis in mice. Bingana RD[1], Silva RO[1], Oliveira FFB[1], Sousa FBM[2], Carmo LD[1], Chaves LS[3], Barros FCN[3], Ribeiro RA[1], Barbosa ALR[2], Freitas ALP[3], Soares PMG[4], Souza MHLP[1], Medeiros JVR[2] [1]UFC – Farmacologia, [2]UFPI – Biotecnologia, [3]UFC – Bioquímica, [4]UFC – Morfologia

Introduction: Marine algae are rich sources of sulfated polysaccharides (PLS), which are recognized as having several biological activities. Recent studies have shown that PLS extracted from *G. caudata* demonstrated gastroprotective and anti-inflammatory effects. **Aims**: The present study aimed to investigate the effect of a sulfated polysaccharide fraction from marine algae *G. caudata* (GC) in the zymosan- and CFA-induced arthritis models in mice. **Methods**: Mice (25-30g, n=8) received saline or GC (3, 10 and 30 mg/kg, *i.p.*) 1 h before the injection of zymosan (30 µg/art). Mechanical hypernociception was evaluated by the electronic Von-Frey at baseline and 2, 4 and 6 h after zymosan injection. After 6 h of arthritis induction, the animals were sacrificed and synovial fluid was collected for determination of myeloperoxidase (MPO) activity, leukocyte count, IL-1β and nitrate/nitrite (NO_3/NO_2) levels. Joint edema was evaluated by measuring the diameter articular and vascular permeability through extravasation of Evans blue dye. In other experimental design, the mice received saline or GC (30 mg/kg, *i.p.*) 1 h after complete Freund's adjuvant (CFA) (1 mg/ml; 20 µl/paw) for determination of mechanical hypernociception and paw edema acute (between 2 and 8 h) sub-chronic (between 24 and 72 h) and chronic (between 6 and 10 days), index arthritis (at 72 h and 10 days) and body weight. **Results**: Pretreatment with GC significantly reduced zymosan-induced mechanical hypernociception (4.05 ± 0.31 for GC 3 mg/kg, 2.96 ± 0.35 for GC 10 mg/kg, 1.73 ± 0.38 for GC 30 mg/kg *vs.* 5.42 ± 0.48 Δg for zymosan group; $p < 0.05$) in a dose-dependent manner and also reduced joint edema (0.21 ± 0.09 *vs.* 0.62 ± 0.05 mm; $p < 0.05$), compared to the zymosan group. Likewise, GC (30 mg/kg) significantly decreased MPO activity (23.86 ± 4.95 *vs.* 59.57 ± 4.41 UMPO/ml; $p < 0.05$), leukocyte (3.73 ± 0.88 *vs.* 21.16 ± 3.38 *x* 10^3 cells per cavity; $p < 0.05$) and neutrophils (1.45 ± 0.28 *vs.* 18.22 ± 3.22 *x* 10^3 cells per cavity; $p < 0.05$) count, IL-1β (1857.0 ± 578.1 *vs.* 4776.0 ± 991.1 pg/ml; $p < 0.05$) and nitrate/nitrite (NO_3/NO_2) (6.83 ± 1.87 *vs.* 14.08 ± 1.73 µM; $p < 0.05$) levels in the synovial fluid, compared to the zymosan group. In addition, postreatment with GC (30 mg/kg) was effective in the inhibition of mechanical hypernociception and paw edema in acute (82.2% and 40.6% of inhibition, respectively; $p < 0.05$), sub-chronic (73.3% and 39.8% of inhibition, respectively; $p < 0.05$) and chronic (80.6% and 38.5% of inhibition, respectively; $p < 0.05$) phases in the CFA-induced arthritis model. **Conclusions**: GC promotes anti-inflammatory effect by modulating neutrophil migration, NO and IL-1β levels, culmining in the reducition of paw edema and mechanical hypernociception. **Financial Support**: CNPq, FUNCAP and FAPEPI. This study was approved by the Ethics Committee in Animal Research of the UFPI (Protocol Nº 068/14).

09.040 Effects of *Tityus serrulatus* **scorpion venom on bronchial epithelial cells.** Rigoni VLS[1,2], Vieira RP[3], Silva JLV[4], Nogueira-Pedro A[5,6], Kwasniewski FH[7], Zamuner SR[1] [1]Uninove – Medicina, [2]Unifesp-EPM – Biofísica, [3]Uninove – Ciências da Reabilitação, [4]Uninove – Farmácia, [5]Unifesp-EPM – Bioquímica, [6]FCF-USP – Análises Clínicas e Toxicológicas, [7]UEL – Ciências Patológicas

Introduction: The scorpion envenomation is considered a public health problem worldwide. Tityus serrulatus is the specie that causes the main accidents in Brazil. Envenomation by Tityus serrulatus range from local pain to severe systemic reactions such as cardiac dysfunction and pulmonary edema. **Aim**: The aim of this study was to analyze the effect of T. serrulatus scorpion venom (TsV) on bronchial epithelial cells. **Methods**: The human bronchial epithelial cells line (BEAS) was used. The cells were grown in culture medium BEBM (Lonza) supplemented with 10% fetal bovine serum, incubated at 37°C with 5% CO_2 for 24 hours for cell attachment; after that, the cells received the TsV venom in concentrations of 10 and 50 µg/mL and were incubated for 1, 3, 6 and 24 h. Cultured cells were observed with light microscopy before and after 24 hours of TsV incubation. Cell viability was analyzed by MTT and cell permeabilization by LDH release assay. Cell viability also was verify by FACsCalibur cytometer, approximately $1x10^6$ cells per sample were incubated with 50 µL of annexin buffer, 3 µL of annexin V-FITC and 5 µg/mL of 7-amino-actinomicyn D (7-AAD) for 20 min protected from light. After incubation, cells were washed and then resuspended with 200 µL of annexin buffer for data acquisition on flow cytometer. A total of 50.000 events were acquired on cytometer. Results were expressed as mean ± s.e.m. and subjected to t-Test statistical analysis ($P < 0.05$). The procedures were performed in triplicate. **Results**: Cells treated with TsV (50 µg/mL) showed a reduction in the number of cells compared to control as well as a decrease in cytoplasmic volume (plasmolysis). Cells treated with TsV (10 and 50 µg/mL) reduced significantly the viability ($P < 0.01$) at 3 hs (50.1 ± 0.1 and 49.2 ± 2.2 %, respectively) and 24 hs (51.1 ± 2.2 and 26.7 ± 2.5 %, respectively). At 24 hs after TsV (50 µg/mL) incubation the cells had an increase ($P < 0.01$) of LDH release (1.0 ± 0.04 mmol NADH/min) compared to control (0.6 ± 0.04 mmol NADH/min). In the absence of TsV (control), 95.4 ± 0.2 % cells were viable and only 2.3 ± 0.1 % death cells ($P < 0.0001$), in the presence of TsV at 24 hs (50 µg/mL), 35.2 ± 0.4 and 60.3 ± 0.4 % ($P < 0.0001$) were the viable and death cells, respectively. **Conclusion**: TsV is citotoxic bronchial epithelial cells and this cell may play a role in the envenomation caused by TsV.

09.041 Antinociceptive and antidepressant-like effects of the *Vitex megapotamica* **in rats.** Rubin MA[1], Hamann FR[1], Rossato MF[1], Mello CF[2] [1]UFSM – Bioquímica e Biologia Molecular, [2]UFSM – Farmacologia e Fisiologia

Introduction: *Vitex megapotamica* (Spreng) Moldenke, popularly known in Brazil as "tarumã", is a native tree from Brazil, Uruguay, Paraguay and Argentina of the *Lamiaceae* family. Folk medicine reports support that *Vitex megapotamica* leaf infusion is indicated to treat rheumatism, skin disorders, hypercholesterolemia and hyperglycemia. **Aims**: This study investigated whether the leaf crude extract or ethyl acetate fraction from *Vitex megapotamica* exhibits antinociceptive and antidepressant effects in adjuvant-induced chronic inflammation and depression model. **Methods**: The leaves of *Vitex megapotamica* were collected in Sobradinho, (Rio Grande do Sul, Brazil) in March 2010. A voucher specimen number SMDB 12.526 was deposited at the Federal University of Santa Maria. The crude extract was obtained by maceration of leaves in ethanol/water (70: 30 v/v). Chronic inflammation was induced by the intraplantar administration of 100 µL of complete Freund's adjuvant in rats. The effect of oral crude extract of *Vitex megapotamica* (VmE; 3–30 mg/kg, p.o.) or ethyl acetate fraction (Eta; 1.1 mg/kg, p.o.) on nociception (von Frey test of mechanical allodynia and ongoing pain score), inflammation (paw edema and local myeloperoxidase activity), immobility (forced swimming test - FST), locomotor activity (open field) and gastrointestinal transit was evaluated. Naloxone (2 mg/kg, i.p.) was used to test the involvement of opioid mechanisms in the currently described effects of VmE. **Results**: Crude extract, as well as ethyl acetate fraction caused antinociception (86.0 ± 23.0%; 89.0 ± 40.4% reduction of pain, respectively, $p<0.05$) in the von Frey test and antidepressant-like (138.2 ± 25.2 %; 142.8 ± 8.4 % reduction of immobility time, respectively, $p<0.05$) effect in the FST. The effects of the crude extract were prevented by naloxone (74 ± 16.5% and 79.2 ± 15.8 % prevention of the antinociceptive and antidepressant-like effects of VmE, respectively, $p<0.05$). The VmE extract (10 mg/kg, p.o.) did not alter locomotor activity, gastrointestinal function, paw edema or myeloperoxidase activity when compared to vehicle group. Brum et al (Brum, Molecules, 18, 8342, 2013) have shown the presence of flavonoids, particularly rosmarinic acid, in the ethyl acetate fraction of *Vitex megapotamica* leaves, which is reported to have antinociceptive and antidepressant-like effects. Therefore, it is possible that rosmarinic acid contributed to the antinociceptive and antidepressant effect of Eta. **Conclusion**: *Vitex megapotamica* induces antinociception and antidepressant-like effect that involve the opioid system, without anti-inflammatory activity. The results support the use of VmE as analgesic and antidepressant. **Financial Support**: This study was supported by CNPq, FAPERGS, CAPES, PRPGP-UFSM. The experiments were performed with the approval of the Ethics Committee of the Federal University of Santa Maria (process number 116/2013).

09.042 Antiulcer effect of *Solanum stipulaceaum* **Will ex. Roem & Shult.** Oliveira DF[1], Lima CAA[2], Estevam CA[2], Batista JS[2] – [1]UFS – Enfermagem, [2]UFS – Fisiologia

Introduction: - Solanum stipulaceaum Will ex. Roem & Shult is an endemic and native plant from Brazil. It is popularly known as “Jurubeba-roxa” and used to treat digestive system disorders. In addition, previous studies have showed that the essential oil and aqueous and ethanolic extracts of Solanum stipulaceaum have gastroprotective effect. **Aim**: To investigate the gastroprotective action of crude hydroethanolic extract of Solanum stipulaceaum (HEE) and determine which fractions obtained from this extract have gastroprotective effect on ethanol-induced gastric ulcers in rats. **Methods**: In these experiments were used rats Wistar (250 – 300 g) divided randomly in 10 groups (n=10). After 18 hours of food deprivation, the animlas were orally pretreated with HEE (at doses of 100, 200, and 400 mg/kg), and with fractions obtained from HEE: chloroform fraction (CF) at doses of 50, 100, and 200 mg/kg, ethyl acetate fraction (EtAcF) at dose of 200 mg/kg and hydromethanol fraction (HMeF) at dose of 200 mg/kg. Two other groups were orally pretreated with ranitidine (50 mg/kg), and Tween 80 at 5% (control group). One hour after the above treatments, gastric ulcers were induced by oral administration of ethanol (0.4 ml/100g). After 30 min, the animals were sacrificed and their stomachs were removed and photographed. The ulcers were quantified from images obtained and the results were analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni’s test. The images were analyzed by Avsoft Bioview 4.0 Software. **Results:** The results are showed as the mean ± SEM of ulcerated area percentage (%AU). The ethanol administration produced gastric ulcer in all control animals (%AU = 37.9 + 7.6%). On the other hand, ranitidine reduced significantly the ulcer formation (%AU = 0.8 + 0.3%). HEE also produced gastroprotective effect at doses of 200 and 400 mg/kg (%AU = 14.7 + 4.0 and 4.4 + 1.2%, respectively). All fractions obtained from HEE presented antiulcer effect at the dose of 200 mg/kg (%AU CF = 1.2 ± 0.6, %AU AcEtF = 9.5 ± 3.1 and %AU HMeF =10.6 ± 3.2). Moreover, CF also produced gastroprotection at doses of 50 mg/kg (%AU = 3.1 + 1.2%) and 100 mg/kg (%AU = 3.8 + 1.3%). **Conclusion:** The results obtained indicate that HEE, CF, AcEtF and HMeF present gastroprotective effects. In addition, the results suggest that the active constituents of Solanum stipulaceaum are in higher concentration in the CF. **Support Financial**: CNPq. The present experimental protocol was approved by the Research Ethics Committee of Animal of the Federal University of Sergipe under the protocol number 05/2015.

09.043 Extract assessment *Allium cepa* **L. in diabetic rats streptozotocin-induced.**
Lemos LIC[1], Medeiros MA[1], Silva FS[1], Abreu BA[1], Bortolin RH[1], Meira KV[1], Rezende AA[1], Figueiredo CAV[2], Oliveira T[2], Medeiros KCP[1] [1]UFRN, [2]UFBA

Introduction and Aims: Diabetes Mellitus (DM) is a chronic metabolic disease characterized by hyperglycemia secondary to the reduction in circulating insulin levels or a deficit of tissue effects of this hormone, reflecting the dysfunction of various organs. The onion (Allium cepa L.), in the preparations and known worldwide, has been used in research that has a focus on diabetes to check their potential hypoglycemic and systemic beneficial effect. The aim of this study was to evaluate the treatment effect of Allium cepa extract strain in rats induced with streptozotocin. **Methods**: We used 26 Wistar rats and the DM was induced by streptozotocin (40 mg / kg ip.). Allium cepa extract (400 mg / kg, po) was administered daily for 30 days after installation of MD. Clinical, biochemical profile, pancreatic morphology and morphometry were the parameters analyzed in this study. **Results**: Clinically, diabetic animals showed polydipsia (723 ± 109), weight loss (18 ± 12), hyperglycemia (413 ± 38) and increased lipid profile (91 ±7), classic DM and treatment with the Allium cepa extract strain could decrease significantly cholesterol shape (64 ± 5) and showed no weight loss (9 ± 6) as the diabetic animals. As to morphological appearance, the treatment with the extract was able to improve pancreatic atrophy of diabetic animals. **Conclusion**: The acute treatment Allium cepa extract demonstrates shown to reduce cholesterol and regeneration ability of pancreatic beta cells by an unknown mechanism, and thus can be considered a promising product for future clinical applications associated or not with other therapies. **Financial Support**: CAPES/CNPq Research approved by the Animal Research Ethical Committee of Federal University of Rio Grande do Norte (process number: 054/2014).

09.044 *In vitro* **effects of brasiliensic and isobrasiliensic acids from *Calophyllum brasiliense* Camb. on gastric cell turnover.** Lemos LM[1], Pritchard DM[2], Burkitt MD[2], Martins DTO[1] [1]UFMT – Farmacologia, [2]University of Liverpool – Gastroenterology

Introduction: The stem bark of *Calophyllum brasiliense* (Calophyllaceae) is used in popular medicine to treat chronic dyspepsia. Studies have reported antiulcer and anticancer activity for stem bark extracts of *C. brasiliense*. Brasiliensic (Bras) and isobrasiliensic (Isob) acids were isolated from a hexane extract of *Calophyllum brasiliense* stem bark (HECb). The current study aims to investigate the effect of HECb, Bras and Isob on cell turnover *in vitro*. **Materials and Methods**: Trypan blue exclusion assays were used to evaluate AGS cell viability. $1x10^5$ AGS cells/well were seeded in a 96-well plate, incubated overnight at 37°C, 5% CO_2. They were exposed to HECb (12.5-100 µg/mL) or etoposide (50 µM), for 24-72h. Attached and floating, viable and unviable cells were counted and results were expressed as absolute number of cells. To evaluate the effect of these compounds on apoptosis and proliferation, primary gastric gland cultures were generated from male C57BL/6 mice. Dissociated glands were seeded, on glass coverslips. After 48h they were treated with HECb, Bras, Isob (12.5-100 µg/mL) or IFN-γ (0.15 µg/mL) for 24h. Immunoflourescent staining was performed using anti-cleaved caspase-3 antibody and a Click-iT EdU kit, with DAPI counterstaining. Results were expressed as percentage of positive cells/gland. For flow cytometric studies, AGS cells were seeded ($1x10^6$ cells/well), incubated overnight, treated with HECb, Bras and Isob (12.5-100 µg/mL) for 24-48h, and stained with propidium iodide to evaluate cell cycle arrest. 10,000 events were recorded per sample, results were expressed as percentage of recorded events. **Results and Discussion**: The number of AGS cells observed during cell culture increased from 28,300 cells/mL at 24 h to 112,400 cells/mL at 72 h. HECb treatment maintained the number of cells between 14,300 and 32,300 cells/mL, despite increasing concentration and duration of treatment. The number of floating, unviable cells was increased approximately 3 fold ($p<0.01$) by 50 µg/mL HECb, compared to control. Immunofluorescence of primary murine gastric gland cultures identified an apoptotic rate of 2.8% for untreated cultures. HECb and Bras increased apoptosis 20 and 25 fold ($p<0.001$) respectively at 100 µg/mL. Isob increased apoptosis 15 and 25 fold at 50 and 100 µg/mL, respectively, compared to control. The proliferation rate of untreated gastric glands was 8.48%. All three plant derivates reduced proliferation in this model, with maximum effect observed at 100 µg/mL ($p<0.001$). Compared to untreated cells (39.48% G1, 29.32% S and 31.02% G2/M), treatment of AGS with 25 µg/mL of HECb, Bras or Isob increased the number of cells in G1 phase (25% higher than untreated, $p<0.05$), while 50 µg/mL maintained the G1 increase, increased S phase (19%, $p<0.05$) and reduced cells in G2/M (66%, $p<0.01$) in relation to control. Treatments for 48h confirmed this trend. **Conclusion**: HECb, Bras and Isob induced both a pro-apoptotic, and an inhibitory effect on proliferation in primary murine gastric gland cultures and AGS cells *in vitro*. Our preliminary data demonstrate that this effect is due to cell cycle arrest in G1/S phase. Further studies will elucidate the mechanism by which this cell cycle arest occurs. **Financial support**: CNPq, FAPEMAT, INAU, The Wellcome Trust. Ethical approval number 23108.031167/13-1 by Ethic Committee for Animal Experimentation of UFMT.

09.045 Use of *Tibouchina granulosa* tea wound healing of diabetic mice. Sobrinho AP[1], Amorim JL[1], Ferreira LLC[2], Fernandes PD[3] – [1]UFRJ – Laboratório de Farmacologia da Dor e Inflamação, [2]Instituto Vital Brazil – Fitoterápicos, [3]UFRJ – Farmacologia e Inflamação

Introduction: Tibouchina granulosa it is a ornamental plant used in Brazil and popularly named "quaresmeira". Personal observation from our group indicate that infusions of leaves demonstrate wound healing effect when directly applied to rodents. **Objectives**: Characterize and evaluate the wound healing effect of lyophilized infusions of quaresmeira leaves. Methods T. granulosa leaves were collected in Cachoeiras de Macacú (RJ). A voucher specimen is deposited in the herbarium of IB/UFRJ and received the number 37.931. Leaves were dried, ground in a knife mill. Infusions were prepared, lyophilized and stored at -20 ^{0}C until use. Diabetes were induced in Swiss Webster mice (female, 30-35 g, n = 10-15) through iv injection of alloxan (65 mg/kg). After 1 week, diabetic mice had part of their back trichotomized and an area of 15 mm diameter was exposed by a pouncher. For a period of 14 consecutive days the wounds were treated daily with 10, 30 or 100 mg/kg of a solution prepared with lyophilized material. On days 0, 3, 7, 10 and 14 the wounds were photographed and the image processed using ImageJ software. The results are presented as the mean ± SD of wound size (Percentage and Arbitrary Units - AU). Statistical analysis was performed with ANOVA followed by Bonferroni's test (*p<0.05). The experimental protocols were approved by COBEA/UFRJ (#DFBCICB015-04/16). **Results**: normal animals (non-diabetic) already show a decrease of 40% wound after 3 days and after 14 days 98% of the wound already shrank: day 3 = 39.5 AU ± 8.3 (44.7% shrinkage); day 7 = 21.2 AU ± 11 (70.3% retracement); day 10 = 9 AU ± 6.8 (87.4% of shrinkage); day 14 = 1.2 AU ± 1.8 (98.3% retracement). Diabetic animals had significant shrinkage of the wound only after the 10th day: day 3 = 55.3 AU ± 11.3 (0% shrinkage); day 7 = 36.4 AU ± 8.9 (21.2% of shrinkage); day 10 = 23 AU ± 12* (50.2% of shrinkage); day 14 = 4.4 UA ± 5.4* (90.5% shrinkage). Diabetic animals treated with different doses showed the following trend: 10 mg/kg: day 3 = 63.6 AU ± 17.6* (36.4% shrinkage), day 7 = 47.9 AU ± 10.5* 52% shrinkage); day 10 = 18.9 AU ± 3.3* (81.1% shrinkage) and day 14 = 5.2 AU ± 2.2* (94.8% shrinkage of wound); 30 mg/kg day 3 = 90.7 AU± 15.8 (9.3% shrinkage); day 7 = 69.6 AU ± 32.6 (30.7 % shrinkage); day 10 = 32.6 AU ± 11.7 (67.4% shrinkage); day 14 = 9.3 AU ± 2.4 90.7% shrinkage) and 100 mg/kg day 3 = 73.4 AU ± 9* (27% shrinkage); day 7 = 23.1 AU ± 14.9* (77% shrinkage); day 10 = 7.2 AU ± 3.7 * (92% shrinkage); day 14 = 0.8 ± 0.3 * AU (99.2% shrinkage). Conclusions: The results suggest that the tea T. granulosa have a significant healing effect reducing the time required for wound shrinkage in diabetic animals and may be a new therapeutic option in folk medicine. Thanks: Alan Minho (technical support); Instituto Vital Brazil (donation of animals); CNPq e FAPERJ (funding).

09.046 Effect of heparin in cutaneous lesions induced by *Bothrops jararacussu* snake venom. Borges PA[1], Teixeira RGS[2], Nogueira TA[2], Oliveira FL[3], Calil-Elias S[2], Melo PA[1] [1]UFRJ – Farmacologia e Química Medicinal, [2]UFF, [3]UFRJ

Introduction: Envenomations by snake venom are characterized by prominent local effects, including edema, hemorrhage, blistering and dermonecrosis. The intensity loads and the heterogeneous structure of heparin are responsible for their interaction with several proteins such as growth factors. Heparin has been studied in other models of tissue regeneration with treatment of burns and diabetic foot wounds. Due to lack of studies about skin lesions by ophidian, we studied acute skin alterations induced by Bothrops jararacussu venom and treatment with heparin. Methods and **Results**: Male Swiss albino mice weighing 25 +/- 3 g were used in this study. The lesion was induced in the abdomen by an intradermal injection of the venom (3 mg/Kg). Treatment with heparin (10 mg/kg) was administered subcutaneously during three days after the venom injection. The animals were sacrificed at 3 days after the venom injection. The mobilization of inflammatory cells from the bone marrow (lympho-hematopoietic organ), spleen (effector organ) and blood (migratory vehicle) by flow cytometry was investigated. The cellularity in the blood (Control: 2,15 x 10^6 (+/- 0,5) cells/mL; Venom: 5,1 x 10^6 (+/- 0,8) cells/mL; Heparin: 1,47 x 10^6 (+/- 0,5) cells/mL) and bone marrow (Control: 16,4 x 10^6 (+/- 2,1) cells/mL; Venom: 20,9 x 10^6 (+/- 1,8) cells/mL; Heparin: 11,8 x 10^6 (+/- 2,1) cells/mL) were increased on envenomed mice and significantly prevented by heparin treatment. The cellularity in the spleen was not significantly affected by poisoning (Control: 33,0 x 10^6 (+/- 3,3) cells/mL; Venom: 28,3 x 10^6 (+/- 5,2) cells/mL; Heparin: 30,3 x 10^6 (+/- 4,5) cells/mL). Considering this result, we investigated the cell types involved with these responses against the venom. B. jararacussu venom modified the phenotypical distribution of Mac1^{High}Gr-1^{-} monocytes, Mac-1^{High}Gr-1^{Low} immature neutrophils and Mac-1^{High}Gr-1^{High} mature neutrophils. In the bone marrow (Monocytes= Control: 0,46 x 10^5 (+/- 0,15) cells/mL; Venom: 1,34 x 10^5 (+/- 0,19) cells/mL; Heparin: 0,49 x 10^5 (+/- 0,11) cells/mL. Mature neutrophils= Control: 48,9 x 10^5 (+/- 6,1) cells/mL; Venom: 66,9 x 10^5 (+/- 4,3) cells/mL; Heparin: 33,5 x 10^5 (+/- 3,7) cells/mL) and the blood (Monocytes= Control: 5,33 x 10^5 (+/- 0,8) cells/mL; Venom: 10,74 x 10^5 (+/- 1,7) cells/mL; Heparin: 3,0 x 10^5 (+/- 1,2) cells/mL. Mature neutrophils= Control: 12,5 x 10^5 (+/- 1,9) cells/mL; Venom: 26,6 x 10^5 (+/- 2,8) cells/mL; Heparin: 9,4 x 10^5 (+/- 2,3) cells/mL) the venom induced an increase of monocytes and mature neutrophils and the heparin treatment significantly prevented this effect. In contrast, the spleen myeloid cells were poorly modified after the induction with venom. Data is reported as mean (+/- standard error), n=6, $p < 0.05$. All procedures described were reviewed and approved by the Ethics Committee for the Use of Animals of the Federal University of Rio de Janeiro (CEUA-UFRJ). **Conclusion**: These data suggested that B. jararacussu venom induced an intense cellular response 3 days after the venom injection in the skin and heparin treatment was effective in reduced this response.

09.047 Hypolipidemic effect of a grape skin extract of *Vitis vinifera* (ACH09) in C57BL/6 mice fed a high-fat diet. Santos IB, da Costa GF, Costa CA, de Bem GF, Cordeiro VSC, Soares de Moura R, Resende AC UERJ – Farmacologia e Psicobiologia

Introduction: The prevalence of obesity over the past decades has shown rapid rise worldwide. Hepatic steatosis, characterized by increased levels of lipids (triglycerides) in the liver is frequently associated with obesity and is the early stage of nonalcoholic fat liver disease (NAFLD). Recent studies from our group have shown that the hydro-alcoholic grape skin extract (ACH09) rich in polyphenols lowers blood glucose in experimental model of diabetes induced by alloxan. The aim of this study was to evaluate the beneficial effects of preventive treatment with ACH09 on hepaticmetabolic disorders observed in an experimental model of obesity.**Methods**: The Ethics Committee of Animal Experiments of the UERJ approved the experiments (protocol: CEA/025/2010). Male mice C57BL/6 at 30 days of age were separated in four groups and received the following diets for 12 weeks. The Control group: standard diet; ACH09 group: standard diet + 200 mg/kg/day orally; hyperlipidic group (HF): diet with 60% fat and hyperlipidic group + ACH09 (HF + ACH09): diet with 60% fat + 200 mg/kg/day orally. We also determined the food intake, body weight, mass of visceral fat, plasma levels of total cholesterol, triglycerides, glucose and insulin, and hepatic levels of cholesterol and triglycerides. Expression of insulin receptor (IR), PI3-K, pAKT, Glut 2, proteins involved in the synthesis of fatty acids and cholesterol (AMPK, pAMPK, HMG-CoA reductase, FAS, SREBP-1c, pACC), as well as the expression of proteins involved in the excretion of cholesterol (ABCG5 and ABCG8 transporters) were evaluated in liver homogenates by western blotting. Hepatic oxidative damage was also determined by lipid peroxidation and carbonyl protein, and the antioxidant enzyme activities (SOD), (CAT) and (GPx) were evaluated by spectrophotometry.**Results**: The food intake, body weight and the mass of visceral fat were increased in the HF group and the treatment with ACH09 reduced all these parameters.The increased plasma and hepatic levels of cholesterol and triglycerides wereassociated with the development of hepatic steatosis,which was improvedby ACH09. Plasma levels of glucose and insulin resistance were increased in HFgroup and reduced by ACH09. These findings correlated with reduced expression of insulin signaling cascade proteins in the HF group and increased by treatment with ACH09. Increased lipogenesis in the HF group was characterized by the increased expression of SREPB-1c and AMPK proteins and reduction of pAMPK and pACC proteins without changing the HMG-CoA reductase and FAS expressions. The treatment with ACH09 reduced the expression of protein-1c SREPB and increased pAMPK expression without changing pACC. Moreover, ACH09 increased the expression of ABCG5 and ABCG8 transporters and showed an antioxidant effect in hepatic tissue by decreasing the formation of malondialdehyde and carbonyl protein and increasing the antioxidant enzymeactivities. **Conclusions**: In conclusion, ACH09 can modulate the expression of the insulin signalingcascade, as well as the proteins involved in lipogenesis, and excretion of cholesterol preventing the development of hepatic steatosis. These effects associated with the antioxidant action of ACH09 may protect against the phenotypic and metabolic characteristics of obesity.**Financial Support**: CNPq and FAPERJ.

09.048 Hemodynamic responses to *Bothrops fonsecai* **snake venom: Lack of neutralization by commercial Bothropic antivenom.** Tamascia ML[1], Collaço RCO[1], Cogo JC[2], Rodrigues-Simioni L[1], Hyslop S[1] [1]FCM-Unicamp – Farmacologia, [2]UNIVAP – Pesquisa e Desenvolvimento (IP&D) / Serpentário do Centro de Estudos da Natureza (CEN)

Introduction: *Bothrops fonsecai* is a pitviper with a distribution restricted to high altitude Atlantic Forest in the Serra da Mantiqueira, southeastern Brazil. The venom of *B. fonsecai* has similar enzymatic properties and biological activities (edema, hemorrhage, necrosis and neuromuscular blockade *in vitro*) to other *Bothrops* species. In this work, we examined the effect of *B. fonsecai* venom on hemodynamic parameters in anesthetized rats and the ability of commercial bothropic antivenom (CBA) to neutralize these responses. **Methods**: Male Wister rats (350-400 g) were anesthetized with isoflurane (2% in air) and a carotid artery was cannulated for blood pressure measurement. A femoral vein was cannulated for injection of venom or venom/CBA mixture in a fixed volume of 100 □l that was washed in with 100 □l of 0.9% saline. Blood pressure, heart rate and electrocardiogram (ECG) were monitored continuously using PowerLab software (ADInstruments, Australia). Respiratory rate was determined manually. The neutralizing capacity of CBA produced by the Instituto Butantan (against a pool of *Bothrops* venoms: *B alternatus, B. jararaca, B. jararacussu, B. moojeni* and *B. neuweidi*) was tested at a venom: antivenom ratio of 5: 1 (1 ml of CBA neutralizes 5 mg of reference *B. jararaca* venom). For neutralization, the venom: antivenom mixture was incubated at 37 ^{o}C for 1 h prior to injection. Cross-reactivity of CBA with venom components was assessed by immunoblotting using standard procedures; *B. jararaca* venom was used as a positive control. The results (mean±SEM) were analyzed using ANOVA followed by the Tukey test, with $p<0.05$ indicating significance. The protocols were approved by an institutional Committee for Ethics in Animal Use (CEUA/UNICAMP, protocol no. 2648-1). **Results**: Venom caused immediate hypotension that was maximal at 4.3±1 min post-injection (from 130±4 mmHg to 62±9, 127±4 to 44±2 and 125±7 to 45±2 mmHg for 0.1, 0.3 and 1 mg/kg, respectively; n=4-6; $p<0.05$ vs. pre-venom values). With the lowest dose, blood pressure recovered to initial (pre-venom) values after 30 min, whereas the higher doses caused cardiorespiratory arrest (death) after 5.2±1 min for 0.3 mg/kg and 1.6±0.1 min for 1 mg/kg. There were no changes in respiratory rate and heart rate decreased only near death with the highest dose (from 414±11 to 156±55 beats/min). At the manufacturer's recommended ratio, CBA failed to inhibit venom (0.1 and 0.3 mg/kg)-induced hypotension (57±3 and 40±3 mmHg, respectively; n=4-5) or death by 0.3 mg/kg (7.8±1 min). Immunoblotting showed that CBA reacted less with *B. fonsecai* venom than with *B. jararaca*, especially for components <30 kDa and >66 kDa. **Conclusion**: *Bothrops fonsecai* venom causes hypotension and cardiorespiratory arrest in anesthetized rats. These effects were not prevented by pre-incubation with CBA, a finding that agreed with the lower cross-reactivity of CBA with *B. fonsecai* venom compared to *B. jararaca* venom. **Financial support**: CAPES, CNPq

09.049 Effect of methanolic extract, fractions and sub-fractions of *Garcinia achachairu* **on the blood pressure of anesthetized rats.** Januário AGF[1,2], Peruzzo MM[2], Mariano LNB[3], Niero R[3], Nardi GM[2,1] [1]Unoesc – Biotecnologia, [2]Unoesc – Farmacologia, [3]Univali – Ciências Farmacêuticas

Introduction: *Garcinia achachairu Rubsy* (Clusiaceae) is popularly known as "achachairu", and is used in Bolivian folk medicine for its healing, digestive, and laxative properties, and in the treatment of gastritis, rheumatism and inflammation. Despite its widespread therapeutic use, there is a lack of data regarding its effects on the arterial blood pressure (BP), "in vivo". **Aim**: The present study we evaluated the effect of methanolic extract, fractions and sub-fractions of G. achachairu on the BP of anesthetized rats. **Methods**: Barks of *G. achachairu* were collected in Camboriú-SC, in May 2010. Plant material were extracted with methanol and partitioned successively with ethyl acetate (EtOAc), dichloromethane (DC) and n-butanol (n-BuOH), providing fractions. A voucher specimen was deposited at the Barbosa Rodrigues Herbarium (Itajaí-SC) under number HRB-52637. Protocol 1: In order to verify the effect of *G. achachairu* on BP, methanolic extract was administered by oral route at dose of 300 mg/kg. After 1 hour, animals were prepared for recording the mean arterial pressure (MAP) and phenylephrine (10 µg/kg/min, e.v.) were infused over one hour. Protocol 2: For purposes to determine the effect of fractions and sub-fractions of *G. achachairu* on BP, the animals were prepared like above, and received three consecutive doses (3, 10 and 30 mg/kg, e.v.) of EtOAc, N-BuOH and DC fractions. The same way, sub-fractions derived from EtOAc were administered in the doses of 0.3, 1 and 3 mg/kg, e.v. Control animals were treated only with vehicle (saline or water, with DMSO 10% plus Tween 10 %). Protocol 3: In order to verify that nitric oxide (NO) is involved in the hypotensive effect *G. achachairu*, the hypotensive effect of EtOAc were evaluated in presence, or absence, of L-NAME (20 mg/kg, s.c.). All animal experiments protocols were approved by Ethics Committee on Animal Experiments of the Universidade do Oeste de Santa Catarina (CEUA protocol nº 26/2013). Data were analyzed with GraphPad Prism® using Analysis of Variance (ANOVA) followed by Dunnett's *post hoc* test with $p<0.05$. **Results**: The results showed that methanolic extract of *G. achachairu* reduced basal BP about 15 mm Hg (92.3 ± 7.4 mmHg) if compared with non-treated animals (107.7 ± 8.7 mmHg). After phenylephrine infusion, the BP of control animals increase to 145.4 ± 5.4 mmHg. However, the animals treated with *G. achachairu*, the BP was 19.6% lower (116.9 ± 4.2 mmHg). EtOAc fraction presented a dose-dependent reduction in MAP, about 20 mmHg in the high dose of 30 mg/kg. BuOH and DC fraction showed reduction on BP, but were less effective. All fractions promoted a slight decrease on BP, but only at high doses. Epicatechin, single isolated compound, did not change blood pressure. After L-NAME injection, MAP increase about 165 mmHg and this treatment was effective to block the hypotensive effect of EtOAc. **Conclusion**: Under our experimental conditions, the data obtained suggest that a single oral administration of 300 mg/kg of G. achachairu extract reduced MAP significantly. EtOAc was more effective than BuOH and DC fractions in reduction of BP. Results suggest that compounds present ia EtOAc fraction acts synergically to decrease MAP, and its hypotensive effect was result to increase of NO production. **Financial Suport**: Unoesc, Fapesc/CNPq.

09.050 Anti-inflammatory and anti-ulcer activities of *Achyrocline alata* (Kunch).
Silva GGO[1], Arfux CRB[1], Menegatti CF[1], Duarte LC[1], Souza TB[2], Moreno SE[1] [1]UCDB – Biotecnologia, [2]Universidade Católica Dom Bosco – Acadêmico

Introduction: A. alata (Asteraceae) has long been used in traditional medicine due to its protective effects against gastric ulcer and inflammatory diseases. Previous studies based on phytochemical analyses of natural extracts showed the presence of flavones, flavonoids, cafeic and chlorogenic acids and other compounds with pharmacological properties. **Aims**: The present study was carried out to determine the putative anti-inflammatory and anti-ulcer activities of A. alata extracts on Swiss mice. **Methods**: A. allata leaves were collected in Dourados/MS (herbarium number: DDMS 5201).The leaves were air-dried at room temperature and extracted with hexane in Soxhlet extractor. The extract was concentrated and dried. Hexane extract (HE) was not analyzed for chemical composition. HE from A. alata (1, 5 and 10 µg, s.c) were used in order to check its anti-inflammatory activities through neutrophil migration into peritoneal cavity induced either by carrageenan (Cg;1 mg, i.p), thioglicolate (Tg; 4%, i.p) zymosan (Zy;100 µg, i.p) or LPS (200 ηg, i.p). The same doses of A. alata were use in order to determine the effect on the vascular inflammatory phenomenon were also evaluated by paw edema assay and Evans blue exudation induced by carrageenan (1%, s.p) in mice. In order to test a putative role of the plant extract on gastroprotection we have used the indomethacin-ulcer (30mg/kg, v.o) model, in which lesions of the gastric wall were determined by image analysis program (ImageJ). **Results**: Our results showed that all doses of A. alata extract (1, 5 and 10 µg, s.c) inhibited neutrophil migration in 80% induced by Tg, Cg, Zy and LPS, when compare to non-treated mice. A. alata extract also showed anti-edematogenic activity, however, this effect was observed only with 10µg of the extract, which was able to reduce the mice paw volume induced by Cg in 45% when compared with control mice. In addition to these observations, A. alata (10µg) also reduced the Evans blue extravasation in 50%, suggesting a decrease in vascular permeability. We have also shown that pre-treated mice for 14 days with the 1, 5 and 10 µg of plant extract did not induce gastric damage in all concentrations evaluated, that were similar that observed in control group treated with saline. On the other hand, pretreatment with A. alata extract significantly attenuated the indomethacin-induced gastric damage in a dose-dependent manner, with maximum effect with 5µg (80%) when compared with non-treated group. **Conclusion**: These data demonstrate that, in different models of inflammation, treatment with A. alata extract improved inflammatory parameters such as cell migration into the inflamed site, as well as, edema and vascular permeability. The results also showed that A. alata has significant activity against gastric damage. **Financial Support**: Capes/CNPq. Research approval by the Animal Research Ethical Committee: CEUA/UCDB: 015/2013

09.051 A new perspective for F(ab')2 antibodies fragments on Venom:Antivenom Analysis using SE-HPLC. Collaço RCO[1], Randazzo-Moura P[2], Cogo JC[3], Sanny CG[4], Rodrigues-Simioni L[1] [1]Unicamp – Farmacologia, [2]PUCSP – Farmacologia, [3]UNIVAP – Estudos da Natureza, [4]Oklahoma State University – Biochemistry and Microbiology

Introduction: Size-exclusion chromatography on venom-antivenom study includes separation, detection and quantification of antigens, antibodies and antigen-antibody complexes allowing the calculation of parameters as reactivity and affinity. **Aims**: It has been used to evaluate the binding of North American pitviper venoms to a commercial antivenom containing F_{ab} fragments and in this work, it has been extended in this study to $F(ab')_2$ antibody fragments. **Methods**: The *B. fonsecai* venom was donated for Dr. Jose Carlos Cogo. The Commercial bothropic Antivenom (CAv) was obtained by Butantan Institute (Sao Paulo, SP, Brazil) and it is produced by horses hyperimmunization through a *pool* of *Bothrops jararaca*, *B. jararacussu*, *B. alternatus, B. neuwiedi* and *B. moojeni* venoms. The Specific *B. fonsecai* Antivenom was obtained by i) rabbits hiperimmunization using *B. fonsecai* crude venom; ii) antibodies identification by double immunodiffusion; iii) ammonium sulfate fractionation of hyperimmune plasma and; iv) antibodies purification by affinity chromatography. The venom-antivenom bindings study was performed using a SE-HPLC system. The elution profiles were divided on three regions: 1º) the high molecular mass substances (venom: antivenom complexes) region; 2º) the non-reactive venom compounds region, and 3º) the low molecular mass substances. Changes in the elution profiles are dependent upon the relative concentrations of venom and antivenin in the reaction mixtures. The venom-antivenom bindings were evaluated using the dose-response functions EC_{50} (affinity) and Max_h (binding capacity). **Results**: Both antivenoms demonstrates the general principle that venom-antivenom interactions: an increase in high molecular weight by venom-antivenom immune complexes formation with a concurrent decrease in venom and antivenom peaks in the SEC elution profiles. Some venom-antivenom immune complexes may be lost during sample preparation; this finding is consistent with $F(ab')_2$ fragments being able to form very large immune complexes that might be retained during filtration of the samples prior to SEC and confirmed through the elution profile evaluation: the difference between the control and reaction runs was on the higher molecular mass region. The CAv showed a higher binding capacity than SAv, but SAv had an affinity 20 times higher than CAv. **Conclusion**: SAv has lower binding capacity but higher affinity to *B. fonsecai* venom. The $F(ab')_2$ antivenom probably forms very large venom-antivenom complexes and some are lost during the sample preparation. SEC methods developed to study F_{ab} type antivenom-venom interactions were able to analyze the bindings parameters, but it need to be revised to facilitate analysis of $F(ab')_2$ antivenom-venom interactions. **Financial support**: FAPESP, CNPq, UNICAMP, OSU.

09.052 Inhibition of snake venom phospholipasic activity by using distinct neuromuscular junction protocols. Schezaro-Ramos R[1], Randazzo-Moura P[2], Cogo JC[3], Rodrigues-Simioni L[1] [1]FCM-Unicamp – Farmacologia, [2]PUCSP – Ciências Médicas, [3]UNIVAP – Estudos da Natureza

Introduction: Snake venoms phospholipase A_2 (PLA_2) acts cleaves the sn-2 ester glycerophospholips linkage, causing neuromuscular blockade. Some usual procedures are used to inhibit the PLA_2 activity: low temperature bath, substitution of Ca^{2+} by Sr^{2+} of nutritive solution and chemical modification with p-bromophenacyl bromide (p-BPB). Bothrops fonsecai (B. fonsecai), an endemic pitviper restrict to high altitude forests on southwestern Brazil, it has a venom primary compounded by PLA_2 (~30% of venom proteins) and metalloproteases P-I and P-III (~42%). **Aims**: To analyze the different phospholipasic inhibition protocols under neuromuscular junction parameters. **Methods**: In this study, we used B. fonsecai venom as a research tool. Extensor digitorum longus of mice preparations (EDL) were treated with B. fonsecai (100µg/ml) at different bath temperatures (24° or 37°C) or nutritive solution composition (Tyrode solution with Ca^{2+} 1.8mM or Sr^{2+} 4mM), or had its phospholipasic activity inhibited by p-BPB (0.6µM, 24h, 23°C). All muscles were examined histologically. The phospholipasic activity of all the protocols was measured by rate of phosphatidylcholine hydrolysis. **Results**: B. fonsecai caused total neuromuscular blockade at 86 ± 4min at 37°C, in the presence of Ca^{2+} 1.8 mM. The substitution of Ca^{2+} by Sr^{2+} inhibited the total blockade, producing 82.9 ± 7.1% of blockade 120 min after of venom addition. Reducing the bath temperature to 24°C, the blockade after 120 min of venom addition was only 61.2 ± 7.6%. In terms of phospholipasic inhibition, lower temperature, Sr^{2+} substitution and p-BPB were partially effective (47.1, 11.4 and 33.3 mols HCl/min, respectively), compared with 171.6 mols HCl/min of the total venom. **Conclusion**: All the PLA_2 inhibition protocols reduced the activity of the B. fonsecai venom, showing difference in efficiency, what is in accordance with their specificities different for venom protein inhibition. Financial Support: CNPq, Fapesp, Unicamp. This work was approved by the Animal Ethics Committee (CEUA/Unicamp protocol No. 3311-1).

09.053 Gastroprotective activity of *Cissampelos sympodialis* Eichl. (Menispermaceae) involves the maintenance of reduced glutathione levels. Sales IRP, Pessoa MMB, Nascimento RF, Formiga RO, Machado FDF, Barbosa-Filho JM, Batista LM UFPB – Ciências Farmacêuticas

Introduction: *Cissampelos sympodialis* Eichl. (Menispermaceae), popularly known as “milona”, “orelha-de-onça” or “abuteira”, is an endemic medicinal plant in Brazil that is found in the Northeast and Southeast. The choice of the present study was based on chemotaxonomic criteria because this species is rich in alkaloids (such as warifteine, metilwarifteine and milonine) and it has already showed anti-inflammatory activity in previous studies. **Aims**: To evaluate the gastroprotective activity of ethanolic extract (EtOHE-*Cs*) and total alkaloid fraction (FAT-*Cs*) obtained from aerial parts of *Cissampelos sympodialis* and the possible mechanism involved in this effect. **Methods**: It was used Albino male Wistar rats (*Rattus norvegicus*), weighing 180-250 g, fasted for 24 hours and treated (p.o.) with vehicle Tween solution 80 (12%) (negative control), carbenoxolone 100 mg/kg (positive control) and EtOHE-*Cs* or FAT-*Cs* (62.5 125, 250 and 500 mg/kg). Then, they were subjected to ethanol-induced ulcer protocol (MORIMOTO, Y. Jpn. J. Pharmacol., 57, 495, 1991). The stomachs were photographed to determine the ulcerative area (UA) by AVSoft Bioview Spectra 4.0® software, and after they were frozen at -80°C and subsequently from the gastric tissue homogenate to determine the total protein levels (reaction with bicinchoninic acid) and non-protein sulfhydryl compounds (reaction with 2,2’-Dinitro-5,5’-dithiobenzoic acid). The data were analyzed using ANOVA, followed by Dunnett’s test and Tukey’s test. **Results and Conclusions**: In ethanol-induced gastric ulcer model, the EtOHE-*Cs* (62.5 125, 250 and 500 mg/kg) showed protective effect in gastric mucosa with ulcerative area (UA) of 24.22 ± 6.85 mm² (percent inhibition: 69%) ($p < 0.001$), 21.42 ± 4.23 mm² (percent inhibition: 75%) ($p < 0.001$), 5.22 ± 0.92 mm² (percent inhibition: 94%) ($p < 0.001$) and 1.88 ± 0.36 mm² (percent inhibition: 98%) ($p < 0.001$), respectively, when compared to the negative control group (UA: 90,15 ± 6.25 mm²). The TAF-*Cs* (62.5 125, 250 and 500 mg/kg) also showed gastroprotective effect with ulcerative area (UA) of 9.28 ± 0.91 mm² (percent inhibition: 88%) ($p < 0.001$), 7.53 ± 1.42 mm² (percent inhibition: 90%) ($p < 0.001$), 4.41 ± 0.94 mm² (percent inhibition: 94%) ($p < 0.001$) and 3.91 ± 0.63 mm² (percent inhibition: 95%) ($p < 0.001$), respectively, when compared to the negative control group (UA: 79.00 ± 9.04 mm²). Treatment with EtOHE-*Cs* (500 mg/kg) increased levels of reduced glutathione (GSH) in 6.71 ± 0.64 nmol of GSH/mg of protein ($p < 0.01$) than compared to the negative control group (3.97 ± 0.80 nmol of GSH/mg of protein). The TAF-*Cs* (250 mg/kg) also increased levels of GSH in 5.00 ± 0.74 nmol de GSH/mg of protein ($p < 0.01$) than compared to the negative control group (3.58 ± 0.36 nmol de GSH/mg of protein). Thus, *C. sympodialis* presents gastroprotective activity which may be related to the alkaloids of this species and this activity involves possibly antioxidant mechanisms as the maintenance of GSH levels. **Acknowledgments**: CNPq/CAPES/PgPNSB /CCS/UFPB. **Research approval by the Animal Research Ethical Committee**: CEUA-UFPB 008/2015.

09.054 Evaluation of the antibacterial activity of *Struthanthus marginatus* **(Desr.) Blume.** Silva RV[1], Arruda MO[2], Carmo MS[2], Freire SMF[1], Monteiro Neto V[2] [1]UFMA – Farmacologia, [2]Ceuma – Biologia Parasitária

Introduction: The plants of the genus *Struthanthus* are known as "ervas-de-passarinho" and parasitize tall trees. The species *Struthanthus marginatus* (Desr.) Blume are popularly used in the treatment of gastric and respiratory diseases. This work analyzed the antibacterial activity of extracts and fractions obtained from the leaves of *S. marginatus* to evaluate the pharmacological potential of this species. **Methods**: The plant material was collected in São José de Ribamar - MA. The powder, obtained from the dried and crushed leaves, was submitted to extraction with water 72ºC for 30 min or maceration in 70% ethanol (1: 9), to obtain the aqueous extract (AE) and the hydroalcoholic extract (HAE), respectively. The extracts were concentrated under vacuum (55ºC) and partitioned, obtaining the aqueous (AF), butanol (BF), hexane (HF), chloroform (CF) and residual (RF) fractions. The extracts and fractions were tested in the agar diffusion and broth microdilution methods against bacterial species: *Acinetobacter baumannii*, *Bordetella pertussis*, *Enterococcus faecalis*, *Escherichia coli*, *Helicobacter pylori, Klebsiella pneumoniae*, *Listeria monocytogenes*, *Pseudomonas aeruginosa*, *Salmonella enterica* serotype Typhimurium, *Staphylococcus aureus* and *Streptococcus pyogenes*. Antibiofilm effects of BF were assessed against *P. aeruginosa* and *S. aureus* biofilms. **Results**: The extracts and fractions of *S. marginatus* formed halos of inhibition against Gram-positive and Gram-negative bacteria, but did not inhibit the growth of *K. pneumoniae* by the agar diffusion method. In the microdilution method, the extracts and fractions inhibited the growth of bacteria tested, except of *E. coli*. The strains of *E. faecalis*, *S. aureus*, *L. monocytogenes*, *P. aeruginosa* and *H. pylori* were the most sensitive to antibacterial activity of *S. marginatus*. All extracts and fractions showed bactericidal activity against *H. pylori* strain and only FC showed no bactericidal activity against *S. aureus* strain. The FB showed inhibitory and bactericidal activity against strains of *S. aureus* and *P. aeruginosa*, and was able to reduce the biofilm formed by these species. **Discussion**: The antimicrobial activity of *S. marginatus* may be related to compounds like flavonoids, tannins, steroids and terpenoids identified in its extracts and fractions, and reported to have antibacterial and antibiofilm activity. These results support the ethnopharmacological use of *S. marginatus* against disturbances caused by pathogenic bacteria and show its potential as source for the development of phytochemicals with antimicrobial activity. **Financial support**: FAPEMA and CAPES

09.055 Polysaccharide fraction isolated from *Passiflora edulis* inhibits the inflammatory response and the oxidative stress in mice. Sousa FBM[1], Silva RO[2], Damasceno SRB[2], Brito TV[1], Fontenele AM[1], Braúna IS[1], Junior JSC[1], Maciel JS[3], de Paula RCM[3], Freitas ALP[3], Medeiros JVR[1], Silva DC[4], Barbosa ALR[1] [1]UFPI – Biotecnologia, [2]UFC – Farmacologia, [3]UFC – Bioquímica, [4]UNIVASF

Introduction: *Passiflora edulis*, commonly known as 'Maracujá', is a member of the Passifloraceae family, originated in the tropical regions of the Americas. Studies have reported various pharmacological activity of extract, fruit pulp and polysaccharide fraction from *P. edulis*, including antioxidant effects and prevention of colitis. **Aims**: The aim of the study was to investigate the anti-inflammatory, antioxidant and antinociceptive actions of PFPe, a polysaccharide fraction isolated from the dried fruit of the *Passiflora edulis*. **Methods**: Mice (25-30g, n=8) were pretreated with PFPe (0.3, 1 or 3 mg/kg, *i.p.*) 1 h before induction of paw edema by carrageenan, histamine, serotonin, compound 48/80 or prostaglandin E2 (PGE2). Vascular permeability was measured after compound 48/80 injection into the paw. The action of the PFPe on the TNF-α, IL-1β, myeloperoxidase (MPO), glutathione (GSH) and malondialdehyde (MDA) levels was also evaluated. To assay nociception, we examined acetic acid-induced writhing, formalin-induced paw licking and response latency in the hot plate test. **Results**: Pretreatment with PFPe significantly inhibited ($P < 0.05$) carrageenan-induced paw edema, with maximal effect in the dose 3 mg/kg (60.6% of reduction at 3 h). PFPe (3 mg/kg) also significantly decreased ($P < 0.05$) the inflammatory response caused by serotonin (58% of reduction), histamine (85.3% of reduction), compound 48/80 (59.4% of reduction) or PGE2 (62.2% of reduction), measured at the edema peak, and compound 48/80-induced vascular permeability (0.215 ± 0.009 *vs.* 0.179 ± 0.005 µg of Evans blue/mg of paw). In addition, PFPe significantly reduced ($P < 0.05$) the MPO activity (9.18 ± 0.91 *vs.* 5.38 ± 0.78 U/ml), MDA (41.83 ± 1.78 *vs.* 29.32 ± 3.26 nmol/ml), IL-1β level (1046.00 ± 34.53 vs. 297.30 ± 44.47 pg/ml) and increase GSH (93.67 ± 44.68 *vs.* 435.47 ± 4.86 µg/ml) concentrations. In the nociception tests, PFPe reduced acetic acid-induced writhing (29.50 ± 1.32 *vs.* 22.00 ± 2.41 writhing) and formalin-induced paw licking in the first (neurogenic pain: 58.50 ± 8.87 *vs.* 37.00 ± 4.60 s) and second phase (inflammatory pain: 49.50 ± 11.87 *vs.* 5.40 ± 2.76 s) of the test and did not increase the response latency time. **Conclusions**: Our results suggest that PFPe has anti-inflammatory and and antinociceptive activity in mice via multilevel regulation of inflammatory mediators and neutrophil migration that is partly caused by reduction in IL-1β and oxidative stress. **Financial Support**: CNPq and FAPEPI. This study was approved by the Ethics Committee in Animal Research of the UFPI (Protocol Nº 020/2012).

09.056 Cytotoxic and apoptogenic properties of *C. oblongifolia* **Mart. ex Hayne and** *C. duckei* **Dwyer oleoresin and leaf extract on human gastric carcinoma cells.**

Lemos M[1], Silva JJM[1], Rogez HLG[2], Veneziani RCS[3], Ambrósio SR[3], Banderó Filho VC[4], Sasse A[4], Sheridan H[4], Bastos JK[1] – [1]FCFRP-USP – Ciências Farmacêuticas, [2]CVACBA-UFPA – Engenharia de Alimentos, [3]Unifran – Ciências Exatas e Tecnológicas, [4]TBSI-Trinity College Dublin – Pharmacy and Pharmaceutical Sciences

Introduction: The species of *Copaifera* genus, popularly known as "Copaiba", are of trees native to the northern and north eastern Brazil. The leaves, stem bark and the oleoresin of this plant are used in folk medicine to treat several diseases. *Copaiba* oleoresin is used for the treatment of urinary, lung and gastric disorders, as well for its properties as healing and emollient. Previous studies in our laboratory have shown the gastroprotective activity of oleoresins and leaf extracts of *C. oblongifolia* and *C. duckei*. **Aims**: In the present study, the effects of *C. oblongifolia* and *C. duckei* oleoresin and leaf extract on the growth of an AGS human gastric carcinoma cell line were investigated. **Methods**: The AGS cells were cultured in Dulbecco's minimal essential medium/Ham's F12 (DMEM/F12) supplemented with 10% fetal bovine serum (FBS) medium and incubated with different concentrations of oleoresins and leaf extracts. Camptothecin and paclitaxel dissolved in DMSO 2% were used as a positive control, DMSO 2% was used as a solvent control and saline was used as a negative control. Cell viability was evaluated by acid phosphatase assay after 24, 72 and 120 h incubation. Apoptotic cells were determined using propidium iodide by flow cytometry, after 24, 72 and 120 h incubation. All experiments were performed in triplicate, with three replicates per experiment. **Results**: *C. oblongifolia* and *C. duckei* oleoresins and leaf extracts inhibited the growth of AGS cells in a time and dose-dependent manner. The oleoresins of *Copaifera* spp. proved to be more toxic than the leaf extracts. The IC_{50} values determined after 120 h were 20.04±0.50, 31.49±5.62, 43.93±2.74 and 65.15±1.54 µg/mL, for oleoresins of *C. oblongifolia* and *C. duckei*, and leaf extracts of *C. oblongifolia* and *C. duckei*, respectively. Camptothecin and paclitaxel exhibit IC_{50} values 27.87±0.74 and 11.64±0.75 nM after 120 hours of treatment. Oleoresins induced cell death as can be seen in in the flow cytometry histogram of treated cells compared to control cells, indicating that apoptotic cell death is involved in *Copaifera* spp. toxicity. **Conclusion**: *C. oblongifolia* and *C. duckei* oleoresins and leaf extracts exert cytotoxic and proapoptotic effects in AGS cell lines containing active ingredients which can be considered as potential chemotherapeutic agents for gastric cancer treatment. **Financial support**: FAPESP Doctoral Project n. 2012/09727-8 and Thematic Project n. 2011/13630-7. PDSE/CAPES Project BEX 7277/14-8. TCD award n. 203327.13221

09.057 The role of kinin system in *Lonomia obliqua* – induced acute kidney injury: contribution of bradykinin B1 receptor, coagulation system activation and vascular alterations. Berger M[1], Beys-da-Silva WO[2], Santi L[2], Moraes JA[3], Marcon R[4], Vieira MAR[5], Yates JR[6], Calixto JB[4], Barja-Fidalgo C[3], Guimarães JA[1] [1]HCPA-UFRGS, [2]Univates – Biotecnologia, [3]UERJ – Biologia Celular, [4]UFSC – Farmacologia, [5]UFMG – Fisiologia e Biofísica, [6]The Scripps Research Institute – Chemical Physiology

Introduction: The *Lonomia obliqua* caterpillar envenomation is considered a common and serious occupational disease, especially in rural areas of southern Brazil regions. *L. obliqua* venom is highly nephrotoxic and acute kidney injury (AKI) is the main cause of death among envenomed victims. **Aims**: This study evaluates the pathophysiological mechanisms involved in renal dysfunction. **Methods**: Here we use an *in vivo* model to characterize the *L. obliqua*-induced AKI. A multidisciplinary approach was employed including methods of renal biochemistry, pharmacology, morphology, cell biology and a global proteomic analysis to identify the molecular pathways of kidney injury. **Results**: According to our results, the pathophysiological mechanism of venom-induced AKI, it seems to be complex involving three main issues: the heme-derived tubular cytotoxicity; vascular alterations, including systemic hypotension, increase in vascular permeability and glomerular fibrin deposition; and the activation of renal kinin system. Acting together these mechanisms are directly related to the functional alterations observed in envenomed animals such as renal hypoperfusion, inflammation, tubular necrosis and the sudden loss of basic renal functions, including filtration and excretion capacities, urinary concentration and maintenance of body fluid homeostasis. The activation of renal kallikrein and the bradykinin receptor B1 (B1R) play a crucial role in *L. obliqua*-induced AKI, because both the pharmacological blockade of B1R or systemic kallikrein inhibition are able to prevent the renal functional and histopathological alterations and also ameliorates the venom-induced blood incoagulability observed in envenomed animals. The main mechanisms underlying these beneficial effects are associated with a decrease in renal inflammatory response (reduction of pro-inflammatory cell migration and pro-inflammatory cytokine levels) and tubular degeneration. The B1R blockade also protect against both free heme and venom-induced smooth muscle vascular cell alterations mainly by the decrease in intracellular reactive oxygen species production and downregulation of NF-κB signaling pathway. **Conclusions:** Our findings show a consistent evidence linking kinin system with the *L. obliqua*-induced AKI and indicate that the inhibition of kinin components, mainly kallikrein inhibition or B1R antagonism, could be a therapeutic alternative to control the progression of renal injury. This work was approved by the Animal Research Ethical Committee of UFRGS (number 26570-2014). **Financial Support**: CAPES-DRUG DISCOVERY - AUXPE: 0163/2015

09.058 The anti-ulcer and anti-proliferative activities of the hexane extract and candidate isolates brasiliensic and isobrasiliensic acids of *Calophyllum brasiliense*: A mechanistic evaluation of their properties. Castilho GRC[1], Lemos LMS[1], Oliveira RG[1], Miyajima F[2], Martins DTO[1] [1]UFMT – Ciências Básicas em Saúde, [2]University of Liverpool – Pharmacology

Introduction: *Calophyllum brasiliense* Cambèss (Calophyllaceae) is an evergreen tree that is native to subtropical and tropical regions of Americas, from Brazil to Mexico, and traditionally used to treat gastric disturbances in folk medicine. The chromanones brasiliensic (BRA) and isobrasiliensic (ISO) acids isolated from its stem bark hexane extract (HECb) are reported to be potent anti-proliferative and protective agents of the gastric mucosa. **Aim**: To mechanistically investigate the anti-ulcer/anti-proliferative properties of HECb, BRA and ISO by *in vitro* assays. **Methods**: HECb was obtained by maceration of stem bark in hexane for 7 days. BRA and ISO were isolated from HECb by a series of chromatographic processes. For the first experiment, AGS cells ($2x10^6$/well) were plated out and left overnight prior to the treatment with HECb (12.5–50µg/mL) for 24h. The cells were then infected with *Helicobacter pylori* (*H.pylori)* for 1h, trypsinized and washed with PBS. Following cell lysis, the cytosol fraction was subject to electrophoresis and transferred to a nitrocellulose membrane. Membranes were subject to a sandwich immunoreaction using antibodies targeting intracellular factors p-ERK and p-p38 and revealed by chemiluminescence. For the second experiment, AGS cells were treated with HECb, BRA, or ISO (12.5-50µg/mL) and infected with *H.pylori* for 24h. Prostaglandin E_2 (PGE_2) levels were measured from cell supernatants using a commercial ELISA kit. The cells were trypsinized and stained with DCFH-DA to quantitate intracellular ROS by flow cytometry. **Results**: As expected, infected AGS cells displayed higher expression of p-ERK than uninfected controls (p<0.001). Interestingly, in infected cells all HECb concentrations tested prevented ERK phosphorylation, inhibiting p-ERK expression by 88-92% (i.e. 8-12% relative to the baseline levels, p<0.001), which is comparable to the results observed with the control inhibitor PD98025 (14%). When compared to infected untreated cells, the relative expression of p-p38 in infected cells treated with HECb at 12.5 and 25 µg/mL and inhibitor SB203580 (10 µM) was 21%, 59% and 15%, respectively (p<0.001). Similarly, infected AGS cells displayed higher levels of PGE_2 compared to the uninfected control group (426.1 ± 103.4 vs 64.1 ± 2.6, p<0.001). HECb, BRA and ISO significantly inhibited *H.pylori*-induced PGE_2 release in all tested concentrations since treated infected cells produced PGE_2 levels resembling those displayed by indomethacin-treated and uninfected groups. The relative levels of ROS increased from 1.3% of viable cells within the uninfected group to 7.1% of viable cells within the *H. pylori* infected group (p<0.001), however, neither HECb, BRA, nor ISO seemingly abrogated ROS increase in our tests. **Conclusions**: The anti-proliferative activity of HECb is associated with its inhibitory action on ERK and p38 phosphorylation pathway. The gastric protection conferred by HECb, BRA and ISO is at least in part attributable to the inhibition of *H.pylori*-induced PGE_2 release and this seems to be independent to the ROS production. **Financial support**: CNPq, FAPEMAT, INAU.

09.059 Evaluation of acute toxicity and hypoglycemic effect of *Amasonia campestris* **in animal model**. Nascimento AA, Guimarães Junior BS, Alvez CM, Ribeiro RB, Santos AM Unifap – Experimentação Animal

Introduction: The *Amasonia campestris* is a family Lamiaceae plant popularly used as a complementary therapy in the treatment of *Diabetes mellitus*. The aim of this study was to evaluate the acute toxicity and the hypoglycemic activity of the methanol extract of the roots of *Amasonia campestris* (EMAC). **Methods**: There was a preliminary bioactivity of the extract against *Artemia salina* to obtain the median lethal concentration test (CL_{50}) and then evaluated the acute toxicity as recommended by RE 90/2004 of the National Health Surveillance Agency - ANVISA. Male Wistar rats (n=12) were divided into two groups received, respectively, EMAC (2000 mg/kg, p.o.) and the vehicle (control). General signs of toxicity and body weight gain, feed consumption and water and extract the degree of mortality were observed for 14 days. At the end of this period, hematological and biochemical parameters, as well as, macroscopic analysis and calculation of the mass relative to the vital organs (heart, liver, lung and kidney) were also determined. For the oral glucose tolerance test (TOTG) 18 normoglycemic animals, divided into 3 groups received different treatments: vehicle (negative control), Metformin 500 mg/kg (positive control) and EMAC (400 mg/kg) (test). After glucose load, blood glucose was determined at 30, 60, 90 and 120 minutes. In the test animals with diabetes induced by alloxan (120 mg/kg). A negative control group were used (vehicle), positive control group (Glibenclamide, 10 mg/kg) and two test groups EMAC (200 and 400 mg/kg, respectively). In these groups glucose samples were taken at the times 0h, 6h, 24h, Day 4 and Day 6 after their treatments. The blood test for cholesterol and triglycerides tests was made at the end of the sixth day. All experimental procedures were approved by the Animal Studies Committee of the UNIFAP (n. 0001/2015). **Results**: The CL_{50} determined by bioassay *A. salina* was 248.57 mg/ml showing that holds the plant active constituents. In animals exposed acutely to EMAC (2000 mg/kg, p.o.) was not observed deaths, behavioral changes, changes in water consumption and feed or changes in biochemical and hematological parameters evaluated in the control group. Macroscopically and relative mass of the bodies showed normal, the exception of a small change in kidney relative weight. Oral tolerance test group treated with extract showed significant reduction in blood glucose in time 90 minutes ($p=0.00241$). During the 6 day treatment with induced diabetic animals 6 hours after the first administration was observed a significant reduction in blood glucose at a dose 400 mg/kg ($p<0.05$) that this statement actually repeated on the fourth day of treatment ($p<0.001$). Animals that received extract (200 and 400 mg/kg, respectively) showed a similar weight gain those treated with glibenclamide. EMAC (400mg / kg) induced lowering cholesterol without significantly altering triglyceride levels in animals evaluated. Conclusions: We can consider that the methanol extract of *Amasonia campestris* has low toxicity and has hypoglycemic properties, requiring further study to better assess the response-dose relationship and elucidate the mechanism of action. **Financial support**: CNPq/PIBIC/CAPES

09.060 Antispasmodic effect of dichloromethane phase from ethanol extract of *Serjania caracasana* (Jacq.) Willd. (Sapindaceae) on ileum rat. Gonçalves ACB[1], Marcolin LSA[2], Silva VA[3], Rigoni VLS[4,3], Silva FL[5], Barbosa-Filho JM[6], Nouailhetas VLA[4], Silva JLV[7] [1]Uninove – Farmácia, [2]Uninove – Ciências Médicas, [3]Uninove – Mestrado Medicina, [4]Unifesp – Biofísica, [5]USP – Química, [6]UFPB – Ciências Farmacêuticas, [7]Uninove – Ciências da Saúde

Introduction: Serjania caracasana also known as “timbó”, is found in states of Brazil. It presents few studies, but previous results showed that the ethanolic extract and n-hexane (Sc-Hex) and n-buthanol (Sc-BuOH) phases obtained from aerial parts of S. caracasana (Sc-EtOH) presented antispasmodic effects on ileum rats. A chemical study of S. caracasana showed putative molecules. **Aim**: To observe effect of the dichloromethane phase (Sc-CH_2Cl_2) obtained from Sc-EtOH partition on pre-contracted rat ileum. **Methods**: The ileum isolated from 24 h fasting rats (250 – 300 g), then prepared on glass baths containing modified Krebs solution, at 37 °C, 1 g/force resting tension and bubbled O_2. The isometric contractions (control) were stimulated by addition of carbachol (1 µM), followed by Sc-CH_2Cl_2 addition (9, 27, 81, 243, 500 and 730 µg/mL), and recorded by acquisition analogy system (AQCAD). The results were calculated by percentage (E_{max} %) of control response to contractile agens and value IC_{50} was calculated by non-linear regression. These procedures were approved for ethics committee in animal use of Federal University of São Paulo (CEUA 4195060514/14). The data were expressed as mean ± SEM and analyzed by GraphPad Prism software, tested for significance by T-test or ANOVA one-way ($p < 0.05$). **Results and Conclusion**: Sc-CH_2Cl_2 (81, 243 and 500 µg/mL) inhibited in a concentration-dependent and significant manner the contractions (n = 4) induced by carbachol (E_{max}= 62.7 ± 12.6; 47.6 ± 5.6 and 20.2 ± 3.7 %, respectively) and presented IC_{50} of 161.3 ± 40.7 µg/mL. Those results suggest that Sc-CH_2Cl_2 phase also present antispasmodic effects, as soon as Sc-BuOH and Sc-Hex, probably due to having active metabolites.

09.061 Evaluation of the gabaergic system in the anesthetic effect of S-(+)-Linalool in silver catfish (*Rhamdia quelen*) evaluation of the gabaergic system in the anesthetic effect of S-(+)-linalool in silver catfish (*Rhamdia quelen*). Bianchini AE[1], Garlet QI[1], Silva LL, Heinzmann B[2], Baldisserotto B[1] [1]UFSM – Farmacologia e Fisiologia, [2]UFSM – Farmácia Industrial

Introduction: The linalool is a depressant effect terpenoid on the central nervous system whose action has been described in silver catfish (*Rhamdia quelen*). The isomer S-(+)-linalool is major constituent of essential oil of *Lippia alba* (HELDWEIN C., Vet. Anesth. and Analg. 41: 621, 2014). The study aimed to evaluate the involvement of the GABAergic system in anesthetic action mechanism of S-(+)-linalool in silver catfish. **Methods**: The S-(+)-linalool was obtained from the essential oil (EO) of *L. alba* grown in CESNORS campus, Frederico Westphalen - RS, Brazil and identified by Dr. Gilberto D. Zanetti (vouchers SMDB No. 10050 - Department of Biology, UFSM). The EO of *L. alba* was obtained from the fresh leaves by the hydrodistillation process with a Clevenger type, according to European Pharmacopoeia (2007). Column chromatography was used for the isolation of S-(+)-linalool. Juvenile (12. 71 ± 0.38 g; 11.04 ± 0.12 cm) were housed in continuously aerated 250 L tanks for 2 weeks before experiments with parameters of water (temperature: 19.76 ± 0.11 °C; pH 7.15 ± 0.12; dissolved oxygen levels: 6,86 ± 0,33 mg L^{-1} and total ammonia levels 0.12 ± 0.02 mg L^{-1}) and adequate food. These procedures were approved by the Ethical and Animal Welfare Committee of the UFSM (Process no. 074/2014). An initial group of 18 animals were divided into 1L tanks, one at a time, and anesthetized with 5 mg L^{-1} of propofol according to method previously described (SCHOETTGER R.A., Invest. Fish Control, 13: 1, 1967). After induction, the animals were separated into two groups (n = 9 per group). One group was transferred to an anesthetic-free aquarium with water and the second group was placed in an aquarium containing 100 mg L^{-1} the picrotoxin previously solubilized in Tween 80 at 0.033%. The same procedure was performed with 153.5 mg L^{-1} de S-(+)-linalool, previously solubilized in ethanol 95% (1: 10). **Results**: The animals anesthetized with propofol and recovered in picrotoxin (about 26 min) showed less recovery time compared to only exposed to water (about 30 min), however the anesthetized group linalool showed no significant difference between groups recovered in water and picrotoxin (about 17 and 16 min, respectively). **Discussion and Conclusion**: The results for the group anesthetized with propofol are important for validation methodology. However, linalool mechanism of action seems not to involve the GABAergic system. Therefore, further studies are needed to elucidate the mechanism of action of linalool in fish. Financial Support: CNPq/Capes/FAPERGS/UFSM.

09.062 Chemoprotective effect of apple juice in liver and blood of rats exposed to cadmium. Moura CFG[1], Ribeiro FAP[2], Gollucke APB[2], Oshima CTF[1], Ribeiro DA[2,1]
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Introduction: Cadmium is a non-essential heavy metal able to trigger several chronic diseases, including cancer. **Aim**: This study evaluated the potential chemoprotective and antioxidant effect of apple juice against toxicity induced by cadmium in liver and blood by histopathology, genotoxicity and mutagenicity by comet and micronucleus tests, oxidative stress by 8OHdG immunoexpression and antioxidant enzymes expression by real time-PCR. **Methods**: A total of 15 Wistar rats were distributed into three groups, with five animals each, as follows: I. Control group (negative control; IM injection of distilled water and, after 15 days, administration of 1 mL of distilled water for 15 days). II. Cadmium group (intramuscular injection, IM, 1.2 mg/kg cadmium chloride) III. Apple juice group (IM injection of 1.2 mg/kg of cadmium chloride and, after 15 days, administration of 1 mL of concentrated apple juice for 15 days by gavage). **Results**: Histopathologically, apple juice was able to ameliorate the liver architecture diminishing the damage caused by cadmium. Additionally, comet assay in hepatocytes and blood cells were reduced in animals treated with concentrated apple juice (1,08±0,29; 1,53±0,3, respectively, $p<0.05$) when compared with cadmium group (3,6±1,07; 2,85±0,35, respectively, $p<0.05$). The total number of micronucleated cells in bone marrow (72.25±19.69) and in the liver (40±7.61) also showed a considerable decrease when compared with animals exposed only to cadmium (446.5±29.94). Apple juice was also able to reduce the 8-OHdG levels and decrease the oxidative stress in liver (244.8±15.2) when compared to cadmium group (895.8±18.95). Concerning the antioxidant enzymes superoxide dismutase cupper-zinc, superoxide dismutase manganese, and calatase, only catalase showed a decrease on its expression after apple juice intake and cadmium exposure (15.73±4.88; cadmium group: 24.96±5.14, $p<0.05$). **Conclusion**: In summary, our results show a high potential hepatoprotective, anti-genotoxic, anti-mutagenic and antioxidant action of apple juice, as a result of its polyphenolic compounds. **Financial Support**: National Counsel of Technological and Scientific Development - CNPq Ethical Committee Approval CEP-UNIFESP: CEUA 484411

09.063 Antinociceptive activity of extracts and secondary metabolites of *Renealmia alpinia.* Benjumea D[1], Cortés N[2], Osorio E[2], León F[3], Cutler S[3], Gómez-Betancur I[1] – [1]Universidad de Antioquia – Ofidismo/Escorpionismo [2]Universidad de Antioquia – Investigación en Sustancias Bioactivas [3]The University of Mississippi – BioMolecular Sciences

Renealmia alpinia is native to the American continent and can be found from Mexico to Brazil, and in the Caribbean islands. It is known as "matandrea" in Colombia, and it has been commonly used in traditional medicine to treat painful diseases and ailments. Based on its traditional uses, it is of interest to evaluate the pharmacologic effects of this plant and its secondary metabolites. *Materials and methods:* Methanol and dichloromethane extracts of wild *R. alpinia* (leaves) were obtained and chemically compared by High Performance Thin Layer Chromatography (HPTLC). The antinociceptive activity of these extracts were examined using an *in vivo* assay (Siegmund test) (act number 44 of the Ethics Committee for experimental animals). Additionally, the dichloromethane extract of *R. alpinia* was fractionated and pure compounds were isolated by chromatographic **Methods**: The structure elucidation of isolated compounds were performed by NMR experiments and spectroscopic techniques and comparison with the literature data. Purified compounds were evaluated for their *in vitro* binding affinity for opioids and cannabinoids receptors. **Results**: The dichloromethane extract of the plant's aerial part afforded six compounds: pinostrobin (**1**), naringenin 7,4'-dimethyl ether (**2**), 2',6'-dihydroxy-4'-methoxychalcone (**3**), 4-methoxy-6-(2-phenylethenyl)-2H-pyran-2-one (**4**), naringenin 7-methyl ether (**5**) and 3,5-heptanediol, 1,7-diphenyl (**6**), which were isolated using chromatographic **Methods**: Their chemical structures were established by physical and spectroscopic techniques. The methanol extract of wild *R. alpinia*, at doses of 200 and 300mg/Kg, showed dose-dependent inhibition percentages of 66.2% and 74.0%, respectively when compared with the control group. Ibuprofen at a dose of 75mg/kg showed a percentage pain inhibition of 91.0, while the dichloromethane extract from *R. alpinia* at doses of 200 and 300mg/kg showed inhibition rates of 67.8% and 86.6%, respectively. The antinociceptive effects observed in mice by extracts were similar. The compounds isolated from *R. alpinia* do not show affinity to opioid or cannabinoid receptors. **Conclusion**: dichloromethane and methanol extracts of *R. alpinia* provide antinociceptive and analgesic effects in an *in vivo* model. These results contribute additional insight as to why this plant is traditionally used for pain management. Also, this is the first comprehensive report of a phytochemical study of *R. alpinia*.