09. Natural Products and Toxinology

09.001 Hypolipidemic potential of aqueous suspension of *Bixa orellana* seeds and its partition chloroform in mice with hypercholesterolemia induced by diet modified. Ferreira JM[1], Sousa DF[2], Pereira NBS[1], Meneses RRC[1], Holanda RTM[1], Araújo VM[1], Morais TMF[1], Dantas MB[1], Fonseca SGC[3], Queiroz MGR[1] [1]UFC – Análises Clínicas e Toxicológicas, [2]UFC – Fisiologia e Farmacologia, [3]UFC – Farmácia

Introduction: In Ceará, *Bixa orellana* seeds are used to reduce serum lipids levels. In previous studies carried out by our group, a decrease in the triglycerides (TG) and total cholesterol (TC) levels was observed. These effects are very interesting and studies to describe the substances and its possible action mechanisms are fundamental. **Objective**: Verify the hypocholesterolemic effect of the aqueous suspension of *Bixa orellana* seeds (ASBOS) and its chloroform partition (CLO) in mice fed with hypercholesterolemic diet (HD). **Methods:** The male Swiss mice (25-35g) were divided (n=6) in: negative control (NC) and positive control (PC), SV10 (simvastatin 10mg/kg), ASBOS200 (200mg/kg), ASBOS400 (400mg/kg), ASBOS800 (800mg/kg), CLO1 (2.3 mg/kg), CLO2 (4.6mg/kg) and CLO3 (9.2 mg/kg). For the increase of TC levels, all the animals except the NC group were fed during 6 weeks with the HD. After the first two weeks, a blood collect was carried out to verify TC increase and after the animals were treated with saline, SV10, ASBOS (200, 400 or 800) or CLO (1.2 or 3) for 28 days. The parameters were determined glucose (GL), TC, TG, AST and ALT, after 6-8h fasting. The results were expressed mean ± S.E.M and the groups were compared for ANOVA (Tukey post-test), adopting as the significance criterion $p<0.05$. The described protocol was approved by the Ethics Committee on Animal Research of Universidade Federal do Ceará with the number 048/07. **Results:** The ingestion of HD didn't change the GL and ALT values of groups studied. The TC (mg/dL) increase was 64.8% when compared with the NC (239.8±6.4 *vs.* 145.5±6.8). ASBOS200 (215.2±4.2), ASBOS400 (220.2±2.2), ASBOS800 (213.0±7.6), CLO3 (199.8±5.5) and SV10 (209.0±7.2) promoted the reduction of TC levels (mg/dL) after 28 days of treatment, when compared to PC group (239.8±6.4). The values of TG of PC decreased in relation to NC in 95.4% (199.3±11.5 *vs.* 102.0±7.2mg/dL). In the treated groups with CLO1, CLO2 or CLO3 showed the increase of AST (U/L) (NC: 88.8±7.4; PC: 97.3±3.8; CLO1: 106.8±5.4; CLO2: 110.0±3.8; CLO3: 110.3±4.3). **Discussion:** The HD was composed by 1% cholesterol + 0.1% cholic acid + 10% *Cocus nucifera* oil (Wilson *et.al.*, J. Nutr. Biochem., V.18, p.105, 2007). The cholic acid in addition to increase the effect of dietary cholesterol, elevating its absorption, can have inhibited conversion of cholesterol to bile acids, promoting lipids accumulation (MACHADO *et.al.*, Ciênc. Tecnol. Aliment. v.23, p.270, 2003). The reduction of TG values can be justified for the presence of coconut oil, a medium chain fatty acid which may have promoted a protector action. The ASBOS and the CLO can be favored the cholesterol conversion in bile acids, reducing their blood levels. More studies are necessary to elucidate the exact mechanism of ASBOS and CLO action on the TC levels. **Financial support:** FUNCAP

09.002 Evaluation of acute oral toxicity of a naturally occurring sesquiterpene. Nogueira Neto JD[1], Oliveira RFAM[1], Sousa DP[2], Freitas RM[1] [1]UFPI – Bioquímica e Farmacologia, [2]UFS – Química de Produtos Naturais e Sintéticos Bioativos

Introduction: This study evaluated the safety of nerolidol through studies of acute oral toxicity. **Methods:** 2 months old male Swiss mice were used, 25-30 g, from the Central Animal Facility of the Center for Agrarian Sciences, Federal University of Piauí. The animals received water and (Purina ®) *ad libitum* diet, kept in controlled lighting (cycle 12 h light / dark) and kept at a temperature of (26 ± 1 ° C). A total of 60 mice were used, divided into 6 groups (n = 10 mice / group). The control group was treated with 0.05% Tween 80 dissolved in 0.9% saline solution orally (po; vehicle). The other five groups were treated with doses of nerolidol 1000 - 5000 mg / kg orally. After treatment with a single acute dose, the animals were observed for 14 days. During the period of observation the number of animal deaths was recorded in each group, and the weight of each animal was recorded and evaluated every 2 days, the consumption of water and food, and the production of feces of all animals. After 14 days the animals were anesthetized with sodium pentobarbital (10-15 mg/100 g body weight, ip), blood collection was performed via orbital venous plexus puncture using Microhematocrit tubes and needles. The blood was placed in two types of pipes, an anticoagulant HB (Laborlab ®) for determination of hematology parameters, and the other without anticoagulant to obtain serum for biochemical evaluation, and subsequently performing a morphological analysis of the macroscopic major organs. The project was approved by the Ethics Committee for Animal Experimentation of the Federal University of Piauí (Protocol number 06/2011). **Results:** No significant change was recorded in relation to the body mass of the animals, and no change in water consumption, feed and production of feces. After oral administration of nerolidol, slight modifications were observed in the parameters, such as states of mind and arrangement of each animal, downwards, and piloerection. After the observation period the animals were euthanized and dissected for macroscopic morphological analysis of the major organs. During this analysis, no gross morphological changes in the stomach, liver, kidneys, lungs, brain and heart of mice treated with nerolidol was found. During treatment, these changes were normalized and LD50 was observed at 72 hours of 4472.14 mg / kg and 14 days of 3,456.29 mg / kg, with a confidence interval of 95%. **Discussion:** In the Hippocratic test of the analyzed substance, discrete, reversible and observed only at the highest dose, clinical signs of toxicity appeared. With regard to hematological and biochemical analyzes, there were no significant changes. This study demonstrated that nerolidol has a high LD50 with minimal expression of toxic effects and perceived influence on the locomotor system, which means it needs to be further investigated in future studies to elucidate its possible mechanism of action. **Financial support:** CNPq, CAPES, FAPEPI.

09.003 Study of antimicrobial action of aqueous extracts of joint *Plantago major* L. (Plantaginaceae) and *Punica granatum* L. (Punicaceae) and interference in action of amoxicillin *in vitro*. Gontijo LS, Damasceno EMA, Fernandes MFG, Teles DG, Costa MM FIPMoc

Introduction: The use of medicinal plants to treat diseases is a practice of many people, being held since antiquity. Several studies are conducted to determine the efficacy of these plants, the existence of unknown interactions and interference in the combination thereof, either beneficial or cause damage to the body. In order to discover the existence of these interactions or interference with the action of the antibiotic amoxicillin was tested in this research, *Punica granatum* (Pg) and *Plantago major* against *Staphylococcus aureus* and *Escherichia coli*. **Methods:** The raw materials (dried bark of *P. granatum* and dried leaves of *P. major*) were obtained from different sources, the Municipal Market of Montes Claros and Curvelo region, and its aqueous extract obtained by decoction. The Minimum Inhibitory Concentration (MIC) of the extracts was determined by broth microdilution. For the interference test was performed the disk diffusion test. To investigate the existence of interactions was performed disk diffusion test described by Kirby-Bauer. We used these test concentrations of the extracts that showed antimicrobial activity in the MIC test. **Results and discussion:** In the test broth microdilution, *P. granatum* had MIC of 6.25% against *S. aureus* and 25% against *E. coli.* Catão et al. (2006) noted that the MIC of the ethanolic extract of *P. granatum* was 10%, against strains of *S. aureus* source outpatient. This difference can occur due to extraction techniques have not as effective or even because the source ambulatory strains are more likely to undergo mutation and to acquire resistance compared to ATCC strains, which may also occur with other extracts. As for the extract of *P. major* there was no colorimetric satisfactory result for both bacteria in no dilution, which can be interpreted as absence of antibacterial activity or the existence of interference of a substance from the extract. The disk diffusion test confirmed the existence of interactions, and only four results, significant (Amoxi + PgPura, Amoxi + PgDiluição1 and Amoxi + PgDiluição2 against *E. coli* and Amoxi + PgPura against S. *aureus*), because only these combinations variation of the halo was less than 2 mm compared to halos formed by the respective controls, in accordance with the view of Canton; Becker (2010) in his work on the interference of extracts in the action of antibiotics used in the clinic, where they claim there is synergism when the difference between the inhibition of microbial growth tests, compared to controls, is ≥ 2 mm. It follows therefore that there is indeed interaction of extracts and extracts together with amoxicillin, which can also happen with other antibiotics and to generate rich population. **Financial Support:** No support. **References:** CANTON, M.; BECKER, S.O. *Rev. Bras. Farmacogn.*, v.20, p.348, 2010. CATÃO, R.M.R. et al. *Rev. Bras. Anal. Clin.,* v. 38, p.111, 2006.

09.004 Effect of the alkaloid indigo in dextran sodium salt-induced colitis (DSS).
Almeida ACA[1], de-Faria FM[2], Dunder RJ[2], Manzo LP[2], Socca EAR[2], Luiz-Ferreira A[2], Souza-Brito ARM[1] [1]IB-Unicamp – Biologia Estrutural e Funcional, [2]FCM-Unicamp – Farmacologia

Introduction: Inflammatory bowel diseases (IBD) are characterized by uncontrolled inflammation of the mucosa. The limitations in efficacy and safety encountered with the current medical approaches for IBD continue to drive the search for better therapeutic agents. The aim of this study was to evaluate the effect of indigo in intestinal inflammation. Indigo, a bis-indolic alkaloid is found in species of the genus *Indigofera*, known as "anileira." Our group demonstrated that indigo has antiulcerogenic, antioxidant, anti-inflammatory and analgesic activities. **Methods:** Male mice Unib: SW (30-40 g) were divided into four groups: SAL (non- colitic control), IND (non-colitic test), SAL+DSS (colitic control) and IND+DSS (colitic test). Groups SAL+DSS and IND+DSS received solution of dextran sodium salt 3% (DSS) *ad libitum* instead of water for 7 days. During this period, daily, the animals were treated by gavage: vehicle 0.9% NaCl (10 $ml.kg^{-1}$) for groups SAL and SAL+DSS, and indigo (3 $mg.kg^{-1}$) for groups IND and IND+DSS, this dose was selected in another experimental model of intestinal inflammation. On the 8th day, animals were euthanized and the weight/length ratio of the colon was evaluated. Diarrhea, bloody stools and weight change were evaluated to define the disease activity index (DAI), whose data are expressed as median (minimum-maximum). Statistical analysis: ANOVA, one way, followed by Tukey or Kruskal-Wallis test, followed by Duns. The experiments were approved by the Ethics Commission in Animal Use – CEUA (CEUA number 2399-1). **Results:** The treatment with indigo did not alter DAI [SAL: 0 (0-1), IND: 0 (0-0), SAL+DSS: 3.3 (2.7-4), DSS+IND: 2.7 (2.7- 3.7)], but prevented the increase in weight/ length ratio of the colon [SAL: 32 ± 4.3^{a}; IND: 30 ± 2.5^{a}; DSS+SAL: 40 ± 6.4^{b}; IND+ DSS: 31 ± 4.1^{a} $g.cm^{-1}$]. IND+DSS-treated animals did not lose as much weight as SAL+DSS-treated group. **Discussion:** Although DAI was not reduced, treatment with indigo showed beneficial effects in the model of DSS, what motivates further studies on its action in IBD. **Financial support**: CAPES, FAPESP.

09.005 Tocolityc action of the flavonoid 3,6 dimethyl ether (FGAL) isolated from aerial parts of *Piptadenia stipulacea* (Benth.) Ducke involves blocked of Ca_v.
Carreiro JN, Travassos RA, Souza ILL, Vasconcelos LHC, Oliveira GA, Pereira JC, Lira DP, Santos BVO, Silva BA PgPNSB-CCS-UFPB

Introdution: The flavonoid 3,6-dimethyl ether galetin (FGAL) was isolated from aerial parts of *Piptadenia stipulacea* (Benth.) Ducke (Pereira, 2009). In previous studies, was demonstrated that FGAL inhibited in a significant and concentration-dependent manner phasic contractions induced by carbachol and oxytocin on rat uterus (Macedo, 2011), with a higher potency to oxytocin (Macêdo, J Smooth Muscle res., 47, 123, 2011). **Aim:** The aim of this study was to investigate the mechanism of tocolytic action of FGAL on rat uterus. **Methodology:** The rats were euthanized by cervical dislocation and exsanguinations. Segments of the organ were suspended in organ bath and the isometric contractions were monitored. All the experimental protocols were approved by Ethical Committee in Animal Research of LTF/UFPB (Protocol 0303/11). **Results:** FGAL inhibited (pD'_2 = 5.68 ± 0.06) oxytocin cumulative curves and these were shifted to the right, in a non parallel manner (slope = 3.59 ± 0.04), with reduction of E_{max}, suggesting a non-competitive antagonism. In other hand, FGAL relaxed uterus pre-contracted in a concentration-dependent and significant manner by oxytocin (pD_2 = 6.9 ± 0.1) and KCl (pD2 = 5.6 ± 0.06), been more potent to oxytocin. **Discussion:** The opening voltage-dependent calcium channels (Ca_V) is one of the common step to the signaling pathways of oxytocin and KCl to maintain the tonic phase of contraction, we hypothesized that FGAL could be acting by blocking the influx of Ca^{2+} through the Ca_V. To verify this hypothesis we performed cumulative contractions with $CaCl_2$ in depolarizing medium nominally without Ca^{2+} in the presence and absence of several concentrations of FGAL. The flavonoid antagonized the contractions induced by $CaCl_2$, shifted control curve to right in a non parallel manner with reduction of E_{max}, suggesting that FGAL was blocking indirectly Ca_V to promote tocolytic effect on rat uterus. Thus, we concluded that the relaxing action mechanism of FGAL functional level, no involves ocytocin receptors, but blockade of the Ca_V, leading to a consequent reduction of calcium cytosolic concentration and uterine smooth muscle relaxation. **Financial Support**: CnPq

09.006 Developmental toxicity of isolated and associated artesunate and mefloquine in rat. Boareto AC[1], Araújo SL[1], Lourenço ELB[1], Lourenço AC[1], Gomes C[1], Minatovicz B[1], Lombardi N[1], Paumgartten FR[2], Dalsenter PR[1] [1]UFPR – Farmacologia, [2]Fiocruz – Toxicologia Ambiental

Introduction*:* Malaria, caused mostly by *Plasmodium falciparum* and *P. vivax*, remains one of the most important infectious diseases in the world. *P. falciparum*, which is responsible for causing severe forms of the disease, is the cause of nearly all of the 1–3 million malaria-related deaths each year. Pregnant women and the conceptus are at particular risk for adverse malarial outcomes, including death, making immediate drug therapy necessary during pregnancy, even when the potential for teratogenicity is known. Artemisinins combination therapy (ACTs) is the first choice therapy to *falciparum* malaria. Data on safety of ACTs in pregnancy are limited and controversial and the use is not recommended on the first trimester. ***Methods****:* To test the developmental toxicity of isolated and associated artesunate/mefloquine, pregnant rats were treated orally with artesunate (15 and 40 mg/kg/day), mefloquine (30 and 80 mg/kg/day) and artesunate-mefloquine (15/40 and 40/80 mg/kg/day) on days 9-11 *post coitum* (pc). The dams were euthanized on day 20 pc and gestational and fetal parameters were evaluated. ***Results and Discussion:*** Embryolethality and anomalies in embryofetal developmental was significant when artesunate was given alone or in combination with mefloquine. However, the results indicate a reduction of the developmental toxicity of artesunate associated with mefloquine at both evaluated doses. Isolated mefloquine did not induce developmental toxicity. Thus, these results provide a new insight into the reproductive toxicology of the ACTs. All animal studies were carried out in accordance with the Ethics Committee on Animal Experimentation of Federal University of Parana (Protocol number: 308/08). We are grateful to CAPES, CNPq and FUNPAR for financial support and to FIOCRUZ/PDTIS for supplying the drugs.

09.007 Omega-3 and -6 fatty acids affect oxidative damage on mice skin exposed to UV irradiation. Barcelos RCS, Vey LT, Benvegnú DM, Trevizol F, Segat HJ, Dias VT, Roversi K, Bürger ME UFSM – Fisiologia e Farmacologia

Skin is the first physical and biochemical barrier against ultraviolet radiation (UVR) harm (Lee et al., 2006), which constitute the primary cause of oxidative stress (OS) that damages proteins, lipids and DNA (Digiovani, 1992). The skin preservation against UVR exposure is also related to lipids that compose it (Hansen and Jensen, 1985), affecting cell signaling mechanisms (Jump, 2004) and the skin physiological functions (Trommer et al., 2001). Essential fatty acids (EFA) play a important role on fluidity of cell membrane affecting proteins activity (Foster et al., 2010). Here we evaluated the influence of supplementation of soybean oil (SO, rich in n-6 FA) and fish oil (FO, rich in n-3 FA) on the oxidative markers of mice exposed to UVR. The experimental protocol was approved by the Animal Ethical Committee (UFSM protocol nº 40/2010), which is affiliated to the Council for Control of Animal Experiments (CONCEA). Male Swiss mice weaned (n=7) were supplemented with water (control-C); fish oil (FO, rich in n-3 FA) and soybean oil (SO, rich in n-6 FA) for gavage (3g/kg/day) for 90 days. After, animals were exposed to UVR dose (5.44mJ/cm^2). Twenty-four hours after the last UVR, mice were anesthetized and euthanized; the dorsal skin was removed for determination of lipid peroxidation (LP) and protein carbonylation (PC) levels (Levine et al., 1990). UVR exposure increased the LP on skin of C (464%) and SO (108%) groups, but not FO group ($F=61.11$;$p=0.0000$). UVR exposure increased the PC levels of C (177%) and SO (140%), but not FO group. However, SO supplementation increased PC level UVR-induced in relation to C group no exposed to UVR and to FO+UVR group (154%)($F=62.82$;$p<0.001$). Considering that dietary FA may reflect the composition of cell membrane phospholipids (Haag, 2003), skin damages can be related to the type of fat consumed (Purba et al., 2001). Our results shower the harmful effects of on n-6 FA consumption, whose intensity can increase vulnerablility to skin diseases as well as the beneficial effects of FO supplementation which can be useful to prevent skin problems associated with sun exposure. **Acknowledgment** Herbarium® Laboratório Botânico Ltda by FO capsules kindly donated. **Financial support:** Fapergs/PQ Gaúcho 2011;PROAP/PPG-Farmacologia PRPGP-UFSM. R.C.S.B. is grateful to CAPES, L.T.V and M.E.B. are grateful to CNPq,by the research fellowships. **References:** D.B. Jump. Crit. Rev. Clin. Lab. Sci. 41 (2004) 41-78. H.S. Hansen et al. Biochim. Biophys. Acta 834 (1985) 357-63. H. Trommer et al. Eur J Pharm Biopharm 51 (2001) 207-14. J. Digiovani. Pharmacol Ther 54 (1992) 63-128. M. Haag. Canad. J. Psychiat. 48 (2003) 195-203. M. Purba et al. Am. Coll. Nutrit., 20 (2001) 71-80. R.H. Foster et al. Nutrition 26 (2010) 708-718. S.H. Lee et al. Yonsei Med. J. 47 (2006) 293-306.

09.008 Antioxidant activities from *Eugenia punicifolia* extract, a plant used in folk popular medicine of the Amazon Region. Galeno DML[1], Boleti APA[2], Carvalho RP[1], Lima AS[2], Almeida PDO[2], Lima ES[2] [1]UFAM – Ciências Fisiológicas, [2]UFAM – Ciências Farmacêuticas

Natural products with antioxidant potential can be used against diseases induced by free radicals, such as degenerative diseases, atherosclerosis, inflammatory injury, cancer, cardiovascular disease and aging. *Eugenia punicifolia* (Kunth) DC known as "pedra-ume caa" is a shrub largely distributed in the Amazon region and useful in cases such as diarrhea, stomach disturbances, hemorrhage, and as hypoglycemic medicine. The antioxidant activities, total phenolics and flavonoids contents of *Eugenia punicifolia* extract were investigated. Antioxidant capacity was detected by ABTS, DPPH, superoxide radical anion ($O_2^{\cdot-}$) and nitric oxide ($NO^{\cdot}$) radical assay. Fibroblastic Line (3T3-L1) was cultured in DMEM medium and incubated with different concentrations (50, 25, 12.5, 6.25, 3.12, 1.56 µg/mL) of ethanol *E.punicifolia* extract. Cell viability was evaluated by Alamar Blue assay. Antioxidant capacity were measured by 2'7—diclorofluorescein diacetate assay. The antioxidant activities detected by ABTS, DPPH, $O_2^{\cdot-}$ and nitric oxide radical assay showed IC_{50} values 10.5±1.2, 28.8±0.5, 38.1±2.6, 2.3±0.5 µg/mL respectively using ascorbic acid and gallic acid, as the reference antioxidants. The amount of total phenols and flavonoids showed (21.6 ± 1.05mg acid gallic equivalent and 2.62 ±0.48 mg quercetin equivalent). These results showed that *E.punicifolia* extract did not reduced significantly the cell viability of 3T3-L1 cell after 48 hs at 50µg/mL. Intracellular ROS scavenging activity was indicated by a decrease in dichlorofluorescein fluorescence once treated in different concentration of extract. Due to these findings, extract of *Eugenia punicifolia* can be a source of natural antioxidants and may be considerable interest in preventing the ill effects of excessive free radicals generation in the human body. **Keywords**: antioxidant activities, *Eugenia punicifolia*, Amazon plants

09.009 Inhibitory effect of anethole on persistent inflammatory pain. Arruda LLM, Ritter AMV, Estevão-Silva CF, Barbosa PB, Kummer R, Gimenez L, Silva FMS, Cuman RKN, Bersani-Amado CA UEM – Pharmacology and Therapeutic

Introduction: The anethole [Anethole 1-methoxy-4-benzene-(1-propenyl)] is the major component (about 90%) of star anise essential oil (Illicium verum), and appears to be responsible for most of the properties attributed to the oil like antioxidant, antimicrobiane, anti-inflammatory and anesthesic properties. In the present study were evaluated the effect of anethole in pain model of inflammatory origin: persistent inflammation induced by Complete Freund's adjuvant. **Methods:** Animals received intraplantar injection of 20 µL of Complete Freund's adjuvant (CFA) one hour after oral treatment with anethole (250 mg/Kg), saline (10 mL/Kg) or indomethacin (2.5 mg/Kg). All groups were treated daily with single dose for a period of seven days. The development of edema and mechanical hypernociception were determined using a caliper and electronic anesthesiometer (von Frey) everyday. On the last day of treatment (seventh day), animals of all groups were anesthetized and sacrificed for collection of tissue paw for assessment of cytokines levels (TNF-α and IL-1β) and mieloperoxidase activity. Data were presented as mean ± SEM of six animals. The means from different treatments were compared by ANOVA with Tukey's test. $P \leq 0,05$. The protocol for these experiments was approved by the Committee on Ethics and Animal Experimentation of the State University of Maringá (CEAE/UEM 125-2010). **Results**: Intraplantar CFA injection (20 µL) resulted in an intense edema and hypernociceptive response, evaluated by caliper and electronic anesthesiometer, respectively, which persisted for several days. Treatment with anethole, orally, in dose of 250 mg/kg, significantly reduced CFA-induced edema and hypernociception throughout the entire evaluation period (seven days). A similar effect was observed when the animals were treated with the reference drug (indomethacin 2.5 mg/kg). The levels of TNF-α and IL-1β were significantly increased in the paw tissue of mice that received intraplantar CFA injection when compared to control animals (which received only saline injection in the paw). Treating animals with anethole (250 mg/kg) or indomethacin (2.5 mg/kg) significantly reduced the levels of TNF-α and IL-1β in paw tissue. In the group treated with anethole (250 mg/kg), the inhibition percentage was 93% in TNF-α I and 75% in IL-1 β levels. The reduction caused by indomethacin (positive control) was similar. Additionally the MPO activity in paw tissue were increased in seventy day after the intraplantar injection of CFA. Treatment with anethole at a dose of 250 mg/kg for seven days significantly inhibited enzyme activity (98%). Indomethacin (positive control) inhibited enzyme activity by 83%. **Conclusion**: The present results provide evidence of anti-inflammatory and analgesic effects of anethole in persistent inflammation model by a mechanism related to inhibition of production/release cytokine and recruitments of neutrophils. **Supported by**: CNPq; CAPES. **Acknowledgements**: Jaílson Araújo e Célia Miranda

09.010 Anesthetic and sedative activities of essential oil of *Ocimum americanum* in silver catfish (*Rhamdia quelen*). Silva LL[1], Garlet QI[2], Mallmann CA[3], Baldisserotto B[4], Heinzmann BM[5] [1]UFSM – Farmacologia, [2]UFSM – Farmácia, [3]UFSM – Medicina Veterinária Preventiva, [4]UFSM – Fisiologia e Farmacologia, [5]UFSM – Farmácia Industrial

Introduction: Anesthetic and sedative drugs are used in aquaculture to improve fish welfare, minimize movement, handling trauma and pain, and also to attenuate the physiological response to stress (NEIFFER et al. 2009; ZAHL et al. 2012). *Ocimum americanum* L. (synonymy*. canum* Sims), commonly known as hoary basil or manjerona, is a species used in folk medicine in cases of insomnia and anxiety (HASSANE et al. 2011). Thus, the aim of this study was to evaluate the sedative and anesthetic activities of the essential oil (EO) of *O. americanum* L. in silver catfish (*Rhamdia quelen*). **Methods:** Leaves of *O. americanum* (voucher specimen no. SMDB 13163, Herbarium of the Department of Biology of the UFSM) were submitted to hydrodistillation for 3h to obtain the EO. Juveniles of silver catfish (8.09 ± 0.22 g; 9.89 ± 0.11 cm) were placed individually in 1L aquaria containing different EO concentrations (25, 50, 100, 200, 300 or 500 mg L^{-1}, N = 10 for each concentration) diluted in ethanol 95 % (1:10) to determine time of anesthesia induction and the length of the recovery period after exposure (SCHOETTGER et al. 1967). These procedures were approved by the Ethical and Animal Welfare Committee of the UFSM (Process no. 46/2010). Data (median and interquartile range [Q1–Q3]) were submitted to Kruskal–Wallis and Dunn tests or regression analysis ($P < 0.05$). **Results:** Concentrations of 25-100 mg L^{-1} of the EO of *O. americanum* were not able to induce anesthesia during 30 min of exposure. Fish exposed to 200-500 mg L^{-1} reached deep anesthesia (4-8 min) without side effects or mortality. A positive relationship was detected to stages 3b (total loss of equilibrium) and 4 (deep anesthesia), where an increment of EO concentration caused a reduction in the time required for anesthesia induction. The recovery of animals exposed to 25-200 mg L^{-1} occurred quickly (within 1-6 min) without significant differences between concentrations. Larger times of recovery (within 11-14 min) were observed to the highest concentrations tested (300 and 500 mg L^{-1}). **Discussion:** Fast anesthesia (about 4 min) could be obtained in silver catfish with 500 mg L^{-1} of EO of *O. americanum*. The same depression level could be obtained at lower concentrations of the EOs of *Lippia alba* (300 mg L^{-1}) and *Ocimum gratissimum* (150 mg L^{-1}) in similar period (CUNHA et al. 2010; SILVA et al. 2012). Regarding to recovery time, fish exposed to 300 mg L^{-1} of EOs of *O. americanum* showed an intermediary period (about 12 min) when compared to those anesthetized at the same concentration with EOs of *L. alba* (about 6 min) and *O. gratissimum* (about 20 min) (CUNHA et al. 2010; SILVA et al. 2012). **References:** SCHOETTGER R.A. et al., Fish Control U.S. Department International 13:1, 1967; NEIFFER D.L. et al., ILAR J, 50:343, 2009; CUNHA M.A. et al., Aquaculture, 306:403, 2010; HASSANE S.O.S. et al., Phytothérapie, 9:18, 2011; SILVA L.L. et al., Aquaculture, 350-353:91, 2012; ZAHL I.H. et al., Fish Physiol Biochem, 38:201, 2012. **Financial Support**: CAPES, CNPq, Fapergs-Pronex, FIT/UFSM.

09.011 Evaluation of gastroprotective effect of *Struthanthus marginatus* (Desr.) blume in chronic ulcers and gastric secretion models. Silva RV, Morais TMF, Lima JS, Sousa RS, Gomes JPB, Silva SN, Cartágenes MSS, Freire SMF UFMA – Farmacologia

Introduction: *Struthanthus marginatus* (Desr.) Blume (Loranthaceae), commonly known as "erva-de-passarinho" is used in folk medicine for stomach diseases and respiratory problems. The study aimed to assess the gastroprotective effect of aqueous extract of the leaves *in vivo* model of chronic ulcers induced by acetic acid and gastric acid secretion induced by histamine and pilocarpine, in mice. **Methods:** The plant material was collected in the county of São José de Ribamar - MA. The powder (20g), obtained from the dried and crushed leaves, was added 1000 ml of water at 72 ° C. The mixture, maintained at the same temperature for 30 min, was submitted to occasional shaking, then filtered, concentrated and lyophilized, obtaining an extract (EA), with a yield of 20.26%. **Results:** In chronic ulcers induced by acetic acid (30%, 50mL, subserosal layer) treatment orally for 14 days with EA (125mg/kg and 500mg/kg) and cimetidine (100mg/kg) reduced the ulcerogenic index 29%, 65% and 54% respectively, compared to the control group (4.46±0.402%). For the evaluation of gastric acid secretion stimulated by pilocarpine (1mg/Kg, subcutaneous[sc.]) or histamine (60mg/Kg, sc.) as stimuli, the animals were subjected to pylorus ligation for four hours and were analyzed the volume, pH and total acidity of gastric juice. In animals pre-treated with EA (500 mg / kg, intraduodenal [id.]) or atropine (1mg/kg, sc.) that received stimulation of pilocarpine (v = 0,34±0,04mL, acidity = 0,18±0,02[H^+]mEq/ mL/4hr, pH = 3,496 unity) there was a reduction in the volume of gastric secretion in 47% and 68% and total acidity in 70% and 74%, respectively, and the pH increased by 73% and 68%, respectively, when compared to the group that has only been administered pilocarpine. In the groups pretreated with EA (500mg/kg, id.) or cimetidine (60mg/kg), who received oral histamine (v = 0,52±0,08mL, acidity = 0,2±0,03162 [H^+]mEq/ml/4h e pH = 3,37 unity) as a stimulus, there was a reduction in the volume of gastric secretion in 46% and 65% and the acidity total 67% and 76%, respectively, and the pH increased by 70% and 74%, respectively, when compared to the group that has only been administered histamine. **Discussion:** These results contriburtes to the gastroprotective activity popularly attributed to the plant, the mechanism may be related in part to the inhibition of gastric acid secretion, providing basis for further study. The experimental protocols developed in this study were approved by Ethics Committe of Universidade Estadual do Maranhão (CEEA) (Protocol 009/2008). **Financial support:** CNPq and FAPEMA. 0058197-22.2012.8.26.0100

09.012 Local antiophidic activity of the extract of *Bredemeyera floribunda* Willd. Alves NTQ[1], Ximenes RM[1], Jorge RJB[1], Alves RS[1], Soares VCG[2], Costa PHS[1], Abreu ML[1], Menezes DB[3], Havt A[1], Monteiro HSA[1] [1]UFC – Physiology and Pharmacology, [2]Unicamp – Biochemistry, [3]UFC – Pathology

Introduction: In Brazil most of snakebites are caused by snakes of the *Bothrops* genus that can induce local tissue damage like edema and hemorrhaging. Traditional antivenom therapy acts neutralizing the systemic effects, but has limited effectiveness on neutralization of local effects (SANTORO, *Toxicon*, v51, p1440, 2008). The compounds isolated from plants popularly used to treat snake poisoning can be good alternatives to antivenom treatment (MELO, *Brazilian J. Pharmacog*, v17, p29, 2007). **Methods:** This study evaluated the antiophidic activity of the aqueous extract of the roots of *Bredemeyera floribunda* (EBf) through inhibition of phospholipasic A_2 (PLA2) and proteolytic activities by *in vitro* tests in the proportions 1:1 and 1:3 vBju/EBf (w:w), and *in vivo* tests like edema and hemorrhagic activities promoted by venom of *Bothrops jararacussu* (vBju) in mice. The groups of *in vivo* tests were A)venom: 50 µg/animal; B)PBS; C)pre-incubated: 50 µg of vBju+150 mg/kg of EBf, 15 min of incubation at 37ºC; D)pre-treated: EBf 150mg/kg, i.p., 30 minutes before vBju (50 µg); E)post-treated: EBf 150mg/kg ,i.p., 30 minutes after vBju (50µg); F)bothropic antivenom (SAB, amount sufficient to neutralize 500 µg of venom): SAB, i.p., 30 minutes before administration of vBju (50 µg). To hemorrhagic test, the animals received intradermic injections, in the dorsal region, containing aliquots of 50 µL according to the groups. Two hours later, mice were sacrificed, their skins were removed and the hemorrhagic area (mm^2) was determined using scanned photographs and the programme Image J. To the edema test, the contralateral hind paw was injected with sterile saline (30 µl/paw) to volume control. After sub-plantar injection of venom (50µg/paw), paw volume was measured at 10, 30, 60, 120, 240 and 480 minutes, using a hydroplethysmometer (Ugo Basile®). The single dose acute toxicity of the EBf was also determined. Data were expressed as mean ± S.E.M. and analyzed by ANOVA followed by Bonferroni post-test, with *p* set at 0.05. This study was approved by Ethics Committee (protocol no.68/09). **Results and discussion:** The EBf showed to be nontoxic in mice. PLA2 and proteolytic activities of vBju were significantly inhibited *in vitro* by EBf in the proportion of 1:3. The EBf inhibited the edema induced by vBju, a significant reduction of the maximum edematogenic effect at 120 minutes (158.0 ± 14.6 mL) was noted in C, D, and E groups (60.0 ± 7.1 mL; 59.6 ± 5.5 mL; 113.0 ± 7.2 mL, respectively). The positive control, group SAB, showed no significant inhibition of the tested toxic activities (116.3 ± 13.6 mL). The EBf also decreased the hemorrhagic area induced by vBju (229.7 ± 5.83 mm²) in the C, D and E groups (103.5 ± 11.3 mm², 92.7 ± 13.5 mm², 77.8 ± 7.9 mm², respectively). The SAB (225.5 ± 16.3 mm²) showed no significant effect. Triterpene saponins, major constituents of the EBf (BEVENINO, *J. Ethnopharmacol,* v43, p203, 1994), may act by binding to vBju components as *PL*A2, and also to metalloproteases, partly blocking the action of these proteins, reducing edema, phospholipasic, hemorrhaging and proteolytic activities. **Financial support:** CNPq; CAPES.

09.013 Evaluation of *stryphnodendron sp* release using natural rubber latex membrane as carrier. Romeira KM, Silva RMG, Herculano RD Unesp-Assis – Ciências Biológicas

Natural rubber latex from *Hevea brasiliensis* has interesting characteristics related to this work such as: it is easy to manipulate, low cost, can stimulate the natural angiogenesis, is a biocompatible material and presents high mechanical resistance. The aim of this study is to develop a novel sustained delivery system for *Stryphnodendron sp* based on Natural Rubber Latex (NRL) membranes and to study the *Stryphnodendron sp* delivery system behavior. *Stryphnodendron* sp, commonly known as barbatimão, is extensively used in folk medicine for the treatment of diarrhoea, gynecological problems and for healing wounds. The stem bark of this species is mentioned in the Brazilian Pharmacopeia with a content of at least 20% of tannins. Previous studies showed significant cicatrizing properties, anti-inflammatory activity and gastric anti-ulcerogenic effects for the stem bark crude extract. For the extract release study, membranes with *Stryphnodendron* sp were placed in 200 mL of aqueous solution, where the release behavior was observed. Aliquots of this solution were collected during an interval ranging from 10 to 24,000 minutes. The *Stryphnodendron sp* release as a function of time was determined using the UV-VIS method. This work also studied the antioxidant capacity of *Stryphnodendron sp* that will be analyzed through tests, with extracts and fractions, as of kidnapping of free radical DPPH and quantification of phenols and total tanning barks. **References:** Lima JCS, MARTINS DTO, SOUZA-JUNIOR PT. Experimental evaluation of stem bark of *Stryphnodendron adstringens* (Mart) Coville for anti-inflammatory activity. *Phytotherapy Research* 12. (1998). p.218-30. Herculano RD, Silva CP, Ereno C, Catanzaro-Guimarães SA, Kinhoshita A, Graeff CFO. Natural rubber latex used as drug delivery system in guided bone regeneration (GBR). *Materials Research*. 12(2) (2009) pp.253-256. **Financial agencies:** FAPESP

09.014 Increasing the antioxidant capacity of Brazilian beverage by biotransformation of flavonoids. Silva CMG[1], Braga MA[1], Pascoal ACRF[3], Salvador MJ[2], Martinez CAR[3], Carvalho PO[4] [1]Unicamp – Bioquímica, [2]Unicamp – Biologia Vegetal, [3]USF – Biologia Molecular de Tumores, [4]USF – Biotecnologia

Introduction: Flavonoids have been subject of much evidence of their functional properties, especially concerning their antioxidant properties. It is known that these compounds have diverse structures, which although abundants are not always well absorbed in their native form, which can reduce or delay their benefits. In this work we studied the flavonoid biotransformation processes of mate tea (*Ilex paraguariensis*), green tea (*Camellia sinesis*), orange juice (*Citrus sinensis*) and lemon juice (*Citrus aurantifolia*) using microbial enzymes to obtain derivatives with higher antioxidant capacity. **Methods:** The enzymatic reactions were performed in a controlled process using commercial enzymes (hesperidinase from *Penicillium* sp, naringinase from *Penicillium decubens*, glucosidase from *Aspergillus niger* and β-galactosidase from *Aspergillus oryzae*). The best reaction conditions for hydrolysis were determined and optimum pH from 3.8 to 4.5 and optimum temperature 30°C to 40°C was obtained. Antioxidant activities were evaluated by *in vitro* tests before and after the biotransformation reactions: FRAP, DPPH, β-carotene and ORAC. The composition profile of these compounds in the samples was performed by mass spectrometry (ESI-MS). **Results and Discussion:** Mate and green teas before the biotransformation reaction showed antioxidant activity, by ORAC assay, of 3447.36±310.26 and 3557.56±183.21 μmol TEg^{-1} respectively. After the biotransformation reaction the antioxidant capacity increased to 4092.18±10.23 and 4098.25±30.74 respectively. Orange and lemon juices before the biotransformation reaction showed 737.43±20.21 and 367.87±4.82 μmol TEg^{-1}, after the biotransformation reaction these values increased significantly to 1503.25±76.67 and 544.96±24.09 μmol TEg^{-1}. On the mate tea MS spectrum, before the biotransformation reaction, could be observed ionized species of compounds such as quercetin, cafeoylglucose, feruloylquinic acid and rutin. After the biotransformation reaction could be observed an increase in signal intensity (*m/z*) of the aglycone quercetin, from glycosylated quercetin (rutin), which after biotransformation had reduced signal intensity, which proved the action of the enzyme. Green tea spectrum, before biotransformation, showed phenolic acids, dicaffeoylquinic derivatives and glycosides flavonoid. In the profile after the biotransformation reaction could be seen an increase in signal intensity (*m/z*) of the aglycone quercetin and dihydrated quercetin from rutin. On the orange juice spectrum could be observed ionized species of compounds such as hesperidin, hesperetin and tangeritin. However after the biotransformation reaction could be observed an increase in signal (*m/z*) of aglycone hesperetin from hesperidin. And on the lemon juice spectrum could be observed ionized species of compounds such as hesperidin, acacetin and tangeritin. However after the biotransformation reaction could be observed an increase in signal (*m/z*) of aglycone hesperetin from hesperidin, which shows the breakdown of glucose and rhamnose units. **Conclusion:** The enzymes used in the biotransformation reactions produced aglycone flavonoids or less complex molecules, which improve the antioxidant capacity of these drinks. Mass spectrometry confirmed the action of enzymes in the hydrolysis of the flavonoids glycosidic fractions. **Financial Support:** CAPES/FAPESP.

09.015 Hypolipidaemic evaluation of tyramine in mice with dyslipidemia induced for poloxamer-407. Pereira NBS[1], Morais TMF[1], Dantas MB[1], Sousa DF[2], Meneses RRC[1], Rodrigues HG[1], Holanda RTM[1], Damasceno DV[1], Ferreira JM[1], Queiroz MGR[1]
[1]UFC – Análises Clínicas e Toxicológicas, [2]UFC – Fisiologia e Farmacologia

Introduction: Lipid disorders constitute one of the risk factors for developing coronary heart disease and appropriate treatment of this risk factor becomes important for reduction of cardiovascular events. In previous studies, tyramine was capable of reducing plasma glucose levels in a diabetes model, as well of decreasing plasma cholesterol. Thus, this study aimed to evaluate the action of tyramine in a specific model of dyslipidemia induced by poloxamer 407 (P-407). **Methods:** For the development of this model, male Swiss mice were used and divided into 6 groups (n = 6): negative control (NC), positive control (PC), fenofibrate (FENO), tyramine 1 (T1), tyramine 2 (T2), tyramine 4(T4), which were given saline, saline, fenofibrate 200 mg /kg, tyramine 1 mg/kg, 2mg/kg and 4 mg/kg, respectively. For induction of dyslipidemia, 400 mg/kg P-407 was administered intraperitoneally (i.p) in all groups, except for the NC. The treatments were performed 2, 26 and 46 hours after induction by oral via and blood collections were made at 24 and 48 hours time points for verification of biochemical parameters triglycerides and cholesterol. The experimental procedures were approved by the UFC Ethics Committee for Animal Research under protocol number 24/10. The results were analyzed using ANOVA (Tukey post-test), adopting as a criterion of significance $p < 0.05$. **Results and Discussion:** The poloxamer promotes inhibition of lipoprotein lipase and activation of HMG Co reductase, causing increases in both triglycerides and cholesterol (Subramaniam et al., Indian J Exp Biol, 49:282, 2011). The results of this study confirmed the ability of poloxamer to promote both hypertriglyceridemia (NC: 129.5 ± 4.8 vs 168.3 ± 2838 mg/dL in 24 hours; NC: 152.3 ± 8.1 vs CP:760.2 ± 41.9 mg/dL in 48 hours) and hypercholesterolemia (NC: 110.0 ± 7.2 vs 399.7 ± 15.7 mg / dL in 24 hours; CN: 108.7 ± 5.3 vsCP: 275.5 ± 12.1 mg / dL in 48 hours). After the first collection, only tyramine T2 was effective in lowering serum triglyceride levels (2057 ± 158.5 vs. 2838 ± 168.3 mg / dL) and cholesterol (309.0 ± 11.17 mg/dL vs 399 7 ± 15.66 mg/dL). After 48 h, T2 (453.0 ± 35.47 vs 760.2 ± 41.86 mg/dL), T4 (605.8 ± 26.61 vs 760.2 ± 41.86 mg/dL) and fenofibrate (416, 0 ± 21.75 vs 760.2 ± 41.86 mg/dL) significantly reduced triglyceride levels. The same time, cholesterol levels were reduced in T1 (220.5 ± 12.78 vs 275.5 ± 12.11), T2 (205.8 ± 7.07 vs 275.5 ± 12.11) and T4 (216.8 ± 12.79 vs 275.5 ± 12.11) as well as fenofibrate (190.0 ± 9.29 vs 275.5 ± 12.11). These decreases in lipid levels caused by treatment with tyramine corroborate data from Cho et al. (Nutr Res Pract, 5:412, 2011) as well as findings of Lino et al. (Am J Toxicol & Pharm, 2:178, 2007). **Financial Support:** FUNCAP

09.016 ***Rhizophora mangle* as an anti-inflammatory source of drug**: **Role on cytokines in TNBS-induced colitis in rats**. De FariaFM[1], Luiz-Ferreira A[2], SoccaEAR[1], Dunder RJ[1], Almeida ACA[3], Manzo LP[1], Silva MA[4], Vilegas W[4], Souza-Brito ARM[1] – [1]Unicamp – Farmacologia, [2]UFG – Ciências Biológicas, [3]Unicamp – Biologia Estrutural e Funcional, [4]Unesp-Araraquara – Química Orgânica

Introduction: The production of pro-inflammatory cytokines, such as interleukin-6, interleukin-12 and tumor necrosis factor (TNF-α), is increased in patients with inflammatory bowel disease. The TNBS model of colitis induces a transmural lesion with pathological characteristics similar to Chron's disease (Xavier & Podolsky, 2007). In this context and in order to evaluate the significance of the therapeutic use of the procyanidins from the bark of *Rhizophora mangle,* the protocol of TNBS-induced colitis in rats was used. **Methods:**

TNBS-induced colitis was performed according to Morris et al. (1989). Four groups of rats were used (n=8); non-colitic (NC) and colitic non-treated (T); one group received orally, 0,5 mg.Kg^{-1} of *R. mangle* bark butanolic fraction (Bu), another group received 1,5 mg.Kg^{-1} of the ethyl acetate fraction (EA). After two weeks of treatment, colitis was induced by intra-colonic administration of TNBS (10.0 mg) and one week after the induction, damage score and biochemical parameters were evaluated. **Results:** The treatments reduced the size of the lesions, Bu 4.3 ± 0.8 cm (**P*<0.05) and Et 4.4 ± 1.3 cm (**P*<0.05) with colitic non-treated (control group), the damage score was marked only by the Bu treatment, 8.1 ± 0.8 (**P*<0.05) when compared to colitic non-treated group (control). Both treatments, Bu and Et, were capable of decreasing the levels of TNF-α (***P*<0.01) and IL-12 (****P*<0.001), but only Et reduced the level of IL-6 (****P*<0.001) when compared to colitic non-treated group (control). **Discussion:** Overproduction of IL-12, a macrophage-derived cytokine, shifts the immune response in a Th-1 direction; this response is characterized by increased production of TNF-α and IL-6 resulting in a self-sustaining cycle of activation (Danese & Fiocchi, 2011). It has been reported that cytokines (e.g. TNF-α) are one of therapeutic targets of inflammatory bowel disease. Our results suggests that *R. mangle* was able to protect the colon of the rats by the inactivation of IL-12 pathway, once the release of TNF-α and IL-6 were also found decreased. **References:** Xavier, RJ et al. *Nature*, 448:427, 2007; Morris, GP et al. Gastroenterology, 96:795, 1989; Danese, S & Fiocchi, C. *N Engl J Med*, 365:1713, 2011. **Financial agency and acknowledgement:** FAPESP

09.017 Evaluation of antimicrobial activity of ant *Dinoponera quadriceps* venom. Lima DB[1], Fernandes LC[1], Torres AFC[1], Mello CP[1], Menezes RRPPB[2], Costa MFB[2], Sampaio TL[1], Tessarolo LD[1], Quinet YP[3], Nogueira NAP[1], Martins AMC[1] [1]UFC – Análises Clínicas e Toxicológicas, [2]UFC – Fisiologia e Farmacologia, [3]ISCB-UECE

Background: Animal venoms have been widely recognized as one of the main sources of biologically active molecules [1]. The study of the therapeutic potential of toxins is arousing great interest from the scientific community as a source of molecular models for the design of new drugs [2]. The aim of the present study was to evaluate the antimicrobial effect of the ant *Dinoponera quadriceps* venom (*Dq*v) of standard strains. **Methods:** The antimicrobial effect of *Dq*v was evaluated using the broth microdilution method, as described in [3]. Five pure microbial cultures (*Salmonella choleraesuis subsp. choleraesuis sorotype choleraesuis* ATCC 10708, *Staphylococcus aureus* ATCC 6538P, *Escherichia coli* ATCC 11229, *Pseudomonas aeruginosa* ATCC 15442 and *Candida albicans* ATCC 10231, donated by the Laboratory for Reference Materials of the Oswaldo Cruz Foundation, FIOCRUZ), were sub-cultured, and after adjusting the density to 1.5×10^6 CFU/mL. An inoculum of microbial culture was added to different concentrations of *Dq*v in Mueller-Hinton broth for bacteria or Sabouraud-Dextrose broth for *C. albicans*, in 96-well plates. Sterile PBS buffer (pH 7.4) was used as negative control. Antimicrobial agents (amikacin for bacteria and ketoconazole for yeast) were used as a positive control. The plates were incubated at 35ºC for 24 h, and the minimum inhibitory concentration (MIC) was assessed as the lowest sample concentration required to inhibit microbial growth. Three independent experiments were performed in triplicate. In addition, aliquots were removed from the wells without visible turbidity and spread on the surface of Agar Plate count for bacteria or Agar Sabouraud-Dextrose for *C. albicans*. Colonies were counted after incubation at 35ºC for 24 h. The sample concentration that resulted in ≤0.1% of the growth of the initial inoculums was determined as the minimum lethal concentration (MLC). **Results:** The MIC and MBC obtained for the *Dq*v were 3.12 µg/mL and 12.5 µg/mL for *S. aureus*, 3.12 µg/mL and 3.12 µg/mL for *E. coli*, 12.5 µg/mL and 12.5 µg/mL *P. aeruginosa*, 12.5 µg/mL and 25 µg/mL for *S. choleraesuis* and 50 µg/mL and 50 µg/mL for *C. albicans*, respectively. **Discussion:** Several studies have aimed to find novel antimicrobial substances from natural sources. Previously, antimicrobial peptides were isolated from ant *Pachycondyla goeldii* venom, called ponericins. The ponericins showed antimicrobial activity against gram-positive and gram-negative bacteria [5]. Recently, a peptide similar the ponericin was identified from ant *Dinoponera australis* venom [6]. **Conclusion:** The *Dq*v showed a broad-spectrum antibacterial and antifungal effect against the tested strains suggesting the presence of antimicrobial peptides. **Financial agencies:** UFC; CNPq; FUNCAP. **References: 1**. PIMENTA, AMC. *J. Peptide Sci.* v11, p670, 2005; **2.** Mortari, M. R. *Pharmacol Ther*, v114, p171, 2007; **3.** Clinical and Laboratory Standards Institute (CLSI). NCCLS document M7, 2003; **4.** Zelezetsky, I. *Arch. Biochem. Biophys*, v434, p358, 2005; **5.** ORIVELL, J. *J Biochem Chem*, v286, p17823, 2001; **6.** JOHNSON, S. R. *Toxicon***.** v55, n4, p702, 2010.

09.018 Effect of fixed oil of pequi *Caryocar coriaceum Wittm* in zymosan-induced arthritis in rats. Oliveira FFB, Araújo JCB, Ribeiro RA, Vale ML UFC – Fisiologia e Farmacologia

Introduction: Rheumatoid arthritis (RA) is a chronic autoimmune disease presenting acute inflammatory episodes. The most prevalent symptoms of RA are the increased sensitivity to joint pain (hyperalgesia or hypernociception) and joint swelling. Pequi, *Caryocar coriaceum* Wittm, fruit pulp fixed oil (PCCO) has wide application in folk medicine. Preclinical assays with PCCO showed anti-inflammatory, gastroprotective and wound healing properties. Based on this context, this study has evaluated the antinociceptive and anti-inflammatory activity of PCCO in a model of arthritis in rats. **Methods:** The study was approved by the Ethics Committee on Animal Research of the Federal University of Ceará (nº 83/11). Male Wistar rats (180-200g) received an intra-articular injection, in the right knee joint, of zymosan (ZY, 1 mg/50µl) for the induction of arthritis. Articular incapacitation (AI; articular hypernociception) and joint edema (JE) were measured at every hour, during 4 hours, after ZY injection, by the AI test and by the measure of transverse diameter of the articulation, respectively. Evans blue dye extravasation was also evaluated in articular and peri-articular tissue to verify the increase in vascular permeability. Leukocyte influx (LI) and the expression of TNF-α and COX-2, in the synovial tissue, were evaluated 4 hours after ZY injection by cell counting and myeloperoxidase (MPO) activity, in the joint lavage fluid, and by immunohistochemistry in synovial tissue harvested from injected joints. The animals were pretreated with 100, 200 and 400 mg/kg of PCCO or vehicle for 7 consecutive days, orally, before ZY-induced arthritis. Dexamethasone (DX; 4 mg/kg, s.c.), two hours before the injection of ZY, was used as positive control and the negative control group consisted of non treated /non arthritic animals. **Results and Discussion:** Three or four hours After ZY injection the rats developed AI characterized by an increase in paw elevation time (PET). The PCCO treatment, in all doses, significantly decreased ($p<0.05$) the PET when compared to control group at the 4th hour of AI, as well as with DX pretreatment ($p<0.05$). Regarding the LI into articular cavity, all groups treated with the PCCO reported significant reduction ($p<0.05$) of the number of leukocytes counted in the joint lavage, as well as with DX pretreatment ($p<0.05$). The groups treated with 100 and 200mg/kg of PCCO or DX also showed a significant decrease ($p<0.05$) in MPO activity. The immunohistochemical analysis showed a decreased expression of TNF-α and COX-2 in the synovial tissue of PCCO (only 200mg/kg) and DX pretreated animal when compared to control. In relation to JE, the pretreatment with PCCO, in all doses, showed a significant reduction in the transverse diameter of articulation ($p<0.05$), when compared to the control group, and the doses of 100 and 400 mg/kg decreased also the Evans blue extravasation in a significant manner ($p<0.05$). **Conclusion:** Our results showed that the PCCO has antinociceptive effect as well as an anti-inflammatory effect in ZY-induced arthritis, inhibiting joint edema, leukocyte influx and, in a less extension, the expression of COX-2 and TNF-α in synovial tissue.
Financial support: CAPES

09.019 Hypocholesterolemic and hypoglycemic effect of ursolic and oleanolic acid in obese mice. Rodrigues HG[1], Melo CL[1], Melo TS[1], Damasceno DV[1], Araújo VM[1], Freitas AMP[1], Holanda RTM[1], Pessoa ODL[2], Rao VS[3], Queiroz MGR[1] [1]UFC – Análises Clínicas e Toxicológicas, [2]UFC – Química Orgânica e Inorgânica, [3]UFC – Fisiologia e Farmacologia

Introduction: The investigation of natural products is a potential source to the development of new products for therapeutic use. In this scenario, the triterpenoids, such as ursolic and oleanolic acids, have various biological effects. Focusing on this study, obesity is a multifactorial disease that has been considered as the epidemic of XXI century and promotes various other diseases such as hypertension and diabetes. **Objective:** To observe the hypocholesterolemic and hypoglycemic effect of ursolic and oleanolic acids on mice fed with high calorie diet-induced obesity and to compare its effects as the reference drug, sibutramine. **Methods:** Male Swiss mice (20-25g) were divided into groups (n=8): SD (standard diet + Tween 80, 3%), HD (high calorie diet + Tween 80, 3%), HD + UA (high calorie diet + ursolic acid 50mg/L), HD + OA (high calorie diet + oleanolic acid 50 mg/L), HD + SIB (hypercaloric diet + sibutramine 10mg/L). Obesity was induced by DH that had a ratio of 15g of standard diet, 10g of milk chocolate, 10g of roasted peanuts and 5g of cornmeal biscuit. After the ingestion of HD, groups whose weight had increased 20-25% compared to DP, were considered obese. After induced obesity, treatment was carried out with UA, OA, SIB or Tween 80 3% *ad libitum* for 15 weeks. After this period, the animals were submitted to 6-8h fast for blood collection and determination of glucose (GLU), total cholesterol (TC) and HDL-cholesterol (HDL-c) and insulin (INS). The protocol was approved by the Ethics Committee on Animal Research of UFC under number 031/08. The results were expressed as mean ± S.E.M. and the groups were compared by ANOVA (Tukey post-test), adopting significance as $p < 0.05$. **Results:** Groups HD + UA, HD + OA and HD + SIB decreased TC levels (mg/dL) (SD: 112.3±5.5, HD: 207.1±6.3; HD + OA: 141.2±6.6, HD + UA: 154.6±4.3; HD + SIB: 170.4±5.3), while treatment with AU or OA decreased GLU values (mg/dL) (SD: 86.1±3.4; HD: 136.3±13.1, HD + OA: 96.0±6.5, HD + AU: 78.7±11.4; HD + SIB: 137.4±19.1). Only UA promoted an increase of INS plasma levels (ng/mL) (SD: 0.092±0.05, HD: 0.39±0.05, HD + OA: 0.47±0.07; HD + UA : 0.67±0.66; HD + SIB: 0.59±0.06) while UA, OA, or SIB increased levels of HDL-c (mg/dL) (SD: 84.7±6.8 , HD: 88.3±6.1, HD + OA: 111.4±7.5, HD + UA: 131.9±6.2; HD + SIB: 142.3±7.1). **Discussion:** Several triterpenes are amylase inhibitors, reducing the intake calories from carbohydrates, avoiding hyperglycemia (HOU, Phytother Res., v.5, p.614, 2009). The effect on the TC levels may have been by inhibiting the enzyme acyl-CoA cholesterol acyl-transferase (ACAT), as described elsewhere (Hwang, *Phytochemistry*, v.62, p.662, 2003). Ursolic and oleanolic acids showed hypocholesterolemic and hypoglycemic activities and also an increase on the values of HDL-c, and they could be a therapeutical alternative for obesity after more studies. **Financial support:** FUNCAP

09.020 Antioxidant action of lycopene in testicular deleterious effects caused by the mycotoxin zearalenone. Boeira SP[1], Borges Filho C[2], Del Fabbro L[2], Roman SS[3], Jesse CR[2], Oliveira MS[1], Furian AF[4] [1]UFSM – Farmacologia, [2]Unipampa, [3]URI, [4]UFSM – Tecnologia e Ciência de Alimentos

Introduction: Zearalenone (ZEA), a *Fusarium* toxin, is a non-steroidal estrogenic mycotoxin found as a contaminant in cereals cultivated all over the world. ZEA has major effects on reproductive system and such effects have been related to oxidative stress (Zinedine et al., 2007; Boeira et al., 2012). Accordingly, lycopene, a carotenoid antioxidant has been used for treatment of spermiotoxicity after chemotherapy and prostate cancer (Dahan et al., 2008). Therefore, the current study was aimed to investigate the effects of a lycopene pre-treatment on markers of oxidative stress, histopathological and reproductive parameters induced by an acute administration of ZEA. **Methods:** 20 Swiss albino mice were kept for acclimatization during one week under constant temperature and in a 12h light/dark cycle. They received water and food *ad libitum.* Mice were weighed and randomly divided in four groups which received lycopene (20 mg/kg) or olive oil by gavage (10 ml/kg) for ten consecutive days. In the eleventh day mice received ZEA (40 mg/kg) or olive oil by gavage. Forty eight hours after ZEA or vehicle administration the animals received a dose of pentobarbital (180 mg/kg, i.p.), and testes and epididymis were collected. We evaluated the sperm count and motility in epididymis, and the activity of antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione-S-transferase (GST) in testes (Boeira et al 2012). In addition, we analyzed histopathological parameters in testes (Li et al., 2006). This project was approved by the Committee on Care and Use of Experimental Animal Resources (process #071/2011) of the UFSM, Brazil. Data were analyzed by using a two-way analysis of variance (ANOVA), followed by Duncan's Multiple Range Test when appropriate. **Results:** No differences in CAT and SOD activities were observed in all groups, however lycopene prevented the reduction of GST activity induced by ZEA in testes. Moreover, lycopene prevented the drastically reduction of the number and motility of live spermatozoa induced by ZEA. Histopathology evaluation of testes revealed that ZEA induced intense loss of cellular architecture and severe injuries, and histological characteristics were more preserved in lycopene than those from ZEA group. **Discussion:** Oxidative stress is recognized as one of the main contributors to the increased risk of testicular cancer and reproductive disturbances. As a highly effective antioxidant, lycopene may exert its beneficial functions through this important mechanism. In conclusion, this study indicated that ZEA induces reproductive toxicity in male Swiss albino mice, as demonstrated by changes in spermatozoa count and motility and testicular damage as well in the GST activity. In addition, we showed that a ten day treatment with lycopene might prevent this toxicity in mice. **References:** Boeira, S.P. et al., *Toxicon*, in press, 2012. Dahan, K.,et al., J. Soc. Integr. Oncol. 6, 29; 2008. Li et al., Histochem Cell Biol 125, 607; 2006. Zinedine, A et al., Food Chem Toxicol 45, 1; 2007. **Financial Support:** CNPq, FAPERGS, CAPES, UNIPAMPA

09.021 ***Euterpe oleracea*** **Mart. (açaí) extract prevents endothelial dysfunction, oxidative stress, vascular and renal changes in 2 kidneys, 1 clip renovascular hypertension**. Costa CA[1], Carvalho LCRM[1], Emiliano da Silva AF[1], de Bem GF[1], Oliveira PRB[1], Valença SS[2], Pires KMP[2], Ognibene DT[3], Resende AC[1], Soares de Moura R[1] [1]UERJ – Farmacologia e psicobiologia, [2]UFRJ – Farmacologia, [3]UEZO

Introduction: Studies have shown that *Euterpe oleracea* Mart. (açai) is rich in polyphenols and consumption of polyphenol-rich food is associated with protection against cardiovascular risk. This study examined the effect of açaí stone extract (ASE) on the endothelial dysfunction, oxidative stress, vascular and renal structural changes associated with 2 kidney, 1 clip (2K,1C) renovascular hypertension. **Methods:** The experiments were approved by the Ethics Committee of Animal Experiments of the UERJ (protocol: CEA/024/2010). Male Wistar rats used to obtain the 2K,1C hypertensive and control rats 2K (sham), were treated daily with vehicle or ASE (200mg/Kg/dia) for 40 days, and systolic blood pressure (SBP, mm Hg) was measured by plethysmography. The vasodilatory effects of acetylcholine (ACh) and nitroglycerin (NG) were studied in perfused mesenteric arterial bed (LAM) pre-contracted with norepinephrine (30 mM). The antioxidant enzymatic activity of superoxide dismutase (SOD), catalase (CAT), and glutationa peroxidase (GPx), malondialdehyde (MDA) levels, carbonyl protein and nitrite levels were assessed in mesenteric arteries and kidney by spectrophotometry. The expressions of eNOS, (NOX-4) and antioxidant enzymes and metalloproteinase 2 (MMP-2) were assessed by western blot and the activity of MMP-2 was analyzed by zymography in mesenteric arteries. The serum creatinine levels were assessed using a kit by spectrophotometry. The vascular and renal changes were evaluated by light microscopy. **Results:** ASE prevented 2K-1C hypertension and the reduction of acetylcholine-induced vasodilation. The vasodilator effect of NG was not different between groups. In 2K-1C rats, the increased levels of MDA and carbonyl protein were reduced by ASE. The reduced activities of SOD, CAT and GPx in samples of mesenteric arteries, and kidney of 2K-1C rats were recovered by ASE. Expression of SOD-1, 2, TIMP-1 and eNOS and production of NO were decreased in 2K-1C rats and recovered by treatment with ASE. The increased expression of MMP-2 and NOX-4 and MMP-2 activity in 2K-1C rats were reduced by ASE. 2K-1C rats showed an increase in medial thickness of mesenteric artery, which was prevented by ASE. Increased serum levels of creatinine in 2K-1C rats were normalized by ASE. The renal morphological changes in 2K-1C rats were prevented by ASE. **Discussion:** Therefore, treatment with ASE prevents the development of hypertension, improves endothelial dysfunction and prevents the renal and vascular changes in 2K-1C rats. The reduction in antioxidant activity and increased lipid peroxidation and protein carbonyls suggest the involvement of a mechanism of impaired antioxidant defense and increased oxidative damage in 2K-1C rats, which was reversed by ASE. The NO synthesis, the antioxidant action and the inhibitory effect on MMP-2 levels induced by ASE may contribute to these benefic effects of the extract, suggesting that the ASE can be a important tool for the treatment of hypertension and cardiovascular changes present in this model. **Financial Agencies:** FAPERJ and CNPq.

09.022 The effects of hydroethanolic extract of *Smallanthus sonchifolius* leaves on the liver metabolic changes in streptozotocin-induced diabetic rats. Rocha BA[1], Baroni S[1], Comar JF[2], Caparroz-Assef SM[1], Silva MARCP[1], Suzuki-Kemmelmeier F[2], Bersani-Amado CA[1] [1]UEM – Pharmacology and Therapeutic, [2]UEM – Biochemistry

Introduction: The present study determined the anti-hyperglycemic effect of the hydroethanolic extract from S*mallanthus sonchifolius* leaves (yacon extract) on plasma glucose levels and some metabolic changes of STZ-induced diabetes in animals. **Methods:** Diabetes was induced in fasted rats with streptozotocin (STZ - 50 mg/kg ip) and after 12 days, the blood was collected. Rats with glycemia above 300 mg/dL were included in the study. The extract of yacon (400 mg/kg bw) or water was administered by gavage in diabetic and nondiabetic rats for 30 days. At the end of experimental time, plasma glucose levels and activity of glucose-6-phosphate dehydrogenase, AST and ALT was measured. The isolated liver was perfused with Krebs/Henseleit-bicarbonate buffer (pH 7.4), saturated with O_2 and CO_2 (95:5) by means of a membrane oxygenator and heated to 37^{o}C. **Results:** The groups of normal rats treated with the extract did not show a significant change in glycemia (NY = 105.5 ± 4.1 mg/dL) compared to the normal group (N = 91.0 ± 2.7 mg/dL). The glycemia of diabetic rats was 160.9 ± 11.7 mg/dL, i.e., 76% higher than normal rats. Treatment of diabetic animals with the extract significantly reduced the glycemia of diabetic rats (DY = 126.2 ± 7.4). Glucose released from the normal livers was small and almost constant over the 60-minute perfusion time. Livers from diabetic animals, however, initially showed very high rates of glucose release, with a diabetes/ normal ratio equal to 19.3 at time zero. These high rates diminished progressively during the subsequent time and the diabetes/normal ratio was reduced to 2.6 at 60 minutes perfusion time. This decrease most likely represents the progressive reduction of glycogen stores as the result of glycogenolysis. Diabetic rats treated with yacon extract showed reduced glucose release compared to diabetic rats; the treated diabetes/ normal ratio was equal to 13.42 at time zero and 1.7 at 60 minutes perfusion time. The total rate of glucose released, as well as reduced area under the curve of the diabetic treated rats was significantly lower throughout the test. Liver glucose-6-phosphate dehydrogenase activity was decreased in diabetic rats compared to normal rats ($P<0.05$). The enzyme activity of diabetic rats treated with yacon extract was normalized. On the other hand liver AST and ALT activities were significantly elevated in diabetic rats in comparison to normal rats. After 30 days of treatment with yacon extract, there was a significant reduction in liver AST activity with respect to diabetic group, but these parameters were not recovered to the normal level ($P<0.05$). However, there was not a significant reduction in liver ALT activity in diabetic rats treated with the extract. **Conclusion:** This study has advanced our understanding of the beneficial effects of the yacon extract in this experimental model of diabetes, which may be related to: (a) reduced glucose release in substrate-free perfused liver, (b) decreased AST activity and (c) increased glucose-6-phosphate dehydrogenase activity. To our knowledge, this is the first study showing the beneficial effects of yacon extract on some metabolic changes of STZ-induced diabetes in animals. **Sources of research support:** CAPES/CnpQ; Fundação Araucária-PR.

09.023 Inhibitory effect of anethole on leukocyte migration in the microcirculation of spermatic fascia *in situ*. Estevão-Silva CF[1], Ritter AMV[1], Arruda LLM[1], Barbosa CB[1], Silva FMS[1], Kummer R[1], Perdigão TD[2], Cuman RKN[1], Bersani-Amado CA[1] [1]UEM – Pharmacology and Therapeutics, [2]UEL – f Health Sciences

Introduction: The anethole (AN) [1-methoxy-4-benzene-(1-propenyl)] is the major component of star anise essential oil (*Illiciumverum*) corresponding to 90% of the total essential oil. It appears to be responsible for most of the properties attributed to the oil. Studies have demonstrated that anethole exhibits antioxidant, antimicrobial, anti-inflammatory, analgesic, and anesthetic properties. The aim of this study was to evaluate the effect of anethole on leukocyte migration using a microcirculation model *in situ*. **Methods:** *In situ* Microcirculation Assay - This assay was performed in the internal spermatic fascia of the scrotum. The rats was treated orally with AN (62.5, 125, 250, 500 mg/kg body weight), indomethacin (5mg/kg), dexamethasone (0.5mg/kg) or saline solution 0.9% (negative control). The carrageenan suspension (100µg) was injected in the scrotum of the rats, 30 min after respective treatments. Two hours later, the animals were anesthetized with chloral hydrate (500 mg/kg body weight, subcutaneously). The number of leukocytes rolling and adhered to the endothelium was determined at 10 minutes intervals, two counts in different fields. In another series of experiments, it was determined the number of leukocytes that migrated to an area of 2500 μm^2 of connective tissue adjacent to post-capillary venules, 4 hours after injecting of carrageenan. The number of leukocytes was determined by an image of 4 different fields for each animal, and the average value was calculated. The protocol for this experiment was approved by the Committee on Ethics and Animal Experimentation of the State University of Maringá (CEAE/UEM 125-2010).Statistical analysis - **Results** were statistically analyzed using one-way analysis of variance (ANOVA) followed by Tukey's test. Statistical significance was set at $P \leq 0.05$. **Results**: In the intravital microscopy, the images obtained two hours after injecting carrageenan (100µg) in the scrotum showed a marked increase in the number of leukocytes rolling and leukocytes adherent to the vascular endothelium. The treatment with AN at doses of 125, 250 and 500 mg/kg led to a significant reduction ($p < 0.01$) in both rolling and adherent leukocytes, compared to the control group treated with saline. Similarly, the AN had significantly decreased ($p < 0.01$) leukocytes migration into the perivascular tissue. The dose of 250 mg/Kg, caused effect similar to controls indomethacin and dexamethasone. **Discussion:** The anethole had an inhibitory effect on leukocyte recruitment *in vivo*, reducing all stages of leukocyte recruitment, i.e., the rolling behavior, adhesion and migration, validating thus the previous data that showed an anti-inflammatory and anti-hypernociceptive activity of these compound. **Supported by:** CNPq; CAPES. **Acknowledgements**: Jaílson Araújo e Célia Miranda.

09.024 Protective effect of a hydroalcoholic extract of *Euterpe oleracea* Mart (açaí) on cardiovascular and metabolic changes induced by maternal protein restriction during pregnancy. Bem GF, Costa CA, Oliveira PRB, Cordeiro VCS, Carvalho LCRM, Souza AV, Vieira AB, Resende AC, Soares de Moura R UERJ – Farmacologia e Psicobiologia

Introduction: Epidemiological and experimental studies have suggested that nutritional changes during intrauterine development results in permanent alterations in the structure, physiology and metabolism. Our group demonstrated that the hydro-alcoholic extract of the seeds of açaí (ASE) rich in polyphenols induces vasodilator and antihypertensive effects in different models of experimental hypertension. Thus, the aim of this study was to evaluate the effect of gavage treatment with ASE (200mg/Kg/day), on the metabolic, morphological and oxidative stress alterations in adult sixteen-week-old offspring, whose mothers were fed a low protein (LP) diet during pregnancy. **Methods:** The experiments were approved by the Ethics Committee of Animal Experiments of the UERJ (protocol: CEA/024/2010). Four groups of rats were fed experimental diets: control (20% protein); control + ASE (20% protein + ASE); LP (6% protein); LP + ASE (6% protein + ASE) during pregnancy. After weaning, all male pups became to feed a control diet and were sacrificed at four months old. Systolic blood pressure (SBP) was measured by plethysmography and vasodilator effect of acetylcholine (ACh, 1-1000 pmol) was studied in perfused mesenteric arterial bed (MAB). Body weight, serum cholesterol, triglycerides, high-density lipoprotein (HDL), albumin, urea, creatinine, insulin and renin levels were determined by kit and insulin resistance (IR) was calculated by HOMA index. Oxidative damage, nitrite levels and antioxidant enzyme activity: superoxide dismutase, catalase and glutathione peroxidase were measured in plasma and kidney homogenate. The number of glomeruli in the kidney was also measured. **Results:** Body weight was lower in LP group and recovered by treatment with ASE. SBP and plasma renin levels were higher in LP animals and reversed by treatment with ASE. The vasodilator response to ACh (1-1000 pmol) was reduced in LP group and increased by treatment with ASE. LP group showed increased urea, creatinine, cholesterol, triglycerides, glucose, insulin levels and HOMA index which were reduced by ASE. LP group demonstrated decreased albumin levels wich was increased by ASE. There was no difference in HDL levels in both groups. Malondialdehyde and protein carbonyl levels were increased and nitrite levels were decreased in LP group. These results were reversed by treatment with ASE. Antioxidant enzymes activities in plasma and kidney were lower in LP group and increased by ASE treatment, except for catalase activity, which showed no significant difference between groups. LP group showed a reduction in the number of renal glomeruli and treatment with ASE prevented renal changes found in this model. **Discussion:** This study provides further support for the early gestational environment playing a critical role in programming later life metabolic disorders, hypertension and oxidative stress. The administration of ASE protected the adult offspring whose mother was exposed to a LP diet during pregnancy, from hypertension, endothelial dysfunction, hyperglycemia and oxidative stress. **Financial Support:** CNPq and FAPERJ.

09.025 Effect of estragole in experimental models of acute inflammatory response in rodents. Silva FMS, Arruda LLM, Ritter LMV, Estevão-Silva CF, Kummer R, Freitag AF, Damião MJ, Bersani-Amado CA, Cuman RKN UEM – Pharmacology and Therapeutics

Introduction: Estragole is a phenylpropanoid derivative that is largely used in the food and beverages industry as flavoring. The estragole demonstrated sedative, anticonvulsant and myorelaxant activities. In this study we evaluated the effect of estragole (EST), on acute inflammatory response in experimental models of inflammatory in rodents. **Methods:** Pleurisy was induced by injection of 0.25mL of a carrageenan suspension (200 µg) in the intrapleural cavity in rats treated with EST in a dose of 125, 250, 500 and 750 mg/Kg orally 30 minutes before irritant agent injection. Indomethacin (5 mg / kg) was used as reference drug. Four hours after, the animals were euthanized and the exudate of pleural cavity was collected and the volume of exudate and leucocytes number was determined. The topic inflammatory activity was evaluated and compared with croton oil-induced ear edema in mice. The animal's right ear were topically treated with EST (2,5 and 5,0 mg/ear), croton oil (CO) (5% v/v) and dexametasone (DEX)(0,1 mg/ear). All drugs were diluted or dissolved in acetone (vehicle). Four hours after, the ear weight (edema volume) and the myeloperoxidase (MPO) activity were evaluated. The MPO activity was evaluated in the supernatant of homogenates of the ear sections. The supernatant was added to a 96-wells microplate, followed by addition of a buffer solution containing o-dianisidine dihydrochloride and bidistilled water, potassium phosphate buffer and 1%H2O2. The enzyme activity was determined by measuring the optical absorbance (460 nm) ELISA reader. **Results** were statistically analyzed by using one-way variance analysis (ANOVA) followed by Tukey's test ($p<0.05$). The protocol for these experiments was approved by the Committee on Ethics and Animal Experimentation of the State University of Maringá (CEAE/UEM 126-2010). **Results:** Intrapleural injection of carrageenan induced an acute inflammatory response, characterized by an increase of the exudate volume and the intense cell migration to the pleural cavity. The oral administration of EST reduced significantly the inflammatory edema volume when compared to that of control group: **control**: 0.74±0.03 mL; **EST** $_{125\ mg}$0.59±0.05*mL; **EST** $_{250\ mg}$ 0.57±0.05*mL; **EST** $_{500\ mg}$ 0.57±0.05*mL; **EST** $_{750\ mg}$ 0.54±0.02*mL. However, the EST did not reduce the leukocyte migration into the pleural cavity similarly to that observed with indomethacin treatment. After tropically administration of CO or EST, an increased ear edema was observed when compared to that obtained in ear of mice of control group: **CO:** 0.015±0.002*; **EST$_{2,5mg}$:** 0.020±0.002*; **EST$_{5,0mg}$:**0.018±0.002*; **control:**0.01±0.003*. Mice ear edema was reduced after DEX treatment (0.01 ± 0.001*). An increased MPO activity was observed in EST (2,5 and 5,0mg) and CO groups (0.17±0.10*;0.28±0.14* and 0.20±0.11*; respectively) but not in the DEX treatment group (0.03±0.02*). **Conclusion**: Our study provides evidence that EST has an irritant effect when topically administrated and a systemic anti-inflammatory activity when orally administrated. **Supported by:** CNPq. **Acknowledgements**: Jaílson Araújo e Célia Miranda.

09.026 Vascular reactivity of *Mimosa caesalpiniifolia* Benth. (Fabaceae) in mesenteric rings arteries. Moura LHP[1], Campelo RT[1], Nunes AF[1], Sabino CKB[1], Silva-Filho JC[1], Monção NBN[2], Citó AMGL[2], Oliveira RCM[1], Arcanjo DDR[1], Oliveira AP[1] [1]UFPI – Plantas Medicinais, [2]UFPI – Química

Introduction: The species *Mimosa caesalpiniifolia* Benth (Fabaceae) popularly known as sansão-do-campo, unha-de-gato or sabiá, is a species found in caatinga region in Brazil. The bark and flowers of this species are commonly used to inflammations in general and hypertension (De Albuquerque, *J. Ethnopharmacol*.**,** v.114, p.325, 2007). **Methods:** Male Wistar rats (270 ± 30 g) were used for all experiments (Animal Research Ethics Committee/UFPI CEEAPI 076/2010). After euthanasia procedure, the superior mesenteric artery was removed and cleaned from the connective tissue and fat. Mesenteric rings (1 to 2 mm) were obtained and suspended by cotton threads in organ baths containing 10 ml of Tyrode's solution, at 37°C and gassed with carbogenic mixture (95% O_2 and 5% CO_2). The isometric tension was recorded by a force transducer coupled to a data acquisition system (AECAD 1604, AQCAD 2.8.0., AVS Projects, SP, Brazil). The endothelium integrity was verified by relaxation to acetylcholine (10 µmol/L) in rings pre-contracted by phenylephrine (10 µmol/L) described by Oliveira et al. (Oliveira A P, Vascul. Pharmacol., 44; 338, 2006). All values were expressed as mean ± S.E.M. Student's t-test and ANOVA-one way Newman-Keuls post-test were used in the data analysis and results were considered significant when $p<0.05$ (GraphPad™ Prism 5.0). **Results and Discussion:** Cumulative addition of ethanolic extract of the flowers *Mimosa caesalpiniifolia* (Mc-Fl 0.1 - 750 µg/mL) induced concentration-dependent relaxation in preparations with and without vascular endothelium, (pD_2 = 2.16 ± 0.05; pD_2 = 2.01 ± 0.06 µg/mL, E^+ and E^-, respectively; n=5). A similar effect was obtained in preparations pre-contracted with KCl 80 mM (pD_2 = 2.06 ± 0.04; pD_2 = 2.06 ± 0.04, E^+ and E^- , respectively; n=6). Moreover, the Mc-Fl (9, 81 and 500 µg/mL) inhibited contractions induced by cumulative addition of phenylephrine (10^{-9} – 10^{-5} M) in a concentration-dependent manner (E_{max}: Control = 100 ± 0.00; E_{max}: 9 µg/ml = 98.5 ± 2.1%; E_{max}: 81 µg/ml = 77.8 ± 4.7 %***; E_{max}: 500 µg/ml = 28.2 ± 2.7%***; ***$p<0.05$ vs control n=5). Mc-Fl (243, 500 and 750 µg/mL) also inhibited contractions induced by a cumulative addition of $CaCl_2$ (10^{-6} – 3 x 10^{-2} M) in KCl 60 mM depolarizing Tyrode solution without Ca^{2+} in a concentration-dependent manner (E_{max}: Control = 100.00 ± 0.00; E_{max}: 243 µg/ml = 89.6 ± 8.6 % E_{max}: 500 µg/ml = 45. 4 ± 3.7 %*** and E_{max}: 750 µg/ml = 24.3 ± 4. 6%***; ***$p<0.05$ vs control n=5). The extract of flowers of *Mimosa caesalpiniifolia* promotes vasodilation which can be lead to a reduction in blood pressure. **Financial Support:** This work was supported by UFPI/FAPEPI/CNPq.

09.027 Study of inflammatory and myotoxic effects of *Bothrops* venoms: Effects of dexamethasone and *Eclipta prostrata* (L.). Patrão-Neto FC[1], Tomaz MA[2], Machado MM[1], Rocha-Júnior JR[2], Camilo RL[2], Melo PA[1] [1]UFRJ – Farmacologia e Química Medicinal, [2]UFRJ – Farmacologia das Toxinas

Introduction: We investigated the toxic activities from *Bothrops* genus snake venom using *in vivo* and *in vitro* experimental protocols in mice muscle and tested the protector effect of dexamethasone (DEXA) in different conditions, comparing it with the polyvalent antivenom. We also expanded the investigations on the antiophidic effect of *Eclipta prostrata* (EP) crude extract. **Methods:** *In vivo* experiments were performed in mice **(licence CEUA-UFRJ DFBCICB 023)** with *Bothrops jararaca* and *Bothrops jararacussu* snake venom. We quantified the increase of plasma creatine kinase (CK) activity as well as the CK content in the *Extensor digitorum longus* (EDL) of these animals. We measured the edema and inflammatory response evaluated by the presence of inflammatory cells at the inoculation site when we administrated *B. jararacussu* venom (1.0 mg/Kg). *In vitro* we determined the increase of the rate of CK release from the isolated EDL mouse muscle perfused with appropriated nutrition solution. We also observed the amplitude of the indirect evoked twitch-tension at the isolated mouse phrenic-diaphragm preparation. Data were analyzed statistically by the Student t-test and ANOVA. **Results:** Treatment with DEXA (1.0 mg/Kg) preserved over 50 % of the muscle CK content *in vivo* when evaluated 24 and 72 hours after the injection of *B. jararacussu* venom, and likewise decreased about 20 % of the edema induced by this venom. DEXA reduced in 50 % the presence of inflammatory cells in the muscle. The EP extract (50 mg/Kg) showed antagonized the edema and preserved the muscle CK content, and its association with DEXA showed an additive effect. EP also antagonized the increase of plasma CK activity induced by the *B. jararacussu* venom in 77 %. The association of DEXA with polyvalent antivenom did not show additive or benefic effect. On the *in vitro* experiments, DEXA did not show ability to antagonize the increase of the rate of CK release from the muscles exposed to 25.0 µg/mL of *B. jararacussu* venom, neither to prevent the fall on amplitude of the indirect evoked twitch-tension at the isolated phrenic diaphragm preparation, while the EP extract showed a 100 % protection at concentrations of 50 and 100 µg/mL. **Discussion:** Our results are showing that DEXA was able *in vivo* to decrease the inflammatory response and did not show any protective effect *in vitro*. Otherwise the inflammatory responses were almost completely neutralized by EP. **Conclusion:** Our data together are demonstrating that the inflammation is an important element to be neutralized on the envenomation by snake venoms. **Support:** FAPERJ, CNPq, PRONEX. Key words: *Bothrops* venom, dexamethasone, myotoxicity, inflammation, *Eclipta prostrata*.

09.028 Evaluation of antiobesity effect of ferulic acid in mice submitted to hypercaloric diet. Holanda RTM[1], Melo TS[1], Lima PR[2], Carvalho KMMB[2], Morais TMF[1], Rodrigues HG[1], Melo CL[1], Santos FA[2], Rao VS[2], Queiroz MGR[1] [1]UFC – Análises Clínicas e Toxicológicas, [2]UFC – Fisiologia e Farmacologia

Introduction: Obesity can be defined as the degree of fat storage in the body associated with health risks due to its relationship with several metabolic complications. Studies suggest that ferulic acid (FA) is a compound having therapeutic potential for treating obesity. **Objective:** To evaluate the possible hypoglycemic, hypolipidemic and antiobesity FA effects in animals fed with hypercaloric diet. **Methods:** Mice male Swiss (25-35g) were divided into 4 groups (n = 10), according to the average weight gain for 15 weeks and submitted to: standard diet (SD), hypercaloric diet (HD), HD + FA; HD + sibutramine (SIB). FA was prepared at a dose of 25 mg/L and SIB at a dose of 50mg/L being given "ad libitum". FA or SIB treatment was carried out simultaneous to obesity induction. After 15 weeks, the animal's weight and abdominal fat deposition (AFD) quantity were measured and blood samples were collected to determination of glucose (GLU), total cholesterol (TC) and triglyceride (TG). The protocol described was submitted to the Ethics Committee on Animal Research at the Universidade Federal do Ceará being approved under the number 34/2011. **Results** were expressed as mean ± S.E.M and analyzed by Anova and Newman-Keuls test, considering significant $p < 0.05$. **Results:** Both FA and SIB reduced significantly animal weight (g) (SD: 42.80 ± 0.7, HD: 48.75 ± 1.1; HD + FA: 43.29 ± 1.3, HD + SIB: 41.89 ± 0.8) and AFD rate (mg/10g weight) (SD: 16.46 ± 2.0, HD: 52.00 ± 6.8, HD + FA: 30.94 ± 7.8; HD + SIB: 32.88 ± 3.1). FA and SIB decreased significantly TC plasma levels (mg/dL) (SD: 121.8 ± 3.8, HD: 206.6 ± 13.3, HD + FA: 147.9 ± 8.2; HD + SIB: 171.4 ± 6.3) and TG levels (mg/dL) (SD: 110.2 ± 11.1; HD: 207.3 ± 26.8; HD + FA: 128.7 ± 17.1; HD +SIB: 106.0 ± 6.9). Only FA was capable to reduce GLI plasma levels (mg/dL) (SD: 117.9 ± 8.5, HD: 209.8 ± 16.5; HD + FA: 120.0 ± 5.7, HD + SIB: 178.6 ± 18.3). **Discussion:** The HD provided a final energy content of 21.40 kJ/g while the SD 17.03 kJ/g (Estadella, Nutrition, v.20, p.218, 2004). Studies show the close relationship of abdominal adiposity with glucose intolerance, hyperinsulinemia, hypertriglyceridemia and hypertension (Ribeiro Filho, *Arq Bras Endocrinol Metab*., v.50, p.230, 2006). FA may have reduced the insulin resistance, providing a reduction of the GLU values and correcting the lipid metabolic disorder of TC and TG. The FA has therapeutical potential anti-obesity and comorbidities associated such as diabetes and dyslipidemia. Further studies are needed to evaluate the possibility of this substance to become a therapeutic alternative for the obesity treatment. **Financial support:** CNPq and CAPES.

09.029 Cysteine proteases from *Vasconcellea cundinamarcensis* have antimetastatic activity in colon carcinoma by death and loss of cell adhesion. Dittz D[1], Diniz MLL[1], Viana CTR[2], Salas CE[3], Lopes MTP[1] [1]UFMG – Farmacologia, [2]UFMG – Fisiologia e Biofísica, [3]UFMG – Bioquímica

Introduction: P1G10 is a fraction rich in cysteine proteases, obtained from *Vasconcellea cundinamarcensis*' latex after chromatography on Sephadex G10 resin. The fraction exerts antitumor activity on different animal models, including colon carcinoma. The aim of this study is to evaluate the antimetastatic activity of P1G10 in murine colon carcinoma model, as well as their cytotoxic and profiles in cell adhesion, DNA integrity as part of the mechanism of action. **Methods:** Balb/C mice received CT26.WT cells ($5x10^4$ cells / animal), in the spleen. After 24 hours, the treatment began with P1G10, daily, at doses of 1, 5 mg/kg or saline (control), s. c. for 15 days (CETEA No 103/2007). Then, the animals were sacrificed and the number of metastatic points was determined in lung. The cell cytotoxicity was measured by the MTT assay. Cells were seeded in 96 well plates and exposed to P1G10 (0.1 to 1000 µg/mL) for 72 h and cell viability determined at 530 nm. The ability of P1G10 (0.1 to 100 µg/mL) to inhibit cell adhesion was evaluated in the presence or absence of extracellular matrix components, ECM, (Cytomatrix, Millipore). Cells that have lost adhesion in plates without substrate were evaluated for viability by the Resazurin method. DNA fragmentation of non adherent cells was assayed by labeling with propidium iodide and flow cytometry analysis. **Results and Discussion:** P1G10 5 mg/kg induced a reduction of approximately 75% in the number of metastatic points (6.4 ± 1.6, n = 8) compared to control group (28.5 ± 5.4). In CT26.WT and normal epithelial cells, BHK-21 and CHO, the inhibitory concentration for 50% of cell population (IC-50) for P1G10 was respectively 20, 28 and 52 µg/mL. The loss of adhesion to ECM was observed for P1G10 50 µg/mL and maximum effect for P1G10 100 µg/mL, after exposure for 2 hours. On plates without substrate, the loss of adhesion was observed after 15 min, 24 and 48 hours at concentrations of P1G10 50, 25 and 10 µg/mL, respectively. In all conditions, the cells that had lost the adhesion, remained viable in the supernatant, with levels of DNA fragmentation similar to control (1.66% of sub-diploid DNA). A similar increase on DNA fragmentation was observed for P1G10 50 or 100 µg/mL after 24 hours of exposure (24%, $p<0.05$, ANOVA Bonferroni's *post-test*), 2 folds higher than observed in control group. Thus, it was observed that P1G10 shows antimetastatic activity in murine colon carcinoma. **Results** suggest that part of this effect is associated with the loss of cell adhesion and subsequent induction of cell death. **Financial Support**: CNPq, FAPEMIG and CAPES

09.030 Vascular relaxation induced by the ethanolic extract of *Tapirira Guianensis* Aubl (Anacardiaceae) in the rat aorta. Vidal MC, Ferreira LLDM, Rodrigues AMG, Paes BM, Muzitano MF, Raimundo JM, Konno TUP, Carmo PL UFRJ

Introduction: The Restinga of Jurubatiba National Park (PARNA Jurubatiba), located in the northern Rio de Janeiro state, protects a coastal region of great diversity of habitats and flora. Studies on the pharmacological activities of the plant species present on PARNA Jurubatiba are scarce, including *Tapiria guianensis*, popularly known as pau-pombo. Considering the impact of cardiovascular diseases on global public health and the presence of functional and structural vascular changes in many of these diseases, this study aimed to investigate the effects of ethanol extract of leaves of *Tapirira guianensis* (ETG) on vascular smooth muscle and its mechanisms of action. **Methods:** ETG was examined for its vascular relaxant effect in isolated Wistar rat (220-280 g) thoracic aorta prepared for isometric tension recording. Aortic rings were placed in vertical chambers filled with Krebs-Henseilet solution continuously oxygenated with carbogen gas at 37°C. Contractile response to phenylephrine (10 μM) was measured before and after exposure of aortic rings to increasing concentrations of ETG (1–100 μg/ml). It was tested in aorta with and without endothelium, which was considered intact if acetylcholine-induced relaxation of pre-contracted aorta was greater than 80%. Mechanical removal of endothelium was confirmed by the lack of relaxation in response to acetylcholine (10 μM). Also, some endothelium-intact aortic rings were pretreated with Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME, 100 μM). All protocols were approved by the Animal Care and Use Committee under license Macaé01. **Results:** ETG induced relaxation of the phenylephrine-contracted aortic rings in a concentration-dependent manner, with maximal relaxation, 73.38 ± 5.22%, observed at 10 μg/ml ($P<0.05$; $n=6$). The concentration of ETG necessary to reduce phenylephrine-induced contraction of endothelium-intact aorta by 50% was 1.01 ± 0.12 μg/ml. Endothelium-denudation abolished the ETG-induced vasorelaxation, as well as the pretreatment of endothelium-intact aortic rings with L-NAME, an inhibitor of nitric oxide synthase. **Discussion:** These findings suggest that ETG-induced vascular relaxation in rat aorta is dependent on endothelium and involves the nitric oxide signaling pathway. **Financial agencies:** FAPERJ, FUNEMAC, UFRJ.

09.031 Effect of kaurenoic acid on ovalbumin-induced asthma in mice. Domiciano TP[1], Arakawa NS[2], Ambrósio SR[3], Casagrande R[2], Verri Jr WA[1] [1]UEL – Ciências Patológicas, [2]UEL – Ciências Farmacêuticas, [3]Unifran – Ciências Exatas e Tecnológicas

Introduction Kaurenoic acid (KA) (*ent*-kaur-16-*en*-19-oic acid) is a diterpene and the major compound in *Sphagneticola trilobata* (*Wedelia paludosa*, *Acmella brasiliensis*, Asteraceae), which is popularly known in Brazil as Arnica-do-mato, pseudo-arnica, picão-da-praia and vedélia. KA is traditionally used as a medicine for rheumatic inflammatory diseases and fever. Studies have shown that KA presents several biological effects as vasorelaxant, bactericide and anti-inflammatory. Furthermore, in a model of asthma in guinea pigs, KA inhibited the ovalbumin challenge-induced airway resistance in immunized animals as well as the production of histamine and activity of phospholipase A_2. Bronchial asthma is a chronic inflammatory disease of the airway that involves multiple components and is governed by a variety of cell types, including lymphocytes, mast cells and eosinophils. Th2 lymphocytes play a key role in the regulation of this process through the release of cytokines such as IL-4, IL-5. In guinea pigs, asthma inflammation does not depend on cytokine production. The present study examined the effects of KA on leukocyte infiltration and the expression of Th2 cytokine in ovalbumin sensitized mice. **Methods:** The experimental protocol was approved by the Ethics Committee and Animal Experimentation (process number 9776.2012.93). Male Balb/c mice, weighing 30 to 40g, with 6 animals per group were sensitized by an intraperitoneal injection of 100µg of ovalbumin (OVA) in 1mg of alum on days 0 and 7. The challenge was made with saline solution nebulization of OVA (10mg/ml) through inhalation for 30 minutes using an ultrasonic nebulizer on days 21 and 23. Mice received KA, per oral, treatment (0.1, 1 and 10 mg/kg) or vehicle (2% DMSO in saline) 1 hour prior challenge and every 24 hours after. Mice were sacrificed 24 hours after the last challenge and the bronchoalveolar lavage fluid (balf) was obtained by washing the lung with 1 ml PBS through cannulation of the trachea. The number of total leukocytes, mononuclear and polymorphonuclear cells present in balf was determined by optical microscope. The levels of IL-4 and IL-5 were determined in the balf by ELISA. **Results and Discussion** OVA total leukocytes and eosinophils numbers were significantly elevated compared to saline group. It was observed that KA treatment at the dose of 10mg/Kg significant decreased the total leukocyte numbers in balf by 52%, when compared to OVA group. In OVA group, most leukocytes were eosinophils. A significant decrease in the number of eosinophils was also observed in the KA treated groups ($KA_{0.1}$=71%; KA_1=69%; KA_{10}=73% $P<0.5$). OVA also induced a significant cytokine elevation compared with saline group. Cytokine levels of IL-4 (%), IL-5 (%) were significantly lower in the KA group treated at the dose of 10mg/kg compared to OVA group. These results indicate that oral administration of KA resulted in reduced Th2 cytokine release in balf and leukocyte recruitment. Therefore, suggesting the oral treatment with KA could be used to reduce allergic asthma inflammation. **Financial Support:** SETI/Fundação Araucária and Governo do Estado do Paraná.

09.032 Comparative study of the in vitro cytotoxic activity of two rear-fanged snake venoms against 3T3 fibroblasts. Peichoto ME[1,2,3], Tavares FL[2,4], Jones SWL[2], DeKrey G[2], Mackessy SP[2] [1]INMet [2]University of Northern Colorado – Biological Sciences, [3]UNNE – Ciências Veterinárias, [4]UDC – Veterinária

Introduction: Relatively few studies have investigated the composition and biological activities of the venoms of rear-fanged snakes. Comparative analyses of these venoms may provide a better understanding of their toxicity and may reveal novel compounds with biomedical applications. In this study, we conducted a comparative analysis of the cytotoxic activity against 3T3 fibroblast cell line of the venom of the South American rear-fanged snakes *Philodryas patagoniensis* (PpV), and the venom of the North American rear-fanged snake *Trimorphodon biscutatus lambda* (TblV). Taking into account that one of the most significant differences between both opisthoglyphous "colubrid" snake venoms tested is the presence of phospholipase A_2 (PLA_2) activity in TblV only (Peichoto et al., Toxicol. Lett. 196S, S347), we also investigated the cytotoxicity induced by trimorphin, a PLA_2 from this venom. **Methods:** Both venoms were dissolved in PBS (pH 7.4) and filtered through a Millipore filter (0.22 µm). Trimorphin from TblV was purified in two chromatographic steps: size exclusion chromatography on a TSKgel G2000 SWXL column (TOSOH Bioscience LLC, Tokyo, Japan) and reversed-phase chromatography on a Protein and Peptide C_{18} column (VYDAC, Hesperia, CA, USA), using a previously described procedure (Peichoto et al., *Toxicon* 58, 28, 2011). In order to verify their possible cytotoxic effects, both venoms and the trimorphin fraction were incubated in a culture of 3T3 fibroblasts for the time of 72 hours. Cell proliferation/viability was determined colorimetrically by the MTT (bromuro de 3-(4,5-dimetiltiazol-2-yl)-2,5-difeniltetrazolio) assay, according to the manufacturer's instructions (ATCC, Manassas, VA, USA). **Results**: Both crude venoms induced cytotoxic activity on 3T3 fibroblasts. PpV induced a dose-dependent response, producing 96.6 ± 5.1% cytotoxicity at a concentration of 12 µg/mL. However, TbLV showed a more complex response, exhibiting an intriguing hormetic effect. Thus, TbLV at a concentration of 24 µg/mL induced cytotoxic activity (96.6 ± 6.3%), but lower concentrations stimulated proliferation of fibroblasts. Trimorphin from TbLV produced a hormetic dose-response effect comparable to the whole venom, inducing a cytotoxic activity of 87.3 ± 9.7% at a concentration of 5 µg/mL. For this reason, this isolated protein may be considered as the main responsible for the hormetic cytotoxicity induced by the whole venom. **Discussion:** This is the first report showing the *in vitro* effect of rear-fanged snake venoms on fibroblast proliferation. These findings may help to understand the local tissue damage induced by these venoms, which is characterized by a complex superposition of pathophysiologic phenomena, such as hemorrhage, inflammation and repair. Moreover, trimorphin may be considered as a potential tool to study the hormetic effect of cell death and proliferation. **Financial Support**: A postdoctoral fellowship for MEP by the Fulbright Commission and CONICET is gratefully acknowledged. Additional financial support was provided by CONICET (PIP 114-200801-00088, to MEP) and ANPCyT (PICT-2010-1908, to MEP) from Argentina, and by a Bioscience Discovery Evaluation Grant from the Colorado Office of Economic Development and International Trade (to SPM).

09.033 Chemical composition and cytotoxic activity of sap essential oil from two *Mangifera indica* L. fruits varieties. Ramos EHS[1], Moraes MM[2], Militão GCG[3], Câmara CAG[2], Silva TG[1] [1]UFPE – Antibióticos, [2]UFRPE – Ciências Moleculares, [3]UFPE – Fisiologia e Farmacologia

Introduction*: Mangifera indica* L. is a perennial arboreal tree belonging to the family Anacardiaceae. Originally native from southeastern of Asia, this plant had been domesticated for centuries before spreading to other parts of the tropical world. In Brazil is known commonly as *manga* and it was introduced first in Northeast Regions by Portuguese at eighteenth century. Two varieties *manga-espada* and *manga-rosa* are the most common cultivated in Pernambuco. The aim of the present study was to describe the chemical composition of the essential oil obtained from sap fruits of two *M. indica* varieties and its cytotoxicity against four cancer cell lines. **Methods:** Essential oils were obtained by exsudate hidrodistillation from *Mangifera indica* var. rosa and espada originated from UFRPE – Brazil Campus, and analyzed by GC-FID and GC-MS. Antiproliferative activity was evaluated on HEp2 cell line (human larynx carcinoma), HT-29 (human colon adenocarcinoma), NCI-H292 cell line (human lung carcinoma) and HL-60 (human leukemia cells) after 72h on treatment by MTT assay. **Results and Discussion:** Twenty-seven components were indentified in the essentials oils. The main compounds obtained from the espada variety sap essential oil was terpinolene (73.6±0.2%) followed by minor amount of δ-3-carene (5.7±0.0%). While the sap essential oil of the rosa variety was characterized by a high amount of *β*-pinene (40.7±0.3%), terpinolene (28.3±0,1%) and *α*-pinene (11.5±0.1%). Essential oils were evaluated for their cytotoxic activities against four cancer cell lines, HEp2 cell line (human larynx carcinoma), HT-29 (human colon adenocarcinoma), NCI-H292 cell line (human lung carcinoma) and HL-60 (human leukemia cells) by MTT method. Variety rosa essential oil was more effective against HL-60 cell line (IC_{50} = 11.6 µg/mL), followed by Hep2 (IC_{50} = 24 µg/mL), NCI-292 and HT29 (both with IC_{50} = 28.9 µg/mL). Espada variety was effective against all cell lines (IC50 ranging between 2.9 and 7.7 µg/mL for HL60, HT29 and Hep2) and IC_{50} of 12.3 µg/mL for NCI 292. Our results demonstrated that the two oils tested inhibited the tumor cell growth being variety Espada sap essential oil the most active. The results obtained in this study can be considered as starting point for further research to identify the compounds responsible for the biological activities. **Financial support:** CNPq, UFPE and CAPES.

09.034 Pharmacological characterization of the leaves, stems and roots of *Coriandrum sativum* L. (coriander). Begnami AF[1], Ruiz ALTG[2], Carvalho JE[2], Rehder VLG[2] [1]FOP-Unicamp, [2]Unicamp – CPQBA

Introduction: The discovery of drugs derived from medicinal plants has played na extremely important role in the treatment of cancer, and currently 50% the chemotherapy come from source natural. *Coriandrum sativum* L. (Umbelliferae/Apiaceae) popularly known as coriander is used in the manufacturer of spice, cosmetics, perfumery and also in folk medicine as an anxiolytic, diuretic, carminative and moderator of appetite. A previous study of the leaves of this species showed potential antiproliferative activity against cell lines, which motivated us to study the other parts of the plant. The aim of this study was to evaluate the antiproliferative activity and perform a phytochemical screening of the leaves, stems and roots, fresh and dried of coriander. **Methods:** *Coriandrum sativum* was purchased in regional trade of Campinas (CEASA), leaves, stems and roots were separated and a fresh portion was reserved while another portion was subjected to drying in na oven at 40°C, and both extracted with dichloromethane, yielding extracts of fresh leaves (EDFf), fresh stalks (EDTf) and fresh roots (EDRf). And dried extracts of leaves (EDF), stalks (EDT) and dried roots (EDR). All the extracts were subject to test *in vitro* antiproliferative activity against nine human tumors cells line: melanoma (UACC-62), breast (MCF-7), resistant ovary (NCI-ADR/RES), kidney (786-0), lung (NCI-H460), prostate (PC-3), ovarian (OVCAR-03), colon (HT-29) and leukemia (562). The extracts were tested at concentration of 0.25-250µg/mL, using Doxorrubicin as positive control[1]. **Results and Discussion:** Among the extracts tested, the comparative assessment of the results revealed a similarity between the profiles *in vitro* antiproliferative, highlighting the EDTf that showed selectivity against all the strains studied. Thus, the study showed that besides the leaves, other plant parts such as stalks and roots also exhibit antiproliferative activity and may be used as alternative source in phytochemicals process. Bibliographic citation: [1] Shoemaker, RH. Nat. Rev. Cancer, 6,813, 2006. **Acknowledgments**: CAPES.

09.035 The comparison of hypolipidemic potential of aqueous suspension and hydroalcoholic extract of *Passiflora edulis* in mice with hyperlipidemia induced by triton WR-1339. Oliveira GP[1], Neto JNFG[1], Ferreira JM[1], Sousa DF[2], Meneses RRC[1], Oliveira KS[1], Holanda RTM[1], Rodrigues HG[1], Lemos TLG[3], Queiroz MGR[1] [1]UFC – Análises Clínicas e Toxicológicas, [2]UFC – Fisiologia e Farmacologia, [3]UFC – Química Orgânica e Inorgânica

Introduction: Nearby 80% the world population utilizes preferentially, the traditional medicine on primary health care. Ingestion of dietetic fibers found in fruit and legumes, is an alternative for reduction of risk factors for cardiovascular disease (Callegaro, *Cienc. Tecnol. Aliment*., v.25, p.271, 2005), including dyslipidemias. The flour obtained of the *Passiflora edulis* (Family *Passifloraceae*) peel fruit and other preparations have been used to treat dyslipidemia, although scientific studies are needed to confirm this effect. **Objective:** To compare the possible effect hypolipidemic between aqueous suspension (ASPE) and hydroalcoholic extract (HEPE) of *Passiflora edulis* peel fruit in mice with hyperlipidemia induced for Triton WR-1339. **Methods:** The *Passiflora edulis* peel fruit (rich in pectin) obtained in a particular plantation in Fortaleza - Ceará - Brazil was dried and powdered. After, this material was used to prepare ASPE and HEPE. Male Swiss mice (25-35g) were divided (n=6) in: negative control (NC), Triton (Triton 400mg/kg), GEMF100 (triton + gemfibrozil100mg/kg), ASPE100 (triton + ASPE100mg/kg), ASPE200 (triton + ASPE200mg/kg), HEPE100 (triton + HEPE100mg/kg), HEPE200 (triton + HEPE200mg/kg). In the study of hypolipidemic effect of ASPE and HEPE were done two distinct experiments. The hyperlipidemia was induced for a single administration i.p. of Triton 400mg/kg in all animals, except the NC. The animals were treated with saline, GEMF, ASPE or HEPE, by oral way, three times, being an 1h before, 22h and 46h after Triton injection. On 24h and 48h after the dyslipidemia induction, the blood was collected to determine levels of total cholesterol (TC) and triglycerides (TG). The protocol was approved by Ethics Committee on Animal Research of UFC under number 20/08. The results were expressed in mean ± S.E.M. and the groups were compared for ANOVA and Newman-Keuls post-test (significance criterion $p<0.05$). **Results:** GEMF100 and ASPE100 reduced the TG (mg/dL) on 24h: (Triton: 5725.0±136.2; ASPE100: 4068.0±399.0; ASPE200: 5344.0±82.9; GEMF: 3725.0±42.3) and on 48h (Triton: 1958.0±178.9; ASPE100:1387.0±105.0; ASPE200:1908.0±182.5; GEMF: 1229.0±164.5). On 24h only GEMF100 reduced TC (mg/dL) levels: (Triton:529.0±17.9; ASPE100: 482.3±30.1; ASPE200: 527.2±19.6; GEMF:438.0±23.9), while on 48h the decrease was with GEMF100 and ASPE100 (Triton:419.3±17.7; ASPE100:351.0±17.4; ASPE200:466.0±37.5; GEMF:294.3±15.7). The HEPE was able to promote a decrease on values of TC only after 48h, don't verifying effects over the TG. **Discussion:** The triton is a surfactant that can promote hyperlipidemia in experimental animals. The found resulted with the ASPE could be because soluble pectin present on passion fruit bark. On experimental evidences has demonstrated that soluble fibers ingestion delayed the gastric emptying (Chandalia, *New Engl. J. Med*., v.342, p.1392, 2000), promoting satiety and reducing carbohydrates and lipids absorption. The absence of effects of HEPE can be because incapacity of hydroalcoholic solution to extract the pectins. More studies are necessary for elucidation the exact mechanism of action of constituents *Passiflora edulis* bark on lipid metabolism. **Financial support**: FUNCAP

09.036 Toxins from the spider *Phoneutria nigriventer* inhibit nociceptive and inflammatory responses in the mouse model of hemorrhagic cystitis induced by cyclophosphamide. Silva RBM[1], Sperotto NDM[2], de Souza AH[3], Gomez MV[3], Morrone FB[4], Campos MM[1] [1]PUCRS – Medicina e Ciências da Saúde / Toxicologia e Farmacologia, [2]PUCRS – Farmácia, [3]UFMG – Neurociências, [4]PUCRS – Biologia Celular e Molecular – Farmacologia Aplicada

Introduction: Hemorrhagic cystitis (HC) is an inflammatory process of the urinary bladder, allied to diffuse bleeding and pain, representing one of the most challenging clinical issues for the urologists. Main causes of HC include the use of chemotherapeutic agents such as cyclophosphamide (CPA) (Decker, *J Pediatr Urol*. Aug;5(4):254, 2009). Venom peptides are exceptional sources for drug development to treat inflammation and pain. This study investigated the anti-inflammatory and antinociceptive effects of Tx3-3 and Tx3-6, two peptide toxins isolated from *Phoneutria nigriventer* venom, which inhibits P/Q- and N-type high-voltage-dependent calcium channels (VDCCs), respectively (Leão, *Neuropharmacol*. Jul;39(10):1756, 2000). **Methods:** Male Swiss mice (25 to 30 g) were used. HC was induced by a single administration of CPA (300 mg/kg, i.p.). Immediately after, mice were housed in individual plastic cages to observe the spontaneous behavior for 4 h, for 2 min, every half-hour. In addition, visceral pain behavior of mice was scored according to the following scale: 0 = normal; 1 = piloerection; 2 = strong piloerection; 3 = labored breathing; 4 = abdomen licking; and 5 = abdomen stretching and contractions (Martins, *Br J Pharmacol*. Jan;165(1):183, 2012). The animals were euthanized 6 h following CPA administration and we performed the gross examination of bladders, to determine the presence of edema and hemorrhage. The wet weight of bladders was also registered at this time-point. The treatment with the reference compound Mesna (60 mg/kg, i.p.) was given 30 min prior CPA. The toxins Tx3-3 (10, 30 and 50 pmol/site) and Tx3-6 (100 pmol/site) were administered intrathecally 2 h post-CFA injection. Statistical evaluation was performed using analysis of variance (ANOVA) followed by Newman-Keuls post-hoc test. Values of $P< 0.05$ were considered as indicative of significance. All the experimental procedures were approved by the Local Ethics Committee (08/00074, CEUA, PUCRS). **Results:** The intrathecal administration of Tx3-3 (10, 30 and 50 pmol/site) and Tx3-6 (100 pmol/site) produced a significant inhibition of the nociceptive behavior evoked by CPA (25 ± 9%, 38 ± 3%, 68 ± 6% and 18 ± 4, respectively). Interestingly, Tx3-3 (30 and 50 pmol/site) was able to visibly reduce the edema, the hemorrhage and the bladder wet weight following the application of CPA. **Discussion:** Our results show that pharmacological blockade of both P/Q- and N-type CCVDs attenuates CPA-induced HC and the above-mentioned bladder inflammatory alterations. The blockage of P/Q and N-type CCVDs by Tx3-3 and Tx3-6, respectively, might represent valuable strategies to control the pathogenic alterations of HC. **Financial Support:** PRPPG/PUCRS, CAPES-AUX-PE *Toxinologia*, CNPq and FINEP/PUCRSINFRA #01.11.0014-00.

09.037 Hypolipidemic potential evaluation of cinnamic acid esters isolated from carnauba wax in dyslipidemia induced by triton WR-1339; Meneses RRC[1], Arruda-Filho ACV[1], Melo TS[1], Ferreira JM[1], Damasceno DV[1], Pereira NBS[1], Sousa DF[2], Queiroz MGR[1], Vieira IGP[3], Guedes MIF[4] [1]UFC – Análises Clínicas e Toxicológicas, [2]UFC – Fisiologia e Farmacologia, [3]PADETEC-UFC, [4]UECE – Nutrição

Introduction: The mixture of cinnamic acid esters (CAE) has been isolated from carnauba wax. These esters are structurally similar to gamma oryzanol, a substance reported in the literature as having pharmacological properties: cardioprotective agent, and antioxidant prevention of atherosclerosis. **Objective:** To evaluate CAE hypolipidemic effect on dyslipidemia induced by Triton WR-1339. **Methods:** Swiss male mice (30-40g) were divided (n=6): negative (NC) and positive (PC) controls, gemfibrozil 100mg/kg (GEMF), CAE 10mg/kg (CAE10), CAE 50mg/kg (CAE50) and CAE 100mg/kg (CAE100). Dyslipidemia was induced by a single intraperitonial (i.p.) administration of Triton 400mg/kg in all animals except in NC. Animals were treated with saline (NC, PC), CAE or GEMF orally, three times, 1hour before, 22 hours and 46 hours after i.p. of Triton. 24 and 48h after dyslipidemia induction, blood samples were collected to determine glucose (GLU), total cholesterol (TC), triglyceride (TG), aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea (UR) and creatinine (CR). The protocol was approved by the Ethics Committee on Animal Research from UFC with the number 038/08. **Results** were analyzed by ANOVA and Newman-Keuls test and expressed as mean ± S.E.M., considering significant $p<0.05$. **Results:** CAE100 decreased GLI (mg/dL) at 24h (NC:139.9±7.0; PC:238.7±5.9; CAE10: 241.1±12.7; CAE50:219.5±8.2; CAE100:182.4±12.6; GEMF:205.5±15.7) whereas in 48h, there was no significant change. CAE50, CAE100 and GEMF demonstrated a TC reduction (mg/dL) in 24h (NC: 130.8 ± 6.4; PC: 545.3 ± 30; CAE10: 513.1 ± 62; CAE50: 346.8 ± 41; CAE100: 317.8 ± 41; GEMF: 395.5 ± 35) and in 48h (NC: 118.4 ± 5.6; PC: 340.4 ± 32.9; CAE10: 271.9 ± 30; CAE50: 232.9 ± 38; CAE100: 189.3 ± 18; GEMF: 232.5 ± 22). TG (mg/dL) were reduced in groups treated with CAE 100 or GEMF in 24h (NC: 95±3.6; PC:4818±200; CAE10:4521±224; CAE50:4027±428; CAE100:3303±437; GEMF:2834±203) while in 48h the reduction occurred in groups CAE50, CAE100 and GEMF (NC:153.0±18.0, PC: 1618±150; CAE10: 1546±186; CAE50:1094±155 ; CAE100: 672 ±173; GEMF: 829±116). AST, ALT, UR and CR were not altered by CAE administration at doses and periods studied. **Discussion:** Among the surfactants triton seems to form a layer on the lipoproteins surface (VLDL), avoiding hydrolysis caused by lipoprotein lipase (LPL), leading to hypertriglyceridaemia (Hall, Am J Vet Res, 61:941, 2000). Moreover, it stimulates hepatic synthesis and cholesterol efflux from tissue to the circulation (Friedman, *Am J Physio*l, 190:439, 1957). It is suggested that CAE apparently acts on a path of lipoprotein metabolism previously modified by Triton WR-1339. CAE isolated from carnauba wax had a hypolipidemic action in protocol of dyslipidemia induced by Triton. There were not liver and kidney changes. More studies are needed to elucidate a possible mechanism of action of this compound. **Financial support:** FUNCAP and Banco do Nordeste

09.038 Evaluation of renal, hepatic and pancreatic functions of hypercholesterolemic animals treated with aqueous suspension of *Passiflora edulis*. Oliveira KS[1], Neto JNFG[1], Morais TMF[1], Dantas MB[1], Pereira NBS[1], Damasceno DV[1], Araújo VM[1], Freitas AMP[1], Lemos TLG[2], Queiroz MGR[1] [1]UFC – Análises Clínicas e Toxicológicas, [2]UFC – Química Orgânica e Inorgânica

Introduction: Dietary fiber ingestion can be efficiently in reducing and preventing risk factors for cardiovascular disease (MARLETT, J Am Diet Assoc., v.102, p.993, 2002). In addition to studies of the pharmacological activity of these substances is necessary to investigate the maintenance of normal functions in the homeostasis of major organs such as kidneys, liver and pancreas. **Objective:** To evaluate renal, hepatic and pancreatic functions of hypercholesterolemic animals treated with aqueous suspension of *Passiflora edulis* (ASPE). **Methods:** ASPE was obtained after drying (60ºC) and pulverization of the *Passiflora edulis* fruit peel. The fruits were collected of a particular plantation localized in Fortaleza - Ceará - Brazil. Male mice Swiss (25-35g) were divided (n=6) in: standard diet (SD), hypercholesterolemic diet (HD), HD + simvastatin 20mg/kg (SV20), HD + ASPE 100mg/kg (ASPE100), HD + ASPE 200mg/kg (ASPE200). For the hypercholesterolemia induction, all animals were fed with HD for 4 weeks, except for the SD group, and diet was maintained until the end of the experiment. A blood collection was carried out to investigate the increase in total cholesterol (TC). The treatment was conducted, orally, with saline (SD and HD groups), SV20, ASPE100 or ASPE200 for 28 days. A new blood collection was performed to determine TC, AST, ALT, creatinine (CR), amylase (AMY) and lipase (LP) values. The protocol was approved by the Ethics Committee on Animal Research of the UFC under number 020/08. The results were expressed as mean ± S.E.M. and the groups were compared by ANOVA (post-test Newman-Keuls), adopting as a significance criterion $p<0.05$. **Results:** TC values were significantly reduced after treatment with SV20 or ASPE100. HD, SV20, ASPE100 or ASPE200 were not able to change AST (U/L) values: (SD: 126.3±2.9; HD: 121.8±0.3; SV20: 98.3±4.1; ASPE100: 125.7±2.1; ASPE200: 134.8±6.3) or the ALT (U/L) values: (SD: 42.0±0.8; HD: 45.5±1.3; SV20: 39.5±1.1; ASPE100: 40.8±2.3; ASPE200: 50.3±2.0). CR (mg/dL) was not modified in any experimental group (SD: 0.22±0.03; HD: 0.20±0.04; SV20: 0.25±0.04; ASPE100: 0.22±0.03; ASPE200: 0.21±0.03). HD, SV20, ASPE100 or ASPE200 did not have their plasma levels of LIP and AMI modified. **Discussion:** HD was composed of 1% cholesterol + 0.1% colic acid and 10% *Cocus nucifera* oil (WILSON, *J Nutr Biochem*., v.18, p.105, 2007). Experimental evidence has demonstrated that soluble fiber ingestion slows gastric emptying (CHANDALIA, New Engl. J. Med, v.342, p.1392, 2000). It is suggested that the ASPE, which is rich in pectins, may have promoted a decrease of lipids absorption, reducing TC. Furthermore, ASPE did not change the parameters of evaluation of renal, hepatic and pancreatic functions in period and doses studied. This evidence shows that fiber and pectin may have action in biological systems without changing mechanisms and functions important for homeostasis. **Financial support:** FUNCAP

09.039 Hypolipidemic and antioxidant potential of betulinic acid in mice with triton WR-1339-induced dyslipidemia. Dantas MB[1], Feitosa ML[2], Sousa FCF[2], Maia AIV[3], Freitas AMP[1], Morais TMF[1], Ferreira JM[1], Araújo VM[1], Pessoa ODL[3], Queiroz MGR[1] [1]UFC – Análises Clínicas e Toxicológicas, [2]UFC – Fisiologia e Farmacologia, [3]UFC – Química Orgânica e Inorgânica

Introduction: Increased plasma cholesterol and triglyceride levels may lead to dyslipidemia, a risk factor for the development of cardiovascular disease, alone or in association with diabetes and obesity (Shukla *et al.*, *Bioorg. Med. Chem. Lett.*, v.21, p.3475, 2011). **Objective:** To evaluate the hypolipidemic effect of betulinic acid (BA) on dyslipidemia induced by triton WR-1339® and analyze its modulating effect on oxidative stress. **Methods:** Male Swiss mice (25-35g) were assigned to 6 groups with 8 animals each: negative control (NC), positive control (PC), 200mg/kg fenofibrate (FENO) and 5, 10 or 20mg/Kg betulinic acid (BA5, BA10 and BA20). Dyslipidemia was induced by a single intraperitoneal administration of 400mg/kg Triton, except in NC. The animals received BA or FENO one hour before, or 22 or 46 hours after, triton administration. At 24 and 48 hours, following 6-8 hours of fasting, blood was collected to determine total cholesterol (TC) and triglyceride (TG) levels. By the end of the experiment, the animals were euthanized and the liver was removed to assess the antioxidant activity. The production of thiobarbituric acid-reactive substances (TBARS) and the superoxide dismutase (SOD) activity were measured. The results were expressed as mean ± SEM. The groups were compared with the Newman-Keuls test followed by ANOVA, with the level of statistical significance set at 5% (p<0.05). The study protocol was previously approved by the UFC Committee on Animal Research and Ethics under entry 03/11. **Results:** At 24 h, BA10, BA20 and FENO promoted a decrease in TG (mg/dL) (PC: 6395.0±401.5; FENO: 5159±115.9; BA5: 6004.0±123.3; BA10: 5172.0±273.1; BA20: 5321.0±314.0). At 48 h, TG remained decreased only in groups treated with BA10 and FENO (PC: 3165±330.0; FENO: 1874.0±287.1; BA5: 2702.0±258.6; BA10: 1826.0±136.7; BA20: 2264.0±263.0). At both 24 h and 48 h, only FENO reduced TC levels. Triton administration increased TBARS (µmol MDA/mg tissue) by 168.7% (NC: 14.5±6.8; PC: 38.8±7.1) and reduced SOD (U/g tissue) (NC: 509.7±37.6; PC: 336.4±58.8). Treatment with BA10 (11.9±2.1) and BA20 (8.8±1.2) reduced MDA formation (µmol MDA/mg tissue) compared to PC (38.8±7.1). SOD (U/g tissue) increased in groups treated with BA10 (502.6±30.6) and BA20 (543.1±30.9) compared to PC (336.4±58.8). **Discussion:** Triton increases plasma lipid levels by impairing the absorption of lipoproteins and stimulating cholesterol synthesis in the liver through an increase in HMG-CoA redutase activity and inhibition of lipoprotein lipase (LPL) activity (Mandukhail *et al.*, Lipids Health Dis., v.9, p.88, 2010). Other studies suggest oxidative stress can also reduce LPL activity and thereby change lipid metabolism (Yang *et al.*, Nutrition, v. 22, p. 1185, 2006). BA appears to increase LPL activity, but further studies are required to clarify the pharmacological mechanism involved. **Financial support:** CAPES

09.040 Chemical composition of the essential oil of *Aloysia triphylla* in different seasons. Parodi TV[1], Silva LL[2], Gressler LT[1], Cunha MA[1], Zeppenfeld CC[1], Heinzmann BM[2], Baldisserotto B[1] [1]UFSM – Fisiologia e Farmacologia, [2]UFSM – Farmácia Industrial

Introduction: *Aloysia triphylla* (Verbenaceae) is native species of South America with important place on herbal market due to the sensory, anxiolytic (Pascual et al., 2001) and anesthetic (Parodi et al., 2012) properties of accumulated essential oil of its leaves. Differences in the content and composition of the essential oil of lemon verbena have been reported previously can affect the properties mentioned. **Methods:** The EO was extracted from the aerial parts of *A. triphylla* (SMDB n° 11169) collected in different seasons (spring, summer, autumn and winter) by hydrodistillation according to European Pharmacopoeia (2007) using Clevenger apparatus for 3h. The EO was extracted from plants collected in different seasons (spring, summer, autumn and winter). Analyses of EOs were performed by gas chromatography-mass spectrometry (GC / MS) and the constituents were identified based on the retention index (RI) determined by using a calibration curve of a homologous series of n-alkanes (C_8-C_{32}) and by comparing of the fragmentation pattern of the mass spectra with a database and literature data (Adams, 2001; NIST, 2002) and with the equipment library. **Results and Discussion:** Sixty two compounds that differed quantitatively between collection seasons were identified in 2009 and fifty in 2010. The aliphatic alcohol nonadecanol (0.35%) was identified only in the EO obtained in winter harvest of 2009. Regardless of the year of extraction during the year 2009, the main components were limonene, 2-pinen-4-ol, Z and E-geraniol, α and β-citral, spathulenol, caryophyllene and caryophyllene oxide. Throughout 2009, limonene had the lowest rates (2.7%) in the winter while the rates were higher for the E-geraniol (4.41%), caryophyllene (12.68%) and caryophyllene oxide the rates were higher (10.05%) in the same period. Along 2010, the following components prevailed: limonene, 2-pinen-4-ol, pulegone, Z-geraniol, α and β-citral, geranyl acetate, caryophyllene, α-curcumene, spathulenol and caryophyllene oxide. Limonene had the lowest rates in autumn and winter (2.6 and 2.28%). This reduction was the same during this period for caryophyllene (2.38 and 1.91%) and caryophyllene oxide (1.61 and 1.52%). The reduction of spathulenol proportion was higher during spring (0.38%). The α and β-citral remained predominant in the constitution of the EO and did not show quantitative changes due to seasonal variation during the two years of evaluation. Gil et al., 2007 found citral is the most noticeable compound present in the EO from *A. triphylla*, together with limonene, geraniol, caryophyllene, α-curcumene and spathulenol. Furthermore EO presented quantitative and qualitative changes in the chemical composition in relation to seasonal variation during two years. Taveira et al. (2003) found that the biosynthesis of essential oils is influenced by climatic factors, the harvest season and stage of plant development, which interferes with the production of secondary metabolites. **References:** Adams RP, 2001; European Pharmacopoeia, 6th ed. 2007; NIST, 2002; Gil et al. Food Chem, 56, 8664, 2007; Parodi TV et al. *Comp Biochem Physiol* C, 155,3,2012; Pascual ME et al. J *Ethnopharmacol*, 76, 201, 2001; Taveira FSN et al. *Biochem Syst Ecol*, 31, 69, 2003. **Sources of research support:** Capes, CNPq, Fapergs-Pronex.

09.041 Antibacterial activity and cytotoxicity induced by derivatives nitrocompounds *in vitro*. Santos DC, Souza KGS, Mendonça LCV, Vale JKL, Borges RS, Monteiro MC UFPA –Microbiologia e Imunologia Clínica

Introduction: In recent years, several nitrocompounds were synthesized and tested against various microorganisms; they present mainly immunosuppressive and antimicrobial activity. However, several studies have reported a high toxicity of these compounds in clinical practice, so there are a constant search for new organic nitrocompounds with a better efficacy and lower toxicity. This study aimed to evaluate the antimicrobial potential of four nitro derivatives front of gram-positive bacteria (*Staphylococcus aureus* and *Enterococcus faecalis*) and gram-negative (*Pseudomonas aeruginosa* and *Escherichia coli*). **Methods:** In this study, we tested four derivatives of nitrofeniletenos called 7A, 7B, 7C and 7D. The synthesis of the nitrocompounds was performed in the Laboratory of Pharmaceutical Chemistry, Faculty of Pharmacy / UFPA-PA. For antimicrobial assay, it was used the standard strains *Staphylococcus aureus* (ATCC 6538), *Enterococcus faecalis* (ATCC 29212), *Pseudomonas aeruginosa* (ATCC 25853), *Escherichia coli* (ATCC 8739). The bacterial inoculum preparation and *in vitro* antimicrobial drug screening were performed by broth microdilution method, as described by the Clinical and Laboratory Standards Institute (2008). Recent culture, in log phase, of bacteria was used to prepare the cell suspension adjusted to 0.5 McFarland standards corresponding to the suspension containing 1×10^6 CFU/mL. Briefly, all compounds were prepared from the stock solutions of 10mM, and they were used in concentrations of 5.0; 2.5; 1.25 and 0.625 mM. Compounds were dispensed in 100µl volumes and 100 µL of the fungal suspension into the wells of the microdilution plates and incubated at 37ºC for 24 hours. After this time, MTT was added to the microplates and after 4 hours was performed the reading of the wells to determine the minimum inhibitory concentration (MIC).. To obtain the minimum fungicidal concentration (MFC), volumes of 10 µl from each drug concentration were spread onto Mueller Hinton agar (MHA) plates. Colony-forming unit (CFU) was counted after incubating the plates at 35°C for 48 h. The MFC was the lowest drug concentration that resulted in either no growth or fewer than three colonies (99.9% killing). The cell viability was evaluated by MTT assay. **Results and Discussion:** Of the nitrocompounds tested, the compound 7B showed the better antimicrobial activity against gram-positive bacteria, while the compound 7D was effective against gram-negative. To *S. aureus*, the compound 7B presented values of MBC of 2.5 mM and MIC of 0.625 mM. Regarding the *E. faecalis*, the compounds 7B and 7D showed similar values of MBC = 2.5 mM and MIC = 0.625 mM. To gram-negative bacteria, the compounds 7B, 7C and 7D were effective against *P. aeruginosa*, obtained values of MBC = 3.75 mM and MIC =2.5 mM. For E. coli, the compound D showed a high antimicrobial activity showing MBC= 2.5 mM and MIC = 0.625 mM. To cell viability, the compound D showed a lower cytotoxicity *in vitro*, since all concentrations remained 100% viable cells. Furthermore, the compound C led to 80% of cell viability in concentration of 2.5 mM. **Financial support:** CAPES/CNPq, FAPESPA; UNIVERSAL/CNPq, UFPA.

09.042 ***Passiflora incarnata*** **treatment during gestation and lactation**: **Toxicity and antioxidant evaluation in Wistar dams**. Boll KM[1], Bortolasci CC[2], Veríssimo LF[3], Zaminelli T[3], Bacchi AD[3], Higachi L[2], Barbosa DS[4], Moreira EG[3] [1]HU-UEL – Farmácia, [2]UEL – Ciências da Saúde, [3]UEL – Ciências Fisiológicas, [4]UEL – Patologia

Introduction: *Passiflora incarnata* is marketed in many countries as a phytomedicine. Even though the physician prescribing directions of most marketed phytomedicines recommend them to be used under medical supervision, reproductive and developmental studies are sparse and not mandatory for regulatory purposes. **Methods:** In this study, we conducted a reproductive toxicity evaluation of *P .incarnata* administered to Wistar rats (30 or 300 mg/kg, gavage) during pregnancy and lactation. Moreover, considering that antioxidant properties have been attributed to flavonoids present in the genus *Passiflora*, we have also evaluated the antioxidant/pro-oxidant balance in the plasma of these dams and conducted an *in vitro* test to evaluate antioxidant potential. The experimental procedures performed in this study were approved by the Ethics Committee for Animal Experimentation of the Universidade Estadual de Londrina (CEEA - UEL 16/2010). **Results and Discussion:** *P. incarnata* treatment did not influence dams´ body weight as well as reproductive (gestation length, post-implantation loss, litter size, litter weight) and hepatic function (albumin, AST, ALT, GGT) parameters. The antioxidant property of *P. incarnata* was evidenced both *in vivo* (increase in the total antioxidant plasmatic potential) and *in vitro* (decrease in neutrophil-induced respiratory burst). The results from the present study indicate that under the experimental conditions evaluated, *P. incarnata* treatment during gestation and lactation presented antioxidant effect in the absence of maternal reproductive toxicity. **Keywords:** *Passiflora incarnata*, reproductive toxicity, pregnancy, lactation, oxidative stress**. Acknowledgements:** This work was supported by CNPq (fellowship to TZ and LH), Fundação Araucária (fellowship to LFV) and CAPES (fellowship to CCB and ADB). We also thank Herbarium for the Passiene™ donation.

09.043 Mechanisms underlying the vasorelaxant action of ethanolic extract of *Mandevilla moricandiana* (Apocynaceae) leaves in rat aorta. Ferreira LLDM, Paes BM, Gomes MVS, Konno TUP, Muzitano MF, Raimundo JM UFRJ

Introduction: Many species of the *Mandevilla genus* have been studied for their biological activities. Among the effects described are antioxidant, anti-inflammatory and vasorelaxant in isolated rabbit vessels1,2. However, the phytochemical and pharmacological profile of *Mandevilla moricandiana* has not been investigated yet. The aim of the present study was to evaluate the effects of ethanolic extract of *Mandevilla moricandiana* leaves and its fractions on vascular smooth muscle and to investigate the possible mechanisms of action. **Methods:** Thoracic aorta from male Wistar rats (220–280 g) was prepared for isometric tension recording. Aortic rings were placed in vertical chambers filled with Krebs-Henseilet solution and were stabilized under 1 g resting tension for 90 min. Then, the contractile response to phenylephrine (10 μM) was measured before and after exposure to increasing concentrations of crude extract or fractions (1-100 μg/ml). To investigate the mechanisms involved in the vasodilatory activity, aortic rings with intact endothelium were pretreated for 15 min with Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME, 100 μM), inhibitor of endothelial nitric oxide synthase (eNOS); 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ, 10 μM), inhibitor of soluble guanylate cyclase (sGC); indomethacin (10 μM), cyclooxygenase inhibitor; atropine (10 μM), muscarinic antagonist; or wortmannin (300 nM), phosphatidylinositol 3'-kinase (PI3K) inhibitor. All protocols were approved by the Animal Care and Use Committee under license Macaé01. **Results**: The maximal relaxant effect of ELM was 84.35 ± 1.97% at a concentration of 30 μg/ml ($P<0.05$; n=6). The concentration of ELM necessary to reduce phenylephrine-induced contraction of endothelium-intact aorta by 50% was 0.90 ± 0.07 μg/ml. ELM-induced vasorelaxation was abolished in endothelium-denuded aorta. Similar results were obtained in endothelium-intact aortic rings pretreated with L-NAME or ODQ. Wortmannin significantly reduced ELM maximal relaxant effect to 31.16 ± 0.64% ($P<0.05$; n=4). Neither indomethacin nor atropine produced significant changes on the relaxation response. Partitioning of ELM yielded 5 fractions: n-hexane (HF), dichloromethane (DF), ethyl acetate (EF), butanol (BF) and residual water (WF). HF, DF and EF have been tested in endothelium-intact aorta, however only EF significantly reduced phenylephrine-induced contraction. The maximal relaxant effect of EF was 48.51 ± 5.97% at 30 μg/ml ($P<0.05$; n=4). **Discussion:** Our findings suggest that ELM relaxes vascular smooth muscle via endothelium-dependent NO-cGMP signaling, which is in part related to the activation of the Akt-eNOS-sGC pathway. EF, but not HF and DF, seem to contribute to the relaxant effect of ELM. **References**: [1]Matos WM. Neuropeptides 40: 125, 2006. [2]Calixto JB. *Br J Pharmacol* 88: 937, 1986. **Financial agencies**: FAPERJ, FUNEMAC, UFRJ.

09.044 Vasodilatory activity of ethanolic extract of *Kielmeyera membranacea casar* (Clusiaceae) leaves and its mechanism of action in the rat aorta. Paes BM, Ferreira LLDM, Souza PBN, Konno TUP, Guimarães DO, Muzitano MF, Raimundo JM – UFRJ

Introduction: Cardiovascular diseases are the number one cause of death globally and systemic arterial hypertension (SAH) is the most prevalent and the major risk factor for these diseases (WHO, 2011). The use of vasodilators allows the control of SAH by promoting relaxation of vascular smooth muscle, whose tone is regulated by several factors derived from vascular endothelium. Thus, the objective of this study was to investigate the mechanisms involved in the vasodilator effect of the ethanolic extract of *Kielmeyera membranacea* Casar leaves (ELK). **Methods:** Aortic rings, with or without endothelium, from male Wistar rats (220–280 g), were prepared for isometric tension recording. Aortic rings were placed in vertical chambers filled with Krebs-Henseilet solution and were stabilized under 1 g resting tension for 90 min. Then, the contractile response to phenylephrine (10 μM) was measured before and after exposure to increasing concentrations of ELK (1-100 μg/ml). To investigate the mechanisms involved in ELK-induced vasodilation, aortic rings with intact endothelium were pretreated for 15 min with Nω-nitro-L-arginine methyl ester hydrochloride (L-NAME, 100 μM), inhibitor of nitric oxide synthase; 1H-[1,2,4]oxadiazolo[4,3-a]quinoxalin-1-one (ODQ, 10 μM), inhibitor of soluble guanylate cyclase; or atropine (10 μM), muscarinic antagonist. All protocols were approved by the Animal Care and Use Committee under license Macaé01. **Results:** ELK relaxed pre-contracted endothelium-intact aortic rings in a concentration-dependent manner. The maximal relaxant effect of ELK was 70.5 ± 7.9% at a concentration of 30 μg/ml ($P<0.05$; n=6). The concentration of ELK necessary to reduce phenylephrine-induced contraction of endothelium-intact aorta by 50% was 3.9 ± 0.5 μg/ml. To test whether the vasodilation is endothelium-dependent, the vascular effects of ELK were tested in endothelium-denuded aortic rings. Denudation of endothelium abolished the relaxant effect of ELK. Pretreatment of endothelium-intact aorta with L-NAME and ODQ also inhibited the ELK-induced vasodilation. On the other hand, atropine had no effect on it. **Discussion:** Our results suggest that ELK induces endothelium-dependent vascular relaxation of rat aorta, which is mediated by nitric oxide and cGMP (cyclic guanosine monophosphate) production, without involvement of muscarinic receptors. **Reference:** WHO. Fact sheet nº 317. 2011. **Financial agencies:** FAPERJ, FUNEMAC, UFRJ.

09.045 Effect of β-pinene obtained from *Citruslatifolia tanaka* essential oil on neutrophil *in vitro* chemotaxis. Kummer R, Silva FM, Estevão-Silva CF, Ritter AMV, Rocha BA, Arruda LLM, Grespan R, Cuman RKN UEM – Farmacologia e Terapêutica

Introduction: The fruits of the genus *Citrus* (Rutaceae) have appreciable content of essential oil, being the main constituents: limonene, a-pinene, b-pinene, p-cymene, g-terpinene, linalool, nerol e citral. Studies have demonstrated the biological activity of essential oils, such as: anti-inflammatory, anthelmintic, anti-infective, antinociceptive and immunomodulatory properties. This study evaluated the effect of β-pinene (PIN), a terpenoid on the chemotaxis activity of neutrophils *in vitro*. **Methods:** The fruits were purchased in the city of Maringa-PR. The essential oil of the fruits of Tahiti lime (*Citrus latifolia* Tanaka) was extracted from distillation in Clevenger apparatus. The PIN was identified by GC-MS and NMR. To evaluate the PIN effects on chemotaxis, neutrophils were isolated from the peritoneal cavity of mice, 4 hours after Zymosan injection (1mg/cavity, i.p). The cell number was adjusted to $1x10^6$ cells /mL in RPMI/BSA 0.01% and pretreated with PIN (1, 3, 10, 30, 60 or 90 µg/mL) for 30 min. The cells were placed in a chemotaxis Boyden chamber (48 wells; Neuro Probe, Inc., Cabin John, MD–USA) and as stimuli to neutrophils migration fMLP (10^{-6}M) was used and medium RPMI 1640 as control of migration. The cells were incubated 1 h at 37 °C, 5% CO_2. After cells incubation, the membrane was washed and stained using the *Instant Prov* (Newprove). Neutrophils were counted by optical microscopy (1000X), five fields in each well. The results were expressed as the number of neutrophils per field. Data were presented as mean ± SEM of 3 separate experiments. The means from different treatments were compared by ANOVA with Tukey's correction. Statistical significance was set at $P \leq 0.05$. The protocol (number 066/2010) regarding this study was approved by the Ethical committee in Animal Experimentation (CEAE/State University of Maringá). **Results:** A significant inhibition of cell migration was observed with treatment PIN induced by fMLP (10^{-6}M). The chemotaxis induced by **fMLP** (31±2.7 cells/field) was inhibited by PIN in different concentrations: **$PIN_{1µg/mL}$**: 19.2±1.8* cells; **$PIN_{3µg/mL}$**: 8.8±1.0* cells; **$PIN_{10µg/mL}$**: 13.1±0.80* cells; **$PIN_{30µg/mL}$**: 11.2±1.4* cells and by **$PIN_{60µg/mL}$**: 13.8±2.0* cells. Only one of the tested doses showed no inhibitory effect: **$PIN_{90µg/mL}$**: 21.5±1.3 cells. **RPMI** was used as negative control: 4.9±1.1 cells/field. **Discussion:** Our study provides an evidence that PIN treatments inhibit the *in vitro* neutrophil chemotaxis, suggesting an possible anti-inflammatory effect of this compound. **Supported by:** CNPq, CAPES and Fundação Araucária. **Acknowledgements**: Jaílson Araújo e Célia Miranda.

09.046 Cardiotoxic effects of microcystin–LR in mouse isolated hearts. Siqueira-Lece F[1], Ricardo HD[1], Tomaz MA[1], Machado MM[1], Tavares SM[1], Strauch MA[1], Silva-Gonçalves T[1], Azevedo SM[2], Soares RM[2], Melo PA[1] [1]ICB-CCS-UFRJ – Farmacologia das Toxinas, [2]ICF-CCS-UFRJ – Ecofisiologia e Toxicologia das Cianobacterias,

Background: Microcystin-LR (MC-LR) is related to envenoming of animals and humans following blooms of cyanobacteria and the release of large quantities of the toxin in lakes and rivers used as water supplies. There are no previous studies showing the cardiac effects of MC-LR under oxidative stress conditions, such as ischemia and reperfusion (I/R). The main goal of this study was to analyze the effect of MC-LR on isolated mouse heart cardiac function. **Methods:** We assessed the effects of MC-LR in mouse isolated hearts perfused with an appropriated nutritional solution by using the modified Langendorff preparation. We analyzed the tension developed, the electrocardiographic records (EKG), the damaged area and the Creatine Kinase (CK) activity in the perfusate. The preparation was perfused under control conditions and after 15 minutes of stabilization, we added different concentrations MC-LR (0.1-0.3 µg/mL) to the bathing media after 10 min of I/R. At the end of the experiments, hearts were gently sliced and exposed to 1% triphenyl tetrazolium chloride (TTC) to assess damaged areas (Am Heart J, 593: 101, 1981). Electrical and contractile properties were analyzed by the WINDAQ program. The experiments were performed in according with ethic principles of CEUA-UFRJ (DFBCICB 033). **Results**: The heart exposure at MC-LR 0.1µg/mL did not show any changes. At concentrations 0.3µg/mL MC-LR decreased more than 60% on the cardiac tension and on the QRS waves, after 70 minutes compared to control hearts exposed to nutritional solution (n=4). The analysis with TTC test did not show image evidences of damage, although the CK released in the perfusate increased more 100%. The I/R protocols did not change the control records or the TTC image or CK analysis. However, the hearts exposed to 0.1µg /mL after I/R, showed a decrease of 50% of the cardiac tension without changes on the EKG wave sizes or CK released. **Conclusions:** Our experiments showed that MC-LR alone has cardiotoxic effect above 0.3 µg/mL and its cardiotoxic effects is increased by I/R conditions which stress the cardiac tissue. Our results have shown for the first time the direct damage and effects on cardiac function by MC-LR and this effect is increased by oxidative stress conditions. **Financial support**: CAPES, CNPq, PRONEX, MCT/INCT - INPeTam and FAPERJ

09.047 Anticonvulsant and sedative effects of hydroethanolic extract of *Himatanthus drasticus* Mart. stem bark. Pinto BAS[1], Flister KFT[2], Machado KRG[3], França LM[4], Moraes DFC[1], Borges ACR[2], Paes AMA[2], Olea RSG[3] [1]UFMA – Farmácia, [2]UFMA – Ciências Fisiológicas, [3]UFMA – Química

Introduction: Himatanthus drasticus Mart. (Apocynaceae) is a large tree popularly known as "janaúba" and commonly used in Brazilian folk medicine for treatment of anxiety and stress-related disturbs. Thus, the present work sought to investigate the hypnosedative and anticonvulsant effects of hydroethanolic extract from stem barks of Himatanthus drasticus in mice. **Methods:** Powdered barks were macerated in 70% ethanol (1:3) for 72 h, rendering the hydroethanolic extract (HEE), which was concentrated under vacuum and kept at 4°C for posterior use. Initially, acute toxicity protocols were used for determination of LD_{50}. Hypnosedative effect of HEE (HEE 10, HEE 30 and HEE 100 mg/kg, ip) was assessed as its capacity to potentiate the pentobarbital-induced sleep (PBB, 50 mg/kg), while anticonvulsant activity for the same doses was assessed through both pentylenetetrazole- (PTZ, 80 mg/kg) and strychnine-induced (STR, 2 mg/kg) seizures models. In all protocols, control animals (CTR) were administrated with vehicle (saline 0.9%, ip) by the same routes. Data were expressed as mean ± SEM and groups compared by one-way ANOVA followed by Newman-Keuls for $p< 0.05$ (n= 7- 10 per group). All procedures involving animals were approved by Animal Studies Committee of UFMA (23115-006060/2010-18). **Results:** Administration of HEE to adult mice resulted in DL_{50} = 3.4 g/kg and observation of intense ptosis, hind paws abduction, decreased ambulation, sedation and catalepsy. In a less extent degree, it was observed analgesia as well as decreased ear and corneal reflexes. HEE showed significant sedative effect since it shortened the time for PBB-induced sleep at all doses (HEE 10 – 2.9 ± 0.1; HEE 30 – 2.6 ± 0.1 and HEE 100 – 2.5 ± 0.1 min) as compared to CTR (3.5 ± 0.13 min) and prolonged the total sleep time from 63.3 ± 3.3 min (CTR) to 124.5 ± 22.04, 131.3 ± 5.1 and 143.5 ± 13,8 min, respectively. When analyzed the time for the onset of PTZ-induced seizures, HEE delayed it from 83.6 ± 2.9 sec (CTR) to 460.4 ± 76.4 and 558.3 ± 127.5 sec in the doses of HEE 30 and HEE 100, respectively. Similar results were found on the latency to death (CTR: 165.3 ± 33.8; HEE 10: 581 ± 130; HEE 30: 737.4 ± 135.4 and HEE 100: 916.6 ± 72.1 sec). On the other hand, HEE had no effect on STR-induced seizures. **Discussion:** Taken together, our data show that HEE from H. drasticus stem bark strongly promotes hypnosedative and anticonvulsant effects. Moreover, such effects seem to be mediated by GABA-dependent mechanisms, since it was able to potentiate barbiturate effects, while impaired the response to PTZ. Nevertheless, HEE did not interfere on glycine-dependent mechanisms of STR. Therefore, we present, for the first time, evidences that reinforce the ethnopharmacological use of H. drasticus and open up a promissory field on the search for new useful compounds to neurological disorders. **Financial support:** FAPEMA, CAPES and UFMA.

09.048 Isobrucein B, a quassinoid from *Picrolemma sprucel* Hook. f., reduces the release of proinflammatory cytokines and nitric oxide from mouse macrophages: Possible effect by inhibition of NF-kB activation. Silva RL[1], França RFO[1], Lopes AH[1], Vieira SM[2], Amorim RCN[3], Cunha FQ[1], Pohlit AM[3], Cunha TM[1]
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Introduction: The Isobrucein B (IsoB) is a quassinoid obtained from *Picrolemma sprucei*, a plant found in Brazilian Amazon. Teas derived from the roots and leaves of this plant are used to treat gastritis. In a previous work from our group, the IsoB was isolated and tested in model of indomethacin-induced gastritis, displaying anti-inflammatory and gastroprotective effects. However, the molecular mechanism of IsoB anti-inflammatory effect remained unknown. In the present study, we evaluated the possible mechanism involved in the anti-inflammatory effect of IsoB. **Methods:** Initially, tests were conducted to verify the effect of IsoB on the production of pro-inflammatory cytokines *in vitro* by mouse macrophages. Peritoneal cells were collected of naïve C57BL/6 mice or pretreated with 3% thioglycollate (i.p.), 4 days before harvesting. Harvested cells were incubated with lipopolysaccharide (LPS-1 µg/mL) for 4 hours in the presence or absence of IsoB (0.001 - 10 µM). The concentration of TNF, IL-1β and KC/CXCL1 were measured in the supernatant by ELISA. *In vitro* production of nitric oxide (NO) by macrophages was indirectly determined by Griess method, 24 hours after LPS incubation. In attempt to investigate whether inhibition of NF-kB is the mechanism of IsoB, we performed a Luciferase activity Assay using macrophages RAW 264.7 stable transduced with the NF-kB-dependent luciferase reporter (RAW-Luc). RAW-Luc cells were stimulated with different stimuli to activate NF-kB (LPS 1 µg/ml, Peptideoglican 10 µg/ml, TNF 10 ng/ml and PMA 1 µg/ml) in presence or absence of IsoB. Confocal Microscopy was used to detect nucleus NF-kB p65 (RelA) subunit translocation, in RAW 264.7 cells, after activation with TNF in presence or absence of IsoB (10 µM). Anti-rabbit IgG alexa Fluor 488 was used as secondary antibody in confocal analysis, nucleus were counterstained with DAPI. Cytotoxicity of IsoB was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), lactate dehydrogenase (LDH) and Trypan Blue cytotoxicity assays. **Results:** The results show that IsoB (0.1-10 µM) inhibits the production/release of pro-inflammatory cytokines and NO from macrophages stimulated by LPS in a concentration-dependent manner. Furthermore, IsoB also inhibited luciferase activity, NF-kB promoter dependent, and NF-kB p65 nucleus subunit translocation in cells to all the described activators. No cytotoxic activity was detected in all tested concentrations of IsoB . **Discussion:** The present results indicate that IsoB displays anti-inflammatory effects by reducing the production/release of pro-inflammatory cytokines such as TNF, IL-1β and KC/CXCL1, and other mediators such as NO. Thus, our results suggest that IsoB may directly inhibit the NF-kB signaling, which plays a crucial role in the development of inflammatory response. **Ethical Commission of Ethics in Animal Research**: Protocol n° 077/2012. **Supported by**: CNPq, FAPESP.

09.049 Hypotensive effect induced by alcohol free-lyophilized red wine garziera (GASH) from Vale do São Francisco in different models of hypertension. Luciano MN[1], França-Silva MS[2], Ferreira-Costa HG[1], Braga VA[2], Medeiros IA[2] [1]UNIVASF – Farmacologia Experimental, [2]UFPB – Biotecnologia

Introduction: Alcohol free-lyophilized red wine induces an endothelium-dependent vasorelaxant effect due, at least in part to a secondary increase in the concentration of nitric oxide and this effect might be associated to phenolic compounds found in the GASH (Luciano *et al*, 2011). The aim of this study was to investigate the hypotensive effect induced by alcohol free-lyophilized GASH (Adega Garziera, grape Shiraz, 2005) in spontaneously hypertensive (SHR) and NO-deficient hypertensive (L-NAME) rats. **Methods:** All experiments were reviewed and approved by the Ethics Committee of Animal Experiments of the Centro de Biotecnologia of the Universidade Federal da Paraíba (0310/08). Under ketamine (75 mg/kg, i.p.) and xylazine anesthesia (10 mg/kg, i.p), the lower abdominal aorta and inferior vena cava were canulated via left femoral artery and vein using a polyethylene catheter for cardiovascular parameters recording and drug administration, respectively. **Results:** In SHR conscious animals, GASH at dose 75 mg/kg (i.v) produced hypotension (∆mmHg = -39.40 ± 11.62) and bradycardia (∆HR = -96.60 ± 44.34) followed by hypertension (∆mmHg = 69.40 ± 15.82) and tachycardia (∆HR = 75.40 ± 35.95) (n=6). In L-NAME hypertensive rats, GASH at dose 75 mg/kg (i.v) produced hypotension (∆mmHg = -44.40 ± 5.05) and bradycardia (∆HR = -108.00 ± 64.30) followed by hypertension (∆mmHg = 55.00 ± 4.83) and tachycardia (∆HR = 150.00 ± 66.88) (n=6). **Discussion:** In the present study, corroborating with the results obtained in normotensive rats (date not show), GASH produces a biphasic effect initially characterized by hypotension and bradycardia followed by hypertension and tachycardia, when administered acutely (i.v). The hypotension is greater and the bradycardia is lower in SHR and L-NAME hypertensive rats when compared to normotensive rats. **References:** Luciano, M.N; J *Cardiovasc Pharmacol*; 57 (6): 696, 2011. **Financial Support:** CNPq/CAPES/CBiotec-UFPB/UNIVASF.

09.050 Comparison of the effects of betulinic acid and sibutramine on leptin and ghrelin levels in animals with obesity induced for high calorie diet. Araújo VM[1], Melo CL[1], Melo TS[1], Ferreira JM[1], Oliveira GP[1], Dantas MB[1], Meneses RRC[1], Rao VS[2], Pessoa ODL[3], Queiroz MGR[1] [1]UFC – Análises Clínicas e Toxicológicas, [2]UFC – Fisiologia e Farmacologia, [3]UFC – Química Orgânica e Inorgânica

Introduction: Obesity is a multifactorial disease considered a public health problem. This pathology is associated with increased morbidity and mortality. The available drug treatment has several adverse effects which may complicate the therapeutic. The study of natural products, such as betulinic acid (BA), emerges as a possible alternative in improving the therapeutic arsenal antiobesity. **Objectives:** Evaluate the effect of BA on plasma levels of leptin and ghrelin in animals with obesity induced for high calorie diet. **Methods:** Male mice Swiss (20-25g) were divided into groups (n=10): SD (standard diet + tween 80.3%); DH (high calorie diet + tween 80.3%); DH + BA (high calorie diet + betulinic acid 50mg/L); DH + SIB (hypercaloric diet + sibutramine 50mg/L). The animals were subjected to SD or DH and simultaneously treated for 15 weeks with the substances described. After this period, blood samples were collected for determination of ghrelin and leptin hormones. The animals of groups fed with DH were considered obese when increase in body weight 20-25% compared to SD. This protocol was submitted and accepted by the Ethics Committee on Animal Research of the UFC under number 031/08. The results were analyzed by ANOVA and Tukey test and expressed as mean ± S.E.M., considering significant $p < 0.05$. **Results:** BA and SIB reduced body weight (g) (SD: 39.8 ± 0.51; DH: 49.38 ± 1.07; DH + BA: 42.88 ± 1.52*; DH + SIB: 44.00 ± 0.68*) and abdominal fat (mg/10g animal live weight) (SD: 201.80 ± 29.22; DH: 887.40 ± 121.10; DH + BA: 332.80 ± 57.00*; DH + SIB: 540.40 ± 93.93*) compared to DH. BA (62.5%) was more effective in reducing abdominal fat that the SIB (39.19%). DH intake increased leptin and decreased ghrelin values in relation to the SD group. However, only the group DH + BA increased levels of leptin (ng/mL): (SD: 0.28 ± 0.07; DH: 2.13 ± 0.49; DH + BA: 3.50 ± 0.36*; DH + SIB: 1.78 ± 0.47) and decreased ghrelin values (ng/mL): (SD: 0.65 ± 0.10; DH: 0.45 ± 0.08; DH + BA: 0.20 ± 0.03*; DH + SIB: 0.29 ± 0.04) when compared to DH. **Discussion:** There are reports in the literature of natural products with function in reducing abdominal fat as observed in this study. This may help to prevent the development of metabolic syndrome, considering the close relationship between these parameters (Ribeiro Filho, Arq Bras *Endocrinol Metab*., v.50, p.230, 2006). Leptin and ghrelin hormones have central physiological role in food intake and energy expenditure (Klok, Obes. Rev., v.8, p.21, 2007), showing a possible neural mechanism of action of the BA to control obesity. However, further studies are needed for this triterpene to be used as a therapeutic tool in treating obesity. **Financial support:** FUNCAP

09.051 Proteolytic fraction from *Vasconcellea cundinamarcensis* latex shows antitumoral effect and alters leukocytes properties in an inflammatory tumor microenvironment. Braga AD[1], Santos VG[1], Oliveira-Lima OC[2], Marques SM[2], Salas CE[3], Andrade SP[2], Carvalho-Tavares J[2], Lopes MTP[1] [1]UFMG – Farmacologia, [2]UFMG – Fisiologia e Biofísica, [3]UFMG – Bioquímica e Imunologia

Introduction: A latex fraction named P1G10, rich in cistein proteases, from *Vasconcellea cundinamarcensis*, a plant native of South America, has shown a variety of effects upon physiopathological systems. In previous studies, P1G10 showed an antitumor and antimetastatic effects as well as anti-inflammatory effect in carragenan-induced paw edema and cell migration models. In ascitic Ehrlich tumor model, P1G10 reduced the levels of TNF-α in the supernatant of ascitic fluid. Based upon this, we proposed to investigate whether the antitumoral effect of P1G10 may be explained by an anti-inflammatory activity on the tumor inflammatory microenvironment. **Methods:** The antitumor activity of P1G10 was evaluated using an inflammatory tumor model - 4T1 breast adenocarcinoma. Female Balb/c mice were injected *s.c* with $2{,}5x10^6$ cells in the left flank region. Mice received daily injections of P1G10 *s.c* (1, 3 or 5 mg/kg) from the third to the 20^{th} day after tumor cells inoculation. In the 21^{st} day, mice were euthanized and the tumor weight was measured. The activity of P1G10 on the rolling and adherent leukocytes to tumor vessels was evaluated by intravital microscopy (epi-illumination microscopy system - Excitation 510-560 nm and Emission 590nm). Female Balb/c mice bearing 4T1 adenocarcinoma and treated with P1G10 *s.c* (1 mg/kg), as described above, were injected with Rodamina 6G *i.v* (0,3mg/Kg) and had the tumor exposed surgically. **Results:** P1G10 reduced the tumor mass about 52%, in all doses tested. In the dose of 1mg/kg, P1G10 reduced the leukocyte adhesion to small vessels (<60 µm Ø) at 46% (6.45 ± 0.54 *vs* 11.98 ± 1.42 – control, $p< 0.05$) and to large (>70 µm Ø) at 51% (4.65 ± 1.15 *vs* 9.75 ± 0.71 – control, $p<0.05$). This reduction in leukocytes adhesion was accompanied by an increase of 70% in the number of peripheral leukocytes (7.40 ± 0.92 *vs* 4.35 ± 0.43 - control, $p<0.001$) and by an increased (158%) of NAG activity in the tumor (162.3 ± 37.17, $p<0.05$) related to the control group (62.76 ± 4.13). **Discussion:** The results show that P1G10 is capable of inhibit tumor growth of inflammatory 4T1 breast adenocarcinoma. This ability is probably associated to a reduction in leukocytes adhesion to tumor vessels, however the increase of peripheral leukocytes number, minimizing the inflammatory process. Furthermore, the reduction of tumor mass by P1G10 perhaps is associated with an augment of macrophages activity, revealed by the activity of the enzyme NAG. **Protocol number of the Animal Ethics Committees:** 55 / 2012**. Financial Support**: CNPq, FAPEMIG and CAPES.

09.052 Relaxing and contractile effects of *Pereskia grandifolia* Haworth (Cactaceae) in vascular and non-vascular smooth muscles of rats. Silva TLC[1], Maba IK[1], Souza P[1], Crestani S[1], Kazama CC[2], Gasparotto Junior A[2], Silva-Santos JE[3]
[1]UFPR – Farmacologia, [2]UNIPAR – Farmacologia, [3]UFSC – Farmacologia

Introduction: *Pereskia grandifolia* Haworth (Cactaceae), popularly known as ora-pro-nobis, is native from Northeastern Brazil. Its popular usage includes the treatment of hypertension. However, this effect remains to be confirmed. In this context, we evaluated the activity of the hydroethanolic extract *of P. grandifolia* (HEPG) on the tonus of vascular (aorta) and non-vascular (trachea) smooth muscles of rats. **Methods:** Female Wistar rats were anesthetized with ketamine/xylazine (100/20 mg/kg; i.p.) and had their thoracic aorta and trachea removed, cleaned and sectioned in rings. The rings were mounted in baths containing physiological nutritive solution (37 °C, aerated with 95% O_2/5% CO_2), connected to force transducers and a digital polygraph (MacLab System®, ADI Instruments, Australia). A stabilization period of 60 min was respected between each exposition for drugs. The functionality of endothelium was verified by the ability of acetylcholine (Ach; 1 µM) to relax aortic rings pre-contracted by phenylephrine (Phe; 1 µM). Concentration response curves to HEPG (3 - 3000 µg/ml) were performed in pre-contracted vessels. The same protocol was repeated in vessels previously incubated with L-NAME (a nitric oxide synthase inhibitor). In addition, aortic rings were preincubated with HEPG (3000 µg/ml) and contracted by phenylephrine (1 nM - 3 µM). We also evaluated the relaxing activity of HEPG (100-1000 µg/ml) in isolated tracheal rings pre-contracted by Ach (1 µM). Since in trachea HEPG induced contraction instead relaxation, its contractile capacity was investigated in the presence or absence of atropine (muscarinic antagonist). All procedures were submitted and approved by Ethics Committee for Animal Use of the Biological Sciences Sector from UFPR (CEUA/BIO-UFPR), under authorization number 593. **Results and Discussion:** HEPG was able to induce maximal relaxation of 72.5 ± 5.1% at the highest concentration tested in aortic rings with functional endothelium, an effect not observed when the endothelium was removed. Interestingly, previous incubation with L-NAME (100 µM) did not change the relaxation induced by HEPG, suggesting the involvement of nitric oxide independent mechanisms. Incubation of HEPG completely inhibited Phe-induced contraction in aortic rings. The HEPG was unable to induce relaxation in isolated tracheal rings, but induced significant contraction in this tissue, an effect unchanged by atropine, discarding the involvement of muscarinic receptors. The results suggest that HEPG has one or more compounds able to cause contraction in trachea and relaxation in aortic rings from rats. These effects are endothelium-dependent and, at least in part, seem to involve the blockade α1-adrenergic receptors. Additional studies must be conducted to allow a better understanding regarding the therapeutic potential of *Pereskia grandifolia* as a phytomedicine. **Acknowledgements:** UFPR/CNPq.

09.053 Analgesic and anti-inflammatory effect of ethyl acetate fraction of methanolic extract of leaves of *Rheedia longifolia* Planch & Triana. Nascimento DD[1], Calheiros AS[1], Siqueira AM[1], Souza CZ[1], Azeredo JA[1], Bérenger ALR[2], Figueiredo MR[2], Frutuoso VS[1] [1]IOC-Fiocruz – Imunofarmacologia, [2]Fiocruz – Produtos Naturais

Introduction: Previous studies with *Rheedia longifolia* Planch & Triana leaves extract, showed significant analgesic activity, associated with low toxicity. This extract was fractionated, originating the ethyl acetate fraction (RhFAcEt) which contains amentoflavone and showed a dose-dependent analgesic effect, with ED50 of about 1 mg/kg. **Methods:** Swiss Webster mice (CEUA License 033/09) were treated with RhFAcEt (1 mg/kg, p.o.), 1h before carrageenan (300 µg), bradykinin (3 nmol) or histamine (100 nmol) intraplantar injection. The analgesic effect was evaluated by von Frey filaments and paw volume was measured by pletismometer. To perform pleurisy, mice received intrapleural injection of carrageenan (300 µg) 1h after treatment with RhFAcEt (1 mg/kg, p.o.) and the inflammatory response observed 4h after stimulation. For safety evaluation mice were treated with RhFAcEt (10 mg/kg, p.o.) for 28 days. To determine amentoflavone analgesic potency, mice received 0.8% acetic acid i.p. 1h after treatment (0.01; 0.1 and 1 mg/kg, p.o.) and writhing number was counted for 10 min. Diclofenac (50 mg/kg, p.o.) and hidroxizine (10 mg/kg, p.o.) were used as standard drugs. All results were represented by mean of two reproductive blind experiments ($n \geq 5$) and considered significant when $p < 0.05$ by ANOVA, Student Newman-Keuls Multiple Comparison Test. **Results and Discussion:** RhFAcEt reduced nociception and paw edema induced by bradykinin (25%, 50%) or histamine (61%, 51%) respectively. Despite its analgesic effect (35%), RhFAcEt did not reduced carrageenan edema, even with higher dose (10 mg/kg). Whereas RhFAcEt was unable to inhibit the edema induced by carrageenan, regardless of their efficacy against histamine and bradykinin, we can assume that this fraction does not affect the production/release of other mediators involved in the response of carrageenan like serotonin, prostaglandins and NO. Mice challenged with carrageenan and treated with RhFAcEt showed reduction in the total number of leukocytes (38%), mainly neutrophil (55%) in their pleura comparing to those non treated. These results were accompanied by significant reduction of TNF-α (20%), IL-1β (59%) and IL-6 (65%) in relation to control group. We confirm by the writhing model the dose-dependent antinociceptive effect of amentoflavone, with potency (ED50 ≈ 0.6 mg/kg) similar to RhFAcET, suggesting that this substance is primarily responsible for the pharmacological activity of this fraction. No deaths neither toxic effect was observed after 28-day treatment with RhFAcEt. This fraction did not induced gastric lesions, even with prolonged treatment, which suggests that RhFAcEt does not present the characteristic ulcerogenic action of NSAIDs. Maintaining normal levels of blood glucose and creatinine rule out the occurrence of metabolic and kidney disorders respectively. Similarly, the enzymes pyruvic transaminase and alkaline phosphatase were not changed throughout the treatment, being an important indicator of maintenance of liver and cardiac function. In conclusion, our results demonstrated the analgesic effect of amentoflavone and the antinociceptive and anti-inflammatory activity of RhFAcEt with efficacy and safety. **Financial Support: CNPq**

09.054 Antispasmodic evaluation of *Lippia microphylla* CHAM. (Verbenaceae) on rat ileum. Santos MS[1], Jacinto KR[2], Rigoni VLS[3], Tavares JF[4], Nouailhetas VLA[5], Silva JLV[1] [1]Uninove – Farmácia-Bioquímica, [2]Uninove – Ciências da Reabilitação, [3]Uninove / Unifesp/Biofísica, [4]UFPB – Ciências Farmacêuticas, [5]Unifesp – Biofísica

Introduction: Species of *Lippia* genus are used to treat gastrointestinal and respiratory disorders in Brazilian folk medicine, the essential oils and phenol compounds are active principles responsible for pharmacological activities (PASCUAL et al., J *Ethnopharmacol*, 76, 201, 2001).*Lippia microphylla* is called as “alecrim-pimenta” (ALBUQUERQUE et al., J Ethnopharmacol, 114, 325, 2007). As relaxing (rat trachea, aorta and mesenteric) and gastrointestinal effects are related to *L. microphylla*, we thus decide to evaluate the crude ethanol extract from aerial parts of *L. microphylla* (LM-EtOH) on contractions of isolated rat ileum. **Methods:** *L. microphylla* was collected in "Cariri Paraibano" in the State of Paraíba (Brazil) and dried for herbaria sheets. It was identified by Agra et al. (6118) and voucher specimen was deposited in the collection of reference of the "Laboratório de Tecnologia Farmacêutica" in the Federal University of Paraíba. Aerial parts from plant were macerated on ethanol followed by concentration and evaporation resulting in a crude ethanol extract (LM-EtOH). Ileum was isolated from five Wistar rats (250-350g) after 24 h fasting. Ileum fragments were suspended in glass cubes (5 mL) in presence of modified Krebs solution on basal tension 1g, O_2 aeration and at 37°C. Contractions were induced either by carbachol (10^{-6}M) or KCl (40mM) in the absence or presence of the LM-EtOH (3, 9, 27, 81, 243 µg/mL). LM-EtOH (1, 3, 9, 27, 81 µg/mL) effects were also accessed on pre-contracted ileum by both contractile agents. Tissue contractile responses were monitored by force isometric transducers and recorded through acquisition data system AQCD. The IC_{50} and EC_{50} values were determined by adjusting data to non-linear regression curve. These procedures were approved for ethics committee in research of Federal University of São Paulo (CEP 0038/10). Data were expressed as mean **±** SEM and analyzed by GraphPad Prism 5.0 software using one-way ANOVA following Dunnett´s post-test or Student t-test, with $p < 0.05$ as level of significance. **Results and discussion:** LM-EtOH (3 - 243 µg/mL) inhibited in a concentration-dependent manner and a same potency the ileum contractions (n = 4) induced either by carbachol (IC_{50} = 34.4 ± 4.9 µg/mL) or KCl (IC_{50} = 34.9 ± 2.3 µg/mL). LM-EtOH (1 – 81 µg/mL) relaxed in a same potency ileum pre-contracted either by KCl (EC_{50} = 21 ± 2.8 µg/mL, n = 3) or carbachol (EC_{50} = 32 ± 3.6 µg/mL, n = 5). As LM-EtOH had same potency in relation to the agonist (carbachol) and depolarizing agent (KCl), it is proposed that *L. microphylla* has non-selective spasmolytic effect and acting on a common pathway of the two contractile agents. As both agents induced contraction due to activation of voltage-dependent calcium channels (Ca_V) in intestinal smooth muscle and following extracellular Ca^{2+} entrance, it is proposed that LM-EtOH is a Ca^{2+} channel blocker. **Financial support:** UNIFESP; UNINOVE.

09.055 Standard *Hypericum perforatum* extract inhibits Ehrlich tumor cells-induced in mice. Corrêa M, Calixto-Campos C, Zarpelon AC, Casagrande R, Verri Jr WA UEL – Patologia

Introduction: Ehrlich tumor cells derive from a murine breast carcinoma cells and have been used as a murine model to study the proliferation mechanisms and clinical signs of cancer. *Hypericum perforatum*, known as St. John's swort, is a herbaceous perennial that has been widely used in folk medicine. *H.perforatum* inhibits neuropathic pain and tumor cell proliferation. Therefore, in the present study it was evaluated whether *H perforatum* inhibits Ehrlich tumor cells-induced pain. **Methods:** We used Swiss mice weighing 20-25g from Universidade Estadual de Londrina. They were kept in a vivarium of the Department of Pathology in acrylic boxes and had free access to water and food with light and dark cycle (12/12 hours). Ehrlich tumor cells were removed from the peritoneal cavity of mice, and injected in the hind-paw o f mice at $1x10^6$ cells in 25µL. We used three groups of mice: saline plus vehicle, tumor group plus vehicle and tumor group plus treatment with *H. perforatum* (commercial standard extract.). The treatment was performed at a dose of 300mg/kg per animal for 12 days. After 3 hours of treatment the mice were monitored by parameters of mechanical hyperalgesia, thermal hyperalgesia and edema (analog caliper), and carried out at intervals of 48 hours. At the 12^{th} day, animals were euthanized and paws collected for myeloperoxidase activity. Based on mechanical hyperalgesia, the experiment was repeated and paws were collected for testing of myeloperoxidase on 4^{th} day. Paw flinching was analyzed with $1x10^7$ Ehrlich tumor cells. The present study was approved by the ethics committee of the UEL Nº 13280.2011.64 Of. Circ. CEUA No. 166/12. **Results:** There was reduction of mechanical (up to 43,11%) and thermal (up to 11,49%) hyperalgesia in mice treated with *H. perforatum* in comparison with vehicle treated mice. The paw edema results were saline (0,083 ± 0,031), tumor (3,075 ± 0,142), tumor *H. perforatum* treatment (3,042 ± 0,244) at the 12^{th} day. The same profile was observed in the previous days with no difference comparing treated and Hp treated tumor group. The myeloperoxidase activity was reduced at the 4^{th} day (51.23%), but not at 12^{th} day after tumor injection. There was also a reduction in the number of flinches (75.92%) by the treatment with *H. perforatum.* **Discussion:** There was no inhibition of paw edema, which is an indicative, although no t conclusive, that the dose of the extract used did not interfere with the tumor proliferation. The mechanical hyperalgesia was diminished by *H. perforatum* treatment at all time points evaluated while thermal hyperalgesia was reduced between 4-10^{th} days, indicating that different mechanisms might be involved in mechanical and thermal hyperalgesia in this model. The myeloperoxidase activity was reduced only in the 4^{th} day after tumor injection, which was also the peak of anti-hyperalgesia by the *H. perforatum* extract, and a progressive reduction of the Hp effect was observed, which corroborates the ineffectiveness over myeloperoxidase activity at 12^{th} day. Moreover, there is an increasing inflammatory response in this model. Therefore, the standard *H. perforatum* extract inhibited mechanical and thermal hyperalgesia and overt pain-like behavior induced by Ehrlich tumor cells. It also inhibited the local inflammatory response. Further investigation is necessary to determine the mechanisms of action of *H. perforatum*. **Financial support**: CNPq and Fundação Araucaria.

09.056 Central effects of aqueous extract of the leaves of *Passiflora edulis f. flavicarpa* in mice. Lima LA[1], Ayres ASJ[1], Rachetti VPS[1], Zucolotto SM[2], Gavioli EC[1]
[1]UFRN – Biofísica e Farmacologia, [2]UFRN – Farmácia

Introduction: *Passiflora edulis* is the most important economic species in the genus *Passiflora* due to its consumption as fresh fruit and in preparing juice. Leaves of *P. edulis* are popularly used in folk medicines for anxiety, nervousness and insomnia. However, *P. edulis* exhibits a considerable morphological variability, thus these species have been differentiated mainly by the fruit color. The population with yellow fruit was named as *P. edulis* f. *flavicarpa*, whereas *P. edulis* f. *edulis* has purple fruits. Previously the ethanol extract of *P. edulis flavicarpa* cultivated in China evoked anxiolysis in mice (Li et al., J. Ethnopharmacol. 133:1085-90, 2011). Herein, we investigated the effects of the aqueous extract of the leaves of *P. edulis flavicarpa* (PEF) in anxiety and mood in mice. **Methods:** Experiments were performed in male Swiss mice (30-35g), which were acutely treated with the extract of PEF (300 and 1000 mg/kg, orally, 60 min prior test) or standard drugs (diazepam 1 mg/kg - DZP - or nortriptyline 30 mg/kg - NTP, intraperitoneally, 30 and 60 min prior test, respectively). *P. edulis flavicarpa* was collected in Antônio Carlos/SC, and identified by Dr. DB Falkenberg (Dept of Botany, UFSC); the voucher specimen was deposited under the number FLOR 33886. Dried leaves were extracted by infusion (90°C; plant:solvent, 1:10, w/v; 10 min), the aqueous extract was filtered and lyophilized. Distinct groups of mice were submitted to the elevated plus maze (EPM) and forced swimming (FST) tests. The EPM is an apparatus composed of two open and two enclosed arms elevated from the floor. Animals were placed individually in the center of the apparatus, and the time spent in and the entries into open and enclosed arms were recorded for 5 min. In the FST, mice are placed in a cylinder with water at 25 °C, 6 min, and the time they spent immobile is recorded during the last 4 min of observation. This study was approved by Local Ethics Committee (Protocol N°: 032/2010). **Results and Discussion:** The acute administration of DZP increased the percentage of time spent in the open arms of the elevated plus maze test compared to control (mean±SEM; Control: 11.2±7.6; DZP: 42.8±3.1; n=5; P=0.004; t=4.00; T test). The administration of PEF extract evoked similar results as DZP (control: 22.3±3.7; PEF300: 35.1±3.6*; PEF1000: 33.9±4.3, n=13; F(2,36)=3.43; *P=0.04 vs. control; ANOVA, Dunnett´s test). No alterations in locomotion were detected after the treatment with PEF, as evaluated by the number of entries into enclosed arms in the EPM. In the forced swimming test, NTP significantly reduced the mouse immobility time (Control: 184.3±13.9; NTP: 75.8±24.5; n=5; P=0.004; t=4.04; T test). The acute administration of the PEF extract reduced immobility time only at the higher dose tested (control: 188.4±5.4; PEF300: 177.3±10.0; PEF1000: 151.7±12.0*, n=13; F(2,37)=3.85; *P=0.03 vs. control; ANOVA, Dunnett´s test), thus suggesting antidepressant-like actions. Taken together, the anxiolytic actions of the aqueous extract of PEF was confirmed in this specie of *Passiflora* cultivated in Brazil. Indeed, this is the first evidence of the antidepressant actions of the aqueous extract of PEF.
Financial support: BNDE

09.057 Determination of leishmanicidal activity, with a possible mechanism of action and cytotoxicity from reduced silver nanoparticles (AgNPs) with resin of *Anacardium occidentale* L. Lima DS[1], Rodrigues KAF[1], Amorim LV[1], Quelemes PV[2], Oliveira JMG[1], Carvalho FAA[3], Mendonça RZ[4], Leite JRSA[2] [1]UFPI – Medicinal Plants, [2]UFPI –Biodiversity and Biotechnology, [3]UFPI – Bioquímica e Farmacologia, [4]IBu – Parasitology

Introduction: *Anacardium occidentale* L. (Anacardiaceae) is popularly known as "cashew tree" and is used in folk medicine as anti-inflammatory, antimicrobial and antileishmanial. Nanotechnology is a promising research field, allowing the improvement of therapeutic effects of various substances, through the study of their physicochemical characteristics and by development of new substances and formulations. The aim of this study was to determine the toxic activity of the resin from *A. occidentale*, stabilized on silver nanoparticles, over *Leishmania amazonenesis* in the search for better leishmanicidal effect. **Methods**: The resin from *A. occidentale*, collected in Ilha Grande-PI/ Brazil, solubilized in ultrapure water (5% w/v) and precipitated with ethanol PA (1:4 v/v) and subsequently dried in a desiccator containing silica glass. The solution of AgNPs (54µg/mL) was prepared from $AgNO_3$ reduced with cashew gum to 0,3% (m/v) and ultrapure water in glass reactor at temperature of 75 °C ± 5. The leishmanicidal activity (54 to 0.42 µg/mL) was evaluated using 1 x 10^6 promastigotes per well in 96-well plates and then counted in a Neubauer haemocytometer at 24, 48 and 72 h. (Oliveira-Silva et al., Am. J. Trop. Med. Hyg., 78, 745, 2008). The cytotoxicity assay (27 to 3.37 µg/mL) was performed using VERO cells in microdilution plates of 96 wells, using trypan blue dye with an exposure time of 48 h. The nitric oxide production (27 to 0.42 µg/mL) was measured by determination of nitrites with Griess reagent 1% at a ratio of 1/1 using peritoneal macrophages of BALB/c. The statistical analyses were performed by ANOVA followed by Tukey test with $p < 0.05$. All procedures were approved by CEEA/UFPI under the number 001/2012. **Results and Discussion:** After analysis of the results at 24, 48 and 72 hour of exposure was reached an IC_{50} of 10,77; 9.26, 6.33 µg/ml, what demonstrated antileishmanial activity more effective in a stabilized form in AgNP than in the conventional manner (França et al., Rev. Soc. Bras. Med. Trop. 26, 155, 1993). The nitric oxide production decreases at the respective percentages of 62.4, 58.74, 48.59, 31.93, 26.73, 12.79, showing that antileishmanial activity does not act by this action mechanism. For cytotoxicity, the concentration of 27 µg/mL showed the highest amount of cells stained with trypan blue, with a reduction in cell viability by 25% compared to control. These results demonstrate that the cytotoxicity is similar to that shown in *A. occidentale*, and is not increased by the particles of $AgNO_3$. **Conclusion**: Studies have indicated that it is possible to improve the *A. occidentale* leishmanicidal potential employing the AgNPs technology, creating new possibilities of applications of nanomaterials in pharmacological activities. This work has also shown that cytotoxicity is not increased in this new formulation and that their mode of antileishmanial action is independent of the nitric oxide production. **Financial Support**: CAPES.

09.058 Phytochemical and pharmacological studies of *Mandevilla moricandiana* (Apocynaceae). Gomes MVS, Leal LA, Mello RJ, Ferreira LLDM, Raimundo JM, Konno TUP, Leal ICR, Muzitano MF UFRJ

Introduction: Natural products are used by humans since early times because of its many medicinal properties. Flavonoids, in turn, form a special class of metabolites that includes a major group of natural products with many biological activities, thus becoming an important target of study. *Mandevilla moricandiana*, a plant collected in "Restinga de Jurubatiba" ecosystem, has no pharmacological studies yet, illustrating the importance to evaluate the therapeutic potential as well as its phytochemical characterization, especially among bioactive flavonoids. **Methods:** 248.88 g of *M. moricandiana* leaves, previously dried and powdered, were submitted to maceration in ethanol/water (7:3). The crude extract (CE) has been submitted to liquid-liquid partition with *n*-hexane, dichloromethane, ethyl acetate, *n*-butanol, yielding 5 fractions (including residual water). All fractions were subjected to HPLC-DAD, C-18 Supelcosil column (25 cm x 4.6 mm) in gradient mode in water/acetonitrile. In parallel, CE and fractions was also evaluated for their potential pharmacological activities in anti-hypertensive, anti-inflammatory and antioxidant assays. For the anti-hypertensive assay, the vasodilator effect was investigated by recording isometric tension of aortae isolated from male Wistar rats (n = 6, positive control phenylephrine). For anti-inflammatory assay, male mice were used (n = 6), where the CE was solubilized in DMSO and used at doses of 5, 10 and 20 mg / kg intraperitoneally, and then evaluated by the methods of the hot plate test , abdominal writhing test and the formalin test. All protocols were approved by CEUA-CC (Protocol Macaé01). For the antioxidant assay, the DPPH method (300 mM), using a spectrophotometer (518 nm) to measures the antioxidant capacity, using the solution of DPPH and solvent as negative, and a solution of *Ginkgo biloba* as positive control. All the biological assays were performed by researcher collaborators. **Results**: The HPLC analysis of rutin showed lambda max (196, 265, 348 nm) and CE showed flavonoids peaks, according UV spectrum, in ethyl acetate fraction, representing 30% of total flavonoids, mainly flavones, flavonols and chalcones subclasses. A calibration curve was obtained using the flavonoid rutin and HPLC-DAD analysis. CE showed a total flavonoid content of 3.59% (w / w) expressed in rutin equivalents. Both *in vitro* and *in vivo* showed very promising results regarding to antihypertensive (70% reduction) and anti-inflammatory (75% reduction) assays with CE. The significant amounts of flavonoids in the extract together with the pharmacological activities, suggest a biological effect of flavonoids derivatives present in *M. moricandiana*. CE has been submitted to chromatographic procedures in order to isolate bioactive flavonoids since it showed a good result in antihypertensive assay (60% reduction in antihypertensive assay). **Discussion:** Many studies indicate the range of effects produced by the flavonoids as an antioxidant, anti-inflammatory, anti-hypertension, hypolipidemic, among others. Based on these results, we suggest that flavonoids may be responsible for the pharmacological effects observed. Moreover, these results become the starting point to follow with purification and identification (s) component (s) responsible for such effects. **Acknowledgments**: FAPERJ, FUNEMAC, UFRJ-Macaé, LAPRON / IMMT

09.059 Effects of latex proteins from *Calotropis procera* on the irinotecan-induced intestinal mucositis. Bitencourt FS[1], Aragão KS[1], Luz PB[1], Alencar RN[1], Lima-Júnior RCP[1], Ramos MV[2], Ribeiro RA[1], Alencar NMN[1] [1]UFC – Fisiologia e Farmacologia, [2]UFC – Bioquímica e Biologia Molecular

Introduction: Intestinal mucositis (IM) is a commonly side effect of irinotecan (CPT-11) based cancer chemotherapy. There is an incidence of IM associated-diarrhea in up to 25% of patients. However, there is not a pattern clinical management of this side effect. *Calotropis procera* (CP), a plant found in Africa, Asia and South America and abundant in the Northeast of Brazil shows anti-inflammatory activities in animal models. Thus, we aimed to evaluate the anti-inflammatory effects of a protein fraction from CP in CPT-11-induced IM. **Methods:** Swiss mice (n=10, 23±2g) were treated for 4 days with saline (Sal, 5 mL/kg, i.p.) or CPT-11 (75mg/kg, i.p.). In other experimental groups, CP (1, 5 and 50mg/kg/day, i.v.) was administered for 6 days, 30 min before the CPT-11. On the 7th day, we evaluated the total leukocyte count ($x10^3$/mL) and diarrhea (by scores). After sacrifice, the duodenum was collected for measurement of myeloperoxidase activity (MPO), morphometric analysis (villi/crypt), IL-1b level (pg/mL), and *in vitro* contractility (% contraction in relation to KCl 60mM). ANOVA/Bonferroni or Kruskal Wallis/Dunn was used as statistical tests. $P<0.05$ was accepted. Ethics Committee 99/10. **Results and Discussion:** CP attenuates diarrhea scores and MPO activity at 5mg/kg (diarrhea: 1[0-2]; MPO: 4.05±1.07) and 50mg/kg doses (diarrhea: 1[0-3]; MPO: 5.57±1.50) when compared with CPT-11 (diarrhea: 3[2-3]; MPO: 24.45±3.0, respectively). In addition, CP decreased over contractility (5mg/kg: 165.1±57.7), IL-1b level (5mg/kg: 143.5±41.5 and 50mg/kg: 182±65.7) and villi/crypt ratio (5 mg/kg: 2.79±0.17) increased in comparison with only CPT-11 treated mice (contractility: 906.1±225.4, IL-1b: 806.1±247.6 and villi/crypt: 1.63±0.17). However, CP did not change leukopenia induced by CPT-11 at doses tested. These findings show anti-inflammatory and anti-diarrhea effects of latex from *Calotropis procera* in CPT-11-induced IM. New approaches are being undertaken to elucidate the possible mechanism of action involved. **Acknowledgements:** CNPq and CAPES.

09.060 Effects of *Salvia officinalis* L. essential oil on *in vivo* and *in vitro* leukocytes migration. Nogueira de Melo GA[1], Grespan R[2], Fonseca JP[2], Farinha TO[2], Silva EL[2], Bersani-Amado CA[2], Cuman RKN[2] [1]UEM – Análises Clínicas e Biomedicina, [2]UEM – Farmacologia

Introduction: *Salvia officinalis* L. (Lamiaceae), popular name sage, is a common household plant grown in many parts of the world, including Brazil. Despite the use in folk medicine, few studies showing the anti-inflammatory effects of the essential oils and their compounds are available. In this work, we evaluated the effects of sage essential oil (SO) on the inflammatory response using in vivo and in vitro leukocyte migration assays. **Methods**: The SO (5, 10, or 25 mg/kg), indomethacin (5 mg/kg) or saline (0.9%) were administered orally 30 min before carrageenan injection (100 mg) in the internal spermatic fascia. The number of rolling, adherent and migrated leukocytes was determined. The in vitro leukocyte chemotaxis was performed in a modified Boyden's. Leukocytes were incubated before chemotactic stimulus with SO (10-4, 10-3 or 10-2 µl/ml) diluted in Hank's Buffered Salt Solution (HBSS) or with dexamethasone (10-5 M) and were allowed to migrate toward casein (50 mg/ml) or medium alone. The filter was removed, fixed, and stained (Harris's hematoxylin). Leukocytes were counted under a light microscope on at least 5 randomly selected fields. The experimental protocols were approved by the Ethical Committee in Animal Experimentation of the State University of Maringá (CEAE/UEM 041/2008). **Results and Discussion:** SO administration (10 or 25 mg/kg) reduced the number of leukocyte rolling and adhesion to scrotal chamber after 2 hours of carrageenan injection. Oral dose of the SO (10 or 25 mg/kg) also diminished the number of leukocytes migrated to the perivascular tissue 4 hours after the inflammatory stimulus. All doses tested (10-4, 10-3 or 10-2 µL/ml) resulted in a significant reduction of leukocyte chemotaxis (39.44 ± 1.02%, 49.06 ± 1.79%, and 52.67 ± 2.06%, respectively) induced by casein suggesting that SO has a direct effect on inhibition of leukocytes migration by independent manner to that in the effects on endothelial-leukocytes interactions. The data showed direct and systemic SO effects on leukocytes migration as an important mechanism of the anti-inflammatory action of sage. **Financial support**: CAPES and CNPq

09.061 Inhibitory effect of eugenol on experimental model of collagen-induced arthritis. Grespan R[1], Paludo M[1], Aguiar RP[1], Silva EL[2], Bersani-Amado CA[1], Cuman RKN[1] [1]UEM – Pharmacology and Therapeutics, [2]UEM – Chemistry

Introduction: This study was designed to test the efficacy of eugenol, a compound obtained from the essential oil of clove (*Syzygium aromaticum*) in collagen-induced arthritis (CIA), a well characterized murine model of rheumatoid arthritis. Macroscopic clinical evidence of CIA manifests first as periarticular erythema and edema in the hind paws. **Methods:** Male DBA1/J mice were injected, intradermally, with an emulsion of bovine collagen type II (100 µg) and complete Freund's adjuvant (4mg/ml). For all experiments, mice received daily, orally, 100 µg of eugenol (Biodinâmica Laboratory) or vehicle (saline in 1% Tween80) from the disease onset (day 25) until the end of the experiment. Besides, in the experiment where signs of arthritis were monitored, other group of mice was treated daily, orally, with indomethacin (1mg/kg) from day 25 until the end of the experiment. To evaluate leukocyte migration, the articular cavities of knee joints were washed twice with 5 µl PBS/EDTA and diluted to a final volume of 100 µl. Total cell counts and differential cell counts were performed, stained with Rosenfeld's stain and results were expressed as the number of mononuclear cells per cavity. To measure cytokine concentrations, articular tissues from ankle joints were harvested and triturated in 500 µl of PBS/EDTA by tissue-trimmer. Articular homogenates were centrifuged and supernatants collected for determination of IFN-g, IL-10, TGF-b, and TNF-α by ELISA (R&D system). Besides, cell viability was measured using an MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide] test and expressed in terms of relative absorbance of eugenol-treated cells (1, 3, 10, 30 or 90 µg/ml) versus control cells. **Results** were expressed as mean ± SEM. The means were compared by *Students t test* for two groups or by ANOVA followed by Tukey's post hoc test for multiple group comparisons ($p \leq 0.05$). **Results and Discussion:** Treatment with eugenol starting at the onset of arthritis (day 25) ameliorated clinical signs of CIA when compared with control group which received vehicle. Even though eugenol started to produce beneficial effects on the inflammation observed in CIA at the beginning of treatment, a statistically significant reduction in clinical score of CIA by eugenol become apparent after 35 days of disease induction. Treatment with indomethacin recorded the lowest score during all experiment. Furthermore, eugenol significantly reduced mononuclear cell migration (665.0 ± 166.5 cells/cavity) to the knee joint when compared with the vehicle-treated arthritic mice (6861 ± 975.4 cells/cavity) and also lowered the levels of cytokines (TNF-α, IFN-g and TGF-b) within the ankle joints. Eugenol treatment did not affect the *in vitro* cell viability as assessed using the MTT assay, even when cells were exposed to the highest concentration (90 µg/ml) of eugenol, the cells remain viable (> 80%). In conclusion, the results presented herein give additional insight into the previously described anti-inflammatory beneficial effects of eugenol, suggesting that this compound may be an alternative and/or supplemental treatment for chronic inflammatory diseases such as rheumatoid arthritis. **Financial Support:** CAPES. **Acknowledgements**: Jaílson Araújo Dantas and Célia Regina Miranda.

09.062 Contractile activity of *Lachesis muta* (Bushmaster) venom in rat ileum and stomach. Stroka A[1], Dias L[1], Rodrigues MAP[1], Brunieri LVP[1], Rennó AL[1], Sousa NC[1], Melgarejo AR[2], Hyslop S[1] [1]Unicamp – Farmacologia, [2]IVB – Zoologia Médica

Introduction: Systemic envenoming by bushmasters (*Lachesis* spp.) results in coagulopathy, bradycardia, hypotension and "neurotoxicity" involving the activation of autonomic cholinergic (muscarinic) mechanisms; muscarinic activation leads to bradycardia, prolonged hypotension, sweating, vomiting, abdominal pain and diarrhea, the latter three involving the digestive tract (Jorge MT *et al., Toxicon***35,** 545, 1997; Pardal PP *et al., Trans. R. Soc. Trop. Med. Hyg.***98,** 28, 2004). In this work, we examined the contractile activity of Peruvian *L. muta* venom in rat ileum and stomach. **Methods:** Male Wistar rats (300-400 g) were anesthetized with 2% isoflurane, exsanguinated and segments of the ileum and stomach were removed and mounted under 1 g of tension in organ baths containing 10 ml of 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); the solution was aerated continuously with 95%O_2-5%CO_2. Contractile responses were measured with a PowerLab system (ADInstruments). After stabilization for 30 min, venom (one concentration per tissue segment) was added to the organ bath in the absence and presence of atropine (non-selective muscarinic antagonist, 10 mM) or pyrilamine (histamine H_1 receptor antagonist, 1 mM). Creatine kinase (CK) was quantified using commercial kits (BioClin, Belo Horizonte, MG) in aliquots of KHS obtained before and after venom addition. 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. 2695-1). **Results:** Venom (5-500 mg/ml) caused a concentration-dependent increase in ileum contractile activity, with tension increasing from 1.0±0.1 g to 1.3±0.1 g (~30%), 2.9±0.5 g (~190%) and 3.0±0.3 g (~200%) with 5, 50 and 500 mg of venom/ml, respectively ($p<0.05$ compared to basal; n=5). CK release increased from 7.5±2.0 U/ml to 14.6±1.4 (95%), 63±8 (740%) and 147±30 (1860%) U/ml for the three concentrations indicated above, respectively ($p<0.05$ compared to basal; n=4). Venom also increased the contractile activity of rat stomach, with an increase from 1.8±0.2 g to 4.6±0.6 g (156%), 5.4±0.5 g (200%) and 5.1±0.6 g (183%) for 50, 150 and 500 mg of venom/ml, respectively. In addition, venom increased the rate of spontaneous contractions in stomach strips. Pretreatment with atropine and pyrilamine virtually abolished the ileal responses to venom (50 µg/ml) (decrease in tension from 2.9±0.5 g to 1.1±0.3 g and 1.3±0.3 g, respectively; $p<0.05$, n=5 each). **Conclusion:** These results indicate that *L. muta* venom stimulates neurotransmission in the digestive tract by activating muscarinic and histaminergic receptors. Such stimulation could partly explain the clinical manifestations after envenoming. The release of CK suggests that the venom also causes direct muscle damage. **Financial support**: CAPES, CNPq, FAPESP.

09.063 Histological evaluation of brain in adult offspring of mothers treated with scorpion venom *Tityus bahiensis* during the lactation period. Martins AN, Nencioni ALA, Dorce VAC IBu – Farmacologia

Introduction: Previous studies with scorpion venom *Tityus bahiensis* demonstrated that, when injected into rats during pregnancy, it causes neuronal loss in pups at the adulthood as well as behavioral changes. It also causes behavioral and reflexology changes in pups whose mothers were treated during lactation. Since the effects of inoculation of this venom during lactation period are unknown, in this study we aimed to verify some changes which might compromise the development of the hippocampus with regard to cell proliferation. **Methods**: The experimental protocol of this study was approved by the Ethics Committee in Animal Experimentation of the Instituto Butantan under number 460/08. Pregnant females were separated into a control group injected with saline (1.0ml/kg sc) or experimental group injected with venom (2.5 mg/kg sc) on the 16^{th} postnatal day. Pups were separated to perform histology in 22^{th} (n = 6) and 60^{th} (n = 6) days of life. Animals were deeply anesthetized with CO_2 and perfused using saline injected directly into the left ventricle of the rat, followed by injection of formalin to fix the tissue. These animals were decapitated and had their brains removed and stored in formalin solution until embedded in Paraplast. The brains were embedded in Paraplast and sliced on the thickness of 10 µm. The slices were stained with cresyl violet solution and analyzed on optical microscope at 40x magnification. The hippocampal areas CA1, CA3 and CA4 were analyzed. For statistical analysis we use "t" test. **Results**: In CA1 hippocampal region of adult rats from mothers treated on the 16^{th} day of lactation, the number of cells in the control group (C) was 62.1 ± 4.9 and in experimental E was 58.9 ± 8.5. In CA3 region was C: 39.4 ± 5.1 and E: 52.4 ± 5.1. In CA4 was C: 36.5 ± 3.3, and E: 33.3 ± 3.8. For rats in the postnatal period (22 days old) from dams treated on the 16th day of lactation the results obtained in CA1 were C: 74.4 ± 5.0 and E: 87.0 ± 3.2, in the CA3 C: 45.4 ± 2.4 and E: 47.5 ± 4.2 and in CA4 C: 34.7 ± 2.4 and E: 39.7 ± 1.5. **Discussion:** Inoculation of *Tityus bahiensis* scorpion venom into mothers on the 16^{th} day of lactation causes no change in the number of hippocampal cells despite earlier experiments had demonstrated behavioral and reflexological changes. **Financial support**: FAPESP (11/10222-5 and 2011/04498-8).

09.064 Diuretic activity and hypotensive effect of a butanolic fraction of *Scutia buxifolia* in normotensive and spontaneous hypertensive rats. Silva RCMVAF[1], Crestani S[2], De Souza P[2], Boligon AA[3], Athayde ML[3], Gasparotto Junior A[4], Marques MCA[2], da Silva-Santos JE[5] [1]UFPR – Farmacologia, [2]UFPR, [3]UFSM, [4]UNIPAR, [5]UFSC

Aim: *Scutia buxifolia* Reiss (Rhamnaceae), known as "coronilha", is used in folk medicine against cardiovascular diseases, such as hypertension. Previous studies *in vitro* demonstrated that the butanolic fraction (BuOH) obtained from barks of *S. buxifolia* induces full relaxation in rat aortic rings. This study aimed to evaluate the hypotensive effect of this fraction in normotensive and spontaneous hypertensive rats (SHR), as well as its diuretic activity. **Methods and Results**: All procedures were approved by the Institutional Ethics Committee from UFPR under protocol number 454. Diuretic activity was determined in normotensive animals kept in metabolic cages. Anesthetized rats had the left carotid artery cannulated and connected to a pressure transducer coupled to a MacLab® recording system, and an application program (Chart, v 4.1) from ADI Instruments. The rats were orally treated with different doses of the crude extract (10-30-100 and 300 mg/kg) or BuOH of *S. buxifolia* (3-10-30 and 100 mg/kg). The urine was collected and its volume was recorded at intervals of 2h for 8h. Electrolytes contents, pH and density were measured at the end of the experiment (8 h). Each group of normotensive male *Wistar* rats received the oral treatment with a single dose of BuOH or vehicle and had the mean arterial pressure (MAP) measurement at different times. In another set of experiments, normotensive rats received intravenous injection of BuOH (10 mg/kg), during continuous infusion with saline (10 µl/min), phenylephrine (an α1-adrenergic agonist; 20 nmol/kg/min), or L-NAME (a non-selective nitric oxide synthase inhibitor, 7 mg/kg/min), or after a single *bolus* administration of ODQ (a soluble guanylate cyclase inhibitor, 2 mg/kg). SHR animals received three different doses of BuOH (1, 3 and 10 mg/kg) and had their MAP measured. Administration of the crude extract promoted only a slight increase in urine volume (no significant), however the BuOH (10 mg/kg) significantly increased diuresis by 100 ± 55% when compared to the control group, an effect accompanied by augmented excretion of Na^+ and Cl^-, but without changes in urine density or pH. The oral acute administration of BuOH (10 mg/kg) significantly reduced the MAP of normotensive rats, with maximal effect (14 ± 3 mmHg) at 3 h after its administration. In animals subjected to a continuous infusion with phenylephrine the intravenous administration of BuOH at 10 mg/kg reduced the MAP by 20 ± 6,9 mmHg, but this same dose was unable to reduce the MAP of rats subjected to either a continuous infusion with L-NAME or a single *bolus* injection of ODQ. In addition, administration of BuOH fraction (10 mg/kg) was able to cause reduction in the MAP of SHR animals (32 ± 8 mmHg). **Conclusion**: Our results indicate that the oral administration of the butanolic fraction obtained from the barks of *S. buxifolia* is able to promote a diuretic and a potent hypotensive effect in both normotensive and spontaneously hypertensive rats. The stimulation of nitric oxide production and subsequent guanylate cyclase activation appear to be the main mechanisms involved in the hypotensive effect. **Sources of research support**: Rita de C. V. de A. F. Da Silva receives a fellowship from CAPES/Brazil.

09.065 Synthesis of lapachol analogues using Suzuki-Miyaura coupling methodology and evaluation of the antiophidic activity. Strauch MA[1], Gomes SLS[2], Machado MM[1], Cruz JMT[1], Silva AJ[2], Costa PRR[2], Melo PA[1] [1]UFRJ – Farmacologia e Química Medicinal, [2]UFRJ – Produtos Naturais

Objectives: Serotherapy against snakebite was discovered more than one hundred years ago, but the antivenin are not available all over Brazil or in some parts of the world. The use of plants in folk medicine is common mainly in the Brazilian Amazon area. We have investigated the antiophydic activity of lapachol, isolated from *Tabebuia impetiginosa* and synthetic structurally related naphthoquinones (LQB 166 and LQB 180), in different experimental protocols against *Bothrops atrox* venom. **Methods and Results**: We investigated the effects of the natural product and its analogues on venom proteolytic and collagenase activities (n=10) (Garcia et al., 1988; Chavira et al., 1984). Hemorrhagic activity were performed in mice (n=5) by i.d. injection of venom alone or preincubated with analogues (Melo et al.,1994). Lapachol and its analogues LQB 166 and LQB 180 inhibited the proteolytic activity in 78.9%, 95% and 98.7%, respectively. Collagenase activity was inhibited by lapachol, LQB 166 and LQB 180 in 63.89%, 63.03% and 83.93% respectively. The Hemorrhagic activity was inhibited for LQB 166 in 35% at a dose of 10 mg/kg. The experiments were performed in according with ethic principles of CEUA-UFRJ (DFBCICB 024). **Conclusion**s: Our studies indicate that Lapachol and analogues present relevant inhibition of *Bothrops* atrox venom enzymatic activities. **Financial Support**: CAPES, CNPq, PRONEX and FAPERJ

09.066 Evaluation of subchronic toxicity of the biofilm acetylated of manioc starch (BIOAC) in Wistar rats. Jesus DR[1], Espanhol CAA[2], Prando TBL[2], Sabatini DR[2], Lourenço ELB[3], Gasparotto Junior A[1] [1]UNIPar – Ciência Animal, [2]UNIPar – Farmácia, [3]UNIPar/UFPR – Farmácia/Farmacologia

Introduction: Looking to improve the conservation of foods like fruits and vegetables, currently we have been studied different ways and products to increase their durability. An alternative is to apply films (pellicle) edible to retard degradation of the same. The biofilm acetylated from of manioc starch was effective for this application, therefore besides retaining all the natural characteristics of the food, it's easy to obtain, cheap and doesn't cause environmental damage to the packaging used nowadays. Even with all its apparent advantages, there isn't available date on the toxicity of this composite. However there are no toxicological studies about its chronic use. Therefore, the objective of the present study was to evaluate the pre-clinical oral toxicity after repeated doses of the biofilm acetylated of manic starch (BIOAC) in Wistar rats. **Methodology:** Three doses of the BIOAC (30, 100 and 300 mg/kg) or vehicle (distillate water) were orally administered to male and female Wistar rats for 28 days. The animals were weighted daily and clinical signs of systemic toxicity were evaluated during the experiments. One day after the last treatment the animals were killed, blood was collected for the hematological and biochemical analysis, and later, organs were removed to determinate the relative weight and histopathological analysis. All experimental procedures adopted in this study were previously approved by the Institutional Ethics Committee of the Universidade Paranaense (nº 20768/2011). **Results and Discussion:** No signs of toxicity or death related to the treatment were observed. There were also no significant changes in body weight gain, hematological or biochemical parameters, relative organ weight and histopathological analysis of the liver, kidneys and spleen. The results gathered in this study show the absence of oral toxicity after repeated doses during 28 days of the BIOAC in Wistar rats in the doses used. However, other studies are necessary for a complete evaluation of the safety of this biofilm, as oral toxicity studies after repeated doses during 90 days, and genotoxicity and carcinogenicity studies. **Financial support:** DEGPP/ Universidade Paranaense – UNIPAR.

09.067 Evaluation of *in vitro* antibacterial and antifungical activity of crude extract and fractions of *Harpagophytum procumbens*. Schaffer LF[1], Denardi LB[2], Mario DAN[2], Boligon AA[1], Athayde ML[2], Wagner C[1], Alves SH[2], Fachinetto R[1] [1]UFSM – Farmacologia, [2]UFSM – Ciências Farmacêuticas

Harpagophytum procumbens, popularly known as “devil's claw”, is a plant used as a medicinal herb; it is indicated for adjunctive treatment of inflammatory origin diseases (GRANT et al., 2007). However, its mechanism of action is not well elucidated, and other effects in addition to its anti-inflammatory have been studied (FIEBICH et al., 2011). Currently, the large use of antimicrobial agents has caused high increase in resistance of many pathogens, requiring the development of new therapeutic options. Thus, the evaluation of antimicrobial activity of medicinal herbs is an alternative to search for new treatments (WECKESSES et al., 2007). Therefore, the purpose of this study was to investigate the in vitro antimicrobial activity of crude extract and different fractions of the root powder of *H. procumbens*. The root powder was commercially obtained. The crude extract was obtained by extraction with ethanol 70% to exhaustion of the plant for a week. The fractions were obtained by the fractionation with solvents of increasing polarity (chloroform, ethyl acetate and n-butanol). The following tests were performed to evaluate the antimicrobial activity of the crude extract and fractions of *H. procumbens* against: *Escherichia coli, Aeromonas sp., Staphylococcus aureus, Streptococcus agalactiae, Salmonella pullurium, Candida albicans, Candida glabrata, Candida parapsilosis, Candida krusei e Candida dubliniensis.* The minimal inhibitory concentration (MIC) of each fraction against the tested microorganisms were determined by the broth microdilution method using the CLSI (Clinical and Laboratory Standards Institute) M27-A3(2008) and M07-A8(2009) standardized reference method for yeast and bacteria, respectively. The concentrations of the crude extract and fractions used for the tests ranged from 3.9 to 2000 µg/mL. The experiments were performed in triplicate. The tests with the crude extract resulted in the following MICs: *C. albicans* MIC=62.5µg/mL; *C. dubliniensis* MIC=125µg/mL; *C. krusei* MIC=500µg/mL; *Aeromonas* sp. MIC= 500µg/mL; *E. coli* MIC= 1000µg/mL and *S. aureus* MIC=1000µg/mL. For the butanolic fraction, *C. krusei* and *E. coli* MIC= 500µg/mL and against *S. agalactiae* and *S. pullurium* MIC=1000µg/mL. For ethyl acetate fraction *C. krusei* and *S. agalactiae* MIC= 500µg/mL, against *E. coli* and *S. aureus* MIC=1000µg/mL. For chloroform fraction against *E. coli* and *S. agalactiae* MIC= 500µg/mL, against *Aeromonas* sp. and *S. pullurium* MIC= 1000µg/mL and against *C. krusei* and *S. aureus* MIC=2000µg/mL. Against *C. parapsilosis* and *C. glabrata* MICs ≥2000µg/mL. The study showed that *H. procumbens* has *in vitro* antifungal and antibacterial activity against the tested microorganisms, and that the crude extract showed the best antimicrobial activity exhibiting MIC= 62.5 against *C. albicans.* References: CLSI- CLINICAL Laboratory and Standards Institute- M27-A3, 2008; CLSI- Clinical Laboratory and Standards Institute- M07-A8, 2009. FIEBICH, B.L. et al. Phytother. Res. v. 26, p. 806, 2012 GRANT, L. et al. Phytother. Res. v.21, p. 199, 2007 WECKESSES, S. et al. Phytomedicine. v.14, p. 508, 2007. **Sources of financial support**: CAPES, CNPq, FAPERGS

09.068 Different effects of bothropstoxin I and II on NA^+/K^+-ATPase and CA^{2+}-ATPase from type serca of murine fast-twitch muscle extensor digitorum longus. Ayres RO, Feijó PR, Tomaz MA, Melo PA, Cunha VMN, Quintas LEM ICB-UFRJ – Farmacologia e Química Medicinal

Introduction: Snakebites constitute a significant risk to public health in Latin America and myonecrosis is one of the main disabling consequences. Experimentally, the injection of *Bothrops jararacussu* venom causes rapid necrosis and a subsequent regeneration in the *extensor digitorum longus* (EDL) of mice. The damage to the sarcolemma promotes depolarization, influx and mobilization of Ca^{2+} ions, contraction of the fiber and rapid efflux of cytosolic enzymes. We showed previously that EDL ion transport ATPases essential to intracellular calcium homeostasis are significantly affected by the crude venom, with an overall increase of Na^+/K^+-ATPase and decrease of Ca^{2+}-ATPase (Schaffazick et al., *Toxicon* 55:52, 2010). We aim to study the effect of perimuscular injection of *B. jararacussu* venom myotoxins BthTX I and BthTX II on the expression of Ca^{2+}-ATPase SERCA and Na^+/K^+-ATPase isoforms in mouse EDL and to assess the ability of these myotoxins to affect the activity of these ATPases *in vitro* and *in vivo*. **Methods:** The use of animals was approved by CEUA-CCS (DFBCICB022). Adult Swiss mice divided into 3 groups received an injection of 50 ml of each myotoxin (1 mg/g) or saline solution (PSS) on the right paw. The EDL muscles were removed 1, 3, 7 and 21 days after administration of myotoxins and homogenized. Western blot analysis was carried out using specific antibodies against α1 and 2 isoforms of Na^+/K^+-ATPase, and SERCA1 and 2 isoforms of Ca^{2+}-ATPase. The colorimetric method of Fiske and Subbarow (1925) was used to determine the *in vitro* effect of myotoxins on the activity of preparations enriched in Ca^{2+}-ATPase (rat EDL) and Na^+/K^+-ATPase α1 (rat kidney) and α2/α3 (rat brain) isoforms. **Results:** We observed a reduced expression of Na^+/K^+-ATPase α2 isoform in the first day post-injection ($n=3$, $p<0.05$; One-way Anova followed by Tukey post-test). No changes were seen in Na^+/K^+-ATPase α1, SERCA1 and 2 expressions. While *in vitro* BthTX I increased rat kidney (α1) Na^+/K^+-ATPase activity in a very high concentration (40 mg/mL), 0.1 mg/mL BthTX II significantly inhibited Ca^{2+}-ATPase activity (90%; $n=4$, $p<0.05$; paired Student *t*-test*)*. Experiments performed with different proportions of associated myotoxins suggest that there is no potentiation. **Discussion:** The isolated myotoxins seem to act differently from the crude venom, with a lower degenerative potential thus mildly affecting the expression of P-type ATPases. On the other hand, SERCA emerges as an important target for *B. jararacussu* venom and its myotoxins may contribute to myonecrosis The effect of myotoxins on the Ca^{2+}-ATPase activity *in vivo* and their effect when administered in association are in progress. **Sources of research support:** PIBIC/CNPq, CAPES, FAPERJ.

09.069 Inhibition of the intracellular Ca^{2+} stores and of the $Ca2^{+}$ sensitization in the vasorelaxant effect induced by geraniol. Feitoza PR[1], Fraga BP[2], Cunha PS[2], Araújo AAS[2], Nunes RS[2], Marchioro M[2], Medeiros IA[3], Santos MRV[2], Ribeiro ÊAN[1]
[1]ESENFAR-UFAL, [2]UFS – Fisiologia, [3]UFPB – Tecnologia Farmacêutica

Introduction: Geraniol is a monoterpene found in essential oils of various herbs with activity on the cardiovascular system. The objective of the present study was to evaluate the vasorelaxant effect induced by geraniol in rat mesenteric artery. **Methods:** Male Wistar rats (200 – 300g) were euthanized by exsanguination under anesthesia and superior mesenteric artery was removed and cut in rings (1-2 mm), which were mounted in organ baths containing 10 mL of Tyrode's solution at 37°C and gassed with carbogen. For isometric tension recordings, each ring was fixed in a force transducer connected to an acquisition system. **Results and Discussion:** In mesenteric artery rings with functional endothelium pre-contracted with 10 μM of phenylephrine(control), geraniol (10^{-8} – 10^{-2} M) was able to induce relaxation in a concentration-dependent manner (Emax = 110 ± 5%; n = 6) which was not attenuated after removal of endothelium (Emax = 108 ± 6%; n = 4). In endothelium-denuded rings pre-contracted with KCl 80 mM, geraniol produced relaxation that was significantly ($p<0.05$) higher than those obtained in rings with functional endothelium pre-contracted with phenylephrine (Emax = 142 ± 14%; n = 6). Furthermore, the incubation with geraniol was able significantly to antagonize the phasic contractions induced by phenylephine and caffeine in without calcium solution, and the tonic contractions induced by sodium orthovanadate, a tyrosine phosphatase inhibitor. **Conclusions**: Taken together, these results suggest that geraniol produces a vasorelaxant effect by an endothelium-independent mechanism in the rat mesenteric artery. This effect appears to involve inhibition of the Ca^{2+} release through intracellular Ca^{2+}stores sensitive to phenylephrine and caffeine, and inhibition of Ca^{2+} sensitization. **Acknowledgments**: CNPq/FAPITEC, Brazil. License number of Animal Ethics Committees: 23065.009481/2011-2/

09.070 Investigate the role of the sympathetic nervous system in the bradycardic and hypotensive response induced by the alpha-terpineol in spontaneously hypertensive rats. Tenorio EP[1], Ferreira AKB[1], Alves JC[1], Sabino CKB[2], Ferreira Filho ES[2], Oliveira AP[2], Ribeiro EAN[1] [1]ESENFAR-UFAL, [2]UFPI – Plantas Medicinais

Introduction: Alpha-terpineol, is a monoterpene that can be isolated from *Alpinia speciosa* or *Salvia Officinalis*. This monoterpene has proven the following biological activities: gastroprotective, and vasorelaxant effects. The objective of this study was to evaluate the hypotensive activity of alpha-terpineol in spontaneously hypertensive rats and to evaluate the role of the sympathetic nervous system in this response. **Methods:** Spontaneously hypertensive rats male (250-350g) were anesthetized with sodium pentobarbital (45 mg/kg, i.p) and catheters were inserted polyethylene within the abdominal aorta and inferior vena cava via the left femoral artery and vein for blood pressure measurements and administration of drugs, respectively. The results were expressed as mean ± SEM and considered significant when ** p <0.01 and *** p< 0.001 in "t" student. All experiments were approved by the ethics committee on animal research nº. 0106/08. **Results and discussions**: In non-anesthetized rats (n=7) alpha-terpineol (1, 5 and 10 mg/kg i.v) promoted hypotension (-15,2±2,1;-33,4±3,4 and-36,0±3,1 %MAP, respectively) independently of dose and bradycardic effect from 5 mg /kg and 10 mg/kg (4,5±0,9; -77,6±2,1 and -58,8±8,2 % HR). After blocking with atropine 2 mg/kg i.v (n=5) a muscarinic receptor antagonists, the hypotensive response wasn´t affected, however there was change in heart rate (-3.52±0,8***;-12,99±5,8 ***, -19,32±7,6** % HR). Pretreatment with hexamethonium 20 mg/kg i.v (n=5) there was a potentiation of hypotension at doses of 1 and 5 mg/kg (-26.9 ±3,7 **; -64,1±6,0*** %PAM). But in anesthetized rats with sodium pentobarbital 45 mg/kg, i.p (n=5) bradycardia was significantly attenuated. **Conclusion:** The results show no sympathetic nervous system involvement in these effects. However, the results show that there is participation the muscarinic receptors in the mechanism associated with the bradycardiac effect induced by alpha-terpineol. **Financial Support:** CNPq and FAPEAL

09.071 Antimicrobial activity *in vitro* of ethanolic extract of stem of *Maytenus erythroxylon* in pathogenic bacteria. Lucena KL[1], Frade ADS[2], Duarte MC[3], Farias RLGP[1], Nascimento JS[1] [1]UFPB – Fisiologia e Patologia, [2]FCM-PB, [3]UFPB – Biotecnologia

Introduction: *Maytenus erythroxylon* belongs to the family Celastraceae[1] has antiulcerogenic activity and so far in phytochemical studies was isolated from leaves of *Maytenus erythroxylon* a triterpene of the class Friedelane, compound 3β-friedelinol. Triterpenes friedelane are characteristic of the genus Maytenus[2]. According to the literature terpenes may have antimicrobial activity and antifungal[3]. The objective of this study was to evaluate the in vitro antimicrobial activity of ethanolic extract of the stem of *M. erythroxylon* on bacteria pathogenic to humans. **Methods:** The botanical material was identified by Profa. Dra. Maria de Fátima Agra (UFPB); a voucher specimen is deposited at the Herbarium Profº. Lauro Pires Xavier, UFPB. The stalk of the plant was dried in an oven at 40 ° C, crushed in a mill mechanic and subjected to exhaustive maceration with ethanol for 72 hours, three times. The extractive solution was concentrated in rotaevaporator at 35 ° C. For performing the microbiological assays, we selected isolates of *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Enterococcus sp., Klebsiella pneumoniae and Acinetobacter spp*. clinical source. In sterile Petri dishes, were seeded suspension of micro-organism (containing 1.5 x108UFC/mL based on the scale of Mac Farland) using "swabs" sterilized. Then, were drilled wells with a capacity of 50 microliters using sterile tubes, which were deposited in 50 microliters aliquots of the ethanol extract at concentrations of 0 - 25 - 50 - 75 - 100mg/mL in triplicate. The negative control used was distilled water. The Petri dishes prepared were incubated at 36 ° C for 24 hours. The results were evaluated by measuring the diameter of the zones of inhibition in mm, formed around the wells. Extracts were considered active when presented inhibition zones greater or equal to 10mm. **Results and Discussion:** The results indicated that *M. erythroxylon* showed inhibitory activity against *S. aureus* and *Enterococcus sp*., producing inhibition zones 12.6 and 12mm, respectively, the concentration of 100mg/mL, 11.3 and 11.6 mm, respectively at a concentration of 75mg/mL, 10 and 10.3 mm respectively to the concentration 50 mg / mL and 8.6 and 9mm respectively at a concentration 25mg/ml. On the other hand, the other bacteria tested *(E. coli, P. aeruginosa, K. pneumoniae and Acinetobacter spp.)* were not sensitive to the extract analyzed. The ethanolic extract of the stem *M. erythroxylon* has compounds that exert antibacterial action against some of the different microorganisms tested, confirming its use in folk medicine against infections. And also suggest that the extract of *M. erythroxylon*, mainly consisting of terpenes, shows selectivity against Gram-positive and no effect on the Gram-negative bacteria. **Financing:** CNPQ. **References:** [1]Carvalho-Okano, R.M. Estudos taxonômicos do gênero Maytenus Mol. emend. Mol (Celastraceae) do Brasil extra amazônico, 1992; [2]Duarte. Triterpeno Friedelano isolado das folhas de *Maytenus erythroxylon,* 2010; [3]Andrade. Avaliação fitotóxica de extratos de *Cenchrus echinatus* – Timbete. 2007

09.072 Participation of glutamatergic system in the effects of a toxin isolated from *Tityusserrulatus* scorpion venom. Freitas MM[1], Nencioni ALA[1], Lebrun I[2], Dorce VAC[1] [1]IBu – Farmacologia, [2]IBu – Bioquímica e Biofísica

Introduction: Studies performed in our laboratory showed that the scorpion toxins are able to cause seizures and neuronal loss when injected in hippocampus. These studies showed that *Tityus serrulatus* scorpion toxins induce the release of neurotrasmitters. Glutamate apparently, is the main responsible by the excitotoxicity observed. It was previously demonstrated that the toxin IV-IV from T*. serrulatus* venom causes behavioral and electrographic effects when injected into hippocampus. **Objective**: The aim of this study is to investigate the participation of glutamate in the central effects of toxin IV-IV using their receptors antagonists: MK-801, AP-3, MCPG, CNQX e AP-5. **Material and Methods**: Toxin IV-IV was purified from fresh venom of the scorpion *T. serrulatus*. All the experimental procedures were conducted with prior permission of the Institutional Ethical Committee for Experiments on Animals (protocol number 677/09). Male Wistar rats (220 – 250g) were anesthetized and positioned in a stereotaxic frame. Stainless steel guide cannulas and bipolar twisted electrodes were chronically implanted in the hippocampus. One day after surgery the animals were injected intrahippocampally with, AP-3 (0.1 mg/rat) MCPG (0.2 mg/rat), CNQX (2.0 mg/rat) and AP-5 (1.0 mg/rat) prior to 1µg/µl of toxin (n=6), MK-801 (1.0 mg/kg, IP) or ringer solution (control group) prior to 1µg/µl of toxin (n=6-9). After the injections, continuous eletroencephalografic recording (EEG) and observations of animals' behavior were performed for a period of 4h. Seven days after the injections the animals were sacrificed and perfused. The brains were removed and prepared for histological analysis. ANOVA followed by Tukey's test were employed for statistical analysis ($p < 0.05$). **Results**: The toxin induced yawning, chewing, "wet dog shakes" (WDS), myoclonus, postural loss, limbs paralysis and respiratory distress. More elevated dose caused additionally pulmonary edema, chromodacryorrhea, clonic and tonic convulsion and, sometimes, death. AP-5, CNQX, MCPG and MK-801 reduced and AP-3 totally eliminated all behavioral and electrographic alterations. Only MCPG and CNQX reverted totally the hippocampal damage caused by the toxin. **Discussion:** All the antagonists were partially efficient in block the effect of the toxin. The glutamatergic system is involved in seizures and excitotoxicity caused by the toxin. **Financial support**: FAPESP (proc: 2009/54711-0).

09.073 Study of alterations on isolated rat kidney promoted by different concentrations of *Bothropoides lutzi* venom. Sousa DF[1], [1]Jorge, ARC, [2]Borges-Nojosa DM, [1]Ferreira JM, [3]Queiroz MGR, [1]Bindá AH, [3]Martins AMC, [4]Menezes, DB, [1]Monteiro HAS. [1]UFC – Fisiologia e Farmacologia, [2]UFC – Biologia, [3]UFC – Análises Clínicas e Toxicológicas, [4]UFC – Patologia e Medicina Legal,

Bothrops and *Bothropoides* snakes cause 70% of the ophidic accidents in Brazil and some renal alterations (OLIVEIRA, Rev. Soc. Bras. Med.Trop., v.43, p.662, 2010). However, the renal effects of venoms of various snakes such as *Bothropoides lutzi* are poorly studied. The aim of this work was to study the renal effects of *Bothropoides lutzi* venom (BLV) on isolated kidney of rats comparing different concentrations of this poison**.** Kidneys of Wistar rats (180-250 g; n=6) were utilized and perfused for 120 min (FONTELES, Am. J. Physiol, v. 244, p. 235, 1983) with Krebs-Henseleit solution (KHS) containing 6% of bovine serum albumin previously dialyzed. At 30 min, 0.01, 0.1 and 1.0 mg of BLV was added to the system perfusion after internal control, corresponding the concentrations of 0.1, 1.0 and 10.0 µg/mL, respectively . An external control group with kidneys perfused only with the solution described was carried out too. The effects of BLV on glomerular filtration rate (GFR), urinary flow (UF), perfusion pressure (PP), renal vascular resistance (RVR) and percentage of sodium (%TNa^+), potassium (%TK^+) and chloride (%TCl^-) tubular transport were studied. Histological analyses were performed to observe possible alterations on the organs perfused. The protocol was approved by the Ethics Committee on Animal Research of the UFC under number 068/08. The results were expressed as mean ± S.E.M. and the groups were compared by ANOVA (post-test of Tukey), adopting as a significance criterion $p<0.05$. The results showed that the BLV induced significant changes in all renal parameters studied, mainly the reduction of perfusion pressure ($PP_{control\ 90min}$ = 108.69 ± 5.08mmHg *vs.* $PP_{BLV\ 1.0\mu g/mL\ 90min}$ = 73.55 ± 2.79 mmHg) and renal vascular resistance ($RVR_{control\ 90min}$ = 5.32 ± 0.57 mmHg. mL^{-1}. g^{-1}. min^{-1}*vs.* $RVR_{BLV\ 1.0\mu g/mL\ 90min}$ = 3.39 ± 0.30 mmHg. mL^{-1}. g^{-1}. min^{-1}), while increased urinary flow ($UF_{control\ 120min}$ = 0.160±0.020 $mL.g^{-1}$. min^{-1}*vs.* $UF_{BLV\ 10.0\mu g/mL\ 120min}$ = 0.981±0.103 $mL.g^{-1}$. min^{-1}) and glomerular filtration rate ($GFR_{control\ 120min}$ = 0.697±0.084 $mL.g^{-1}$. min^{-1}*vs.* $GFR_{BLV\ 10.0\mu g/mL\ 120min}$ = 3.152 ± 0.342 $mL.g^{-1}$. min^{-1}) in at least one of the concentrations studied. The poison is highly toxic to the renal tubules, and this fact was demonstrated by the decrease %TNa^+(reduction of 36% when the concentration of 1 µg/mL was compared with the control group) , %TK^+(reduction of 27% when the concentration of 1 µg/mL was compared with the control group) and chloride %TCl^- (reduction of 34% when the concentration of 1 µg/mL was compared with the control group). In the other concentrations were also observed reductions in the transport of electrolytes. All renal structures can be affected by animal toxins, mainly in bothropic bites. Tubular damages were also observed by histological analysis in all concentrations studied (cell death). The main renal structures affected by BLV were renal tubules, probably by direct and indirect action of this poison (SITPRIJA, V. Nat Clin Pract Nephrol. 2008 v. 4, p. 616, 2008). So BLV promoted nephrotoxicity, damaging renal tissue independently of concentrations such as demonstrated by the renal parameters studied and histological analysis of renal tissue. **Financial support**: CAPES

09.074 Involvement of the nitric oxide pathway in endothelium-dependent vasorelaxation induced by 5,7,4'-trimethoxyflavone in isolated rat superior mesenteric arteries. Oliveira Filho AA[1], Dias LMA[1], Alustau MC[1], Assis KS[1], Assis TJC[1], Furtado FF[2], Queiroz TM[1], Machado NT[1], Fernandes HMB[1], Maia GLA[1], Barbosa Filho JM[1], Medeiros IA[1] [1]UFPB – Ciências da Saúde, [2]UFCG – SAÚDE

Introduction: *Praxelis clematidea* is a member of the Asteraceae family, native from South America. The flavonoid 5,7,4´-trimetoxyflavone (TMF) was isolated from *Praxelis clematidea* leaves ethanolic extract. Since few studies were related in the literature reporting the cardiovascular activity of the compound, the present study investigated the endothelium-dependent vasorelaxation effect induced by TMF in isolated rat superior mesenteric arteries. **Methods:** All protocols of this study were approved by the CEPA/UFPB (Protocol nº 0204/11). Isolated rat superior mesenteric rings (1-2 mm) were suspended by cotton threads for isometric tension recordings in a Tyrode's solution at 37 ºC, gassed with a 95% O_2 and 5% CO_2, under a resting tension of 0.75 g and isometric tension changes were measured continuously by a sensitive myograph system. **Results and Discussion:** In rings pre-contracted with phenylephrine (Phe, 1 µM), TMF (10^{-12}-10^{-3} M) caused concentration-dependent relaxation in the presence of functional endothelium ($E_{máx}$ = 100.8 ± 2.6%; pD_2 = 5.44 ± 0.12; n = 6), and this effect was significantly inhibited after removal of endothelium ($E_{máx}$ = 102.5 ± 4.9%; pD_2 = 4.50 ± 0.10; n = 6). The vasorelaxant effect induced by TMF was significantly attenuated in the presence of a NOS inhibitor (L- NAME 100 µM; pD_2 = 4.52 ± 0.08, n = 5), a soluble guanylate cyclase inhibitor (ODQ 10 µM; pD_2 = 4.36 ± 0.11, n = 5) or a NO scavenger (PTIO 300 µM; pD_2 = 4.62 ± 0.09, n = 5). The NO precursor, L-arginine (1 mM), completely reversed the effect induced in the presence of L-NAME (pD_2 = 5.85 ± 0.14, n =5), suggesting participation of the NOS/NO/CGs pathway in the relaxation produced by TMF. Pre-incubation with PGI_2 inhibitor (indomethacin, 1 µM) did not change the vasorelaxant response induced by TMF, indicating the non-involvement of COX metabolites. To evaluate the involvement of potassium channels, the preparations were pre-incubated with Tyrode's modified solution, KCl (20 mM) or with a non-selective K^+ channel blocker, tetraethylammonium (TEA, 3 mM). In the presence of KCl (pD_2 = 4.62 ± 0.08, n = 5) and TEA (pD_2 = 4.28 ± 0.10, n = 5) the response produced by TMF was attenuated, indicating that this compound probably acts by endothelium-dependent potassium channels activation. In addition, in the presence of glibenclamide (10 µM), a K_{ATP} blocker, the response evoked by the compound was not altered. Nevertheless, the pre-incubation of 4-aminopyridine (1 mM; pD_2 = 4.70 ± 0.08, n = 5), a K_V blocker, or TEA (1 mM; pD_2 = 4.48 ± 0.04, n = 5), a BK_{Ca} blocker, significantly attenuated the potency of the flavonoid induced vasorelaxant response, suggesting the involvement of, at least, K_V and BK_{Ca}. These results suggest that endothelium-dependent vasorelaxation induced by TMF is probably due to the NOS/NO/CGs pathway, with consequent activation of K_V and BK_{Ca} potassium channels. **Financial Support:** CNPq and CAPES

09.075 Effects of *Hypericum perforatum* on vacuous chewing movements induced by fluphenazine in rats. Reis EM[1], Busanello A[2], Reckziegel P[1], Leal CQ[3], Figueira FH[2], Fachinetto R[1] [1]UFSM – Farmacologia, [2]UFSM – Bioquímica Toxicológica, [3]UFSM – Farmácia

Introduction: Chronic treatment with antipsychotic can produce serious side effects, known tardive dyskinesia (TD) in humans or orofacial dyskinesia (OD) in rats. TD and OD have been associated with oxidative stress since it is described in literature that in acute treatments with antipsychotics occur an increase in dopamine turnover. Monoamine oxidase (MAO) is the main enzyme responsible for dopamine metabolism. It catalyses the desamination of monoamines and may produce hidroxyl radicals during this process. *Hypericum perforatum,* commonly known as St. John's Wort, is used in the treatment of mild to moderate forms of depression and its antidepressant effect is attributed in part to inhibition of monoamine-oxidase. In this context, we examined the effects of *H. perforatum* in an experimental model of OD induced by acute treatment with fluphenazine and the possible involvement of MAO activity in these models. Furthermore, we evaluated forced swimming test since we choose a sub-antidepressant dose to avoid side effects. **Methods:** This project was approved by Comissão de Ética no Uso de Animais-UFSM (nº 109/2011). *H. perforatum* was obtained commercially (DEG®) and it contained 0,31% of hypericin as informed by the supplier's report. Adult male Wistar rats were divided into four groups: 1) Control (n=5); 2) *H. perforatum* (n=5); 3) Fluphenazine (n=10) and 4) Fluphenazine plus *H. perforatum* (n=10). Fluphenazine enantate (25 mg/Kg) was administered intramuscularly only at first day of experiment. *H. perforatum* was offers to the animals in the place of water at a dose of 300 mg/Kg/day from day 1 up to day 21. Vacuous Chewing movements (VCMs), locomotor activity and the time of immobility in the forced swimming test (FST) were evaluated in day 21. After behavior evaluations, the animals were killed by decapitation and the striatum was used to determine MAO activity. Data were analyzed by one-away ANOVA. **Results:** Fluphenazine treatment increased the prevalence of VCMs (more than 40 VCMs in 6 minutes) in 20% of animals and the co-treatment with *H. perforatum* did not alter this parameter. Fluphenazine treatment decreased locomotor activity and *H. perforatum* treatment increased it. *H. perforatum* or fluphenazine did not change the immobility time in the forced swimming test, as well as MAO activity in striatum. **Discussion:** Our results showed that *H. perforatum* did not alter the prevalence of VCMs induced by fluphenazine in rats. In addition, *H. perforatum* did not showed antidepressant effect in the forced swimming test, as expected, and in the MAO activity in striatum at a dose used in this experiment. More studies with others doses of *H. perforatum* in this experimental model are needed to elucidate the relation of MAO inhibition by medicinal herbal and OD. **Acknowledgments**: CAPES, CNPq, FAPERGS

09.076 Effect of cinnamic acid esters on lipid metabolism of animals fed hypercholesterolemic diet. Damasceno DV[1], Arruda-Filho ACV[1], Melo TS[1], Pereira NBS[1], Holanda RTM[1], Sousa DF[2], Freitas AMP[1], Queiroz MGR[1], Vieira IGP[3], Guedes MIF[4] [1]UFC – Análises Clínicas e Toxicológicas, [2]UFC – Fisiologia e Farmacologia, [3]PADETEC-UFC, [4]UECE – Nutrição

Introduction: Gamma-oryzanol is a mixture of compounds isolated from rice bran. Some pharmacological properties have been described as reduction the levels of serum cholesterol and hepatic cholesterol synthesis. Recently, a mixture of cinnamic acid esters (CAE) has been isolated from carnauba wax. These substances are structurally very similar to the γ-oryzanol. Therefore, there are prospects in the discovery of a new substance with biological activity on lipoprotein metabolism. **Objective**: To investigate the effect of hypolipidemic cinnamic acid esters (CAE) in mice fed with hypercholesterolemic diet (HD). **Methods:** Cinnamic acid esters were obtained after esterification of Cinnamic acid acquired of Sigma-Aldrich®. Male mice Swiss (25-35g) were divided (n=6) in: standard (SD) and hypercholesterolemic diet (HD), simvastatin 20mg/kg (SV20), CAE 10mg/kg (CAE10), CAE 50mg/kg (CAE50) and CAE 100mg/kg (CAE100). For the increasing of total cholesterol (TC), all animals except the SD, were fed for 10 weeks with HD. Hypercholesterolemic animals were treated with saline, SV20, CAE10, CAE50 or CAE100 for 8 weeks. After this period, blood samples were collected for determination of glucose (GL), TC and triglyceride (TG). This protocol was accepted by the Ethics Committee on Animal Research of the UFC under number 38/08. The results were expressed as mean±SEM, and the groups were compared by ANOVA (post-test Newman-Keuls), adopting as significance criterion $p<0.05$. **Results**: TC (mg/dL) levels increase was 75.8% (SD: 143.7±6.5; HD: 252.6±9.4). SV20 (190.3±5.2), CAE10 (203.1±11.6), CAE50 (194.5±7.2) and CAE100 (158.0±11.0) showed a reduction of TC (mg/dL). The groups fed with HD showed reduced levels of TG levels (mg/dL) in relation to SD (SD: 149.9±17.3, HD: 93.0±7.1; CAE10: 95.3±9.0; CAE50: 101.3±5.4 CAE100: 70.6±6.4; SV20: 90.8±7.6). The treatment with CAE10, 50 and 100mg/kg or SV20 promoted GL (mg/dL) reduction (SD: 127.4±6.4; HD: 196.7±10.5; CAE10: 168.1±4.9; CAE50: 156.9±4.8; CAE100: 135.4±6.5; SV20: 164.9±9.5). **Discussion:** HD was composed of 1% cholesterol + 0.1% cholic acid + 10% *Cocus nucifera* oil (Wilson, J Nutr Biochem., v.18, p.105, 2007). The cholic acid, besides of potentializing the absorption of dietary intake cholesterol, also inhibits the conversion of cholesterol to bile acids, favoring the lipids accumulation (Machado, Ciênc. Tecnol. Aliment. v.23, p.270, 2003). There may have been a synergistic action of cholic acid with the cholesterol present in the HD, providing the increasing of TC. It was found a reduction of TG that can be justified by the presence of coconut oil, a medium chain fatty acid that may have promoted a protective action. The CAE may have favored the conversion of cholesterol into bile acids reducing their blood levels. Furthermore, it suggests an inhibition of intestinal absorption of both CT and GLU. CAE100 provided a more significant reduction of TC and GLI than SV. More studies are needed to elucidate the exact CAE mechanism of action on carbohydrate and lipoprotein metabolism. **Financial support:** FUNCAP and Banco do Nordeste.

09.077 Effects of *Bauhinia forficata* on locomotor activity and vacuous chewing movements induced by haloperidol in rats. Leal CQ[1], Peroza LR[2], Busanello A[2], Fachinetto R[3] [1]UFSM – Farmácia, [2]UFSM – Bioquímica Toxicológica, [3]UFSM – Farmacologia

Introduction: Haloperidol (HAL), a typical antipsychotic, has been used in the treatment of the schizophrenia (Andreassen, 2000), and can cause extrapyramidal symptoms, like tardive dyskinesia (TD) in humans and orofacial dyskinesia (OD) in rodents. Oxidative stress has been associated to TD and OD development. Because of this, several antioxidants have been tested in animal models of OD. Leaves of the genus *Bauhinia* (Fabaceae) are known popularly as cow's foot (Yeh et al. 2003), and some studies have indicated that the aqueous extract of the leaves of *Bauhinia. forficata* is a potential source of natural antioxidants (Damasceno et al. 2004; Khalil et al. 2008; de Sousa et al. 2004). The aim of this study was to evaluate the in vitro antioxidant potential of *B. forficata* and evaluate the use of decoction in the motor alterations caused by chronic treatment with haloperidol in rats. **Methods:** *B. forficata* powder was added to distilled water (2.5 g/L), boiled for 10 min, and filtered. The decoction was used for *in vitro* and *in vivo* experiments. The antioxidant activity of *B. forficata* was evaluated *in vitro* using different concentrations of *B. forficata* on pro-oxidants-induced-lipid peroxidation in whole brain (Ohkawa et al, 1979). For *in vivo* experiment, male Wistar rats (±2 months old), were separated in control group (soy oil (i.m.) + water to drink), *B. forficata* group (soy oil (i.m.) + *B. forficata* to drink), HAL group (HAL 38mg/Kg (i.m.) + water to drink) and HAL+*B. forficata* group (HAL (i.m.) + *B. forficata* to drink). The haloperidol was administrated each 28 days and the new decoction of *B. forficata* was given each 2 days to drink. After 16 weeks of treatment, the locomotor activity of the animals was measured in the open-field test (Broadhurst, 1960), and the vacuous chewing movements (VCMs) were evaluated in glass cages (Fachinetto et al. 2007). This project was approved by Comissão de Ética em Uso de Animais of UFSM, under number 025/2011. **Results:** *In vitro* results indicate that decoction of *B. forficata* prevents oxidative damage in brain preparations induced by different pro-oxidants sodium nitroprusside (SNP), Fe^{2+} and Fe^{2+}/ EDTA complex. The treatment with HAL induced VCM and decreased the locomotion, but the co-treatment with decoction of *B. forficata* was not able to reduce neither HAL-induced VCM nor the hipolocomotion. The long-term *B. forficata* treatment caused hyperlocomotion in the rats. **Discussion:** Although the powerful antioxidant activity of decoction of *B. forficata* in brain, it was not able to decreases the neither HAL-induced hipolocomotion and the VCM, suggesting that the mechanism of antipsychotic drugs induce motor deficits are not related only to oxidative stress. **Financial support** by FAPERGS, CAPES, CNPq and UFSM. **References:** Andreassen OA. Prog Neurobiol, 61:525, 2000. Broadhurst PL. Experiments in psychogenetics. In: Eysenk H J (ed). Experiments in personality. London: Routledge & Kegan Paul; 1960. Damasceno DC et al. Phytomedicine, 11:196, 2004. Fachinetto R et al. Psychopharmacology. 194:423, 2007. Khalil NM et al. Biol Res, 41:165, 2008. Ohkawa H et al. Anal. Biochem. 95: 351, 1979. de Sousa E et al. J Nat Prod, 67:829, 2004. Yeh GY Diabetes Care 26:1277, 2003.

09.078 The involvement of oxidative stress in chronic toxicity induced by fumonisin B1 in broilers chicks. Poersch AB[1], Trombetta F[1], Braga ACM[1], Boeira SP[1], Perlin VJ[2], Dilkin P[3], Marchioro A[3], Oliveira MS[3], Mallmann CA[3], Furian AF[1]
[1]UFSM – Fisiologia e Farmacologia, [2]SAMITEC, [3]UFSM – LAMIC

Introduction: Fumonisin B_1 (FB_1) is associated to intake of contaminated maize with fungi of the genus *Fusarium*. It is reported as potential cause of liver cancer in rats (Haschek et. al., 2007). Toxicity mechanism of this toxin is related to inhibition of ceramida sintase causing an acumulation of substrates, sphingosine and sphinganine. Elevated levels these sphingoids bases and reduction in the formation of sphingomyelin may cause the functional impairment and result in development of oxidative stress (López-Carrillo, 2010). Thus, the aim of this study was to determine the toxic action of FB_1 in liver of chicks by oxidative stress markers. **Methods:** 40 broilers chicks Cobb, males, 1day of age were randomly divided in 4 groups which received diet control (C), additive anti-mycotoxins (AD) (aluminosilicate, 0.5 kg/T), FB_1 (100 ppm; FB_1) and AD+FB_1 for 21 days. At the end of treatment, chicks were euthanized using CO_2 for stunning, followed by cervical vessels section. Liver were collected, weighted and homogenate in Tris-HCl (50mM, pH7.4) buffer for the analysis of the activity of antioxidant enzymes superoxide dismutase (SOD), catalase (CAT) and glutathione-S-tranferase (GST), ascorbic acid (AA), non-protein thiols (NPSH) and TBARS content (Boeira et. al, 2012). This project does not require the opinion of the ethics committee because it is developed outside the University; the liver used in the experiments was obtained through donations. Data were analyzed by using a two-way analysis of variance (ANOVA), followed by Dunnett's or Bonferroni's Multiple Range Test when appropriate. **Results:** No differences in liver weight were observed, however AA content and TBARS levels increased in group treated with FB_1. GST activity was not altered by the toxin treatment, but CAT activity increased. SOD activity decreased in the groups treated with AD and AD+FB_1 as well as NPSH levels. **Discussion:** Studies have demonstrated that FB_1 induces liver damage in rats (Haschek et. al., 2007, López-Carrillo et. al, 2010). In this work we demonstrated that chronic treatment with FB_1 to chicks changed oxidative stress markers by increased AA and TBARS content, as well as CAT activity after FB_1 treatment. In addition, a decreased SOD activity and NPSH levels were observed in AD and AD+FB_1 groups. These results are in agreement with the study of Theumer et. al. 2007 which also observed increase of TBARS content and CAT activity in spleen mononuclear cell suspensions treated with FB1. Moreover, Marnewick et al., 2009 reported that SOD activity was not altered in rat liver. Thus such findings suggests that oxidative stress subsequent to inhibition of CS are the targets for the toxic action of FB_1, and this data are of fundamental importance to prevent or reduce the impact caused by FB_1 in the economy and health of animals and humans. **References:** Boeira, S.P. et al., Toxicon, v.60, p.358, 2012. Haschek, W. M. et. al., Animal. Feed Sci. Technol., v. 37, p.299, 2007 López-Carrillo, L. et. al, Salud Publica Mex, v. 52, p.461, 2010. Theumer, M.G et. al., Toxicol., v.268, p.104, 2010. Marnewick, J.L., et. al. Food Chem. Toxicol., v.47, p.220, 2009. **Financial Support:** CNPq, FAPERGS

09.079 Cytotoxic effect of *Bothropsjararacussu* venom in renal tubular cells (LLC-PK1) and antagonism by heparin. Cruz JMT[1], Amaral LS[1], Strauch MA[1], Espindola-Netto JM[1], Machado MM[1], Ricardo HD[1], Melo PA[1], Quintas LEM[1] [1]ICB-CCS-UFRJ – Farmacologia e Química Medicinal

Introduction: Venoms of *Bothrops* are involved in most snakebite in Brazil and they consist of complex mixtures of substances, mostly proteins with different enzymatic activities. Once inoculated in tissues they cause both local and systemic actions, which may lead to severe dysfunction in highly vascularized organs such as kidney, a process that may lead to acute renal failure. This complication is more common among lethal cases envenoming of this genus. Although renal dysfunction has been described in the literature, little is known about cellular and molecular mechanisms involved in this disorder. We evaluated the cytotoxic effects of *Bothrops jararacussu* crude venom on the LLC-PK1 cell line and its antagonism by heparin. **Methods:** The direct action of crude venom of *B. jararacussu* and different concentrations of heparin on the renal LLC-PK1 cell line were studied in vitro. The lesion was characterized quantitatively by LDH release, and viability by staining with trypan blue and the count of damaged cells in a Neubauer hemocytometer. **Results:** Incubation of LLC-PK1 cells with 25 µg/mL and 50 µg/mL of *B. jararacussu* crude venom increase of LDH release after 3 h to 861,5 ± 126,0 and 1130,7 ± 134,8 U/L, respectively compared with control 122,9 ± 22,9 U/L ($p < 0.05$, $n = 12$). The viability analysis after 3 h of incubation with the crude venom (50 µg/mL) showed an increased uptake of trypan blue, indicating a direct and extensive cytotoxicity of this venom. Preincubation of the venom with increasing concentrations of heparin (0.1, 0.3, 0.7, 1, 3, 7, 10, 30 µg/mL) was able to antagonize the cytotoxic activity of the venom in a concentration-dependent manner, with a maximal reduction of approximately 75% (IC50 = 1.6 µg/mL). **Discussion:** Our study demonstrates that the venom of *B. jararacussu* has a cytotoxic action in these kidney cells and that heparin, a polyanionic molecule, has an important protective effect, probably due to its interaction with positive amino acid residues present in venom's proteins. **Financial Support**: CAPES, CNPq, PRONEX and FAPERJ

09.080 Evaluation of anti-inflammatory activity of the basil *(Ocimum americanum L*) essential oil in the zymozan-induced arthritis model. Yamada AN[1], Grespan R[2], Silva-Filho SE[2], Damião MJ[2], Estevão-Silva CF[3], Kummer R[2], Pinho RJ[2], Bersani-Amado CA[2], Cuman RKN[2] [1]UEM – Pharmacology and Therapeutic, [2]UEM – Pharmacology and Therapeutic, [3]UEM – Pharmacology and Therapeutic

Introduction: The plants of Lamiaceae familiy, genus *Ocimum*, have been used in the treatment of many inflammatory disorders. In this study we investigated the anti-inflammatory effect of *Ocimum americanum L.* essential oil (OEO) in the zymosan-induced arthritis model. **Methods:** The OEO was obtained from fresh leaves of *Ocimum americanun* L. by hydrodistillation in a Clevenger-type apparatus. The Balb/c female mice, weighing 22 ± 2 g, were housed at 22 ± 2 ° C, light: dark cycle of 12 hours, with free access to food and water. The protocol regarding this study was approved by the ethical committee in animal research (066/2010)-CAEA/UEM). The animals received an intra articular injection of zymosan (200 µg/10µL of saline), thirty minutes after oral treatment with OEO (50, 150 or 300mg/Kg) or vehicle (1% Tween 80 in 0.9% saline solution). The animals of the control group were injected with saline in the contra-lateral knee joint. Six hours after, the animals were euthanized and the knees joint were exposed by surgical incision. The articular cavity was washing twice with 5µL of PBS containing EDTA. The total leukocyte count was done in Neubauer chambers under optical microscopy (Nikon Eclipse E-200), values were reported as the number of cells per cavity. The influx of leukocytes in synovial membrane, as well as cartilage destruction was evaluated by histological analyses. All sections were stained with Harris' hematoxylin and eosin (H&E) and examined under a microscope (original magnification 40x). A grading scale of 0–3 was used to determine the proportion of infiltrated cells. The score ranged from 0 (infiltrated cells equivalent normal) until 3(densely infiltrated cells compared to normal).and cartilage destruction with score the 0 (for cartilage equivalent normal) at 3 (complete cartilage destruction).Data were presented as mean ± SEM and statistical analyses were performed by ANOVA with Tukey's correction. *P<0.05 were considered significant. **Results** A significant inhibition in the leukocytes recruitment was observed after pretreated with OEO: 50mg/kg (13.4 x 10^4± 2.1cells/cavity); 150mg/kg (7.2 x 10^4 ± 1.5* cells/cavity), and 300mg/kg (9.1 x10^4 ± 2.1*cells/cavity) when compared to values obtained from animals pretreated with vehicle (15.5 x 10^4 ± 5.4cells/cavity). Saline was used as negative control (0.19 x 10^4 ± 0.08 cells/cavity). The analysis histological score showed a significant reduction in the number of infiltrated cells into synovial membrane in animals pretreated with OEO (0.40 ± 0.54) when compared to positive control group (2.40 ± 0.89); negative control (0.20 ± 0.44). The score to cartilage destruction was 0.40± 0.54* in OEO-treated group in comparison to positive control group (1.20± 0.44). **Discussion:** The data showed that OEO treatment reduced inflammatory response in this experimental model, since the leukocytes recruitment into the articular cavity, the infiltration of cells in synovial membrane and the cartilage destruction were reduced, suggesting an important anti-inflammatory effect of this oil. Further studies should be performed to evaluate the mechanism of EO on inflammatory diseases. **Supported by:** CNPq, CAPES and Fundação Araucária.

09.081 Evaluation of the cytokines levels in embryos of mothers treated with *Tityus bahiensis* scorpion venom during the pregnancy. Dorce ALC[1], Freitas LA[1,2], Fusco CBP[1], Frare EO[1], Dorce VAC[1], Nencioni ALA[1] [1]IBu – Farmacologia, [2]IBu – Toxinology

Introduction: Scorpion envenoming is a public health problem. In Brazil, the *Tityus serrulatus* scorpion is considered the most dangerous one; however, a large number of exposures also occur with *Tityus bahiensis*. The objective of this study was to verify the possible changes in the cytokine levels of IL-1α, IL-1β, TNF-α, IL-6, IL-10 and INF-γ in embryos after the treatment of pregnant females with the *Tityus bahiensis* scorpion venom. **Methods:** The procedures were approved by the Ethics Committee on Animal Use of the Butantan Institute (CEUAIB) under protocol number 513/08. For the study, we used embryos and their placentas obtained from the crossing of male and female sexually mature Wistar rats, approximately 90 days old, weighing 260-300g. To evaluate the levels of cytokines, pregnant females were injected with saline (control group) at a dose of 1ml/kg, lipopolysaccharides (LPS) at a dose of 100µg/kg (positive control) or crude venom of the *Tityus bahiensis* scorpion at a dose of 2.5 mg/kg (experimental groups) on the 10^{th} or 16^{th} gestational day. The pups were removed by laparotomy 6 and 24 hours after the mothers had been treated. The samples were macerated by a tissue homogenizer and centrifuged at 10,000 rpm/10min at 4°C. The tissues were placed in a protease inhibitor cocktail Sigma P8340 (100µL of cocktail to 34mL of PBS). The cytokine levels were determined by enzyme immunoassays. **Results**: After 6 hours, in GD10, no alterations were observed in the cytokine levels. In GD 16, an increased in the IL-1α levels (C 56.95±27.39pg/mL, E 64.51±22.06*pg/mL, LPS 184.6±72.97pg/mL) was observed. The levels of the other cytokines in this group were not altered in relation to the control group. After 24 hours, it was observed in GD10 a decrease in the TNF-α levels (C 1514.8±325.2pg/mL, E 193.5±186.0*pg/mL, LPS 395.7±59,3pg/mL). The other parameters observed did not change. In GD16, it was observed a decrease in the levels of TNF-α (C 1424.6±195.2pg/mL, E 110.1±138.5*pg/mL, LPS 319.3±359.7*pg/mL), IL-1β (C 66.6±33.9pg/mL, E 11.5±1.6*pg/mL, LPS 9.3±9.6pg/mL), IL-6 (C 852.8±164.7pg/mL, E 388.4±78.0*pg/mL, LPS 275.3±61.5pg/mL), IL-10 (C 293.5±99.3pg/mL, E 133.2±66.2*pg/mL, LPS 110.4±59.3pg/mL), and INF-γ (C 60.5±21.8pg/mL, E 13.1±2.7*pg/mL, LPS 26.6±1.6pg/mL) in comparison to their control groups. The IL-1α levels did not show significant differences. **Discussion:** The moderate maternal envenomation by the *Tityus bahiensis* scorpion venom caused subtle changes in the cytokine levels after 24 hours in embryos analyzed. **Financial support:** CAPES, CNPq-PIBIC and FAPESP (09/54710-3 and 11/10222-5).

09.082 Acute and subchronic toxicological evaluation of hydroalcoholic extract of *Hibiscus rosa sinensis* L. leaves. Barroso WA, Benevides ROA, Chagas VT, Melo DNS, Sousa AKA, Vieira DA, Silva KP, Ribeiro NLX, França LM, Castro AS, Silva SN, Paes AMA, Câmara AL UFMA – Ciências Fisiológicas

Introduction: *Hibiscus rosa sinensis* L., Malvaceae, is a Chinese shrub widely cultivated throughout the world. This plant is used as anti-spermatogenic, hypoglycemic, antitumor, antihypertensive and antioxidant. Pharmacological effects of *H. rosa sinensis* L. have been confirmed by several studies, but there are scarcing studies on its safety. Thus, this study aimed to evaluate the toxicity of the hydroalcoholic extract made from *H. rosa sinensis* L. leaves (HEH) in rodents. **Methods:** Fresh leaves of *H. rosa sinensis* L. were collected in the campus of Federal University of Maranhão (São Luís, MA, Brazil) in May 2011. The plant was authenticated by the staff of Ático Seabra Herbarium of the School of Pharmacy, where a voucher specimen (#00007) is deposited. Air-dried and powdered leaves (200 g) were macerated in 70% ethanol (1:6, w/v) at room temperature, under continuous stirring for 3 days with solvent renewal every 24 hours. Collected extracts were pooled together and concentrated under vacuum to give the HEH (18% yield). To determine the LD_{50}, male mice (90 ± 5 days) were orally administered with a single dose of HEH (0.5; 1.0 or 4.0 g/kg) and observed for 7 days after gavage. For subchronic toxicity study, oral doses of the HEH (0.5 or 1.0 g/kg) or saline (CTR) were daily administered to male rats during 38 days. At day 30, all animals were housed with female rats and maintained for 8 days for evaluation of reproductive toxicity. After there, both males and females were euthanized. Blood samples, visceral and reproductive organs from overnight fasting males were collected. Female rats had their uteri dissected for implants' counting. All the experimental procedures were approved by the Research Ethics Committee of the State University of Maranhão (#016/2011). **Results** were expressed as mean ± SEM and the differences among groups analyzed by ANOVA followed by Newman-Keuls post-test for $p < 0.05$. **Results and discussion:** The LD_{50} was found to be higher than 4 g/kg. Under lower doses no mortality or signs of clinical abnormality were found. However, signs of passivity and one death were observed under the higher dose. The results of subchronic study showed no significant difference neither in body weight nor in visceral organs or retroperitoneal and periepididymal fatpads weights. Biochemical and hematological parameters did not differ. Reproductive function was unaffected by HEH, as assessed by counting of implants in the female rats. Moreover, reproductive tissues of male rats did not differ from controls. These results suggest absence of acute and subchronic toxicity due to oral treatment with HEH from *Hibiscus rosa sinensis* leaves in rats. However, additional toxicological studies are necessary to further evaluate the safety of this plant. **Financial Support**: FAPEMA and UFMA

09.083 The mechanism of action of the vasodilator effect of *Cecropia glaziovi* Sneth. **Extract**. Lobo KL[1], Santos TC[2], Battisti MA[2], Campos AM[2], Linder AE[1] [1]UFSC – Pharmacology, [2]UFSC – Pharmaceutical Sciences

Introduction: The *Cecropia* genus is distributed throughout Latin America, mostly in Brazil. Popularly, it is used to treat heart failure, high blood pressure, inflammation, cough, asthma, bronchitis and it is also used as a diuretic[a]. The *C. glaziovi* is a common tree in the southeast coast of Brazil. The extract from its leaves produces a sustained and reversible hypotension in normotensive and hypertensive rats[b]. Unpublished data from our laboratory shows that standardized extract of *C. graziovi* [18% of the plant, 27% ethanol (v / v), homogenized for 3 days] induces vascular relaxation. Our goal is to characterize the mechanisms by which *C. glaziovi* extract promotes this relaxation. **Methods:** Aortic rings (~4 mm) were obtained from male Wistar rats (~350g) and mounted in isolated organ chambers for isometric tension recordings under 3,0 g of passive tension (CEUA PP00706). The presence of functional endothelium was assessed by the ability of acetylcholine (ACh; 1 uM) to induce relaxation of phenylephrine (PE; 1 uM)-induced contraction. Only preparations that relaxed more than 50% were considered as endothelium-intact. Endothelium removal was confirmed by the lack of relaxation induced by ACh. Endothelium-intact aortic rings were contracted with PE (1 uM) or KCl (80 mM) followed by the addition of increasing concentrations of *C. glaziovi* extract (0.1 to 100 ug/mL) given at 5-min intervals. The response to the extract was also tested in endothelium-denuded or endothelium-intact rings contracted with PE in the presence of indomethacin (10 uM), a cyclo-oxygenase inhibitor; atropine (10 uM), a muscarinic antagonist; and L-NAME (100 uM), a nitric-oxide synthase inhibitor. Concentration-effect curves (CEC) to PE (1 nM to 1 uM) were performed following incubation with *C. glaziovi* extract (100 mg/mL) or vehicle. The drugs or vehicle were added 30 min before PE. **Results and Discussion:** Similarly to ACh, *C. glaziovi* extract was able to induce relaxation of endothelium-intact but not denuded aortic rings contracted with PE. L-NAME inhibited the relaxation induced by *C. glaziovi* extract (~12 %), whereas indomethacin and atropine had no effect. The relaxation induced by the extract in vessels contracted with KCl was significantly decreased (~47 %) when compared to those contracted with PE (~92 %), suggesting the involvement of the endothelial derived hyperpolarizing factor. The maximal contraction induced by PE in the CEC was significantly reduced in the presence of the extract (~0.88 g) compared to vehicle (~1.77 g), and this may be due to the endothelial derived relaxing factors released by *C. glaziovi* extract. The *C. graziovi* extract does not bind to muscarinic receptors and its vasodilator effect is independent of cyclooxygenase activation. It was also observed that the *C. glaziovi* extract depends on the endothelium, mostly by the endothelial nitric oxide and partially by the endothelial derived hyperpolarizing factor. Given the efficacy of *C. glaziovi* extract to induce vascular relaxation, this effect deserves further investigation. References: a) COSTA, G. M. et al.. *Nat. Prod.Commun,* 6, 913, 2011. b) LIMA-LANDMAN, M.T.R. et al., *Phytomedicine*, 14, 314, 2007. **Financial Support:** CNPq/FAPESC, PPG-FMC-UFSC

09.084 The monoterpene (-)-borneol elicits hypotensive effect in normotensive rats. Silva-Filho JC[1], Ferreira Filho ES[2], Maynard LG[1], Cavalcanti SCH[1], Quintas-Junior L[1], Santos MRV[1], Oliveira RCM[2], Oliveira AP[2] [1]NPPM-UFS – Fisiologia/, [2]NPPM-UFPI, [3]UFS – Fisiologia

Introduction: The monoterpene (-)-borneol is found in essential oils of several medicinal plants. Previously, (-)-borneol showed radical scavenging properties, immunomodulatory effects, antithrombotic and antiplatelet activity and vasorelaxant effects. This study aimed to evaluate the effects of (-)-borneol on the cardiovascular system of normotensive rats. **Methods:** All experimental protocols were approved by Federal University of Piauí Ethics Committee on Animal Experimentation (CEEA/UFPI nº 53/10). For *in vivo* studies, normotensive male Wistar rats (250-300g, n=5) were anesthetized with sodium pentobarbital (45 mg/kg, i.p.) for implantation of polyethylene catheters (PE-10) in inferior vena cava and abdominal aorta. After stabilization period, mean arterial pressure and heart rate were recorded in conscious and freely moving rats before (baseline values) and after i.v. *in bolus* administration of (-)-borneol (1, 5, 10 and 20 mg/kg) for obtainment of dose–response curves. Similar records with (-)-borneol were obtained after pre-treatment during 30 minutes with atropine (2 mg/kg, i.v.), a non-selective muscarinic receptor antagonist, L-NAME (20 mg/kg, i.v.), an inhibitor of the nitric oxide synthase, or indomethacin (5 mg/kg, i.v.), a potent non-selective COX inhibitor, separately. The values of blood pressure and heart rate were obtained through a pressure transducer coupled to an amplifier (AVS projects/SP). The results were expressed as mean ± SEM and considered significant when $p< 0.05$. **Results:** The baseline values of MAP and HR were 119 ± 5 mmHg and 310 ± 15 bpm, respectively. After stabilization, acute administration of (-)-borneol induced hypotension by 22.18 ± 1.8%, 24.58 ± 0.71%, 27.40 ± 2.13% and 38.15 ± 2.8%, respectively (expressed as percentage of baseline values). This response was accompanied by tachycardia (3.65 ± 0.17%, 5.19 ± 1.01%, 6.15 ± 1.55% and 10.82 ± 1.87%, respectively). Pretreatment with atropine, the hypotensive (18.73 ± 2.18%, 22.75 ± 0.47%, 35.36 ± 2.86% and 37.70 ± 2.51%) and tachycardic (0.33 ± 1.79%, 7.82 ± 0.28%, 7.30 ± 0.19% and 7.81 ± 2.08%, respectively) effects did not change and L-NAME attenuated significantly the hypotensive effects at doses of 1 and 5 mg/kg, i.v.) (8.9 ± 1.95%**, 13.36 ± 2.64%**, 19.62 ± 2.26% and 31.57 ± 2.60%) (**p<0.05 vs control, Two-way ANOVA followed by Bonferroni's post-test). After pre-treatment with indomethacin, any parameter was modified (hypotension: 14.92 ± 2.78%, 18.23 ± 2.00%, 22.32 ± 2.25% and 29.93 ± 1.89% and tachycardia: 5.50 ± 1.21%, 3.14 ± 1.64%, 6.17 ± 3.31% and 8.25 ± 4.08%, respectively). The pre-treatment with these drugs did not change the hypotension induced by (-)-borneol. Thus, the (-)-borneol induces hypotensive effect, and tachycardia is probably due to a reflex response. **References:** Juha Š, Folia Biol., 54; 1, 2008. Mitchelson F, Trends Pharmacol. Sci., 5; 6, 1984. Moncada S, Pharmacol. Rev., 43; 109, 1991. Takahashi T, J. Physiol., 504; 479, 1997. Park T J, Biochem. Pharmacol., 65; 83, 2003. Silva-Filho J C, Basic Clin. Pharmacol. Toxicol.,110; 171, 2011. **Keywords:** Hypotension, tachycardia, (-)-borneol
Financial Support: UFPI/UFS/CAPES/FAPEPI/CNPq

09.085 α-lipoic acid reverses the oxidative process induced by DDS-NOH metabolite in erythrocytes *in vitro*. Santos DC, Albuquerque RFV, Malcher NS, Monteiro MC UFPA

Introduction: Dapsone (DDS) is the drug commonly used in multidrug therapy (MDT) of leprosy established by WHO. Clinically, DDS, at daily doses of 100mg, is related to adverse reaction including dose-related hemolysis, methemoglobinemia (MeHgb), peripheral neuropathy, agranulocytosis, aplastic anemia and others. Several reports on the *in vivo* metabolism of these arylamines indicate that 20 to 35% of dapsone is excreted as dapsone hydroxylamine (DDS-NOH). This metabolite is considered to be the inductor to methemoglobin (MetHg), because hydroxylamine metabolites are believed to react with hemoglobin and O2 in a simple coupled oxidation reaction that produces MetHgb. The lipoamida, α-lipoic acid, is a fatty acid found naturally in almost all plant and animal species, which it has anti-inflammatory properties and antioxidants. Thus, this study aimed to evaluate the effect of ALA in MetHg induced by DDS-NOH in erythrocytes *in vitro*. **Methods:** Methemoglobin tests were performed in erythrocyte suspension (hematocrit 40%) from healthy adult volunteers (COMEP/UFPA-protocol 165/11). The percent of total hemoglobin converted to MetHg was then analyzed using spectrophotometry, as described by Malloy and Evelyn, (1938). The MetHg formation was induced by different concentrations of DDS-NOH (2.5, 5.0, 7.5; 10μg/mL) in erythrocyte suspension incubated for 60 min at 37°C. The antioxidant activity of ALA was performed by pre-incubation different concentrations of the compound (10, 100, 200; 1000μM) for 60 min at 37°C. Methylene blue (15ng/mL) was used as positive control on reversal the MetHg induced by DDS-NOH and methanol (solvent) was used as negative control. For statistical analysis we used one-way ANOVA and the Mann-Whitney test, * $p \leq 0.05$. **Results and Discussion:** DDS-NOH at different concentrations significantly increased the MetHg percentage (2,5μg/mL=19,25%; 5.0μg/mL=25.43%; 7.5μg/mL=33.59%; 10μg/mL=41.05%) compared to negative control (methanol= 6.66%), showing that the MetHgb-forming capacity of the DDS-NOH was dose dependent. Pretreatment of the red cells with ALA was able to significantly protect these cells to MetHg formation induced by different concentrations of DDS-NOH (2.5, 5.0, 7.5μg/mL), and the values of % MetHg after treatment with 10 μM ALA were 10.04, 16.48 and 22.51%, 100μM (9.0, 12.96 and 19.27%), 200μM (10.75, 17.43 and 21.48%) and 1000μM (7.25, 10.96, 9.44%). Thus, in this data was observed that the concentration of ALA at 1000μM was more in inhibiting the MetHg formation induced by DDS-NOH, even when compared to positive control, pretreatment of red cells with methylene blue (DDS-2, 5: 6.13%, 5.0: 16.03%, and 7.5mg / mL: 17.86%). Previous study with human erythrocytes of diabetic and nondiabetic patients also demonstrated the antioxidant activity of ALA *in vitro*, these facts show that ALA has a strong antioxidant action that is able to reverse the MetHb formation *in vitro*, and suggesting that this compound could be used in dapsone-combination therapy in leprosy patients. **Financial support:** CAPES/CNPq, FAPESPA-PA; UNIVERSAL/CNPq, UFPA.

09.086 Gastroprotective effects of ethanol extract and fractions of *Neoglaziovia variegata* Mez. (Bromeliaceae) against gastric lesions induced by ibuprofen and ethanol in mice. Viana AFSCV[1], Machado FDF[1], Silva FV[1], Lima JT[2], Oliveira FA[1], Freitas FFBP[1], Oliveira RCM[1], Almeida JRGS[2] [1]NPPM-UFPI, [2]UNIVASF – Ciências Farmacêuticas

Introduction: *Neoglaziovia variegata* Mez., locally known in the Northeast Region of Brazil as caroá. The phytochemical study of this species showed the presence of several secondary metabolites such as saponins, tannins, flavonoids, steroids and triterpenoids. The aim of this study was to analyze the gastroprotective activity of ethanol extract (Nv-EtOH), hexane (Nv- Hex) and chloroformic (Nv-$CHCl_3$) fractions of *N. variegata* Mez. in the model of acute gastric ulcer induced by ibuprofen. In addition, for investigate the involvement of prostaglandins in gastroprotective effect of Nv-EtOH, Nv-$CHCl_3$ and Nv- Hex, the absolute ethanol-induced gastric lesions model were used. **Methods:** Female Swiss mice (25-30 g) were kept under controlled conditions (24 ± 1°C, 12-h dark/light cycle) with access to food and water *ad libitum .They were* divided into groups of 7 animals fasted for 18 h before each experimentation. All experimental protocols were approved by Ethics Committee for Animal Research of the Federal University of Piaui, Brazil, (CEEA-UFPI, 076/10). Mice were orally treated with vehicle, Nv-EtOH (50, 100, 200 and 400 mg/kg), Nv-Hex (200 and 400 mg/kg), Nv-$CHCl_3$ (200 and 400 mg/kg) or cimetidine (100 mg/kg). After 1h, all groups received ibuprofen (400 mg/kg; p.o.). Animals were euthanized 6 h after administration of ibuprofen and stomachs were excised and analyzed for gastric damage according to method described by Bhargava (Eur. J. Pharmacol., v.22, p.191, 1973). In order, for evaluate the involvement of prostaglandins in the gastroprotective effect, the animals received vehicle, Nv-EtOH (400 mg/kg, p.o.), Nv-Hex (100 mg/kg, p.o.) and Nv-$CHCl_3$ (100 mg/kg, p.o.) or carbenoxolone (100mg/kg) 1 hour after of pretreatment with ibuprofen (100 mg / kg). After 1 hour, animals received absolute etanol (0,2 mL, p.o.). The animals were euthanized 30 min. after administration of absolute etanol, stomachs were excised and analyzed. The significance level was evaluated for values of $*p < 0.05$ (ANOVA one way). **Results:** Effect on ibuprofen-induced gastric ulcer, Nv-EtOH decreased the percentage of the lesion at doses of 100 (3.748 ± 0.574%*), 200 (2.246 ± 0.298%*) and 400 mg/kg (1.912 ± 0.680%*) compared to the vehicle (7.424 ± 0.544%). Nv-Hex and Nv-$CHCl_3$ at doses of 200 and 400 mg/kg did not reduce the area of gastric lesions in this model. In the control group, absolute ethanol-induced gastric lesions (9.443 ± 0,687 %) were reduced significantly after pretreatment with Nv-EtOH (400 mg/kg) and carbenoloxone (100 mg/kg) for 1.533 ± 0,285 %* and 1.200 ± 0,368 %*, respectively. Pretreatment with ibuprofen (100 mg/kg), a non-selective COX inhibitor, was able to reverse Nv-EtOH and carbenoxolone (13.883 ± 0.954%* and 10.817 ± 0.736%*, respectively) compared with control group. **Discussion**: Results obtained suggest that Nv-Hex and Nv-$CHCl_3$ -induced gastroprotective effect was not significantly reversed in the presence of ibuprofen. However, ethanol extract (Nv-EtOH) induced gastroprotective effect was reversed in the presence of ibuprofen, suggesting a possible involvement of prostaglandins in this effect. **Support**: UFPI/UNIVASF/CAPES/FACEPE/CNPq

09.087 The gastroprotective effects of *Eugenia dysenterica* DC leaf extract in mice: **The possible role of tannins**. Prado LCS, Mundin AMM, Ferraz CR, Canabrava HAN, Bispo-da-Silva LB UFU – Pharmacology

Introduction: *Eugenia dysenterica,* known in Brazil as “cagaita”, has been used by the general populace to treat some gastrointestinal disorders. Aim of the study: Considering its folk use and that many plants of the Myrtaceae family exhibit protective effects toward the gastric mucosa, we applied a taxonomic approach to select the *E. dysenterica* leaf extract to evaluate its gastroprotective effect. **Methods:** The abilities of *E. dysenterica* leaf extract and carbenoxolone to protect the gastric mucosa from ethanol/HCl-induced lesions were evaluated in mice (CEUA/UFU license number 041/11). The contributions of nitric oxide (NO), endogenous sulfhydryl (SH) groups and alterations in HCl production to the extract’s gastroprotective effect were investigated. We also determined the antioxidant activity of the extract and the possible contribution of tannins to the cytoprotective effects. **Results:** The extract and carbenoxolone protected the gastric mucosa from ethanol/HCl-induced ulcers and the former also decreased HCl production. The blockage of SH groups by NEM and the inhibition of NO synthesis by L-NAME abolished or mildly decreased, respectively, the gastroprotective action of the extract. The phytochemical analyses indicated the presence of tannins in the *E. dysenterica* leaves and its partial withdraw abolished the cytoprotective actions of the extract. Finally, the extract exhibits free radical scavenger activity *in vitro*. **Conclusions:** *E. dysenterica* leaf extract has gastroprotective effects that appear linked to the inhibition of HCl production, to its antioxidant activity, to endogenous SH-containing compounds and to a mild NO-mediated cytoprotection. These pleotropic actions appear strictly related to the tannins contained in the extract. **Financial Support:** PROPP/UFU and (CAPES).

09.088 Antiophidic property of *Cordia salicifolia* and *Lafoensia pacari* plants extracts against effects induced by *Philodryas olfersii* and *Bothrops jararacussu* venoms in neuromuscular preparation. Schezaro-Ramos R[1], Góes MP[1], Collaço RCO[2], Cogo JC[3], Dal Belo CA[4], Rodrigues-Simioni L[2], Moreira AS[5], Randazzo-Moura P[6] [1]UNIP – Farmácia, [2]Unicamp – Farmacologia, [3]CEN-UNIVAP, [4]Unipampa, [5]ICS-UNIP, [6]PUC – Ciências Fisiológicas

Introduction: The species *Philodryas olfersii* (*P. olfersii*) and *Bothrops jararacussu* (*B. jararacussu*) are responsible for the majority envenomous in Brazil. The ophidics accidents caused by *P. olfersii* or *B. jararacussu* snake venom are similar. They can induce serious reactions, as local and systemic effects. In addition to serum therapy several species of plants can be used, such as *Cordia salicifolia* (*C. salicifolia*) or *Lafoensia pacari* (*L. pacari*) extracts, without further knowledge to avoid the venom local damage. The aim of this work was to study the protection of *C. salicifolia* or *L. pacari* extract against biologics effects induced by *P. olfersii* or *B. jararacussu* venoms on mouse phrenic nerve-diaphragm preparations (PND), through conventional myographic technique. This work was approved by the Animal Ethics Committee (CER/UNIVAP protocol nº A025/CEP/2009). **Methods:** The preparations were treated with Tyrode solution (control, n=6), *C. salicifolia* (500µL/mL, n=6), *L. pacari* (400µL/mL, n=5), *B. jararacussu* (100µg/mL, n=5) or *P. olfersii* (50µg/mL, n=5). Pre-treatment were done with: *C. salicifolia* (500µL/mL, 30min before) + snake venom (100µg/mL *B. jaracussu* or 50µg/mL *P. olfersii*) (n=3-5) and *L. pacari* (400µL/mL, 30min before) + snake venom (100µg/mL *B. jaracussu* or 50 µg/mL *P. olfersii*) (n=3-5), all experiments were done at 37^{o}C for 120min. **Results:** *C. salicifolia* and *L. pacari* did not cause any effect on muscle contraction. In contrast *P. olfersii* caused partial and irreversible neuromuscular blockade (54.1±2.9%, $p<0.05$), whereas *B. jararacussu* caused total and irreversible neuromuscular blockade ($p<0.05$). Pretreated preparations with *C. salicifolia* resulted in 72.4% protection against *P. olfersii* and 100% protection against *B. jararacussu*, whereas *L. pacari* resulted in 100% protection against *P. olfersii* and 79.6% protection against *B. jararacussu*. **Discussion:** These results showed that both plants extracts prevents the neuromuscular blockade induced by snake venoms studied. **Financial support**: PUC/SP, UNICAMP.

09.089 Antinociceptive properties of extracts and fractions from the leaves of *Spilanthes oleracea* in mice. Rodrigues MRA[1], Kanazawa LKS[1], Neves TLM[1], Nomura EO[1], Cipriani TR[2], Nascimento AM[2], Baggio CH[1], Werner MFP[1] [1]UFPR – Farmacologia, [2]UFPR – Bioquímica

Introduction*:* *Spilanthes oleracea* L. (Asteraceae) is popularly known as jambu and has been used as a traditional herbal medicine against mouth affection and tooth pain due the presence of alkamides such spilanthol. Here, we investigated the antinociceptive effects of *Spilanthes oleracea* ethanolic supernatant of the aqueous extract (ESAE) and its fractions. **Methods:** Dried powdered leaves of *Spilanthes oleracea* were defatted and depigmented, extracted with water and then the resulting aqueous extract was treated with 3 volumes of ethanol, originating a precipitate and the ethanolic supernatant (ESAE). We obtained several ESAE fractions that were biomonitoring for the most efficient antinociceptive effect (aqueous fraction of ESAE (afESAE), 4B fraction from afESAE and fractions from 4B fraction) using acetic acid (0.6%, 0.45 ml/mouse, i.p.) and orofacial formalin (2.5%, 20 ml) tests. Swiss female mice (30 g) were intraperitoneally treated with ESAE (0.1 - 100 mg/kg); ESAE fraction (0.01 - 100 mg/kg); 4B fraction (4, 10 and 40 mg/kg) and with different fractions of 4B fraction 30 min before the administration of halogens. All experiments were previously approved by the Institutional Ethics Committee of the Federal University of Parana (approval certificate 544). **Results:** ESAE at doses of 0.1, 1 and 10 mg/kg reduced the number of abdominal constrictions induced by acetic acid in 25, 50 and 81%, respectively. ESAE 1, 10 and 100 mg/kg also reduced both phases of the response to formalin, with more pronounced antinociceptive effect at the second phase of nociception (40, 32 and 76%, respectively; control value 159 s). The treatment with afESAE at 0.1 and 1 mg/kg reduced the number of abdominal constrictions by 54 and 79%, respectively, while at 30 and 100 mg/kg reduced the phase II of nociception induced by formalin in 46 and 43%, respectively (160 s). We obtained 7 fractions from afESAE, and the more efficient was the 4B fraction, that reduced the acetic acid-induced writhing reaction in 76% at 4mg/kg and the formalin-induced orofacial reaction in 52 and 65% at 10 and 40 mg/kg, respectively (control value 182 s). Furthermore, 4B alkaline (0.29 mg/kg), 4B acid (0.54 mg/kg) and 4B butanolic (0.54 mg/kg) fractions reduced the number of abdominal constrictions by 67, 59 and 46%, respectively, and only 4B butanolic (0.54 mg/kg) fraction was able to reduce the phase II of formalin test by 63% (127 s). Finally, ESAE (0.005, 0.05 and 0.5 g/kg) did not produce any visible signs or symptoms of acute toxicity and at 1, 10 and 100 mg/kg ESAE did not affect the motor performance on the open-field test. **Discussion**: These results show that the biomonitored fractionation of ESAE revealed the antinociceptive activity of leaves of jambu, effect that was not related to the presence of spilanthol. Additional studies are necessary to guide the isolation of new compounds that could be related to the antinociceptive effect of S*pilanthes oleracea.* **Financial Support:** CNPq (476653/2010-0)

09.090 Blood pressure responses to *vitalius dubius* (Araneae, Theraphosidae) spider venom. Tamascia ML, Silva IRF, Alves-Jr MJ, Hyslop S Unicamp – Farmacologia

Introduction: *Vitalius dubius* Mello-Leitão 1923 (Araneae, Theraphosidae) is a non-aggressive spider found in southeastern Brazil (states of São Paulo and southern Minas Gerais). In this work, we examined the action of *V. dubius* venom on rat arterial blood pressure. **Methods:** Male Wistar rats (350-400 g) were anesthetized with 2% isoflurane in 98% air and a carotid artery was catheterized for blood pressure measurement. A femoral vein was catheterized for injection of venom and chromatographic peaks in 100 µl of 0.9% NaCl washed in with 100 µl). Changes in blood pressure were monitored continuously (PowerLab system, ADInstruments) and, at the end of the experiment, heart, lung and kidney tissue samples were processed for histological analysis. Venom (10 mg) was fractionated (1 ml/min) by gel filtration on Superdex 75 (1 cm x 30 cm column) in 0.1 M sodium acetate, pH 6, containing 0.15 M NaCl and the elution profile was monitored at 280 nm. The major peaks were run on 15% polyacrylamide gels in SDS-PAGE and tested on arterial blood pressure. Peak IV was screened for PLA_2 activity because of the presence of a ~14-15 kDa protein in SDS-PAGE and because of its effect on blood pressure. HPLC of peak IV on a SP-Sepharose column equilibrated with 25 mM sodium phosphate, pH 7.5, and eluted (1 ml/min) with a linear gradient of NaCl (0-0.5 M) yielded three peaks that were tested for PLA_2 and activity on blood pressure (150 µg/kg). Venom PLA_2 activity was measured colorimetrically with egg-yolk phospholipids as substrate. One unit of activity corresponded to the decrease in A_{620} nm caused by 1 mM HCl. The results (mean±SD) were analyzed using ANOVA followed by the Tukey test, with $p<0.05$ indicating significance. The animal experiments were approved by the institutional Committee for Ethics in Animal Use (CEUA/UNICAMP, protocol no. 2166-1). **Results:** Venom caused immediate hypotension in rats (maximum decreases of 30.6±8.5% and 39.2±2.3% for 1 and 3 mg/kg, respectively; n=5-6; $p<0.05$ vs. pre-venom values). Blood pressure recovered after 120 min in rats injected with 1 mg/kg whereas 3 mg/kg caused sudden death after 90 min. The higher dose also caused a progressive decrease in respiratory rate (maximum decrease: 39.6±8.2%; n=5; $p<0.05$ vs. pre-venom value). Gel-filtration resulted in six peaks (I-VI), of which peaks IV and V caused immediate hypotension (maximum decreases of 45.9±2.1% and 28.7±9%, respectively; n=3 each; $p<0.05$ vs. pre-venom values), but did not cause death. Peak IV contained PLA_2 activity (0.7±0.3 units; n=3) and SDS-PAGE of this peak showed a major double band of 14-15 kDa. Ion exchange chromatography of peak IV yielded three peaks, of which peaks IV-1 and IV-2 had PLA_2 activity (0.9±0.4 units and 0.5±0.2 units, respectively; n=3 each) but no effect on blood pressure. Peak IV-3, which accounted for most of the protein content, caused progressive hypotension (maximum decrease: 36.0±9.3%; n=6; $p<0.05$ vs. pre-venom value) and sudden death after 60-90 min. Neither venom nor fractions altered heart rate. Histological analysis revealed damage to cardiac muscle and the renal cortex of rats injected with venom (3 mg/kg) and peak IV-3. **Conclusion:** *Vitalius dubius* venom causes hypotension in anesthetized rats, with peak IV being the most active; this peak contains at least three components, one of which causes sudden death but is devoid of PLA_2 activity. **Financial support:** CAPES, CNPq.

09.091 Diuretic effect of semi-purified fractions obtained from *Achillea millefolium* L. (Asteraceae) in rats. Maba IK[1], Silva TLC[1], De Souza P[1], Crestani S[1], Gasparotto Junior A[2], Marques MCA[1], Silva-Santos JE[3] [1]UFPR – Farmacologia, [2]UNIPar – Farmacologia, [3]UFSC – Farmacologia

Introduction: *Achillea millefolium* L. (Asteraceae), known as "mil folhas", "aquileia", and "botão de prata", is used in folk medicine against cardiovascular diseases. Although previous studies of our group have shown the hypotensive and diuretic activity of the crude extract and the dichloromethane fraction obtained from *A. millefolium* after oral administration, the effects of other semi-purified fractions of this plant remain to be investigated. **Methods:** Aerial parts of *A. millefolium* were extracted with ethanol 90%, concentrated, filtered and the solution lyophilized. It was suspended in EtOH-H_2O (1:1) and then extracted with dichloromethane, ethyl acetate and n-butanol, successively. *Wistar* female rats were treated orally with vehicle (control - CT), butanolic fraction (BtAM, 10, 30 and 100 mg/kg), ethyl acetate fraction (AEtAM, 10, 30 and 100 mg/kg), or furosemide (25 mg/kg), and were allocated in metabolic cages for analysis of diuresis (measured for 8 h). The density, pH, and electrolyte contents (Na^+, Cl^-, K^+, HCO_3^-) of urine were verified at the end of the experiments. All procedures were submitted and approved by the Ethics Committee for Animal Use of Biological Sciences Sector of UFPR (CEUA/BIO-UFPR), authorization number 593. **Results and Discussion:** The treatment with BtAM (10 mg/kg) acutely increased diuresis by 115% in the first hour after its administration, when compared with the control group. This effect was accompanied by reduction of Na^+ and Cl^- excretion (by 80% and 65%, respectively). Administration of AEtAM, at dose of 30 mg/kg, induced an aquaretic effect that remained statistically significant during the 8 h of experiment (for instance, the urinary output was increased by 71,1% at the eighth hour), also accompanied by reductions of 77,5% and 67,8% in Na^+ and Cl^- excretion, respectively. Urinary HCO_3^- excretion was not change by BtAM or AEtAM administration, despite urinary pH being more alkaline for both treatments. None of the treatments changed the density of urine, suggesting that its acute administration do not cause renal damage in rats. These results show that the semi-purified fractions from *A. millefolium* have a diuretic effect when acutely administered by oral route. This diuretic effect can be related with the therapeutic action of this plant in the cardiovascular system. Other studies are being conducted to assess the mechanisms involved in this effect. **Financial support**: Isabella K. Maba received a CNPq PIBIT fellowship.

09.092 Resveratrol inhibits the oxidation of hemoglobin induced DDS-NOH metabolite *in vitro* model. Albuquerque RFV, Santos DC, Malcher NS, Monteiro MC UFPA

Introduction: The strategy for leprosy control is based on the multidrug therapy (MDT), recommended by the WHO, and Dapsone (DDS) is the drug commonly used in MDT Clinically, DDS is related to adverse reaction including dose-related hemolysis, methemoglobinemia (MeHgb), peripheral neuropathy, agranulocytosis, aplastic anemia and others. Reports showed that on *in vivo* metabolism of dapsone, 20 to 35% is excreted as dapsone hydroxylamine (DDS-NOH). This metabolite is considered to be the inductor to methemoglobin (MetHg), because it could react with hemoglobin and O_2 in a simple coupled oxidation reaction that produces MetHgb. Resveratrol (RSV) is a phytoalexin produced naturally by in a variety of plant, including peanuts and grapes. RSV has antioxidant and anti-inflammatory properties in several models *in vivo* and *in vitro*. Thus, we aimed to evaluate the effect of RSV in oxidation of hemoglobin induced by DDS-NOH *in vitro*. **Methods:** Methemoglobin tests were performed in erythrocyte suspension (hematocrit 40%) from healthy adult volunteers (COMEP/UFPA-protocol 165/11). The percent of total hemoglobin converted to MetHg was then analyzed using spectrophotometry, as described by Malloy and Evelyn, (1938). The MetHg formation was induced by different concentrations of DDS-NOH (2.5, 5.0, 7.5; 10µg/mL) in erythrocyte suspension incubated for 60 min at 37°C. The antioxidant activity of RSV was performed by pre-incubation different concentrations of the compound (10; 100; 200; 1000µM) for 60 min at 37°C. Methylene blue (15ng/mL) was used as positive control on reversal the MetHg induced by DDS-NOH and methanol (solvent) was used as negative control. For statistical analysis we used one-way ANOVA and the Mann-Whitney test, * $p \leq 0.05$. **Results and Discussion:** DDS-NOH at different concentrations significantly increased the MetHg percentage (2,5µg/mL=19,25%; 5.0µg/mL=25.43%; 7.5µg/mL=33.59%; 10µg/mL=41.05%) compared to negative control (methanol= 6.66%), showing that the MetHgb-forming capacity of the DDS-NOH was dose dependent. Pretreatment of the red cells with RSV protected these cells to MetHg formation induced by different concentrations of DDS-NOH (2.5, 5.0, 7.5µg/mL), and the values of % MetHg after treatment with 10 µM RSV ranged from (10.51 to 28.71%), 100µM (7.19 to 23.62%), 200µM (10.23 to 26.19%); 1000µM (8.37 to 22.09 %). Furthermore, only the pre-treatment with 100µM of RSV had the same effectiveness as the methylene blue (MB) to inhibit the MetHb formation induced by 2.5 µg/mL of DDS-NOH (RSV=7.19% and MB= 6.13%). At the time curve of the RSV, ours data showed that pre-incubation of erythrocytes with 100µM of the compound in the times of 60 and 90 min were more effective in protecting these cells to oxidation. There is evidence that resveratrol has an antioxidant capacity which depends on the redox properties of its hydroxyl phenolic groups and on the potential for the delocalization of electrons through the chemical structure. In this respect, this work is in agreement with the idea of using resveratrol as a protection against oxidative stress caused by dapsone in leprosy patients. **Financial support:** CAPES/CNPq, FAPESPA-PA; UNIVERSAL/CNPq, UFPA.

09.093 Evaluation of acute toxicity of hydroalcoholic extract of the seeds of *Vatairea guianensis* (Aublet). Alves CM[1], Mariano GRC[1], Silva SL[1], Ribeiro RB[1], Santos AM[1], Burmann APR[2], Medeiros AAN[1] [1]Unifap, [2]Lacen-Ap

Introduction*: Vatairea guianensis* (Fabaceae), popularly known as "faveira" and "fava-impigem", is an original species of the Amazon, used in folk medicine in the treatment of superficial dermatoses. The aim of this study was evaluate the preclinical acute toxicity of the hydroalcoholic extract of the seeds of *V. guianensis* (EHVG). **Methods**: A preliminary cytotoxicity test of the extract using *Artemia salina* Leach was conducted to obtain the median lethal concentration (LC_{50}).Then it was evaluated the acute toxicity as established in the RE 90/2004 of the Agência Nacional de Vigilância Sanitária (ANVISA). Was used 24 Wistar rats (*Rattus norvegicus albinus*), which acutely received the EHVG (2000 mg/kg, po). General signs of toxicity, as well as weight gain, water intake, feed intake and verification of the lethal power of the extract were observed for 14 days after exposure. On the 15th day, blood samples of the animals were collected for the purpose of determination of hematological and biochemical parameters, and then they sacrificed for macroscopic analysis, calculation of the relative mass and histopathological examination of vital organs (heart, liver, lungs and kidneys). Numerical results were expressed as mean ± standard error of mean. Differences between groups were determined using analysis of variance (ANOVA) and method of Turkey. Hematological determinations were analyzed by Student's t-test. For the test with *A. saline* was used the statistical method of PROBIT and the program Microcal Origin 6.0. All protocols were approved by the ethics committee on research on number 007A/2011. **Results and Discussion:** LC_{50} determined by bioassay using *A. salina* was 2692.39 µg/mL, suggesting that the EHVG has low toxicity. The extract of *V. guianensis* did not induce death in the animals, behavioral changes or significant changes in water intake and feed intake when compared with control groups. The weight gain proved to be different between males of the test and control groups, as well as the relative mass of the organs (heart $^{**}p < 0.01$, liver $^{*}p < 0.05$ and kidneys $^{*} p < 0.05$). The blood profile showed significant decrease in the differential counting of segmented cells of males (20.17 ± 1.51; 8.83 ± 1.70 – p = 0.0005***) and females (26.00 ± 2.39%; 13.17 ± 1.25 – p = 0.0008***) and increase in lymphocytes of males (76.33 ± 1.20; 87.83 ± 2.09 – p = 0.0008***) and females (70.00 ± 2.45; 83.17 ± 1.62 – p = 0.0012**) in the test group. Biochemical parameters of the enzyme alanine aminotransferase (ALT) appeared reduced in both males (115.7 ± 18.9; 65.3 ± 6.5 – p = 0.0440*) and females (139.7 ± 20.7; 80.3 ± 10.2 – p = 0.0424*). Alkaline phosphatase (168.3 ± 32.9; 80 ± 10.3 – p = 0.0416*), total bilirubin (0.6 ± 0.04; 0.8 ± 0.03 – p = 0.0061**) and indirect bilirubin (0.33 ± 0.03; 0.55 ± 0.04 – p = 0.0025**) were changed only in females that received the EHVG. Changes in the macroscopic and histopathological examination were not evident. The results show a differential sensitivity between the sexes for various parameters has low acute oral toxicity (2000 mg / kg). However, toxicity studies, sub-chronic and chronic are needed to elucidate the toxicological profile of EHVG. **Financial support:** CNPq/PIBIC/CAPES

09.094 Antispasmodic activity of hydroalcoholic extract *Arrabidaea chica* (HBK) *verlot*. Melo DNS[1], LEAL MM[1], Benevides ROA[1], Sousa AKA[1], Barroso WA[2], ABREU IC[3], Ribeiro RM[3], Amaral FMM[4], Silva SN[3], Cartágenes MSS[3] [1]UFMA – Pharmacology, [2]UFMA – Physiology, [3]UFMA – Pharmacology, [4]UFMA – Pharmacy

Introduction: The *Arrabidaea chica* (HBK) Verlot, belongs to the family Bignoniaceae, known as crajiru, Pariri, is common or find states of northern Brazil. The aim of this paper is to analyze the action of this plant on intestinal peristalsis. **Methods:** The hydroalcoholic extract of leaves of *Arrabidaea chica* Verlot (EAC) was obtained by maceration the dried and powdered drug. Leaves were collected in the Medicinal Garden "Berta Lange Morretes" Federal University of Maranhão and identified with the number of exsiccate 1067, in Atticus Seabra Herbarium of the same institution. Antispasmodic activity was evaluated by observing the effect of the extract at different concentrations in the phasic contractions induced by carbachol in response curve contractions cumulative carbachol and CaCl 2, and KCl-induced tonic contractions of the ileum to carbachol and (in) *Rattus norvegicus* Wistar. It used the statistical program Graphpad Prism 5.0 for evaluating the results using Student's t test and One-way test followed by Newman-Keuls test with $p < 0.05$. The study was approved by the Animal Experimentation Ethics Committee (CEEA-UEMA) under protocol number 002/2009. **Results and Discussion** / **Conclusions**: The doses of 250, 500 and 750 mg / mL extract antagonized the contraction induced phasic 1mM carbachol significantly ($p < 0.05$) concentration-dependent manner, with a reduction of the maximum contraction 35.24; 40.53 and 53.74% respectively. The EAC inhibited in concentration-dependent manner, the contractions induced by curve cumulative carbachol, shifting to the right and reducing the $E_{máx}$. The pD_2 values were -7,572 ± 0,05912 to -6,969 ± 0,1328; -6,592 ± 0,1089 and 6,505 ± 0,1014, at doses of 100, 300 and 500 mg / mL, respectively. When we evaluated the activity of the EAC on the tonic contraction induced by KCl, we observed a relaxation of 76.6 ± 5.0; 46.9 ± 5.3 and 26.9 ± 4.9 % at doses of 100, 300 and 500 mg / mL, respectively. These doses were used in the cumulative curve to $CaCl_2$ and observed the reduction of $E_{máx}$ in three doses and a significant right shift in the dose of 300 mg / mL, with pD_2 of -1,847 ± 0,04661 to 1,603 ± 0,1285. Then, the results suggest that the EAC appears to have a non-competitive antagonism with carbachol and inducing muscle relaxation by blocking Ca_v. **Financial Support**: FAPEMA, UFMA.

09.095 Antimicrobial activity *in vitro* of ethanolic extract of *Agaricus brasiliensis* on pathogenic bacteria. Frade ADS, Lucena KL, Farias RLGP, Nascimento JS UFPB – Fisiologia e Patologia

Introduction: *Agaricus brasiliensis*, known as the mushroom of the sun belongs to the fungi kingdom, Agaricaceae family. It is a species whose properties are attributed to nutraceutical polysaccharides acting as antitumor substances and modulation of the immune system [1]. The aim of this study was to evaluate the in vitro antimicrobial potential of ethanolic extract of *Agaricus brasiliensis* on pathogenic microorganisms. **Methods:** The fungus was Identified by growers fungi located in the state of Sao Paulo, Brazil. The material was dried in an oven at 40 ° C, crushed in a mill mechanic and subjected to exhaustive maceration with ethanol for 72 hours, three times. The extractive solution was concentrated in rotaevaporator at 35 ° C. For performing the microbiological assays, we selected isolates of *Escherichia coli, Staphylococcus aureus, Pseudomonas aeruginosa, Enterococcus sp., Klebsiella pneumoniae and Acinetobacter spp*. clinical source. In sterile Petri dishes, were seeded suspension of micro-organism (containing 1.5 x108UFC/mL based on the scale of Mac Farland) using "swabs" sterilized. Then, there were drilling wells with a capacity of 50µL using sterile tubes, which were deposited in 50µL aliquots of the ethanol extract at concentrations of 0 - 25 - 50 - 75 - 100mg/mL in triplicate. The negative control used was antimicrobial distilled water. The Petri dishes were prepared incubated at 36 ° C for 24 hours. The results were evaluated by measuring the diameter of the zones of inhibition in mm, formed around the wells. Extracts were considered active when presented inhibition zones greater than or equal to 10mm. **Results and Discussion:** From the results, consisted that the *Agaricus brasiliensis* not have antimicrobial activity, because none of the bacteria used were sensitive to the extract at concentrations, which does not diminish the pharmacological importance of the species, since it presents an excellent immunostimulant activity, counteracting the physical and emotional stress. **Financing:** CNPQ. **References:** [1]WASSER, S. P.; WEIS, A. L. General description of the most important medicinal higher basidiomycetes mushrooms. International Journal of Medicinal Mushrooms, v.1, p.351–370, 1999.

09.096 Role of TRPM8 channels in the vasorelaxant effect induced by rotundifolone in the superior mesenteric artery from spontaneously hypertensive rats. Almeida MM[1], Lira DP[2], Barbosa Filho JM[2], Gomes MA[3], Pesquero JL[4], Cruz JS[5], SILVA DF[6], Medeiros IA[1] [1]UFPB – Ciências Farmacêuticas, [2]UFPB – Química, [3]UFMG – Parasitologia, [4]UFMG – Fisiologia e Biofísica, [5]UFMG – Bioquímica e Imunologia, [6]UFBA – Biorregulação

Introduction: The Transient Receptor Potential (TRP) superfamily of cation channels is remarkable since it displays greater diversity in activation mechanisms. It is constitutes targets for plant-derived compounds (D. E. CLAPHAM, Nature, v. 426, p. 517, 2003; J. B. CALIXTO, Pharmacol Therapeut, v. 106, p. 179, 2005). **Aim:** To investigate the role of TRP channels in the vasorelaxant response induced by rotundifolone and menthol in the rat superior mesenteric arteries from 12-week-old spontaneously hypertensive (SHR) and normotensive Wistar-Kyoto rats (WKY). **Methods:** Endothelium-denuded isolated rat superior mesenteric artery rings from SHR and WKY rats were suspended by platinum hooks for isometric tension recordings. The artery rings were pre-contracted with Phe (10^{-6} M) and the relaxation evoked by rotundifolone and menthol was evaluated. The tissue levels of the TRPM8 channel messenger RNA (mRNA) in the superior mesenteric artery from SHR and WKY rats was measured by PCR technique. These procedures were developed in accordance with the Animal Ethics Committee (CEUA/UFPB nº 1201/11). **Results and Discussion:** Rotundifolone caused relaxation of superior mesenteric artery from WKY rats pre-contracted with Phe (10^{-6} M) (Maximum Response = 100.14 ± 3.15 %; pD_2 = 3.48 ± 0.04, n = 7). The pharmacological potency was significantly increased in SHR rats (MR = 102.07 ± 1.47 %; pD_2 = 3.89 ± 0.05, n = 6). The vasorelaxant effect induced by rotundifolone in WKY and SHR rats was attenuated in the presence of BCTC (2 x 10^{-6} M), a TRPM8/TRPV1 channel blocker (MR = 85.72 ± 5.90 %; pD_2 = 3.14 ± 0.04, n = 5 and MR = 97.53 ± 5.71 %; pD_2 = 3.41 ± 0.04, n = 6, respectively). The vasorelaxant effect induced by rotundifolone in WKY and SHR rats was not significantly attenuated in the presence of ruthenium red (10^{-5} M), a non-selective TRP channel blocker, nor after TRPV1 desensitization with capsaicin (10^{-5} M). The tissue levels of the TRPM8 channel messenger RNA (mRNA) in the superior mesenteric artery was significantly increased in SHR rats compared to WKY rats. **Conclusions:** Our findings suggest that rotundifolone induce vasodilatation in superior mesenteric artery from SHR rats involving probably TRPM8 channels activation. Furthermore, the pharmacological potency of rotundifolone was increased in hypertensive rats probably due to alteration in the expression of the TRPM8 channels. **Financial Support:** CNPq/CAPES, UFPB, UFMG, UFBA and FAPESB.

09.097 ***Parahancornia amapa*** **(Huber) Ducke (Apocynaceae)**: **A study of the gastroprotective activity**. Ribeiro RB[1], Silva SL[1], Alves CM[1], Burmann APR[2], Nascimento AA[1] [1]Unifap, [2]Lacen-Ap

Introduction: *Parahancornia amapa* (Huber) Ducke (Apocynaceae) is a plant used by communities of the Amazon region for the treatment of fatigue, lung problems and gastrointestinal disorders as well as analgesic and anti-inflammatory (MONTELES & PINHEIRO, 2007; OLIVEIRA, 2008). The aim of this study was to evaluate the gastroprotective activity of methanol extract from the bark of *P. amapa* (EMPA) in experimental animals (rats). **Methods:** The project was submitted to the Research Ethics Committee of the Federal University of Amapá (CEP/UNIFAP, n.006A/2011). After botanical identification, a voucher specimen was deposited in the herbarium of the Instituto de Pesquisas Científicas e Tecnológicas do Estado do Amapá (HAMAB-18350). Dried and powders barks were submitted to maceration process (in methanol) to yield the crude methanol extract of *P. amapa* (EMPA). That extract was submitted to pharmacological tests to verify its biological activity. For evaluating the antiulcerogenic activity of EMPA, experiments were performed to induce gastric ulcers based on risk factors for the illness in humans such as stress, nonsteroidal anti-inflammatory drugs and alcohol. For each experimental model it was used acidified ethanol, absolute ethanol, indomethacin or acetic acid. **Results:** The models of gastroprotection and antiulcer activity showed statistical significance for the contents of total injured area (mm²) area percentage (%), ulcerative lesion index (ULI) and cure rate (%). Statistical comparisons were performed using one-way ANOVA followed by Dunnett's test (*$p<0.01$; **$p<0.05$) compared with the control group (CTRL). The extract (EMPA) at a dose of 500 mg/kg prevented the formation of gastric lesions induced by acidified ethanol in 99.67%**. In the induction by absolute ethanol, it was evidenced cure rates (%) for lansoprazole and EMPA (100, 250 and 500 mg/kg) groups, values of 71.215 ± 7.280**, 52.045 ± 12.612**, 89.493 ± 3.037**, 98.395 ± 1.572, respectively. The administration of indomethacin (100 mg/kg) induced lesions that were treated with EMPA presented cure rate (%) of 73.76 ± 4.39**, 44.41 ± 8.72*, 76.57 ± 7.17**, 96.46 ± 0.42** in groups pre-treated with cimetidine and EMPA (100, 250 and 500 mg/kg), respectively. The treatment with EMPA (500 mg/kg p.o.) in the model of induced chronic ulcer was able to decrease injured areas at 99.14%** (18.972 ± 3.179** mm^2 of injured area) when compared to CTRL group. **Discussion:** The extract (EMPA) showed dose-dependent gastroprotective activity in different models of induced gastric lesions, which confirms folk indication. Regardless of the inducing stimulus, EMPA was able to prevent ulcerative lesions. EMPA showed significant activity in healing deep ulcers chronically induced. In accordance with the statistical analysis of the models of ulcer induction, EMPA showed better results than the positive controls (lansoprazole and cimetidine). **Keywords:** *Parahancornia amapa*, gastroprotection, antiulcer activity. **Financial support:** CAPES/CNPq/ PIBIC.

09.098 Assessment and quantification of presence of Resveratrol in grape juice obtained in a Brazilian industry. Santos SM[1], Ott FP[1], Oliveira US[1], Weber BD[1], Carneiro AM[2] – [1]UNASP, [2]Superbom – Quality Management

Introduction: The grape has properties with a wide pharmacological applicability, therefore, there many research groups focused on these compounds, especially Resveratrol. Variations in the concentration of this compound in the final products of different industries, has been attributed the different forms of industrialization of juices (Souto, J., *J. Food Comp. Anal.,* v.14, p.441, 2001). The aim of this study was to analyze and quantify the levels of Resveratrol grape juice obtained in industry a Brazilian and compare the result with literature data. **Methods:** In the present study was used integral grape juice of variety Santa Isabel, species *Vitis labrusca*, cultured in Caçador and Rio das Antas – SC, processed in Food Products Industry Superbom. The samples were submitted for analysis and quantification of the levels of Resveratrol by the technique of liquid chromatography-layer high (HPLC) as described by McMurtrey et al., (1994). For this purpose, we used four samples of different batches, in which they were analyzed in duplicate. The results of all analyzes were compared with information of other industries available in the literature. **Results and Discussion:** The Resveratrol is a phytoalexins synthesized mainly in the skin of the grape, especially due to the stress suffered by plant (Souto, J., *J. Food Comp. Anal.,* v.14, p.441, 2001), involved in many biochemical reactions in the body, by having anticoagulant and anti-inflammatory activity, reduced risk of coronary events, by inhibiting of aggregation of fat in the vessel because of its high ability to prevent LDL (lipoprotein low density) oxidation (Giehl, M.R., *Sci. Med.,* v.17, p.145, 2007). In the present study the average results of analyzes and quantification of levels of Resveratrol in samples of grape juice integral, was 1.09 ± 0.04 mg L^{-1} of juice. Studies have shown that grape juices produced in Brazil, has concentrations of Resveratrol that varies between 0.19 to 0.90 mg L^{-1} of juice (Sautter, *Food Sci. Tech*., v.25, 2005). Our results showed a percentage of 21.7% higher when compared with the higher value described above. Thus, our results suggest that high concentration of resveratrol of the samples analyzed may be related the species, cropping system in the region and industrial processing. **Financial Support:** Food Products Industry Superbom.

09.099 Gastroprotective action and antioxidant properties of fractions ethanol extract of *Neoglaziovia variegata* Mez. Machado FDF[1], Oliveira IS[1], Viana AFSCV[1], Piauilino CA[1], Lima JT[2], Almeida JRGS[2], Oliveira FA[1], Oliveira RCM[1] [1]NPPM-UFPI, [2]UNIVASF – Ciências Farmacêuticas

Introduction: Previous studies have shown that ethanol extract of *Neoglaziovia variegata* Mez (Nv-EtOH) presents gastroprotective activity in animals models. The present study aimed at investigating the gastroprotective activity of hexane (Nv-Hex) and chloroformic (Nv-$CHCl_3$) fractions of ethanol extract using ischemia/reperfusion-induced gastric lesions model in rats and to evaluate the antioxidant activity in mice. **Methods:** Female Swiss mice (25-30 g) and Wistar rats (180-220 g), n=8/group, fasted over a period of 18 h before each experimentation. All animal experiments protocols were approved by Ethics Committee of the Federal University of Piauí (CEEA-PI 076/10). In Ischemia and reperfusion induced ulcers model, Wistar rats were orally treated with vehicle, N-acetylcysteine (NAC, 200 mg/kg) or Nv-Hex (50, 100, 200 and 400 mg/kg). After 1h, the celiac artery blood flow was interrupted by a microvascular clamp, under induced anesthesia ketamine and xylazine (50 and 5,0 mg/kg i.p., respectively). After 30 min, the clamp was removed and the reperfusion was established. The animals were euthanized 1 h after induction of the reperfusion. After the protocol ischemia/reperfusion the stomachs area of injury measured by planimetry (mm^2). For the quantification of non-protein sulfhydryl groups (NP-SH), Swiss mice were pre-treated orally with the vehicle but not ethanol (SHAM), vehicle, Nv-Hex (100 mg/kg), Nv-$CHCl_3$ (100 mg/kg) or carbenoxolone (100 mg/kg) 1h before induction of gastric lesions by absolute ethanol (0,2 mL/animal, p.o.) and were euthanized 30 min later. The amount of NP-SH in the gastric mucosa was measured according to the method described by Sedlak and Lindsay (Anal. Biochem., v. 25, p. 192, 1968]. To evaluate the enzymatic activity of catalase Swiss mice were pre-treated with the vehicle but not ethanol (SHAM), vehicle, Nv-Hex (100 mg/kg), Nv-$CHCl_3$ (100 mg/kg) or NAC (200 mg/kg) 1h before induction of gastric lesions by absolute ethanol and were euthanized 30 min later. The catalase activity was measured according to the method described by Beers and Sizer (J. Bio. Chem., v. 95, p.133-140, 1952). The significance level was evaluated for values of $*p< 0.05$ (ANOVA one way). **Results and discussion:** In the ischemia and reperfusion model, the Nv-Hex decreased the area of lesions at doses of 100 mg/kg (4,64 ± 0,37*), 200 mg/kg (2,44 ± 0,63*) and 400 mg/kg (2,12 ± 0.47*), compared with control saline (13,94 ± 1,35). Nv-Hex and Nv-$CHCl_3$ increased the levels of NP-SH at dose of 100 mg/kg (2025,53 ± 209,11 µg/g* and 1573,60 ± 108,98 µg/g*, respectively), compared with respective controls (446,15 ± 16,66 and 891,99 ± 16,98 µg/g, respectively). Any fractions did not increase significantly the catalase activity. **Conclusion:** The results shown that fraction Nv-Hex induced gastroprotective activity in ischemia/reperfusion-induced gastric lesions model and increased levels NP-SH, suggesting a possible antioxidant action. **Support:** UFPI/UNIVASF/CAPES/CNPq.

09.100 Endothelium-dependent vasorelaxant effect of butanolic fraction from *Caryocar brasiliense* Camb. leaves in rat thoracic aorta. Oliveira LM[1], Rodrigues AG[1], Silva EF[1], Castro CH[1], Pedrino GR[1], Carvalho MHC[2], Costa EA[1], Filgueira FP[1], Ghedini PC[1] [1]UFG – Ciências Fisiológicas, [2]USP – Farmacologia

Introduction*: Caryocar brasiliense* Camb. ("pequi") is a native plant from the Cerrado region of Brazil that contains bioactive components widely reported to be antioxidant agents (Khouri et al., Genet Mol Biol 30: 442, 2007; Miranda-Vilela et al., Genet Mol Biol 31: 956, 2008). Previous work has demonstrated that dietary supplementation with pequi decreased the arterial pressure of volunteer athletes (Miranda-Vilela, Thesis, UNB, 171p, 2009). The aim of the present study was to evaluate the vasorelaxant effect of the crude hydroalcoholic extract (CHE) from *C. brasiliense* leaves in rat thoracic aorta, to determine the active organic fractions of CHE, and to investigate the mechanisms of action. In addition, we analyzed the in vivo effect of the extract of the active organic fraction. **Methods:** We evaluated the vasorelaxant effect of the crude hydroalcoholic extract (CHE) of *C. brasiliense* leaves and its organic fractions: hexanic (HF), chloroform (CF), ethyl acetate (EAF), and butanol (BF) in the thoracic aorta from male Wistar rats (200-300 g; n = 5-7) and the effects of BF on blood pressure of anaesthetized rats (halothane 2-3% plus urethane 1.2 g/kg). All experiments were conducted in accordance with the Sociedade Brasileira de Ciência em Animais de Laboratório (SBCAL) and were approved by the local Ethics in Research Committee (Protocol CEP/UFG 22/2011). **Results and Discussion:** We found that the CHE completely relaxed, in a concentration-dependent manner, rat aortic rings that had been precontracted with phenylephrine, and that the BF fraction produced an effect similar to that of the CHE. Aortic relaxation induced by BF was abolished by endothelium removal, by incubation of the nitric oxide synthase inhibitor L-NAME, or the soluble guanylate cyclase inhibitor ODQ. However, incubation with atropine (a muscarinic receptor antagonist) and pyrilamine (a histamine H_1-receptor antagonist) had no effect on the BF-induced vasorelaxation. Moreover, this effect was not inhibited by indomethacin (a cyclooxygenase inhibitor) and tetraethylammonium (a non-selective K^+ channel blocker). The concentration-response curve to calcium in denuded-endothelium rings was not modified after previous incubation with BF, and the vasorelaxation by BF in endothelium-intact aorta rings precontracted with KCl was abolished after incubation with L-NAME. In addition, administration of BF (10, 30, and 100 µg/kg, i.v.) in anesthetized rats resulted in a significant and reversible hypotension. Taken together, the results reveal that *C. brasiliense* possesses both in vivo and in vitro activities and that the vascular effect of BF involves stimulation of the nitric oxide/cyclic GMP pathway. **Financial Support:** CAPES, FAPEG, CNPq

09.101 Essential oil of *Lippia microphylla* Cham. (Verbenaceae) shows spasmolytic effect on guinea-pig trachea and ileum. Oliveira GA, Travassos RA, Souza ILL, Martins IRR, Carreiro JN, Correia ACC, Pereira JC, Ferreira TF, Silva MCC, Tavares JF, Silva BA CCS-UFPB

Introduction: Verbenaceae family consists about 36 genus and 1000 species. In this family, the *Lippia* genus arouses interest, since species from this genus are used in folk medicine for the treatment of respiratory and gastrointestinal diseases (MORTON, Atlas of Medicinal Plants of Middle America, v. I. p. 745, 1981). *Lippia microphylla* Cham. belongs to this genus and it is find only in Guiana and Brazil (LEMOS, Fitoterapia, v. 63, p. 266, 1992). This species is popularly known as "alecrim-do-mato" "alecrim-de-tabuleiro" and "alecrim-pimenta" and it is used by population in the form of decoction or maceration in alcohol as antiseptic and to treat respiratory diseases such as colds, bronchitis, cough and asthma (AGRA, Rev. Bras. de Farmacogn., v. 18, p. 472, 2008.). Recent studies showed relaxing activity on rat aorta and trachea (ANTUNES, XX Congresso Italo-Latinoamericano de Etnomedicina, 2011), and potential gastrointestinal activity from the ethanolic extract of aerial parts of this species (JACINTO, XX Congresso Italo-Latinoamericano de Etnomedicina, 2011). Based on these premises we decided to investigate a possible spasmolytic effect of essential oil from aerial parts of *L. microphylla* (LM-OE) on guinea-pig trachea and ileum. **Methods:** Guinea-pig trachea was suspended in an organ bath containing Krebs solution, at 37 °C, with gas mixture (95% O_2 + 5% CO_2) and 1g of resting tension. After the stabilization period (60 min), the first contraction was induced by CCh 10^{-6} M and the presence of epithelium was verified as described by TSCHIRHART (J. Pharmacol. Exp. Ther., v. 243, p. 310, 1987). In case of epithelium presence, a second contraction was induced by CCh 10^{-6} M and then LM-OE (2-14 µg/mL) was added to organ bath to observe its relaxant effect. Guinea-pig ileum was suspended in an organ bath containing modified Krebs solution in appropriate conditions. Two simple concentration-response curves were induced by CCh or histamine 10^{-6}M, these are considerate the control. LM-OE (1-27 µg/mL) was added in the organ bath, after 15 min, in oil presence, another concentration-response curve was induced by CCh or histamine to verify LM-OE inhibitory effect. All the experimental protocols were approved by Ethical Committee in Animal Use of CBiotec/UFPB (0504/12). **Results:** On guinea-pig ileum, LM-OE antagonized, in a significant and concentration-dependent manner, the CCh-(IC_{50} = 24.4 ± 2.9 µg/mL, n = 5) and histamine-(IC_{50} = 15.8 ± 2.3 µg/mL, n = 5) induced phasic contractions, being significantly more potent to histamine. Suggesting that LM-OE can be acting as an antagonist of the histamine receptors, but more experiments are required to confirm this hypothesis. Furthermore, LM-OE relaxed the guinea-pig trachea pre-contracted by CCh (EC_{50} = 9.9 ± 0.5 µg/mL n = 5) in the functional epithelium presence. **Discussion:** These preliminary results point to justify the ethnomedicinal use of *Lippia microphylla* in the treatment of respiratory and gastrointestinal diseases. However further studies are required to elucidate the action mechanism of this natural product. In the future, perhaps, we will to clarify the efficacy and safety of this species in its use by population. **Financial support:** CAPES, CNPq, PgPNSB/UFPB.

09.102 6-styryl-2-pyron of *Aniba panurensis* (Lauraceae) shows spasmolytic action on rat trachea and aorta rings. Travassos RA[1], Silva MCC[1], Oliveira GA[1], Souza ILL[1], Silva ACL[1], Garcia FM[2], Barbosa Filho JM[1], Silva BA[1] [1]CCS-UFPB – Ciências Farmacêuticas, [2]FMN

Introduction: 6-styryl-2-pyron (dehydrogoniothalamin) is a natural styryl pyrone isolated from ethanolic extract of green fruits of *Aniba panurensis* (Lauraceae) (Barbosa-Filho, Phytochem. v. 26 p. 2615, 1987). Pyrones demonstrate a whole spectrum of bioactivity and have been shown to be antifungal, antibiotic, cytotoxic and phytotoxic (Fairlamb, Bioorg. e Med. Chem. v. 12, p.4285, 2004). The aim of this study was to investigate a possible spasmolytic action of dehydrogoniothalamin (DGT) on isolated rat trachea and aorta rings. **Methods:** Wistar rat (*Rattus novergicus*) trachea and aorta rings were suspended in an organ bath containing Krebs solution in appropriate conditions. Epithelium presence or absence on trachea was verified as described by Tschirhart (J. Pharmacol. Exp. Ther., v. 243, p. 310, 1987) and endothelium presence or absence on aorta was verified as described by Furchgott (Nature, v. 288, p. 373, 1980). Isometric contractions were monitored. The results were statistically analyzed by using the Student's *t*-test and/or ANOVA one-way followed by Bonferroni correction when appropriate. The significance level considered in all tests was $p< 0.05$. The values of pD_2 values were calculated by nonlinear regression and are presented as mean and standard error media in all experiments. All data were analyzed by the program GraphPad Prism® version 5.0 (GraphPad Software Inc., USA). All the experimental protocols were approved by Ethical Committee in Animal Research of LTF/UFPB (0506/05). **Results:** On rat aorta, DGT (3×10^{-5}, 10^{-4} and 3×10^{-4} M) showed spasmolytic activity on this organ pre-contracted by phenylephrine (3×10^{-7} M) in presence (E_{max} = 5.3 ± 3.1, 14.5 ± 3.3 and 60.2 ± 3.5%) or absence (E_{max} = 8.9 ± 1.3, 19.0 ± 1.2 and 44.6 ± 5.9%) of functional endothelium, respectively (n = 3). Moreover, DGT relaxed the rat trachea pre-contracted by carbachol (10^{-6} M) in a significant and concentration-dependent manner in the presence (pD_2 = 5.87 ± 0.09) or absence (pD_2 = 3.93 ± 0.05) of functional epithelium (n = 3), being significantly more potent in epithelium presence. **Discussion:** DGT produced spasmolytic effect on rat aorta, since these effects occurred in very high concentrations, we chose not to continue the investigation of the spasmolytic action mechanism of this pyron in this organ. Furthermore, DGT produced relaxant effect on rat trachea and this effect seems involved epithelium-derived relaxing factors. **Financial support:** CAPES, CNPq, PgPNSB/UFPB.

09.103 Participation of a NANC pathway on spasmolytic effect of the fraction of the total alkaloids from *Solanum paludosum* Moric. root bark on guinea-pig ileum. Silva ACL, Monteiro FS, Oliveira GA, Travassos RA, Pereira JC, Ferreira TF, Souza ILL, Agra MF, Basílio IJLD, Silva BA UFPB – Ciências Farmacêuticas

Introduction: Solanaceae family is formed by 98 genus and 2700 species (OLMSTEAD, Acta Hort., v.745, p. 255, 2007). Solanum genus, belonging to this family, has been studied mainly due to biological activities presented by several of its species (IBARROLA, J. of Ethnopharmacol., v. 70, p. 301, 2000; RIBEIRO, Rev. Bras. Farmacogn., v. 12, p. 34, 2002). *Solanum paludosum* Moric. is an herbaceous species, known popularly as "jurubeba-roxa" in the Northeast of Brazil. (AGRA, Royal Botanic Gardens, p. 341, 1999). Chemical studies of the root bark of this species showed the presence of steroid alkaloids and its glycosides (BASÍLIO, Dissertação, 2008), and about pharmacological studies of the fraction of total alkaloids from root bark (FAT-SP) has shown spasmolytic activity on rat aorta involves NO/cGMP/PKG pathway and potassium channels (MONTEIRO, *J of Ethnopharmacol*., v. 141, p. 895, 2012). Recently we demonstrated that the relaxant effect on FAT-SP in guinea pig ileum involving the upregulation of potassium channels (Brazilian Congress of Pharmacology and Experimental Therapeutics, 2010). Therefore, we decided to assess wich others mechanisms are involved in the relaxant effect of FAT-SP in guinea pig ileum. **Methods:** Guinea-pig ileum was suspended in an organ bath containing modified Krebs solution (pH 7.4) at 37 °C, gassed with 95% O_2 an 5% CO_2 mixture. Isometric contractions were monitored. All the experimental protocols were approved by Ethical Committee in Animal Research of LTF/UFPB (Protocol 0111/09). **Results:** FAT-SP spasmolytic effect (EC_{50} = 61.6 ± 8.6 µg/mL, n = 5) was potentiated significantly in the simultaneous presence of guanethidine 3 x 10^{-6} M and atropine 10^{-6} M (EC_{50} = 30.4 ± 4.7 µg/mL), adrenergic and cholinergic blockers, respectively. We decided to investigate if nitric oxide (NO) pathway participates in this FAT-SP response. Interestingly, the FAT-SP relaxation effect was reduced significantly by L-NAME 10^{-4} M (EC_{50} = 121.1 ± 2.6 µg/mL, n = 3), blocker of nitric oxide synthase. **Discussion:** According to obtained results, we can suggest that in functional level, the spasmolytic effect of FAT-SP on guinea-pig ileum seems to involve a non-adrenergic non-cholinergic pathway (NANC), being NO the major neurotransmission in the gastrointestinal tract. Moreover, NO pathway participation was observed in our experiments. However further studies are necessaries to investigate the more deeply FAT-SP action mechanism. **Financial support:** CAPES, CNPq, PgPNSB/UFPB.

09.104 ***Rinocerophis fonsecai* (*Bothrops fonsecai*) crude snake venom activity and its neutralization by commercial Bothropic antivenom**. Collaço RC[1], Cogo JC[2], Rocha T[3], Tamascia ML[1], Silva IRF[1], Hyslop S[1], Randazzo-Moura P[4], Rodrigues-Simioni L[1] [1]Unicamp – Farmacologia, [2]UNIVAP – Estudos da Natureza, [3]UNIVASF, [4]PUC-SP – Ciências Fisiológicas

Introduction: *Bothrops* sp. venoms induce local damage such as hemorrhage and necrosis and can be neurotoxic, resulting in neuromuscular blockade of nerve-muscle preparations *in vitro*. *Rinocerophis fonsecai* (*Bothrops fonsecai*) venom presents compounds as phospholipases A_2, metalloproteinases and serinoproteinases, however this venom is meanly studied. In this research, the myotoxic and neurotoxic effects in mouse extensor digitorum longus preparations (EDL) and hemorrhagic activity of *R. fonsecai* total venom were studied. The ability of commercial equine bothropic antivenom in neutralizing these effects was also analyzed. **Methods:** Mouse EDL was incubated with venom (3-300µg/ml) for 120min at 37 ºC and the tissues were processed for qualitative histological analysis. Venom PLA_2 activity was measured colorimetrically. The hemorrhagic halo evoked in rats by dorsal intradermic venom administration was also evaluated. For neutralization of neurotoxic, myotoxic, hemorrhagic and PLA_2 activities the samples were previously pre-incubated for 30 min with bothropic commercial antivenom at ratios of 5:1 (venom:antivenom, according to the manufacturer's recommendations) and double of this ratio (5:2). The animal experiments were approved by the institutional Committee for Ethics in Animal Use (CEUA/UNICAMP, protocol no. 2648-1).**Results:** *R. fonsecai* induced neuromuscular blockade in EDL and the time to reach 50% of blockade using 3, 10, 30, 100 and 300ug of venom/ml were: 84.6±6.5, 76.7±11.8, 65.3±11.9, 41.2±6.4 and 46.6±4.2min, respectively (mean±SD; n=5-7; $p<0.05$ compared to control). Histological analysis showed muscle fibers with edema, myofilaments hypercontraction and vacuolization, resulting in ghost fibers. The PLA_2 activity of *R. fonsecai* venom (100µg) was 1.75±0.5mM HCl/min compared to 0.85±0.4mM HCl/min for *Crotalus durrissus terrificus* venom (100µg; positive control; n=5 each; $p<0.05$). The venom also caused a concentration-dependent hemorrhagic halo in rat's skin. Antivenom administration (ratio of 5:1) did not significantly affect the venom neuromuscular blockade (74.6±8.6% after 120min; n=5; 100µg/ml). A partial protection was observed at ratio of 5:2 (37.5±5.5% blockade after 120min; $p<0.05$; n=5). Venom PLA_2 activity was not inhibited by the antivenom (activities of 1.70±0.05mM HCl/min and 1.3±0.13mM HCl/min for ratios of 5:1 and 5:2, respectively; n=3 each). The antivenom protected against the tissue damages, according to the histological analysis and inhibited the hemorrhagic activity of the venom. **Discussion:** *R. fonsecai* venom caused myotoxic, neurotoxic and hemorrhagic effects in EDL preparations. Such effects can be mediated by venom PLA_2; however, hemorrhagic effect can be mediated by metalloproteinases and serineproteinases. The commercial bothropic antivenom protected against hemorrhagic activity of venom, but only partially inhibits the neuromuscular activity. This can be explained by the fact that the *R. fonsecai* venom is not included in the pool used to immunize horses. **Financial support:** CNPq/UNICAMP

09.105 Fucose moieties are essential for the ability of fucosylated chondroitin sulfate to inhibit muscle damage induced by *Bothrops jararacussu* venom. Monteiro-Machado M[1], Strauch MA[1], Tomaz MA[1], Cons BL[1], Ricardo HD[1], Lece FS[1], Fonseca RJC[2], Mourão PAS[2], Melo PA[1] [1]UFRJ – Farmacologia Básica e Clínica, [2]UFRJ – Química Biológica

Introduction: Snakebites by *Bothrops jararacussu* snake induces intense local tissue damage. Phospholipases A_2 are enzymes present in the venom which are responsible for a wide range of activities (Toxicon 45, p.1147, 2005). Some polyanions have been shown to present antivenom properties against this venom (Toxicon 31, p.285, 1993). A new natural polyanion polysaccharide, named Fucosylated Chondroitin Sulfate (fucCS), is involved in many biological activities (JBC 282 (20), p.14984, 2007). We assessed the ability of fucCS and its carboxi-reduced and defucosylated analogues to antagonize the muscle damage induced by *B. jararacussu* crude venom. **Methods:** *In vitro* CK assays were performed with isolated mouse *extensor digitorium longus* (EDL) muscle bathed with venom alone (25 µg/mL) or incubated with fucCS or analogues (10-50 µg/mL). *In vivo* experiments were performed by i.m. venom injection alone or preincubated with fucCS or defucCS (1-10 mg/kg) and the plasma CK activity was evaluated before and 2 hours after injection (1 mg/kg). The phospholipase and hyaluronidase activities were measured using turbidimetric methods. The CK content was evaluated in EDL muscle after a perimuscular injection of venom (1 mg/kg). Histological sections were performed in EDL muscle after crude venom perimuscular injection. All experiments were approved by the Committee of Animal Use of the Rio de Janeiro Federal University (DFBCICB 026). **Results:** It was observed that fucCS inhibits 75% of phospholipase venom activity with IC_{50} = 10 µg/mL (n=10) and 100% of hyaluronidase activity with IC_{50} = 7 µg/mL (n=5), in concentration-dependent manner. Incubation of fucCS with the venom eliminates the increase of plasma CK, *in vivo (*n=4). The EDL muscle was preserved when exposed to venom with fucCS *in vitro* (30 and 50 µg/mL) (n=4). The reduction of the CK content was prevented by fucCS (1-10 mg/kg) (n=4). DeFucCS was unable to protect the phospholipase activity and miotoxicity *in vivo* and *in vitro*. Light microscopy shows that fucCS can inhibit the muscle damage induced by the venom (n=4). **Discussion:** FucCS was capable to inhibit venom activities related to tissue damage, although defucCS does not have this ability. These results indicate that fucCS presents activity against *Bothrops jararacussu* venom and we believe that this antivenom activity may be due to the interaction of negative charges of fucose moieties of fucCS with positively charges toxins present in this snake venom, like others polyanions. **Financial Support:** CNPq, CAPES, FAPERJ and PRONEX.

09.106 Study of topical subacute toxicity of essential oil delta-3-carene extracted from *Myracrodruon urundeuva* Fr. All. Nogueira LM[1], Santos GGL[2], Ferraz IC[2], Ximenes RM[1], Mendonça R[3], Havt A[4], Martins RD[2] [1]UFC – Fisiologia e Farmacologia, [2]UFPE, [3]UFC – Química, [4]UFPE – Fisiologia e Farmacologia

Myracrodruon urundeuva Fr. All. popularly known as aroeira-do-sertão, is a tree that occurs mostly in the Northeast, Southeast and Midwest regions of Brazil (ALBUQUERQUE, U. PJ. Ethnopharmacology, p.156, 2007.). From its leaves we can extract essential oils constituted by mixtures of terpenes and a wide variety of aliphatic low molecular weight hydrocarbons. Essential oils are known to have antioxidant activity and when used on wounds protect these from tissue damage by oxidative stress, as well as accelerating the healing process (NAMSA. J. Ethnopharmacology p. 234, 2009). However, the use of these oils as therapeutic resource requires the knowledge of its safety. So, this study aims to evaluate subacute toxicity of chemotype delta-3-carene of essential oil extracted from leaves of *M. urundeuva* when used on experimental excisional wounds. *M. urundeuva* was cultivated in horticulture sector of Federal University of Ceara and its leaves were collected. The essential oil was extracted by water vapor drag for 2 hours, using a Cleavenger type apparatus, and kept frozen at -20 °C until use. Swiss mice aged 2-4 months (n = 15) were anesthetized with sodium pentobarbital (50 mg/kg, i.p.) and had their dorsal region shaved and cleaned with iodide. The wound was performed on the dorsal region of each animal by excising the skin with a biopsy punch (6 mm). The entire wound was left open. After anesthesia recovery, animals were individual caged, but food and water supply was restored. Animals were divided in three different groups and treated twice a day during the first three days: control group received 30 µL of vehicle (0,5 % Cremophor®); treated group received 30µL of 4% essential oil dissolved in 0,5% Cremophor® solution or 30 µL of 2% essential oil dissolved in 0,5% Cremophor® solution. After 3, 7 and 14 days, blood samples were collected from each animal by orbital puncture to perform measurements of glucose, triglycerides, total cholesterol, protein and total plasma albumin. Measurements were performed using laboratory kits LABTEST following manufacturer protocols. The Ethics Committee of Federal University of Pernambuco approved all animal experiments under the process number 23076.020508/2010-26. This study revealed a significant increase of serum glucose levels in animals treated with the delta-3-carene 2% (142.5±9.46 mg/dL) and 4% (143.0 ±18.87mg/dL) compared to the control group (96.16 ± 1.29mg/dL) only at the 14th day. There were no changes in total cholesterol, but triglycerides were reduced at the 7th and 14th day in both treated groups when compared to the same group at the 3th day. In relation to the measurement of total protein, a reduction of the values in treated animals with essential oil at the 14th day when compared to the same group in the days 3 and 7. The group treated with essential oil at a concentration of 4% had a mean total protein higher than the treated group with 2% essential oil solution at the 14th day. In all groups, after 14 days, there was reduction of plasma albumin, especially in the treated groups with the essential oil at the concentration of 4%, showing statistical significance. These observed changes could be linked to the stages of wound healing. However, reducing triglycerides values in treated groups with essential oil at the 7th and 14th days could be linked to topical treatment with oil until the third day, which may have contributed to increase this parameter at the 3th day. More studies are necessary to better understand these changes.

09.107 Evaluation of a fish oil concentrate in CFA sub-chronic inflammation model in rats. Lobo BWP[1], Teixeira MS[1], Silva NLC[2], Silva LL[2], Lima CKF[2], Miranda ALP[2], Ramos MFS[1], Dellamora Ortiz G[1] [1]FF-UFRJ – Medicamentos, [2]FF-UFRJ – Fármacos

Introduction: Fish oil has been widely described to have acknowledged anti-inflammatory properties. Among fish oil constituents, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are known to be responsible for its effects. Besides being intermediary metabolites of arachidonic acid pathway, it has been recently shown that EPA and DHA seem to be resolvins precursors, being associated not only with reduction of inflammatory process, as well as with pain relief. The present study aimed to evaluate the anti-inflammatory and pain relief properties of a commercial fish oil concentrate (FOC) in CFA sub-chronic inflammation model, comparing its results with a classic anti-inflammatory drug. **Methods:** Inflammation was induced in the right hind paw by intraplantar injection of Complete Freund Adjuvant (CFA) in three different groups (n=6-10 animals/group): first group received FOC by gavage daily, during 7 days before (pre-treatment) and 5 days after (treatment) the induction with CFA (FOC); second group was pre-treated with saline and treated with dexamethasone (1 mg/kg, p.o.) (DEXA); third group (control group - CTRL) received only saline. Edema and pain were evaluated in the day of induction and during the four following days. Pain sensivity was assessed by mechanical stimulation (Von Frey method) and edema by measuring paw width. Blood was collected on the twelfth day by cardiac puncture for lipids evaluation (cholesterol and triglycerides). Subplantar tissue of inflamed paws was removed to evaluate mieloperoxidase activity (MPO), prostaglandin E_2 (PGE_2) and tumor necrosis factor-alpha (TNF-α) levels. Results were expressed in mean ± SEM, % of inhibition compared to CTRL group and statistically analyzed by Student *t* test and ANOVA (*p<0.05; **p<0.01; ***p<0.001). Animal protocols were approved by UFRJ ethical animal care committee (CEUA/FARMACIA01). **Results:** Blood lipids patterns remained unaltered in all groups. The treatment with FOC and DEXA decreased significantly both edema (34.7%** and 55.4%* of inhibition at 5th day) and sensivity to mechanical stimulation (38.8%** and 59.5%** of inhibition of AUC), respectively. We also observed a significant decrease in MPO activity (94.1%* and 77.6%*), in PGE_2 (93.7%*** and 97.0%**) and TNF-α (100%** for both groups) levels. **Discussion and conclusion**: The ensemble of results shows that FOC have anti-inflammatory and anti-hypernociceptive properties, being able to reduce the leucocytes migration and important inflammatory mediators involved in these responses. FOC was as efficient as DEXA in the inflammatory model employed, corroborating its use as adjuvant in the treatment of chronic inflammatory diseases. **Financial support**: FAPERJ, CNPq.

09.108 Bioguided phytochemical study of *Justicia pectoralis* Jacq. var. *Stenophylla leonard* (Acathanceae): Evaluation of bronchodilator activity. Casemiro J[1], Souza CAV[1], Moreira BAA[1], Soares JES[1], Vasconcelos A[2], Lima FJB[2], Brito TS[2], Ferreira LC[2], Roque CR[2], Magalhães PJC[2] [1]UFC – Farmácia, [2]UFC – Farmacologia do Músculo Liso

Introduction: *Justicia pectoralis* Jacq. var. *stenophylla* Leonard, known as "chambá", is a herb used in folk medicine and Public Programs of Phytotherapy, which is indicated for asthma, coughs and bronchitis. Many studies have shown the chemical nature, the low toxicity, the antinociceptive, anti-inflammatory, antispasmodic activities of hydroalcoholic extract, coumarin and umbeliferone obtained from chambá. **Objective:** Investigate the existence of others pharmacological constituents, in addition of coumarine and umbeliferone in extracts of increasing polarity. **Methods:** This study was approved by the Ethics Committee on Animal Research of Federal University of Ceará (53/2009). Plant's stems and leaves suffered washing and drying process in circulating air at the controlled temperature (40^{o}C). The extracts (hydroalcoholic – HA; hexanic – HX; chloroformic – CF; ethyl acetate – EA) were prepared in solvents of increasing polarity. The fractionings of the dried extract were performed by a column chromatograph, followed by comparative analysis by thin layer chromatograph. The pharmacological evaluation was made applying the sensitization and challenge *in vivo* methods (with ovalbumin – OVA), followed by analysis of biochemical and hematological parameters and the activity on tracheal smooth muscles contractility (TSMC) from rats *in vitro*. The differences were considered statistically meaningfully when $p<0,05$. **Results:** The phytochemical profile was performed for different extracts of the plant's aerial and root parts. It shows the presence of the coumarin, in particular HA extract from the leaves and stems and the EA of the roots, while the umbeliferone was found in most of the fractions extracts, except HX fraction. The TSMC increased meaningfully ($p<0,05$, ANOVA) with OVA. Moreover, the extract that appears to contain the main anti-asthmatic action (reversion of tracheal contraction) is the CF extract. The biochemical and hematological parameters have shown no difference between test and control groups. **Discussion:** The pharmacological effects of *J. pectoralis* is related with the HA extract from it, especially due to the antinociceptive, anti-inflammatory and bronchodilator of the coumarinic markers. But plant's extract with increasing solvent polarity allows the extraction of its constituents according to the polarity of the same components and may contain different constituents of those found in HA extract, which may allow the compounds' pharmacological effects' evaluation and their contribution to the plant's theurapitical activity, like that found in CF extract, which had the best response against asthma. **Financial support:** FUNCAP.

09.109 Vasorelaxant effect of extract and fractions from *Solanum sisymbriifolium* in isolated rat mesenteric artery. Simões LO[1], Albuquerque JM[1], Alves QL[1], Ramos M[1], Cechinel-Filho V[2]; Medeiros IA[3], Silva DF[4] [1]UFBA; [2]Univali; [3] UFPB, [4]ICS – Biorregulação

Aim: Natural products research remains to be a successfully tool to produce novel candidates for the treatment of prevalent human diseases (Koehn and Carter, 2005)**.***Solanum sisymbriifolium* is used in folk medicine in several countries in South America as antihypertensive and diuretic (Gonzales Torrez, 1992). Then, the aim of this study was to investigate the vasodilator properties of *Solanum sisymbriifolium*(extract and fractions) on isolated rat mesenteric artery. **Methods and Results:** Isolated rat superior mesenteric artery rings (1-2 mm) were suspended by cotton threads for isometric tension recordings in a Tyrode's solution at 37 ºC, gassed with a 95% O_2 and 5% CO_2, under a resting tension of 0.75g. In rat mesenteric artery rings with endothelium intact, methanol extract of *Solanum sisymbriifolium* aerial parts (0,01 – 300 µg/mL) induced concentration-dependent relaxation of the contractions induced by phenilefrine (10^{-6} M), (Maximum Response (MR) = 103.53 ± 4.87%, EC_{50} = 1.52 (1.12 – 2.07) µg/ml, n=6). After removal of the vascular endothelium, the concentration-response curve was significantly shifted to the right (MR= 63.88 ± 8.6%, EC_{50}=0.72 (0.32 − 1.6) µg/mL; $P<0.05$; n=5). Then, methanol extract of *Solanum sisymbriifolium* was fractioned by solvent-solvent partitioning and studied. The n-hexane (MR = 83.7 ± 4.37 %, EC_{50} = 6.23 (5.09-7.63) µg/ml, n=4) and chloroform fractions of the methanol extract of *Solanum sisymbriifolium* (MR= 96.9 ± 5.1 %, EC_{50}= 20.58 (13.81 – 30.66) µg/ml; n=4) exhibited similar relaxation of mesenteric arteries. Endothelial removal also altered the relaxing effect of the fractions studied, suggesting the participation of endothelium mediators in relaxant effect induced by *Solanum sisymbriifolium.* **Conclusions:** These results suggest thatthe methanol extract and fractions of*Solanum sisymbriifolium*has vasorelaxant properties and this effect seems to involve mediators of vascular endothelium. In summary, these effects might explain, at least in part, the medicinal use of *Solanum sisymbriifolium* in the disorders of cardiovascular systems. **Financial support**: CNPq and FAPESB. Use animal protocols were conducted in accordance with CEPA – Comitê de Ética em Pesquisa Animal - UFPB (CEPA 0105/10). **References**: Koehn, F.E. The evolving role of natural products in drug discovery.N.R.D.D, vol. 4, pp. 206. 2005. Gonzales Torrez, D.M.Catalogo de Plantas Medcinales (y Alimenticias y Utiles) Usadas en el Paraguay. E.P, Asuncio´n, pp. 312 and 452. 1992.

09.110 Venom of *Micrurus lemniscatus* (coral snake) affects survival of neuro-2a cell line, **primary cultured hippocampal neurons and dorsal root ganglia neurons**. Donato MF[1], Freitas ACN[2], Ferreira AF[2], Silveira N[1], Naves LA[1], Pimenta AMC[2], Chaves MM[2], Kuschmerick C[1], De Lima ME[2] [1]UFMG – Physiology and Biophysic, [2]UFMG – Biochemistry and Immunology

Introduction: Snakebite accidents in Brazil are considered a public health issue because their high frequency and severity (CISCOTTO et al. J Proteomics 74, 1810. 2011). Human envenomations by coral snakes (genus *Micrurus,* family Elapidae) despite being relatively rare, there are considered severe by their neurotoxic effects that may lead to death by respiratory paralysis (BRAZIL. Rev Inst Med Trop 29, 119. 1987). A variety of local and systemic manifestations has been described in patients bitten by different species of coral snake, although the main symptoms are neurological signs as anxiety, persistent pain, bilateral ptosis, facial paralysis, decreased visual acuity, dysarthria and dysphagia, paresthesia and hypoxic (MANOCK et al. Transactions of the Royal Society of Tropical Medicine and Hygiene 102, 1127. 2008). The neurotoxic peripheral effect attributed to *Micrurus* venom is the blockade of postsynaptic nicotinic acetylcholine receptors at motor endplates by α-neurotoxins peptides. However, few studies about those central neurotoxic effects have been explored in neuronal models. Therefore, the purpose of this study was to investigate the mechanisms of cell damage in primary cultured neurons and neuronal lineage caused by crude venom of *Micrurus lemniscatus*. **Methods:** Initially, Neuro-2A cell line was incubated with 200, 100, 50, 25, 10, 5, 1 µg of crude venom from *M. lemniscatus* snake diluted in phosphate buffer. Primary culture hippocampal neurons were prepared as described (Banker and Cowan, Brain Res 126, 397.1977). Hippocampi were removed from Wistar male and female rat neonatal (P0-P5). Dorsal root ganglia neurons (DRGs) were prepared as described (Moraes et al. Neurotox. Res. 19, 102. 2011). DRGs were removed from male adults Wistar rats (180-250g). Dissociated neurons were plated on poly-L-lysine and laminin coated glass coverslips 96-well plates ($2x10^4$ cells/well). Using 7- to 8-day-old-cultures (hippocampal neurons) and 2- to 3-day-old-cultures (DRGs neurons), cells were exposed to treatment with 0.5, 0.1, 0.01, 0.001, 0.0001, 0.00001 µg crude venom for 24 hours. The results of the group treated with 3 M KCl were defined as 100 % cell death. Cell survival was quantified by MTT assay as described previously (Mosmann. J Immunol **Methods**: , 65, 55. 1983). MTT solution (5 mg/mL) was added to each well of the plate. After 4 hours of incubation at 37 ^oC, 5% CO_2, DMSO solution was added. The absorbance value (Y) at 570 nm was read using a spectrophotometer and the percentage of cell death was calculated as follows: cell death (%)= ("Y" experiment well/"Y" of positive control well) x 100%. Data were analyzed by Mann-Whitney test. The experiments were approved by the institutional Ethics Committee on Animal Experimentation (CETEA/UFMG, Protocol Nº 169/2011). **Results and Discussion:** Preliminary studies performed on neuroblastoma cell line Neuro-2A show that doses higher than 1 µg/mL of crude venom generated 100% cell death, indicating a probable plateau cytotoxic effect. Thus, lower doses were used and the tests were conducted on primary cultures. The primary cultured cells were susceptible to the toxicity of the venom of *Micrurus* in a dose-dependent manner. In DRG neurons the response against the *Micrurus* venom was not conclusive. We conclude that the venom of *Micrurus* leads to death of different cells of nervous tissue. **Financial support:** CAPES, CNPq, INCTTOX, FAPEMIG, MCT-FINEP.

09.111 **A non-hemorrhagic**, **non-fibrinolytic cysteine-rich venom protein (CRVP) from *Bothrops jararaca* snake venom**. Silva IRF[1], Lorenzetti R[1], Rennó AL[1], Baldissera-Jr L[1], Zelanis A[2], Serrano SM[2], Hyslop S[1] – [1]Unicamp – Farmacologia, [2]CAT-CEPID-IBu – Toxinologia Aplicada

Introduction: Cysteine-rich secretory proteins (CRISPs) are widely distributed among vertebrates and have been implicated in a variety of biological activities. Proteins homologous to CRISPs, known as ***c**ysteine-**r**ich **v**enom **p**roteins* (CRVPs), have been identified in snake venoms. CRVPs can activate ion channels and relax smooth muscle. Although CRVPs have been identified in proteomic and transcriptomic analyses of *Bothrops* snake venoms and venom glands, no such protein has yet been isolated from *Bothrops* venoms. In this work, we characterized a CRVP from *Bothrops jararaca* venom. **Methods:** Venom was initially fractionated by gel filtration (Superdex 75) and the fraction with caseinolytic activity was chromatographed on Q-Sepharose and eluted with a linear gradient of NaCl (0-1.0 M) in 10 mM Tris-HCl, pH 8.0, plus 10 mM $CaCl_2$. The purity of the resulting peak was assessed by HPLC and SDS-PAGE; the latter was also used to determine the molecular mass of the protein. The purified protein was identified by mass spectrometric analysis of fragments generated by tryptic digestion. General proteolytic activity was assayed on casein and (pseudo)elastolytic activity was assayed using elastin-Congo red and n-boc-L-alanine *p*-nitrophenyl ester. Coagulant activity was assayed on rat plasma and fibrinogenolytic activity was assayed on bovine fibrinogen followed by electrophoretic analysis. Fibrinolytic activity was assayed on fibrin plates and was expressed as the diameter (cm) of the lytic halo. Hemorrhagic activity and changes in vascular permeability were assayed in rat dorsal skin (work approved by Committee for Ethics in Animal Use - CEUA/UNICAMP protocol no. 2253-1). **Results:** A combination of gel filtration, anion-exchange and HPLC yielded a protein with a molecular mass of 29.8±0.3 kDa. Mass spectrometric analysis of a tryptic digest identified the fragments KPEIQNEIVDL, HNSLR and SVNPTASNMLK that showed highest homology with ablomin, a CRVP from *Gloydius blomhoffii*. The purified protein was named CRVP-BJ. CRVP-BJ had no caseinolytic or fibrinolytic activity compared to the venom (18.8±0.6 U/mg and 6.3±0.0 cm halo, respectively; mean±SD. CRVP-BJ had low pseudoelastase activity towards n-boc-L-alanine *p*-nitrophenyl ester (7.1±0.9 U/mg vs. 131.5±27.7 U/mg for venom; n=3) but showed greater activity towards elastin-Congo red (162±52 U/mg vs. 414±34 U/mg for venom; n=3). CRVP-BJ (10 mg) showed a- and b-fibrinogenolytic activity that was inhibited by 10 mM EDTA and 5 mM phenanthroline but not by 5 mM PMSF. CRVP-BJ (40 mg) did not clot rat citrated plasma. CRVP-BJ (10 and 30 µg/site) increased vascular permeability (from 21.5±14.5 ml (basal) to 58.1±22.1 µl (10 µg/site) and 73.6±27.8 ml (30 µg/site) vs. 151.7±54.4 µl for venom; n=8 each) but was not hemorrhagic (30 mg) in rat dorsal skin compared to venom (10 mg) which produced a hemorrhagic halo of 1.2±0.2 cm (n=5). **Conclusion:** These results indicate that CRVP-BJ, the first CRVP isolated from *Bothrops* venom, is a non-hemorrhagic protein that may contribute to changes in blood coagulation and vascular permeability *in vivo*. **Financial support:** CAPES, CNPq, FAPESP.

09.112 Tetracycline inhibits hemorrhagic halo induced by *Bothrops erythromelas* venom in mice. Santos JVA[1], Jorge RJB[1], Alves NTQ[1], Nogueira LM[1], Abreu ML[2], Ximenes RM[1], Havt A[1], Monteiro HSA[1] [1]UFC – Fisiologia e Farmacologia, [2]UFC – Medicina

Introduction: *Bothrops erythromelas* (jararaca-da-seca) is widely distributed in north-eastern Brazil. Its venom presents a high level of hemorrhagic, coagulant and proteolytic activity (Furtado, Toxicon, v.29, p.219, 1991). These effects are rarely neutralized by the conventional serotherapy. Thus, researches for new venom inhibitors, which could complement the serotherapy, are necessary (Da Silva, J Ethnopharmacol, v.100, p.145, 2005). Studies have shown that tetracycline inhibits the hemorrhagic activity of *Bothrops asper* venom (Rucavado, Toxicon, v.52, p.754, 2008). However, inhibition of the hemorrhagic activity of *Bothrops erythromelas* venom with tetracycline hasn't been assessed. **Methods**: Groups of Swiss mice (18-20 g, n = 5) received intradermic injections, in the dorsal region, containing aliquots of 50 µL of: a) venom – 50 µg, PBS as diluent; b) PBS c) venom + tetracycline – 50 + 50 µg, 30 min of incubation at 37 ºC, PBS as diluent. Two hours after injection, mice were sacrificed, their skins were removed and the hemorrhagic area was determined using scanned photographs and the program ImageJ 1.37 (National Institutes of Health, Bethesda, MD). The results were submitted to analysis of variance (ANOVA), $p<0.05$. Ethic committee protocol approval: 6808. **Results**: The group that received only PBS solution did not exhibit hemorrhagic area. The treatment with tetracycline, in the pre-incubated group, completely abolished the formation of hemorrhagic area, in comparison to the group that received only venom (PBS: 0 mm^2; venom: 68.78 ± 6.69* mm^2; venom + tetracycline: 0 mm^2). **Discussion:** The inhibition of hemorrhagic activity by tetracycline may be due to its potential as a snake venom metalloproteinase inhibitor. Metalloproteinases are zinc-dependent enzymes involved in the hemorrhagic effects of snake venoms of the genus *Bothrops*. Tetracycline inhibits their action by a chelation mechanism, sequestering the zinc present at the catalytic site of these enzymes (Escalante, Arch. Biochem. Biophys, v.455, p.144, 2006). **Financial Support**: Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico

09.113 Preclinical evaluation of toxicity of the hydroethanolic extract of *Macrosophonia velame* (A. St.-Hil.) M. Arg. Ribeiro RV[1,2], Barbosa MA[3], Lima JCS[1], Martins DTO[1] [1]UFMT – Pharmacology, [2]UNIVAG – Health Sciences, [3]USP – Pharmacology

Introduction: *Macrosophonia velame* (A. St.-Hil.) M. Arg. (Apocynaceae), popularly known as "velamen", is a subshrub typical of Brazilian Cerrado. It is used in the treatments of several ailments by the rural population (De La Cruz, 2008). It is known to possess anti-inflammatory, antinociceptive and antipyretic activities. However little is known concerning the plant's toxicity (Ribeiro et al., 2010). Thus this work was aimed at evaluating acute and subchronic toxicity of the hydroethanolic extract of *M. velame* xylopodium (EH*Mv*). **Methods:** The xylopodia were dried, crushed and macerated for seven days in 75% hydroethanolic solution, concentrated in rotary evaporator and residual solvent was completely eliminated in an oven. In acute toxicity test, mice received by oral route EH*Mv* at doses of 500, 1000, 2000 and 5000 mg/kg, and were observed for 14 days (Malone, 1977). In order to determine the LD_{50}, EH*Mv* was administered orally (3000 to 6000 mg/kg) to mice, and numbers of dead animals were observed after 24 h. Subchronic toxicity was done by daily oral administration to rats of single dose of vehicle or EH*Mv* (50, 200 and 800 mg/kg v.o), and after 30 days the rats were sacrificed and the blood collected for hematological, biochemical, vital organs examination and histopathological analysis. **Results and Discussion:**, In acute toxicity test, EH*Mv* at higher doses caused discrete alterations of clinical signs that were reversed within 24 h of the evaluation. Acute administration of 5000 mg/kg of EH*Mv* caused death in 2/3 of the animals with LD_{50} being 4,176 ± 218.5 mg/kg p.o.. In subchronic toxicity, the clinical parameters altered were weight gain and water consumption at 800 mg/kg, in addition to urine volume at doses of 50, 200 and 800 mg/kg. Among the hematological and biochemical parameters measured, only the number of neutrophils, lymphocytes and creatinine were changed with 800 mg / kg. The acute toxicity and preclinical subchronic toxicity studies demonstrated that EH*Mv* have relatively low toxicity (Ribeiro et al., 2010). **Financial support:** CAPES/FAPEMAT **Selected references**: DE LA CRUZ, M.G. Plantas medicinais de Mato Grosso: a farmacopéia popular dos raizeiros. Cuiabá-MT: Carlini & Caniato, 2008. p. 127-128. Malone, M.H. Pharmacological approaches to natural products screening and evaluation. In: WAGNER, H.; WOLFF, P. (Eds.). New natural products and plant drugs with pharmacological, biological or therapeutical activity. Berlin: Spriger-Verlag, 1977. p. 23-53. Ribeiro R.V., Silva R.M., Lima J.C.S., Martins D.T.O. Anti-inflammatory, antinociceptive and antipyretic effects of hydroethanolic extract from Macrosiphonia velame (A. St.-Hil.) M. Arg. in animal models. Braz J Pharm Sciences. v.46. n.3. p. 515-520, 2010.

09.114 Study of the healing effect of cobrina extract (*Tabernaemontana catharinensis*) in skin injuries induced in rats. Alonso BS[1], Laureano JV[2], Souto PU[1], Freddo RJ[1] [1]Unipampa, [2]UFRGS

Introduction: Among the plants of great popular there is cobrina (*Tabernaemontana catharinensis*), which is a common plant vegetation in southern Brazil, Uruguay and Paraguay. According to reports in literature, cobrina has antitumor, antiinflammatory and analgesic activity being widely used in folk medicine as infusion for topical application to be an antidote to poisonous animal's bites. Although there were papers that report other activities for this plant, little is known about its potential healing effect. **Objective:** To investigate the effect of the dry extract of *Tabernaemontana catharinensis* incorporated in a gel base natrosol in the process of healing of skin inured rats induced by macroscopic and histologic evaluation. **Methods:** The sample of plant material consisted of fresh leaves and twigs that were subjected to soxhlet extraction apparatus, using dichloromethane as the organic solvent. The raw extract was concentrated by rotary evaporator, yielding 3.11%. Then, the extract was washed and dryied with ethanol p.a. at 40°C bath and added to a natrosol gel with a 5% final concentration of solids and the pH was setted in 5,73 ± 0,15. 21 male Wistar rats were used in this study and they were anesthetized with pentobarbital 30 mg/kg (to 3% Hypnol - Fontoveter$^{\hat{a}}$) with an i.p. injection and subjected to a process of deep scarification using a bristle brush metal in a delimited area of 4 cm^2 in the dorsal region of animals, previously exposed by depilation. The procedure was standardized and was repeated 40 times in the caudal direction and 40 times in the axial direction. After that, animals were randomly divided into 4 groups: Group I - (n = 3) receiving no treatment. Group II - negative control (n = 6) treated with gel. Group III - experimental group (n = 6) treated with gel + *Tabernaemontana catharinensis*. Group IV - positive control (n = 6) treated with an ointment containing neomycin sulfate + bacitracin zinc (Nebacetin®). The treatment consisted in applications of 1 g of the tested formulations to the groups II, III and IV, once daily for a 7 days period. At the end of treatment the scars were macroscopically evaluated and photographed to observe the healing process. After, it was proceeded the removal of the dermis for subsequent microscopic evaluation. The experiments were approved to Ethics on Animal Research Committee at UNIPAMPA under protocol # 022/2012. **Results:** The tissue damage inflicted on animals showed an accelerated wound healing and homogeneous tissue regeneration, and no formation of scars at the positive control and test groups. Also, the group treated with *Tabernaemontana catharinensis* showed a slightly less healing time. Regarding the negative control group, we observed the formation of persisted scars until the end of the experiment. **Discussion:** The gel containing dry extract of *Tabernaemontana catharinensis* showed healing potential suggesting that further studies will be needed in order to evaluate the histological samples of the dermis to determine how the tissue repairs itself. **References:** Soares, D.C., Pereira, C.G., Meireles, A. M., Saraiva, E. M. Leishmanicidal activity of the supercritical fluid fraction obtained from Tabernaemontana catharinensis. Parasitology International. Volume 56, issue 2, June 2007, Pages 135-139. Raphael, A. Topic administration of antibiotics in dennatologia, In: LACAZ, C. A. - Antibiotics. Sao Paulo, Editorial Procienx, 1965. Santos MFS, Czeczko NG PAN Nassif, Ribas-Filho JM, Alencar CBS, The Malafaia, Ribas CAPM, Trautwein VM, GS Henriques, Maia JMA, Bittencourt RCA. Evaluation of the crude extract of L. Jatrophagossypiifolia healing of skin wounds in rats. ActaCir Bras. [serial on the Internet] 2006, 21 Suppl 3:2-7.

09.115 Positive inotropic activity of a steroidal compound isolated from *Acnistus arborescens*, Withaphysalin F, in guinea pig atrial tissue. Amorim LS, Gomes VM, Santos IF, Freire MSS, Fonteles MC, Santos CF, Nascimento NRF ISCB-UECE

Patients with heart failure (HF) present impaired cardiac function, when the heart fails to pump, not being able to effectively supply other body tissues. A pharmacologically safe therapy has been still required for long term treatment of HF, which would enhance the survival and life quality of the patients. *Acnistus arborescens* belongs to the Solanaceae family, which has been known as a producer of steroidal compounds, such as withaphysalins. It has not been demonstrated any reports about the effects of these compounds in cardiac tissue. The present work was designed to characterize pharmacodynamically the positive inotropic effect of a mixture of steroidal withaphysalins (ME) and its isolated compounds. The animals (guinea pig) were anesthetized with urethane (800 mg / kg) and pentobarbital (40 mg / kg). After sacrifice in CO_2 chamber, both atria were removed and immersed in modified Krebs-Henseleit solution, at 37 ° C, pH 7.4, aerated with a carbogenic mixture (5% CO_2 and 95% O_2). Tissues were mounted in organ baths, attached to isometric transducers that were connected to a four channels polygraph. After 1 hour stabilization, a stroke control was performed. The parameters evaluated were: heart rate in the right atrium and maximum strength in the left atrium electrically stimulated (supramaximal, 1 ms and 0.1 Hz). For experiments in atria, the groups performed were: ME (n = 5); ME + propranolol (n = 5); witha F (n = 7); Deidrowitha F (n = 4) and Witha F + H89 (n = 4). In the ME + propranolol group were done only right atrium cumulative concentration-response curves. Cumulative concentration-response curves of both ME and its isolated compounds (Witha F and Deidrowitha F) (0.1 to 100 g / ml) were performed on either spontaneous right atria or electrically stimulated left atria preparations. The responses were measured 5 minutes after addition of each concentration and compared with the vehicle (1% DMSO in saline). The current study has been approved by the Ethics Committee No. 12235749-3. ME promoted a significantly positive inotropic effect in left atrium when compared to the control (419.6 ± 28.6 ME, * $p < 0.05$). These results were not observed in the ME isolated compounds group. Witha F showed predominant effect in right atrium (431.43 ± 151.62, ** $p < 0.01$). It was observed a decrease of the maximum effect on contraction force in concentration-response curves of ME by prior administration of propranolol (Propranolol ME + -94.09 ± 7.16, * $p < 0.05$). Neither the mixture, nor its compounds showed positive chronotropism in guinea pig right atrium, which indicates that there is an induction of a positive inotropic effect with no positive chronotropism. Left atrium incubation with H89, a protein kinase A inhibitor, did not affect the inotropic effect of ME or Witha F. The steroidal compounds evaluated, ME and Witha F, showed an increase of atrial tissue strength of guinea pig with no positive chronotropism. Propranolol inhibition of Witha F maximum effect indicates the involvement of adrenergic signaling on Witha F action mechanism. Further studies are needed to fully elucidate the Witha F signaling pathway in atrial tissue strength.

09.116 Anti-hyperglycemic properties of *Averrhoa carambola* L. leaves is related to insulinagogue effect in subchronically-treated hyperglycemic rats. Flister KFT[1], Abreu AC[2], Pinto BAS[2], Silva SN[2], Paes AMA[2], Borges ACR[2] [1]UFMA – Ciências Biologicas, [2]UFMA – Ciências Fisiológicas

Introduction: *Averrhoa carambola* L. leaves are popularly used for the treatment of type 2 diabetes mellitus – associated hyperglycemia in Brazil and abroad. Several reports have described its hypoglycemic effects in animal models of acute diabetes, whereas lack to subchronically characterize such properties. Thus, the present work aimed to assess the effects of repeated administration of the hydroalcoholic extract (HAE) of *A. carambola* leaves to hyperglycemic rats. **Methods:** Dried powdered leaves of A. carambola were macerated in 70% EtOH (1:3) for 72h to render a HAE, which was concentrated under vacuum and kept at 4°C for posterior use. During 30 days, prednisone-induced hyperglycemic rats were orally administered with HAE (250 or 500 mg/kg/day, n=8) while control rats received vehicle (water, 1ml/kg, n=8). Body weight and food intake were taken for ponderal evolution analysis. Periorbital blood samples were collected once a week and assayed for serum glucose levels. At the 31st day, overnight fasted rats were anesthetized and blood samples taken for determination of serum glucose and lipoproteins profiles. Insulin serum levels were also assessed by RIA. All the protocols involving animals were previously approved by Animal Health and Welfare Committee of Universidade Estadual do Maranhão under the judgment number 025/2008. Data were expressed as mean ± SEM and statistical analysis performed by one-way ANOVA for $p<0.05$. **Results:** Daily administration of prednisone resulted in progressive elevation of serum glucose associated to body weight loss even with no effect on food intake. When analyzed the area under the curve (AUC) of progressive serum glucose levels, it was found that both doses of HAE decreased the total area (250: 509±6 AU; 500: 466±12 AU) as compared to untreated hyperglycemic rats (557±9 AU). Noteworthy, AUC of untreated hyperglycemic rats was 43.2% higher than AUC of normoglycemic control rats (557±9 *vs* 389±7 AU). Total cholesterol (TC) and triglycerides (TG) serum levels in hyperglycemic rats were 32 and 25% higher than in normoglycemic animals. HAE (500 mg/kg) not only impaired TC elevation to 11% as increased c-HDL levels in 28% and decreased TG levels in 22% as well. Those effects were further assessed through determination of HOMA1-IR and HOMA1-%B indexes. HAE did not ameliorate insulin resistance of hyperglycemic rats, since there was no change in the HOMA1-IR index. However, hyperglycemic rats treated with 250 or 500 mg/kg of HAE had an increased of HOMA1-%B index in 278 and 515%, respectively. Remarkably, the treatment with HAE caused no hepatic toxicity (data not shown). **Discussion:** Taken together, our data reinforce the characterization of *A. carambola* as a hypoglycemic and/or anti-hyperglycemic herbal medicine. Moreover, our data consistently suggest that HAE harbors some single or complexed compound with expressive secretagogue effect on insulin secretion. **Financial Support:** FAPEMA, CAPES and UFMA

09.117 Antihypertensive effect of α-terpineol on L-Name-induced experimental hypertension in rats. Sabino CKB[1], Ferreira-Filho ES[1], Arcanjo DDR[1,2], Silva-Filho JC[1], Piauilino CA[1], Moura LHP[1], Amaral MPM[1], Oliveira RCM[1,2], Oliveira AP[1,3] [1]UFPI – Plantas Medicinais, [2]UFPI – Biofísica e Fisiologia, [3]UFPI

Introduction: The monoterpene α-terpineol is found in essential oils of several medicinal plants. Previously, α-terpineol showed antihypertensive and vasorelaxant effects in normotensive rats. Whereas animal models of hypertension share many features which are common to human hypertension, and thus, they are very useful for its understanding and treatment, this study aims to evaluate the antihypertensive effect induced by α-terpineol in L-NAME-induced hypertensive rats, a hypertension model which involves endothelial dysfunction, oxidative and inflammatory damages. **Methods:** All experimental protocols were approved by Federal University of Piauí Ethics Committee on Animal Experimentation (CEEA/UFPI nº 53/10). Male Wistar rats (250-300 g) were orally treated with L-NAME (50 mg/kg in drinking water) for 7 days to develop hypertension. Normotensive and hypertensive animals were treated with saline (control) or α-terpineol (100 mg/kg, p.o.) for 8 days. Then, the animals were anesthetized and blood was collected from abdominal aorta artery to the achievement of biochemical tests in serum (Glucose, AST, ALT, cholesterol, triglycerides, urea, creatinine, sodium and potassium). The organs (lung, heart, liver and kidneys) were removed and weighed. Oxidative damage was assessed by measure of GSH and catalase levels in liver homogenate. In another set of experiments, hypertensive rats underwent a process of catheterization of the femoral artery for measuring blood pressure (BP) and heart rate (HR). Then, α-terpineol was orally administered at 25, 50 or 100 mg/kg. Cardiovascular parameters were observed at 0, 10, 30, 60, 90, 120, 150, 180, 210 and 240 minutes. The results were expressed as mean ± SEM and considered significant when $p<0.05$. **Results and Discussion:** There was no significant difference between the organ weights of treated and control animals. Biochemical parameters in serum showed no significant difference compared with control group. Catalase levels increased in hypertensive groups treated with the monoterpene α-terpineol (0.68 ± 0.010***) and GSH levels increased in hypertensive groups treated with saline (0.76 ± 0.08*), respectively. α-terpineol showed significant antihypertensive activity in all tested doses after 180 minutes compared with control group (Control: -11.67 ± 3.5 mmHg; 25 mg/kg: -16.8 ± 6.9 mmHg; 50 mg/kg: -36.25 ± 8.9 mmHg; 100 mg/kg: -31.67 ± 8.7 mmHg; n = 6). **Conclusion:** The monoterpene α-terpineol elicits antihypertensive effect in L-NAME hypertensive rats. Antioxidant mechanisms may be useful in this response. **References:** Ribeiro TP. *Clin Exp Pharmacol Physiol*, 27:811,2010. Zatz R. *Hypertension*, 32:958,1998. **Keywords:** Antihypertensive, monoterpene, α-terpineol, antioxidant **Financial Support:** UFPI/ CAPES/FAPEPI/CNPq

09.118 Effects of solanidane steroidal alkaloids from *Solanum campaniforme* in hemorrhage and skin necrosis induced by *Bothrops pauloensis* venom. Jorge RJB[1], Ximenes RM[1], Alves NTQ[1], Santos JVA[1], Toyama MH[2], Torres MCM[1], Pessoa ODL[1], Evangelista JSAM[3], Monteiro HSA[1] [1]UFC – Fisiologia e Farmacologia, [2]UNESP, [3]UECE

Introduction: Snakebites are a public health problem in tropical regions in the world, an important cause of morbidity and mortality and included by the World Health Organization (WHO) list of diseases neglected (Harrison, PLoS Neglected Tropical Diseases, v.3, n.12, p. e569, 2009). *Bothrops* species venoms quickly develop severe local tissue damage, including swelling, hemorrhage, myonecrosis, skin ulceration and pain (Gutierrez, Toxicon, v. 54, p. 976, 2009). Natural compounds isolated from plants are a good choice to find new lead compounds to improve the snakebite treatment and minimize the sequelae of the victims (Cintra-Francischinelli, Phytotherapy Research, v. 22, n. 6, p. 784, 2008). **Methods:** This study evaluated the anti-hemorrhagic and anti-necrotizing effect of the new six solanidane steroidal alkaloids from dichloromethane fraction of *Solanum campaniforme* against hemorrhagic and necrotizing damage promoted by the venom of *Bothrops pauloensis* (vBp) in mice. Groups of mice (18-20 g, n = 10) received intradermic injections, in the dorsal region, containing aliquots of 50 µL of: a) venom: 50 µg, PBS as diluent; b) PBS c) venom + alkaloids: 50 + 50 µg (1:1 / w:w), 30 min of incubation at 37 ºC, PBS as diluent. Two hours and 72 hours after injection, mice were sacrificed, their skins were removed and the hemorrhagic and necrotic area (mm^2) respectively, was determined using scanned photographs and the programme ImageJ 1.37(National Institutes of Health, Bethesda, MD). The results were submitted to analysis of variance (ANOVA), $p<0,05$. Ethic committee protocol approval: 68/08. **Results:** The group that received saline solution did not exhibited hemorrhagic and necrotic area. Three of the six alkaloids studied had reduced hemorrhagic area (vBp: 91,74 + 12,43 mm², Alkaloid 1*: 57,26 + 5,30 mm²; Alkaloid 2*: 52,50 + 2,04 mm², and Alkaloid 6*: 53,64 + 9,45 mm²) and the alkaloids 2, 3, 4 and 6 (vBp: 23,14 + 3,74 mm², Alkaloid 2*: 10,14 + 1,21 mm², Alkaloid 3*: 9,73 + 1,72 mm², Alkaloid 4*: 9,21 + 2,56 mm² and Alkaloid 6*: 13,18 + 2,44 mm²) had reduced necrotic area **Discussion:** The inhibition of hemorrhagic and necrotizing activity by alkaloids may be due to its potential as a snake venom metalloproteinase inhibitor. Metalloproteinases are zinc-dependent enzymes involved in the hemorrhagic and necrotizing effects of snake venoms of the genus *Bothrops* (Baldo, PLoS Neglected Tropical Diseases, v.4, n.6, p. e727, 2010). These results pointing to a possible direct inhibition of the metalloproteases enzymatic activity or an indirect inhibition due to metallic ions chelation. **Financial Support:** CNPq, CAPES and FUNCAP.

09.119 Structure-activity relationship of the vasodilator activity of lignans in mouse aorta. Maciel LIS[1], Lemos VS[2], Barbosa Filho JM[3], Cortes SF[1] [1]UFMG – Farmacologia, [2]UFMG – Fisiologia, [3]UFPB – Tecnologia Farmacêutica

Introduction: Lignans are natural products which consist of two monomers phenylpropane connected by two carbon-carbon bonds or carbon-oxygen bonds. Previous studies have demonstrated several pharmacological properties of these compounds including cardiovascular effects (Abe et al., Gen. Pharmacol. v. 22, p. 663, 1991; Valsaraj et al., J. Nat. Prod. v. 60, p. 779, 1997; Charlton, J. Nat. Prod. v. 61, p. 1447, 1998; Lopes et al., Planta Med. v. 64, p. 667, 1998, Lee et al., Arch. Pharm. Res. v. 27, p. 1043, 2004; León-Díaz et al., Mem. Inst. Oswaldo Cruz. v. 105, p. 45, 2010). The vasodilatory effect of lignans, their structure-activity relationship and their mechanism of action were investigated in mice aorta. **Methods**: Mice aorta was kept in an organ bath system with Krebs solution (37°C, pH 7.4, 95% O2, 5% CO2). Cumulative concentration-response curves of lignans: grandsin, licarin A and yangambin were obtained to evaluate the effect and mechanism of action of these compounds on vascular reactivity. The experimental protocols were approved by the Ethics Committee for Animal Experimentation - UFMG (protocol 168/2012). **Results**: Grandsin, licarin A and yangambin induced a vasodilator effect, concentration-dependent, in aorta with functional endothelium (pIC50 = 4.2 ± 0.5, > 4.0 and 3.6 ± 0.6, respectively). In the absence of a functional endothelium the vasodilator effect of grandisin did not change. However, the vasodilator effect of licarin A and yangambin were significantly reduced. Preincubation of vessels with L-NAME (300 μM), a non-selective inhibitor of nitric oxide synthase (NOS), significantly reduced the vasodilation induced by licarin A and yangambin, while the vasodilatation induced by grandsin was not changed. The precontraction of vessels with KCl (80 mM) did not affect vascular relaxation induced by grandsin and yangambin. In the absence of extracellular calcium, preincubation with grandsin (100 μM) and yangambin (100 μM) reduced the contraction induced by phenylephrine (0.3 μM). Furthermore, in the absence of extracellular calcium, preincubation with grandsin (100 μM) and yangambin (100 μM) strongly reduced contraction induced by caffeine (10 mM). **Conclusion**s: The relaxation induced by grandsin is independent of vascular endothelium and involves inhibition of the mobilization of calcium stores. The vasodilatation induced by licarin A and yangambin were depend on the endothelium. In the case of licarin A, vascular relaxation depends completely on the activation of NOS, while yangambin also involves the inhibition of the mobilization of calcium stores. **Financial support**: FAPEMIG and CAPES.

09.120 Beta-escin alleviates UV-induced oxidative skin damage in Swiss mice. Segat HJ, Barcelos RCS, Benvegnú DM, Trevizol F, Dias VT, Roversi K, Dolci GS, Bürger ME UFSM – Fisiologia e Farmacologia

Ultraviolet radiation (UVR) generates reactive oxygen species (ROS), whose accumulation is related to oxidative stress (OS) development, which can cause damages to lipids and proteins thus contributing to decrease of cell membrane integrity and consequently cell death. Considering that UV induces OS-mediated adverse effects on skin, topical application of antioxidants is suggested as a useful way to reduce the harmful effects of UVR (Steenvoorden and Henegouwen, 1997). Chemoprevention, defined as ‘‘use of agents capable of ameliorating UVR-induced adverse effects on the skin’’ by natural compounds, represents a new concept in the attempt to control the carcinogenesis process (Zhao et al., 1999). Among many photochemoprotective agents, botanical origin antioxidants are promising. β-escin is a antioxidant natural compound present in *Aesculus hippocastanum* (Sirtori, 2001), and therefore its protective effects on the UVR-induced oxidative damages were evaluated. Experimental protocol was approved by the Animal Ethical Committee (UFSM-40/2010), which is affiliated to the Council for Control of Animal Experiments (CONCEA). Male Swiss mice (28) were randomly designated into 4 groups (n=7): C-UV (non-irradiated and treated with control-emulsion); E-UV (non-irradiated and treated with control β-escin emulsion); C+UV (irradiated and treated with control emulsion); E+UV (irradiated and treated with β-escin emulsion). Topical treatment was performed with β-escin 1% emulsion or control emulsion was applied every day and immediately after the irradiation sessions on the mice shaved dorsal skin. UVR dose (2.72mJ/cm^2) was administered twice other day, yielding a total dose of 5.44 mJ/cm^2. Twenty four hours after the last UV exposure, mice were sacrificed. Dorsal skin was removed for TBARS (Ohkawa et al. 1979), protein carbonyl (Levine et al., 1990) and cell viability (Mosmann, 1983) assays. UVR exposure increased lipid peroxidation (763,58 mMolMDA/gtissue) and protein oxidation levels (4009,620 nmol protein carbonyls/tissue), and decreased survival (64,28%) cell of C+UV group. Topic β-escin (E+UV group) prevented the protein oxidation (2456,18 nmol protein carbonyls/tissue) and cellular viability (86,00%) loss of the dorsal skin UV-induced. Different studies have shown that OS is able to exert an important role on the UVR-induced skin damage, reinforcing the importance of to treat or prevent this. In this study, UVR exposure was able to increase oxidative damages in skin, which were observed by higher levels of lipoperoxidation and protein carbonyls, as well as the lower cell viability, while topic application of β-escin was able to reverse such effects. We infer that antioxidant property of β-escin are related to its chelating effects, resulting in photoprotective effects. **Financial support:** PROAP/PPG-Farmacologia PRPGP-UFSM. R.C.S.B. and M.E.B are grateful to CAPES and CNPq by their fellowships, respectively. **References:** Afaq, F.; Mukhtar, H. Exp. Dermatol. 2006, 15, 678–684. Levine RL, Garland D, Oliver CN, Amici A, Climent I, Lenz AG. Meth Enzymol 1990;86:464–78 Mosmann, T. J Immunol Meth, 65 (1983), pp. 55–63 Ohkawa, H., Ohishi, H., Yagi, K., 1979. Anal. Biochem. 95, 351–357. Sirtori, CR. Pharmacological Research, Vol. 44, No. 3, 2001. D.P. Steenvoorden, G.M. Henegouwen. J. Photochem. Photobiol. B 41 (1997) 1–10. J. Zhao, J. Wang, Y. Chen, R. Agarwal. Carcinogenesis 20 (9) (1999) 1737–1745.

09.121 Influence of hemoglobin content on antioxidant activity of superoxide dismutase and catalase in chicks intoxicated by aflatoxin B1. Trombetta F.[1], Poersch A.[1], Braga A.C.M[1], Dilkin P.[2], Perlin V. J.[3], Marchioro A.[2], Boeira S.P.[1], Oliveira S.M.[2], Mallmann A.C.[2], Furian A.F.[1] [1]Labneuro-UFSM – Fisiologia e Farmacologia, [2]UFSM – Medicina Veterinária Preventiva, LAMIC, [3]SAMITEC
Introduction: Aflatoxin B1 (AFB1), a mycotoxin produced by species of *Aspergillus flavus, A. parasiticus* and *A. nomius*, found in cereals, mainly in corn and peanut (Sirajudeen, et al 2009). This toxin causes cytotoxic effects including hepatic damage, oxidative stress and induces a discoloration of this organ, due to fat accumulation. In this sense, antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) have an important role in the defense against oxidative stress, and the hemoglobin (Hb) content is linked to the enzymatic activity of CAT (Furian, et al. 2007). So, the aim of this study is to check whether the intoxication of chicks by AFB1 alters liver Hb content, and, whether Hb content alters the activity of antioxidant enzymes SOD and CAT. **Methods**: 40 broiler chicks cobb, Males, I day of age, were used. Aflatoxin B1 (2.8 ppm) and additive anti-mycotoxin (aluminosilicate, 0.5kg/T) were added to the diet. Chicks were randomly divided into four experimental groups: diet control, diet with additive, diet with AFB1 and diet with AFB1 and additive. After 21 days, the chicks were euthanized using CO_2 for stunning, followed by cervical vessels section, and liver was weighed, homogenized in Tris/HCl 50 mM (1:10, pH 7.4) and centrifuged for later analysis. Determination of SOD and CAT activity was according to methods described in Boeira et al., (2012), and the Hb content according to Henry (1991). Data were analyzed by two-way analysis of variance (ANOVA), followed by DUNCAN multicomparison test when appropriate. This project does not require the opinion of the ethics committee because it is developed outside the University; and the liver used in the experiments was obtained through donations. **Results:** Treatment with AFB1 reduced the liver Hb content, and the presence of additive anti-mycotoxin reverted partially this decrease. SOD activity was not altered significantly by treatments, or by the Hb content. However, AFB1 decreased CAT activity when the activity was corrected by protein content, and increased CAT activity when it is corrected by Hb content. **Discussion:** Our data suggest that AFB1 reduces liver Hb content, and that Hb content alters directly catalase activity. This fact could be explained due to high perfusion of liver, to the presence of eritrhocythes, and consequently eritrhocythe catalase. In addition, the presence of additive anti-mycotoxin has a beneficial effect on Hb content and CAT activity. These results indicates the necessity of correction of catalase activity by Hb content for drugs that change Hb content, like AFB1. **Key-words:** liver, hemoglobin, mycotoxin, catalase, superoxide dismutase, aflatoxin **Sources of research support:** CNPq, FAPERGS, PRPGP/UFSM. **References:** Boeira, S.P. Toxicon, v.60, p.358, 2012 Furian,A. F. Free Radical Biol. Med., V.43, p.924, 2007 Henry, J. *Clinical Diagnosis and Management by Laboratory Methods.* Philadelphia: W.B. Saunders Co.; p. 553, 1991 Sirajudeen, M. Environ Toxicol, v.26, p.153; 2009