09. Natural Products and Toxinology

09.001

Neutralization of Bothrops snake venoms from Ecuador by antibothropic antivenom manufactured by Instituto Vital Brazil. Strauch MA[1], da Cunha LER[1], Brazil LM[1], Castanheira PNS[1], Castanheira PNS[1], Meirelles LGR[1], Tavares MSH[2], Machado MM[2], Melo PA[2] [1]IVB, [2]UFRJ – Farmacologia das Toxinas

Introduction: Central and South America snakebite are inflicted by species of the family Viperidae, and most of these are of the genus *Bothrops*. These genus snakebites are characterized by edema, hemorrhage and myonecrosis. In this continent some countries, such as Argentine, Brazil and Costa Rica, have capacity to produce many immunobiologicals and a range of products which ensures national and regional support for these envenomations. However, some countries have none antivenom-producing laboratories, and in others, such as Ecuador, the antivenom production is only able to partially fulfill the local demand. Additionally to the locally manufactured products, antivenoms can be imported from Brazil, Mexico and Costa Rica, and have been used in emergency situations. **Methods:** We have investigated the ability of the antibothropic antivenom (SAB) produced by Instituto Vital Brazil (IVB), which are made by horse immunized against *B. jararaca. B. jararacussu*, *B. alternatus*, *B. neuwiedi*, and *B. moojeni*, in paraspecificity cross-neutralization against neutralize the venom species not included in the immunization scheme, *B. atrox* and *B. asper* snake venoms from Ecuador. We performed *in vivo* experiments in Male Swiss mice (30 g) evaluation the myotoxic, edema and hemorrhage effects. We evaluated the myotoxicity of *Bothrops atrox* and *Bothrops asper* venoms by measuring the increase of plasma CK activity induced by intramuscular (i.m.) injection of venoms alone or associated with SAB (12 μL). The hemorrhagic effect was induced by an intradermic (i.d.) injection of 0.1mL of *Bothrops atrox* and *Bothrops asper* venoms (1 mg/kg) alone or associated with SAB (12 μL) in the abdomen of mice and quantified as previously described (Melo *et al.*,1994). The induction of edema was evaluated by an intramuscular injection of 0,1 mL *Bothrops atrox* and *Bothrops asper* venoms (1 mg/kg) alone or associated with SAB (12 μL), the thigh area was measured using a caliper rule (Strauch *et al.*, 2013). **Results and Discussion:** The IVB SAB was able to neutralize 100 % miotoxic activity, 90% hemorrhage activity and 80 % edema activity this venom effects. The Antibothropic antivenom produced in Instituto Vital Brazil was able to confer cross-neutralization of Ecuador *Bothrops atrox* and *Bothrops asper* venoms and could be useful to attend the need of other countries in South America. CEUA/UFRJ: DFBCICB 026. **Financial support**: FINEP, PRONEX, Faperj, CNPq e Capes.

09.002

Silvercatfish central nervous system depression by the essential oil of *Curitiba prismatica*. Garlet QI[1], Silva LL[1], Amaral L[2], Schindler B[2], Watzlawick LF, Longhi SJ[2], Baldisserotto B[1], Heinzmann BM[3] [1]UFSM – Farmacologia, [2]UFSM – Engenharia Florestal, [3]UFSM – Farmácia Industrial

Introduction: Essential oils (EOs) are complex mixtures of secondary metabolites produced by aromatic plants. These volatile extractives are known by its numerous biological activities (ZALACHORAS I., Planta Med. 76: 1647, 2010.). *Curitiba prismatica* is endemic from Southern of Brazil. This species belong to Myrtaceae family and there are not studies involving central nervous system activities for its essential oil (LANDRUM R.L., *Brittonia* 49, 508-536, 1997). Therefore, this study aimed to evaluate the sedative and anesthetic potential its EO in silver catfish (*Rhamdia quelen*). **Methods:** EO of the leaves had been extracted by hydrodistillation process in Clevenger type apparatus for 3h (EUROPEAN PHARMACOPOEIA, 2007). The anesthetic evaluation was performed on silver catfish juveniles (7.53 ± 0.43 g; 9.6 ± 0.26 cm; n=5) and each animal was used only once. These procedures were approved by the Ethical and Animal Welfare Committee of the UFSM (Process no. 46/2010). The concentrations evaluated were 87, 500 or 1000 mg L^{-1} of the EO diluted in ethanol (95%; 1:10) and An ethanol control (9 $mL.L^{-1}$) was performed. The time of anesthesia induction and recovery was observed, following GOMES D.P., Aquac Res. 4, 878-886, 2011. The dissolved oxygen levels, temperature, pH and total ammonia levels were tracked (VERDOUW, H. Water Res. 12: 399, 1978). **Results:** Sedative properties were observed at all concentrations of the EO. An anesthetic effect was noticed at 15 min at concentration of 1000 mg L^{-1} in 40% of the animals. Recovery in anesthetic-free aquaria for the concentrations of 87 and 500 mg L^{-1} occurred about 2 and 14 min, respectively and at 1000 mg L^{-1} was longer than 30 min. The dissolved oxygen levels measured was 7.31 ± 0.22 mg L^{-1}, temperature: 18.53 ± 0.14 ºC, pH: 6.33 ± 0.07 and total ammonia levels: 0.12 ± 0.10 mg L^{-1}. **Discussion:** The use of the EO as an anesthetic is not recommended due to the occurrence of side effects. The high recovery time may be associated with the high lipophilicity of the constituents of the EO (FEMENÍA-FONTA A., *Eur J Pharm Biopharm* 61: 50, 2005.). These values are higher than described for fish anesthetics such as MS-222 and isoeugenol (ZAHL I.H., Fish *Physiol Biochem* 38:201, 2012). However, its sedative effect could be explored extensively in aquaculture without anesthetizing the fish. Furthermore, other studies should be performed with this EO, aiming to elucidate the chemical composition and evaluate other potential biological activities. **Financial Support:** FAPERGS/PRONEX, FINEP, INCT ADAPTA, Capes, FIT/UFSM, PIBITI/CNPq.

09.003

***In vitro* trypanocidal activity of betulinic acid and semi-synthetic derivatives**. Bocalon LG, Tozatti MG, Marçal MG, Ferreira DS, Esperandim VR, Januário AH, Pauletti PM, Silva MLA, Cunha WR Unifran – Ciências Exatas e Tecnológicas

Introduction: Tropical neglected diseases infect people of several countries, especially in low-income ones. There are some treatments for these diseases, but many of them are shown ineffective or uneconomical for the people affected. A large amount of searches for new drugs from natural products have shown triterpenes as potential agent against *Trypanosoma cruzi*, the etiologic agent of Chagas' disease. Recently, our group reported the significant *in vitro* schistosomicidal activity of betulinic acid, a triterpene isolated from *Davilla elliptica*[1].The aim of this work was to evaluate the *in vitro* trypanocidal activity of the betulinic acid, and three semi-synthetic derivatives. **Methods:** Betulinic acid (**1**) was isolated from the ethyl acetate extract of aerial parts of *Davilla elliptica*. The acetyl ester derivative was prepared with an excess of acetic anhydride to give the C-3 acetoxyl derivative of betulinic acid (**1a**). Betulinic acid was treated with CH_2N_2 in Et_2O yielding the C-28 methyl ester derivative (**1b**). The potassium salt derivative of betulinic acid (**1c**) was obtained with 2% KOH in Me_2CO-H_2O. The trypomastigotes forms were added at a concentration of 1×10^6 cells/mL in a 96-well microplate containing RPMI 1640 medium. Thereafter, the trypomastigotes were treated with the compounds at different concentrations (400, 200, 100, 50, 25, 12.5, and 6.25 µM). The plates were incubated at 37°C for 24 h, and the biological activity was evaluated by using the MTT colorimetric method[2] in a microplate reader at 517 nm. RPMI 1640 medium plus DMSO and Benznidazole were used as negative and positive controls, respectively. The assay was performed in triplicate. **Results and discussion:** The results showed that all the derivatives of the betunilic acid were found to potentiate the trypanocidal activity (Table 1). The better result was obtained for the acetyl derivative of betulinic acid (**1a**) with an IC_{50} value of 15.7 µM.The acetyl ester derivative of betulic acid is potentially applicable as a new lead compound for the management of trypanosomiasis.

Table 1. *in vitro* trypanocidal activity of betulinic acid and semi-synthetic derivatives against Y strain of *Trypanosoma cruzi*.

% lysis ± S.D./concentration (µM)								
Samples	**400**	**200**	**100**	**50**	**25**	**12,5**	**6,25**	**IC_{50} (µM)**
1	52.0 ± 1.9		31.7 ± 4.3	15.6 ± 3.0	0		0	
1a	79.0 ± 1.2	46.0 ± 8.0	78.8 ± 5.5	81.7 ± 5.2	69.3 ± 7,1	0	0	285.4
1b	83.4 ± 0.3	88.1 ± 6.7	71.0 ± 4.7	51.9 ± 5.6	34.5 ± 6,5	62.1 ± 2.8	1.0 ± 1.7	15.7
1c	83.0 ± 5.9	81.7 ± 5.9	44.5 ± 9.0	29.1 ± 1.2	24.6 ± 2.8	9.7 ± 4,3	1.2 ± 2.1	51.2
		79.5 ± 2.4				8.4 ± 4.0		93.8

Positive control: Benznidazole (IC_{50} = 29.4 µM)

References: 1.. Bocalon L.G *et al.*, 36ª Reunião Anual da Sociedade Brasileira de Química, BIO-10, 2013. 2. Muelas-Serrano S., *et al.* Setting of a colorimetric method to determine the viability of *Trypanosoma cruzi* epimastigotes. *Parasitol. Res.* 86, 999, 2000. ***Acknowledgements***: *Fapesp* (*#grants12/16182-8*, 11/14199-8, 13/14275-1), *Capes*, *and CNPq.*

09.004

Possible participation of prostaglandins in gastroprotection of ethyl acetate fraction of *Neoglaziovia variegata* (Arruda) Mez. in rats and mice. Lopes KL[1], Machado FDF[1], Oliveira IS[1], Silva-Freitas FV[1], Lima GS[1], Oliveira FA[1], Almeida JRGS[2], Oliveira RCM[1]
[1]NPPM-UFPI, [2]NEPLAME-UNIVASF

Introduction: *Neoglaziovia variegata* (Arruda) Mez. (Bromeliaceae), popularly known as "caroá", is distributed in the caatinga region of the northeastern hinterland. Previous studies with species of the genus *Neoglaziovia* showed the presence of various chemical constituents with antinociceptive, anti-inflammatory and anti-ulcer activities. The best dose observed in earlier studies was 100 mg/kg so obviously the dose was used to evaluate the gastroprotective activity of the ethyl acetate fraction of Neoglaziovia Mez variegata (Nv-EtOAc), using models of gastric lesions induced by absolute ethanol pretreatment with ibuprofen in mice and quantification of mucus in the gastric wall after pylorus ligation in rats. **Methods:** In gastric lesions induced by absolute ethanol, Swiss mice (25-30 g), males and females, fasting of solids for 18 hours, were pretreated with ibuprofen (200 mg/kg, p.o.) 1 h before administration oral vehicle, Nv-AcOEt (100 mg/kg) or misoprostol (50 µg/kg). After 30 min of ethanol administration, the animals were euthanized with an anesthetic overdose and the stomachs were removed and analyzed by planimetry (mm^2). To quantify the production of gastric mucus Wistar rats (250-300 g), male and female, in solid fasting for 18 hours, were subjected to pylorus ligation and treated (i.d.) with vehicle, Nv-AcOEt (100 mg/kg) or carbenoxolone (100 mg/kg) and 4 hours after their stomachs were removed and weighed. Each segment was immediately transferred to Alcian Blue 0.1 % (in sucrose 0.25 M, buffered with sodium acetate 0.05 M, pH 5.8). The free dye was removed by rinsing in sucrose solution 0.25 M. The gastric mucus-bound dye was extracted with 0.5 % magnesium chloride. A 4-mL sample of the blue extract was then vigorously shaken with an equal volume of diethyl ether. The resulting emulsion was centrifuged and the absorbance was recorded at 598 nm. The quantity of Alcian Blue extracted per gram of glandular tissue was calculated. All experimental protocols were approved by the Ethics Committee on Animal Experimentation of the Federal University of Piauí (CEEA/UFPI 008/12). The level of significance was evaluated for values of *$p<0.05$. **Results and discussion:** The results of this study show that ibuprofen, a blocker of cyclooxygenase enzyme, reversed the gastric mucosa protection by Nv-AcOEt (5.92 ± 1.41 to 20.40 ± 3.23*) and misoprostol 50 µg/kg (5.12 ± 0.38 to 15.60 ± 1.81*) suggesting an involvement of prostaglandins in gastroprotective effect produced by this extract. Nv-AcOEt and carbenoxolone produced a significant increase in the amount of mucus in the gastric wall (327.89 ± 23.66* and 306.65 ± 29.28* respectively) in pylorus ligation induced injury model as compared to control group (161.39 ± 4.24). It can be suggested that the increase in mucus production is involved in the gastroprotective effect exerted by Nv-AcOEt, possibly by increasing the production of endogenous prostaglandins. **Financial support:** UFPI/Capes/FACEPI/CNPq

09.005

Effects of *Tityus serrulatus* scorpion venom on endothelial cells. Rigoni VLS[1,2], Vieira RP[3], Silva JLV[4], Zamuner SF[4], Kwasniewski FH[5], Zamuner SR[1] [1]Uninove – Medicina, [2]Unifesp – Biofísica, [3]Uninove – Ciências da Reabilitação, [4]Uninove – Saúde, [5]UEL – Ciências Patológicas

Introduction: The scorpion envenomation is considered a public health problem worldwide. *Tityus serrulatus* is the specie that causes the main accidents in Brazil. Envenomation by *Tityus serrulatus* range from local pain to severe systemic reactions such as cardiac dysfunction and pulmonary edema. The aim of this study was to analyze the effect of *T. serrulatus* scorpion venom (TsV) on endothelial cells. **Methods:** The murine thimic endothelial cell line (tEnd) was used. The cells were grown in culture medium DMEM supplemented with 10% fetal bovine serum, incubated at 37˚C with 5% CO2 for 24 hours for cell attachment; after that, the cells received the TsV venom in concentrations of 10 and 50 µg/mL and were incubated for 1, 3, 6 and 24 h. Cell viability were analyzed by MTT and cell permeabilization by LDH release assay results were subjected to t-Test statistical analysis. **Results and discussion:** Cells treated with TsV at a concentration of 50 µg/mL caused the release of LDH (0.7 ± 0.01 mmol NADH/min) significant ($P<0.05$) greater that baseline control (0.5 ± 0.06 mmol NADH/min) at 6 hs. At 24 hs after incubation of TsV, both concentration of 10 and 50 µg/mL of TsV caused an increase of LDH release (1.1 ± 0.07 and 1.0 ± 0.1 mmol NADH/min, respectively) compared to control (0.7 ± 0.01 mmol NADH/min). No significant changes in cell viability were observed at any time or venom concentration when the MTT assay was used. It is concluded that TsV is cytotoxic for endothelial cells and this cell may play a role in the envenomation caused by TsV. **Financial support**: CNPq

09.006

Neurotoxicity of acanthoscurria juruenicola spider venom in vertebrate neuromuscular preparations *in vitro.* de Moraes DS[1], Sutti R[2], Silva PI[3], Bertani R[4], Hyslop S[1], Rodrigues-Simioni L[1], Rocha-e-Silva TA[2] [1]Unicamp – Farmacologia, [2]FCMSCSP – Ciências Fisiológicas, [3]IBu – Toxinologia aplicada, [4]IBu – Ecologia e Evolução

Acanthoscurria juruenicola (Araneae, Theraphosidae) is a large tarantula from Amazonia which venom has not being yet investigated. In this work we examined neuromuscular action of its venom using mouse phrenic nerve-diaphragm preparation (PND). Under indirect stimulations, the venom caused a progressive blockade (30 µg/ml, 79.0 ± 6.0%, 120 min) and promoted a complete neuromuscular paralysis (100 µg/ml, 13 ± 4 min). The same protocol was tested in low Ca^{2+}, and an increase of the preparation sensibility was observed reaching total blockade under lower concentration (3.0 µg/ml, 30 ± 7.5 min). Despite, with the smallest tested concentration (0.3 µg/ml) it was observed an increase in twitch height (173.5 ± 6.5%, 120 min). Under direct stimulation, muscle twitches in curarized preparations were equally affected by highest venom concentrations (88 ± 1% for 30 µg/ml and 88.5 ± 1.5% for 100µg/ml, 120min), but without total blockade. When exposed to low Ca^{2+} also under direct stimulation the results showed a dose dependent partial blockade (16 ± 5% for 0.3 µg/ml and 24 ± 6% for 3.0 µg/ml, 120 min), without former facilitation seen under indirect stimulation. Furthermore, it was observed some decrease of miniature end-plate potential (MEPPs) frequency with 30 µg/ml of the venom (7 ± 2 for vs. 17 ± 3 for control, 60 min), and the muscle membrane depolarization (-58 ± 3 mV vs. -74 ± 2 mV for control, 120 min). These results suggest that A. juruenicola venom has neuromuscular active venom that affects vertebrate neurotransmission *in vitro* mainly by a neurotoxic effect. Animal Ethics Committees: 2214-1 **Financial support**: Capes

09.007

Effect of ethanolic extract from leaves of *Casearia sylvestris* SW. and its fraction rich in diterpenes on carrageenan-induced pleurisy in rats. Castro RC[1], Carmo EGB[2], Bosquesi CL[3], Pierri EG[1], Santos AG[1] [1]FCF-Unesp-Araraquara – Princípios Ativos Naturais e Toxicologia, [2]UNIRP, [3]UNIFEV

Introduction: *C. sylvestris* Sw. (Salicaceae), guaçatonga, is a species with high occurrence in Brazil. The essential oil (Esteves *et al.*, *J Ethnopharmacol* 101, 191, 2005), the aqueous extract (Ruppelt *et al.*, *Mem Inst Oswaldo Cruz*, 86, 203, 1991) and the hydroalcoholic extract (Albano *et al.*, *J Ethnopharmacol* 147, 612, 2013) from leaves showed inhibitory effect in models of inflammation. However, these studies did not investigate the possible compounds involved. Also, did not use the ethanol extract from leaves (EEtCs) who demonstrated antiulcerogenic effect in rats (Sertié *et al.*, *Pharm Biol*, 38, 112, 2000) and presented predominance of clerodane diterpenes (Ferreira *et al.*, *An Acad Bras Cienc*, 83, 1373, 2011). In this way, the relevance and the objective of this study was to evaluate the anti-inflammatory activity of EEtCs and its rich fraction in clerodane diterpenes on pleurisy model in rats. **Methods:** The EEtCs was produced by maceration with ethanol from dried and powdered leaves of *C. sylvestris*. Dry EEtCs was subjected to fractionation by solid phase extraction (SPE) employing glass column containing silica gel and activated charcoal powder. Three fractions were obtained using the following eluents: 1) hexane/ethyl acetate 95:05 (v/v) (SPECs1); 2) ethyl acetate (SPECs2); 3) methanol (SPECs3). The clerodane diterpenes were identified and quantified in the EEtCs and SPECs2 via HPLC-DAD. The EEtCs and SPECs2 were evaluated on pleurisy model according to the protocol approved by Ethics Committee in Animal Use of the University Center of Rio Preto (CEUA UNIRP 16/2013). Initially the animals received orally the EEtCs (100, 300 and 500 mg/kg), SPECs2 (30 mg/kg), dexamethasone (0.5 mg/kg) or vehicle (control). An hour later, the rats were anesthetized and the carrageenan injected into the pleural cavity (400 μg/pleural cavity). After 4 h, the animals were sacrificed and the pleural cavity rinsed with 2 ml of phosphate buffered saline. Aliquots of fluid were used for counts of total leukocytes (TL), mononuclear cells (MN) and polymorphonuclear cells (PMN) present in the pleural cavity. Statistical differences were considered for $p \leq 0.05$ (ANOVA; Tukey's test; n=6). **Results:** The total concentration of clerodane diterpenes in EEtCs and SPECs2 was 27 and 51%, respectively. Comparing the control to the groups treated with EEtCs and the SPECs2, the dose of 100 mg/kg of EEtCs showed no significant differences, but the doses of 300 and 500 mg/kg of EEtCs and the SPECs2 significantly reduced the number of LT (36, 42 and 47%, respectively) and PMN (41, 49 and 48%, respectively). The group treated with dexamethasone (positive control) significantly reduced the number of LT, PMN and MN (62, 51 and 65%, respectively). **Discussion and Conclusion:** The results demonstrate that EEtCs exhibit anti-inflammatory activity and suggest that this activity is related to the presence of clerodane diterpenes. Thus, it is possible that these diterpenes are involved with both anti-inflammatory and antiulcer activities of this plant, which makes these potential alternative substances for the treatment of inflammatory diseases since one of the main side effects of anti-inflammatory non-steroidal drugs is the appearance of peptic ulcers. **Financial Agency:** CNPq

09.008

Red propolis attenuates ischemic-reperfusion acute kidney injury. Costa MFB[1], Meneses GC[1], Lima DB[1], Libório AB[2], Martins AMC[1] [1]UFC – Fisiologia e Farmacologia, [2]UFC – Medicina Clínica

Introduction: Acute kidney injury (AKI) remains a great problem in clinical practice. Renal ischemia/reperfusion (I/R) injury is a complex pathophysiological process. Propolis is a natural polyphenol-rich resinous substance collected by honeybees from a variety of plant sources that has anti-inflammatory and anti-oxidative properties. Red propolis (RP) protection in renal I/R injury was investigated. **Methods:** Animals were anesthetized with sodium pentobarbital (50 mg/kg i.p.). A midline laparotomy incision was performed, the right kidney was removed and left ischemic renal failure was induced by clamping the renal artery (with a nontraumatic clamp) for 60 min, followed by reperfusion. After 48h, animals were sacrificed to obtain blood samples for biochemical tests. Rats were divided into four groups: 1) sham group, 2) RP group (sham-operated rats treated with RP), 3) I/R group (rats submitted to ischemia) and 4) I/R-RP (rats treated with RP before ischemia). The alcoholic extract of Red Propolis was purchased in PharmaNectar® company (Belo Horizonte – MG, Brazil), at a concentration of 0.25 g/ml. For the experiments, we used the dose of 150 mg/kg. Identification and characterization of the alcoholic extract of Red Propolis was performed by High-Performance Liquid Chromatography analysis (HPLC) equipped with an YMC-Pack ODS-A column and a photodiode array detector. The authentic standards of flavonoids (quercetin, rutin) were purchased from Sigma-Aldrich® (St. Louis – MO, USA). The experimental protocol was approved by the Ethical Committee on Animal Research of UFC (nº 39/13). **Results:** I/R increased plasma levels of creatinine (2.7 ± 0.9 vs. 0.6 ± 0.5mg/dL, $p<0.05$), urea (274.3 ± 91.8 vs. 39.3 ± 6.2 mg/dL, $p<0.05$) and reduced creatinine clearance (CrCl) (0.07 ± 0.04 vs. 1.29 ± 0.46 mL/min/100 g, $p<0.05$), when compared to control animals. These results were associated with a higher absolute excretion of Na+ (AENa+)(1.03 ± 0.39 vs. 0.23 ± 0.01%, $P<0.05$) and K+ (134.4 ± 54.9 vs. 7.5 ± 2.9%, $p<0.05$). RP provided protection against this renal injury by reducing levels of creatinine (1.82 ± 0.5 vs. 2.7 ± 0.9mg/dL, $p<0.05$), urea (181.1 ± 65.6 vs. 274.3 ± 91.8 mg/dL, $p<0.05$) and attenuating the ClCr reduction (0.41 ± 0.14 vs. 0.07 ± 0.04 mL/min/100 g, $p<0.05$). This improvement also was seen in the absolute excretion of Na+ (0.58 ± 0.30 vs. 1.03 ± 0.39%, $p<0.05$) and in absolute excretion of K+ (64.74 ± 54.44 vs. 134.4 ± 54.94%, $p<0.05$) **Discussion:** Acute kidney injury is a multifaceted entity that evolves through different stages, culminating in organ failure. Propolis is a complex honeybee product characterized as being especially rich in isoflavonoids. Several studies have demonstrated the anti-oxidative and anti-inflammatory activity of isoflavonoids, but their effects on renal I/R injury was never studied. In our study, animals were treated on the day of the procedure with a fixed dose of red propolis extract, resulting in a significant attenuation of GFR drop. The protective effect of red propolis was confirmed by a reduced AENa+ in RD-IR group, a marker of functional tubular viability. In conclusion, red propolis protects kidney against acute ischemic renal failure. **Financial support**: CNPq.

09.009

Anti-inflammatory activity of anethole via reduction of macrophage-derived cytokines.

Ponte EL[1], Martin TM[1], Marques-Domingos GFO[1], Murata GM[2], Gorjão R[3], Curi R[2], Assreuy AMS[1] [1]ISCB-UECE, [2]ICB-USP, [3] ICAF-Unicsul

Introduction: Anethole, a terpenoid found in essential oils of medicinal plants (fennel, anise) has anti-carcinogenic (Chainy GB *Oncogene*, v.19, p.2943, 2000), anti-inflammatory (Ponte EL *Pharmacol. Rep*, v.4, p.984, 2012) and wound healing (Cavalcanti JM *J Ethnopharmacol*, v.144, p.240, 2012) properties, among others. Previous studies have shown that anethole inhibits paw edema induced by carrageenan (Cg). This work aims to expand the study of anethole anti-inflammatory activity in the modulation of vascular and cellular events and participation of cytokines derived from macrophages. **Methods:** Male Wistar rats (180–250 g) were used in experimental protocols, approved by the ethics committee of UECE (No: 10130208-8), in the models of paw edema and peritonitis. Animals received anethole (0.01 – 100 mg/kg) or Tween 80 (0.1%) orally (p.o., 100 μl/100g) 1 h prior to inflammatory stimuli. Paw edema was assessed by plethysmometry at zero and 1 – 4 h after subcutaneous (s.c.) injection of 1% Cg as a stimulus. Negative controls received sterile 0.9% NaCl (s.c.). Vascular permeability was assessed by Evans blue (25 mg/kg) intravenous administration (i.v.) 1h before sacrifice. Paws were cut, weighed and incubated in formamide at 37˚C for 72h and quantification of Evans blue by spectrophotometer (A_{600nm}). Peritonitis was induced by intraperitoneal (i.p.) injection of Cg (500 μg), and after 4 h peritoneal fluid was collected for total and differential leukocyte count (optical microscopy), cytokine (ELISA) and nitrite (Griess Method) content. Naive peritoneal macrophages were maintained in CO_2 incubator at 37 ˚C for 24 hours in culture plates containing RPMI 1640 medium, anethole (10-1000 μM) or DMSO (0.2%). Cells were incubated for additional 24 h with LPS (1 μg/ml) and evaluation of cytotoxicity by flow cytometry, measurement of cytokines by ELISA and reactive oxygen species (ROS) by chemiluminescence assays. **Results:** *In vivo* anethole (1 mg/kg) inhibited: a) paw edema from 120 to 240 min, in 40% (61.44 ± 5.51 *vs* Cg: 99.98 ± 20.54 AUC) b) increase in vascular permeability in 58% (0.42 ± 0.07 mg/g *vs* Cg: 0.99 ± 0.32 mg/g), c) total leucocyte migration by 50% (3919 ± 861.7 cells/ml *vs.* Cg: 6675 ± 500.3 cells/ml) d) neutrophil migration by 42% (1088.8 ± 336.6 cells/ml *vs.* Cg: 1883.6 ± 286.5 cells/ml) and peritoneal levels of IL-1β(76%), IL-1α(79%), MIP-3α (64%) and NO (30%). *In vitro* anethole: a) showed no cytotoxicity to macrophages at 10, 25 and 50 μM; b) inhibited production of TNF-α (76% at 25 μM and 86% at 50 μM), IL-1β (81% at 10 μM), 84% at 25 μM and 67% at 50 μM in macrophages stimulated with LPS, c) increased in two times the levels of IL-6 in cultured macrophages, d) reduced in 43% ROS production by macrophages stimulated with PMA. **Conclusion:** Anethole inhibits vascular and cellular events of acute inflammation by reducing the levels of NO and peritoneal macrophage-derived cytokines and ROS. Financial Agencies: FUNCAP.

09.010

Quercetin improves antioxidant response in the gills of silver catfish. Pês TS, Gressler LT, Saccol EH, Londero EP, Ourique GM, Baldisserotto B, Pavanato MA UFSM – Physiology and Pharmacology

Introduction: The silver catfish, *Rhamdia quelen* (Heptapteridae family), is one of the most cultivated species in the southern of Brazil. Products designed to improve the cultivation and production of silver catfish are required due to the importance of this species for aquaculture. The oxidative stress is one of the main challenges in aquaculture, and the use of compounds with antioxidant capacity can be useful. The flavonoid quercetin has several beneficial effects and becomes an important tool to be used on fish farms in order to reduce the physiological changes resulting from stress linked to cultivation (Plakas *et al.*, 1985; Awad *et al.*, 2013). Thus, the aim of this study was to evaluate the oxidative biomarkers in gills of silver catfish fed diets containing quercetin. **Methods:** Three diets (in triplicate) were tested: standard 0 (control) and in the other added different concentrations of quercetin: 0.15 and 0.30 % quercetin in the diet. After 21 days of feed, the animals were euthanized and gills were removed for analysis as follows: lipid peroxidation (LPO) measured by the thiobarbituric acid reactive substances (TBARS) and lipid hydroperoxides (LOOH) levels; enzymatic activity of superoxide dismutase (SOD), glutathione-S-transferase (GST); non-enzymatic antioxidants by determining content of ascorbic acid (AA) and total reactive antioxidant potential (TRAP). The experimental protocol was approved by the Committee on Animal Experimentation of UFSM under registration n° 077/2013. **Results and Discussion:** The results demonstrated that the animals fed diet containing quercetin presented a decrease in LOOH and TBARS levels. The LPO is a marker of oxidative damage to lipids and this result may be attributed to the antioxidant effect of quercetin, which has been proven in other studies (Awad *et al.*, 2013). With the respect of the measures of antioxidant enzymes, there is an increase in SOD and GST activities in fish fed diets containing quercetin when compared to the control. SOD act as a scavenger of the reactive oxygen species such O_2^-. GST has a major role in the detoxification of xenobiotic and ROS through their conjugation to GSH, thus favoring protection against LPO. There was a significant increase in AA content and TRAP in the fishes fed diets containing quercetin when compared to the control. AA has several functions, including its action as an important antioxidant that is able to scavenge oxygen and nitrogen radical species (Harrisson *et al.*, 2009). The decrease on the LPO in the fishes fed diet containing quercetin may be due the increase on the antioxidant response occasioned by this flavonoid. **Conclusion:** In conclusion, these results suggest that supplementation containing quercetin to the diet of silver catfish is recommended because increases the tissue antioxidant response, preventing the oxidative damage. **Acknowledgments:** Authors are grateful to the Conselho Nacional de Desenvolvimento Tecnológico (CNPq) and the Comissão de Aperfeiçoamento de Pessoal de Nível Superior (Capes) and Ministry of Fisheries and Aquaculture/Ministry of Science and Technology/FINEP.

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09.011

Vasorelaxation induced by synthetic derivative of the isoquinoline alkaloids in rat aorta is mediated by the endothelium. Travassos RA[1], Sarmento DM[2], Nascimento GJ[1], Albuquerque JSS[1], Cordeiro MB[2], Rodrigues LC[1], Braga VA[1], Araújo DAM[1] [1]CBiotec-UFPB, [2]CCS-UFPB

Introduction: Isoquinoline alkaloids represent a group of natural products with remarkable importance in biomedical research and drug discovery. Several members of this group exhibit pharmacological and biological properties. Isoquinoline is one of the most widely distributed alkaloids with proven therapeutic potential (Bhadra, Med Res Rev., v.31, p.821, 2010). A new synthetic derivative of the isoquinoline alkaloids, 1-(3-methoxy-4-hydroxyphenyl)-7-methoxy-1,2,3,4,tetrahydroisoquinoline (MHTP), was obtained by the Pictet-Spengler reaction and the vasorelaxant effect of MHTP in rat aorta was investigated. **Methods:** Male Wistar rats (*Rattus norvegicus*) were obtained from Bioterium Prof. Thomas George of Centro de Biotecnologia of UFPB. All rats were euthanized by decapitation with guillotine. The aortic rings about 3-5 mm wide were obtained from the thoracic aorta. To obtain isometric responses, the rings were individually suspended on stainless steel rods in organ baths (10 mL) containing Krebs solution (pH = 7.4) at 37 ºC, bubbled with 95% O_2 and 5% CO_2 mixture and resting tension of 1 g. The relaxation was expressed as the reversal percentage of the initial contraction elicited by contractile agent and EC50 values were obtained by nonlinear regression. All procedures were approved by the CBiotec/UFPB Ethics Committee on Animal Use (Protocol/CEUA nº 0605/13). **Results:** The MHTP relaxed rat aorta pre-contracted with phenylephrine (3 x 10^{-7} M) in a concentration-dependent manner in both intact (EC50 = 7.9 ± 1.6 x 10^{-5} M, n = 5) and denuded (EC50 = 5.7 ± 1.1 x 10^{-5} M, n = 5) endothelium. MHTP spasmolytic effect was attenuated significantly in the presence of L-NAME (EC50 = 4.6 ± 1.0 x 10^{-5} M, n = 5), a competitive nitric oxide (NO) synthase inhibitor, ODQ (EC50 = 5.1 ± 0.8 x 10^{-5} M, n = 3), a selective soluble guanylyl cyclase (sCG) blocker and indomethacin (EC50 = 1.0 ± 0.2 x 10^{-4} M, n = 5), a nonselective inhibitor of cyclooxygenase. **Discussion:** In the functional level, the vasorelaxant effect of MHTP was more potent in endothelium-intact rat aorta, which is suggestive of the involvement of endothelium-derived relaxing factors (EDRF) such as NO and prostacyclin (PGI_2). The fact that the relaxation curves of MHTP have shifted to the right in the present of L-NAME and indomethacin is indicative of the involvement of the endothelial NO synthase and cyclooxygenase enzymes. In addition, we observed that vasorelaxation effect and potency of MHTP were reduced in the presence of ODQ indicating the participation of sCG. In conclusion, the vasorelaxant action of MHTP in rat aorta involves EDRF such as NO and PGI_2, and NO receptors such sCG. Further complementary studies in functional and molecular levels are needed to fully elucidate the mechanism of relaxation produced by MHTP in rat aorta. **Financial Support:** UFPB/CNPq/Capes.

09.012

Study of association of antibiotics with essential oil *Ocimum basilicum or linalool on Multiresistant bacteria*. Silva VA[1], Freitas AFR[1], Lima MA[1], Pessôa HLF[1], Sarmento FQ[1], Coutinho HDM[2], Coutinho HDM[2], Sousa JP[1], Lima EO[1] [1]UFPB, [2]URCA

Introduction: Bacterial resistance to antibiotics has become a serious public health problem. Due to this fact, substances derived from plants could be attractive alternatives. Natural products from plant may change or modulate the action of the antibiotic, enhancing or reducing the activity of this drug (Coutinho *et al.*,2008) . The *Ocimum basilicum* plant belongs to the family Lamiaceae; is popularly known as basil and can be found in various regions of the world. (Carovic-Stanko, 2010; Busatta, 2007). This study aims to determine the pharmacological effect produced by the combination of antibiotics standards and the essential oil of *O.basilicum* or linalool on multiresistant bacterial strains. **Methods:** Standard strains (ATCC 6538) and clinical isolates (M-177) of *Staphylococcus aureus* were used. The drug combination was tested by the microdilution checkerboard technique (Eliopoulos & Moellering, 1991). For this, fractional inhibitory concentration (FIC) indices were calculated as FIC A + FIC B, where FIC A and FIC B represent the minimum concentrations that inhibited bacterial growth for drugs A and B, respectively: FIC A= MIC A combination/MIC A alone and FIC B= MIC B combination/MIC B alone. A mean FIC index was calculated based on the following equation: FIC index = FIC A + FIC B, and the interpretation made as follows: synergistic (<0.5), additive (0.5–1.0), indifferent (>1), or antagonistic (>4.0). **Results and Discussion:** The results of this study showed that the combination of imipenem with the essential oil of *O. basilicum* or with linalool showed synergistic effect on *S. aureus* ATCC 6538 and *S. aureus* M-177, reducing the MIC from 32 to 0.125 µg / mL in two compounds. The association of ciprofloxacin with the essential oil of *O.basilicum* showed antagonistic effect on *S. aureus* ATCC 6538 and *S. aureus* M-177. The association of linalool with ciprofloxacin showed indifferent effect on *S. aureus* ATCC 6538 and *S. aureus* M-177. **Conclusion:** Based on the above, we can conclude that the essential oil of *Ocimum basilicum* and linalool showed synergistic effect when used in combination with imipenem on strains of clinical importance and may be a promising source for the search for new effective drugs in the treatment of diseases caused by bacteria resistant to antibiotics. **Acknowledgments:** Capes; UFPB; PPGPNSB. **References:** BUSATTA, C. *et al.*2007. Evaluation of Origanum vulgare essential oil as antimicrobial agent in Sausage. *Braz J Microbiol*,38:610-16. CAROVIC-STANKO, K. *et al.* 2010. Composition and antibacterial activities of essential oils of seven Ocimum taxa. Food Chem., 119: 196-201. CLINICAL AND LABORATORY STANDARDS.2005 INSTITUTE. Metodologia dos testes de sensibilidade a agentes antimicrobianos por diluição para bactérias de crescimento aeróbico: norma aprovada sexta edição – M7 – A6. Pennsylvania,v.23, n. 2., ELIOPOULOS, G. M.; MOELLERING, R. C., 1991. Laboratory methods used to assess the activity of antimicrobial combinations. In Antibiotics in Laboratory Medicine, 3rd ed, pp. 432–492. H. D. M. COUTINHO.; J. G. M. COSTA.; J. P. SIQUEIRA-JÚNIOR.;E.O.LIMA,"*In vitro* anti staphylococcal activity of *Hyptismar-tiusii* Benth against methicillin-resistant *Staphylococcus aureus*-MRSA strains, *Brazilian Journal of Pharmacognosy*, vol. 18, pp.670–675, 2008

09.013

Effects of silymarin on oxidative stress parameters and monoamine oxidase activity. Dotto MM[1], Oliveira DR[2], Schaffer LF[3], Busanello A[3], Peroza LR[2], Barbosa CP[1], Fachinetto R[4] [1]UFSM – Farmácia, [2]UFSM – Bioquímica Toxicológica, [3]UFSM – Farmacologia, [4]UFSM – Bioquímica Toxicológica e Farmacologia

Introduction: Oxidative stress results from an imbalance between the production of reactive species and antioxidant defense system, a process that has been suggested as responsible for the pathogenesis of neurodegenerative diseases. Silymarin is a standard extract, biologically active, derived from the seeds of *Silybum marianum* (L.) Gaertn, used for the treatment of liver diseases (Borah *et al*, 2013). The aim of this study was to evaluate the *in vitro* antioxidant activity of silymarin and inhibition of monoamine oxidase (MAO). **Methods**: The effect of silymarin on lipid peroxidation induced by pro-oxidants iron sulphate ($FeSO_4$, 5µM) and sodium nitroprusside (NPS, 5µM) (Ohkawa; Ohishi; Yagi, 1979) and on the catalase activity were investigated. We also evaluated the effect of this compound on MAO activity (Villarinho *et al*., 2012). The experimental protocol was approved by internal ethical commission of UFSM under the number 018/2014. **Results and discussion:** Literature data have previously showed the neuroprotective potential of Silymarin against CNS disorders including models of Parkinsonism (Borah *et al*, 2013). However, there are few works investigating the action mechanism of Silymarin. Thus, we demonstrated that Silymarin protected rat brain homogenates from lipid peroxidation induced by Fe^{2+} (IC_{50} = 28.2 ± 8.06 µg/mL) and NPS (IC_{50} = 19.33 ± 4.55 µg/mL) and restored the activity of catalase in the concentration of 30 µg/mL when compared with control (induced by Fe^{2+} or SNP and without Sylimarin). Silymarin had an inhibitory effect on MAO-A (IC_{50} =142.97 ± 17.85 µg/mL) and on the activity of MAO-B (IC_{50} = 71.37 ± 13.47 µg/mL). The inhibition of MAO could contribute to reduce oxidative stress since the metabolism of monoamines generates reactive oxygen species (Youdim; Weinstock, 2004). Furthermore, Ou *et al*., 2006, demonstrated the inhibition of MAO-A prevents cell apoptosis. These effects of Silymarin on MAO activity are particularly interesting since the inhibitory potency was low which could avoid toxic effects of MAO inhibitors. Considering our *in vitro* results, Silymarin could be promissory to treating neurodegenerative diseases. However, further studies should be conducted to investigate the effects of silymarin on *in vivo* models. **Financial support**: CNPq/Capes/FAPERGS/UFSM. **References:** BORAH A. *et al.* Neuroprotective potential of Silymarin against CNS disorders: insight into the pathways and molecular mechanisms of action. *CNS Neurosciense & Therapeutics*. 19:847-853, 2013. OHKAWA H; OHISHI H; YAGI K. Assay for lipid peroxide in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*, 95:351-358, 1979. OU X; CHEN K; SHIH JC. Monoamine oxidase A and repressor R1 are involved in apoptotic signaling pathway. PNAS, 103: 10923-10928. VILLARINHO JG. *et al.* Involvement of monoamine oxidase B on models of postoperative and neuropathic pain in mice. *European Journal of Pharmacology*, 690:107-114, 2012. YOUDIM MBH.; WEINSTOCK M. Therapeutic applications of selective and non-selective inhibitors of monoamine oxidase A and B that do not cause significant tyramine potentiation. *Neurotoxicology*, 25:243–250, 2004. Sources of

09.014

Amides from *Piper* as a diuretic – behind the ethnopharmacological uses of *Piper glabratum* Kunth. Prando TBL[1], Baciquete TF[1], Gasparotto FM[1], Araújo V[1], da Silva GR[1], Lourenço ELB[1], Gasparotto Junior A[2] [1]Unipar – Farmacologia e Toxicologia de Produtos Naturais, [2]UFGD – Farmacologia e Toxicologia de Produtos Naturais

Introduction: Several species of the gender *Piper* are known in Brazilian folk medicine as having diuretic activity (Barbosa-Filho *et al*., 2008; Gutierrez *et al*., 2013). So, we propose to investigate the acute diuretic activity and the possible toxic effects of *Piper glabratum* Kunth popularly known as false Jaborandi. Additionally, we propose to check whether there is any correlation between the biological activities of the crude extract and its 2-methoxy-4, 5-methylenedioxy-*trans*-cinnamoyl-pyrrolidine (MMCP) in Wistar rats. **Methods:** The methanolic extract (MEPG) was fractioned by chromatography column and a pyrrolidine amide (MMCP) was identified by analyses of ^{1}H and ^{13}C RMN spectral data and correlations. Female Wistar rats were separated in different groups (n = 6) and fasted overnight with free access to water. Before the treatments, all animals received physiological saline (0.9% NaCl) in an oral dose of 5 mL/100 g to impose a uniform water and salt load. Then, the first group received vehicle (deionized water) orally and it was used as control. Other groups of rats received, by oral route, MEPG (30, 100 and 300 mg/kg), MMCP (3, 10 and 30 mg/kg), or HCTZ (hydrochlorothiazide, 10 mg/kg). The urine was collected in a graduated cylinder and its volume was recorded for 8 h (expressed as mL/100 g). The urine excretion rate, pH, density, conductivity and content of $Na^{+}K^{+,}$ Cl^{-} and HCO_3^{-} were measured in the urine of saline-loaded animals. Additionally, acute toxicity of the extract was also evaluated. All experimental procedures were previously approved by the Institutional Ethics Committee of the Unipar (authorization 20763-2011). **Results:** Urine volume and electrolytes excretion were not significantly altered by the administration of MEPG. On the other hand, MMCP administration (30 mg/kg) was able to induce a substantial increase in urine volume, urinary HCO_3^{-} and urine pH. All other evaluated parameters did not show significant differences when compared to the control group. Moreover, high dosage of HEPG showed important liver toxicity and elevated mortality when injected intraperitoneally. **Discussion:** the present study does not support the traditional usage of *Piper glabratum* in the Brazilian folk medicine as a diuretic agent. Additionally, we have confirmed that a possible diuretic activity may be associated with the presence of the MMCP, which showed a saluretic/diuretic effect. Furthermore, our results provide evidence for the toxicity profile of the MEPG at high doses and therefore, it should be ingested with caution. Further studies should be conducted to assess the possible mechanisms by which the *Piper* amides exert their diuretic effects and the role of these agents in toxic effects of MEPG. **Acknowledgments:** We are grateful to Diretoria Executiva de Gestão da Pesquisa e Pós-Graduação (DEGPP/UNIPAR-Brazil) for a financial support. **References:** Barbosa-Filho, J. M., *et al.* "Sources of alpha-, beta-, gamma-, delta- and epsilon-carotenes: A twentieth century review," *Rev bras Farmacogn*, vol. 18, pp. 135-154, 2008. Gutierrez, A. M., *et al.* "Alkaloids from piper: a review of its phytochemistry and pharmacology," *Mini Rev Med Chem*, vol. 13, n. 2, pp. 163-193, 2013.

09.015

Ethno-guided investigation of the diuretic effects of native medicinal species. Prando TBL[1], Gasparotto FM[1], Jacomassi E[1], Quindere JR[1], Barbosa LN[2], Lourenço ELB[1], Gasparotto Junior A[3] – [1]Unipar – Farmacologia e Toxicologia de Produtos Naturais, [2]UFPR – Farmacologia, [3]UFGD – Farmacologia e toxicologia de Produtos Naturais

Introduction: Hypertension is the leading cause of disability and death worldwide. Common clinical strategies to achieve the reduction of blood pressure include the use of diuretics. In Brazil, diuretics, especially thiazides are used as first-line drugs in the treatment of hypertension. Despite its high efficiency, its use is associated with a high incidence of adverse effects such as electrolyte imbalance, metabolic disorders, and sexual dysfunction. In recent decades, there has been a growing interest in the use of medicinal plants and their extracts in medical therapy. Furthermore, the use of popular culture as ethnobotanical indicator of possible "compounds" pharmacologically active is very important in the selection of species to be studied and validated in pharmacological trials. So, the aim of the study is evaluate the possible diuretic activity of aqueous extracts (SEI) from *Cuphea carthagenensis* (CC), *Phyllanthus tenellus* (PT), and *Echinodorus grandiflorus* (EG) in an experimental model of diuresis in rats. **Methods:** Aerial parts of CC, PT, and EG were extracted with water, concentrated, filtered and the solution lyophilized to give the CC-SEI, PT-SEI, and EG-SEI. The diuretic activity was determined according to a method previously described with minor modifications (Kau *et al.*, 1984). Before the treatments, all animals (male Wistar rats; n=6) received physiological saline (0.9% NaCl) in an oral dose of 5 mL/100g to impose a uniform water and salt load. Then, the first group received vehicle (deionized water) orally and it was used as control. Other groups of rats received, by oral route, CC-SEI (30, 100 and 300 mg/kg), PT-SEI (30, 100 and 300 mg/kg), EG-SEI (30, 100 and 300 mg/kg) or HCTZ (hydrochlorothiazide, 25 mg/kg). The urine was collected in a graduated cylinder and its volume was recorded for 8 h (expressed as mL/100 g). The urine excretion rate, pH, density, conductivity and content of Na+, K+, Cl- and HCO3- were measured in the urine of saline-loaded animals. All experimental procedures were previously approved by the Institutional Ethics Committee of the Unipar (authorization no. 22833/2012). **Results:** Urine volume were not significantly altered by the administration of CC-SEI, PT-SEI, or EG-SEI. On the other hand, SEI-PT (100 mg/kg) and SEI-EG (300 mg/kg) administration was able to induce a substantial increase in urinary sodium. Moreover, the acute administration of SEI-EG (300 mg/kg) was able to increase significantly the urinary chloride excretion. All other evaluated parameters did not show significant differences when compared to the control group. **Discussion:** The interest in the medicinal plants is growing every year worldwide. If on one hand the indiscriminate use can cause adverse effects, many medicinal plants show to be ineffective for the treatment of pathologies indicated by folk medicine. The present study showed that despite the extensive popular use of these medicinal plants as diuretics, only the *Phyllanthus tenellus* and *Echinodorus grandiflorus* were effective in inducing a saluretic effect. **Conclusion:** The results presented here demonstrate that aqueous extracts obtained from *Phyllanthus tenellus* and *Echinodorus grandiflorus* are able to induce an saluretic effect after acute treatment in Wistar rats. Additional studies are needed to validate or refute the use of these extracts (or their phyto-derived) when a diuretic activity is required. **Acknowledgments:** We are grateful to Diretoria Executiva de Gestão da Pesquisa e Pós-Graduação (DEGPP/UNIPAR-Brazil) for a financial support. **References:** Kau S. T. *et al.* A method for screening diuretic agents in the rat. *J Pharmacol Methods*, v. 11, n. 1, p. 67-75, 1984.

09.016

BPMP-II, a novel *Bothrops pauloensis* venom PI metalloproteinase: inhibition of endothelial cell adhesion and *in vitro* angiogenesis. Achê DC[1], Gomes MSR[2], Naves-de-Souza DL[1], Almeida-Silva M[1], Homsi-Brandeburgo MI[1], Yoneyama KAG[1], Rodrigues RS[1], Borges MH[3], Lopes DS[1], Rodrigues VM[1] – [1]UFU – Genetics and Biochemistry, [2]UESB – Chemical and Physical, [3]FUNED

Introduction: The group of snake venom metalloproteinases (SVMPs) is characterized by a large molecular mass spectrum (20 a 110 kDa) according to the number of structural domains present in the structure. These domains have been characterized due to their specific functions and are named as catalytic domain, disintegrin or disintegrin-like domain, cysteine-rich domain and lectin-like domain (FOX and SERRANO, 2008). SVMPs are reported to induce apoptosis and disrupting the angiogenic process of endothelial cells (I. Tanjoni *et al.*, 2005; A.M. Moura-da-Silva, 2007, P.L. Ho, 2002). Angiogenesis is important in the pathogenesis of a broad range of disorders such as arthritis and cancer. In the present work, we demonstrate some biochemical and functional properties of a new PI SVMP isolated from *Bothrops pauloensis* snake venom (BpMP-II), in addition we evaluated its capacity to inhibit endothelial cell adhesion and *in vitro* angiogenesis. **Methods:** Initially BpMP-II was purified after a combination of three chromatography steps (CM-Sepharose, gel filtration Sephacryl-S300 and íon exchange Capto-Q) and showed molecular mass of 23,000 Da determined by MALDI-TOF. The isoelectric point (pI 6.1) was determined by 2D eletrophoresis and the sequence of some fragments obtained by Mass Spectrometry (MALDI TOF\TOF) presented high structural similarity with other PI-SVMPs. Proteolytic activities assays upon azocasein and fibrinogen were performed in order to ensure the class of the purified toxin. A MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium) assay determined cell viability of BpMP-II treated tEnd cells (mouse thymus Endothelial cells). **Results and discussion:** BpMP-II showed proteolytic activity against azocasein, was able to degrade bovine fibrinogen and was inhibited by EDTA, 1.10 phenantroline and β-mercaptoethanol (specific metalloproteinase inhibitors). BpMP-II did not induce local hemorrhage in the dorsal region of mice (protocol number 046/09) even at high doses and did not affect plasma creatine kinase (CK) levels when administered intramuscularly into the gastrocnemius muscle of mice. Moreover, this metalloproteinase decreased tEnd cells viability at concentrations higher than 20 µg/mL. **Conclusion:** With sub-toxic doses this metalloproteinase affected tEnd cell adhesion and was also able to inhibit *in vitro* angiogenesis. BpMP-II showed very important functional properties suggesting considerable therapeutic potential for this class of protein. **References:** J.W. Fox; v.275; p.3016; 2008. I. Tanjoni; Apoptosis; v.10; p. 851; 2005. A.M. Moura-da-Silva; Curr Pharm Des. v.13; p.2893-905; 2007. P.L. Ho; Biochemical and Biophysical Research Communications, v.294, p.879, 2002. **Financial Suport:** FAPEMIG, Capes, CNPq, UFU, INCT-Nanobiofar

09.017

Primitive ants dinoponera quadriceps venom's have neurotoxic and cardiotonic actions dependent histamine release. Cabral PHB[1], Nascimento NRF[2], Fonteles MC[2], Santos CF[2], Quinet YP[2], Bindá AH[1], Eleuterio EO[2] [1]UFC – Farmacologia, [2]ISCB-UECE

Introduction *Dinoponera* is a genus strictly Neotropical with six known species considered the largest ants in the world, with size between 3 and 4cm and their occurrence is restricted to tropical and sub-tropical areas of South America with biological effects already published such as antinociceptive property, neuroprotective and neurotoxic effects in a seizure model, antimicrobial and antifungal activity and also have been useful to treat rheumatism and back pain. **Methods** The ants were collected in the mountains of Maranguape-CE with the authorization of IBAMA aiming to extraction of venom and their isolation in the form of fractions (DqV), maintained at -20°C. Male Wistar rats (200–250 g), guinea pigs(600-900 g), Swiss mice (35-40 g) and Ross broilers (80-100 g) were used to study cardiac hemodynamic *in vivo* and *in vitro* and for neuromuscular studies. The study protocols were approved by the Ethics Committee of the Ceara State University under the protocol number 11221997-7/45. Systemic hemodynamics was studied by using a Millar pressure-volume microcatheter coupled to a powerlab data acquisition system. Eletromechanic properties of the isolated guinea-pig heart were studied by using a Langendorff system with powerlab data acquisition. The Neuromuscular effects were studied using the Biventer cervicis preparation in organ baths with isometric recordings. Autonomic effects were evaluated by using mice vas deferens strips in organ baths. **Results** The addition of DqV to the isolated heart induced dose-dependent tachycardia of 12.8 ± 9%, 38 ± 10% and 84.4 ± 10%, at dosage of 10,100 e 200 µg/bolus, respectively. Similarly, venom fractions(10,100 e 200 µg) showed positive inotropic effect with increased in pressure in the left ventricle of the order of 72 ± 31%, 237 ± 54% and 225 ± 69%, respectively. The perfusion pressure increased in the higher dose (200 µg as bolus) by 26%. This last concentration also induced atrioventricular block and ventricular premature complexes wich reversed upon several minutes of washout. None of these effects was affected by adding propranolol in the perfusate solution (0.1 or 1µM). In the hemodynamic study, DqV (100 µg/bolus) increased the left ventricle pressure from 118.5 to 125 mmHg while isoproterenol (1µg/bolus) increased it from 114.9 to 124.8 mmHg which was accompanied by increased blood pressure. There are no remarkable effects on heart rate and no arrhythmias were observed. These effects were prevented by the association of promethazine and famotidine. On the other hand, the neurogenic contractions induced by EFS on mouse vas deferens were blocked in a dose-related fashion. This neurotoxic effect was not blocked by naloxone or yohimbine but was blocked by promethazine(10µM). On the other hand, high concentrations of DqV (30 µg/ml) induced a tonic contraction of the biventer-cervicis neuromuscular preparation. **Discussion:** DqV has both cardiotoxic and neurotoxic effects that are dependent on histamine release or alternatively DqV may have histamine as a component. **Acknowledgment**: CNPq

09.018

Involvement of the nitric oxide in the vasorelaxant effect of the water soluble fraction of *Terminalia fagifolia* Mart. & Zucc. in rat aorta. Carvalho EF[1], Nunes AF[1], Nunes PHM[2], Santos RF[1], Chaves MH[3], Oliveira AP[1], Oliveira RCM[1] [1]UFPI – Medicinal Plants, [2]UFPI – Biophysic and Physiology, [3]UFPI – Chemistry

Introduction: *Terminalia fagifolia* Mart. & Zucc. (Combretaceae) is a plant of the Brazilian cerrado, known as "Capitão-do-mato" and "mirindiba", widely used for the treatment of thrush, tumors and gastrointestinal disorders. Previous studies show that the ethanol extract of the stem bark of *T. fagifolia* (Tf-EtOH) and its aqueous fractions (Tf-FAQ) and water soluble (Tf-FSA) have relaxing effect in isolated rat aorta rings, showing dependence on endothelium for the two fractions. **Aim**: To investigate the role of the enzyme in NO-synthase/GCs vasorelaxant effect induced by the water soluble fraction of *T. fagifolia* in rat aorta. **Methods:** The protocols were approved by the Ethics Committee on Animal Experimentation (CEEA/UFPI 008/2012). Wistar rats were used (250-300g), male, originating from the Vivarium Sectorial NPPM/UFPI, kept under controlled temperature conditions (24 ± 1°C) and light-dark cycle of 12h, with free access to food and water. After euthanasia, the artery thoracic aorta was sectioned into rings (3-4mm) free from connective and adipose tissue, which were incubated at 37°C in normal Krebs solution (pH 7.4) aerated with carbogen (95% O_2, 5% CO_2), suspended by cotton threads and attached to force transducers coupled to a data acquisition system (AQCAD/Projects AVS, SP-Brazil) for recording of isometric tension. After stabilization (1.0 gf, 1h), was verified the integrity of the vascular endothelium in the aortic rings by addition of acetylcholine (ACh, 1 µM) on the component and sustained tonic contraction with phenylephrine (PHE, 1 µM) considering its absence (E-) of less than 10% and presence (E +) when the relaxation exceeded 50%. In a second step, in rings with endothelium (E +) were incubated N-nitro-L-arginine methyl ester (L-NAME, 300 µM), 1H-[1,2,4] oxadiazole [4 3-a] quinoxalin-1-one (ODQ, 10 µM) or 2-phenyl-4,4,5,5-tetramethyl-imidazoline-1-oxyl 3-oxide (PTIO, 300 µM). After 30 minutes, contraction was induced with PHE and during the tonic component of cumulatively added to fraction Tf-FSA (0.1-1000 µg/mL). Results were expressed as mean ± SEM pD_2 obtained by non-linear regression. For comparisons of means, was used the Student's t unpaired test, considering significant *$p<0.05$ values. **Results**: The vasodilator effect induced by Tf-FSA in rings incubated with L-NAME/ODQ/PTIO in individual preparations, was significantly attenuated compared to group E + non-blocking, which was also higher than the response in the group E- (E-: pD_2= 2.50 ± 0.02, n=6; E+: pD_2= 2.30 ± 0.03*, n=6; E + L-NAME: pD_2= 2.91 ± 0.02*, n=5; E + ODQ: pD_2= 3.01 ± 0.04*, n=5; E + PTIO: pD_2= 2.57 ± 0.01*, n=5). **Discussion:** The results indicate that there is involvement of the NO pathway in endothelium-dependent induced Tf-FSA fraction of the ethanol extract of *T. fagifolia* in isolated rat aorta vasorelaxant effect. However, additional studies are needed to evaluate the possible involvement of other endothelial mechanisms in this effect. **Financial Support:** UFPI/Capes

09.019

Prolonged diuretic activity and sparing-calcium effect of *Tropaeolum majus* L. – evidence in the prevention of osteoporosis. Barboza LN[1], Prando TBL[2], Silva GR[2], Lourenço ELB[2], Gasparotto Junior A[3] [1]UFPR – Farmacologia, [2]Unipar – Farmacologia e Toxicologia de Produtos Naturais, [3]UFGD – Farmacologia

Introduction: Although several preclinical studies indicate high effectiveness and safety in the use of the hydroethanolic extract obtained from *Tropaeolum majus* leaves (HETM) as a diuretic (Gasparotto Junior *et al.*, 2009; Gasparotto Junior *et al.*, 2011; Gasparotto Junior *et al.*, 2012), the impact of their prolonged use in the presence of low estrogen levels (as occurs in menopause) remain unclear. Thus, the aim of this study was to investigate the diuretic effects of prolonged administration of HETM in ovariectomized rats and evaluate the interrelationship between calcium excretion and bone turnover. **Methods:** Forty-two female Wistar rats were divided into six experimental groups (n=6). An SHAM-operated group was treated orally as a vehicle. Other five groups rats were ovariectomized (OVX) and treated orally with different doses of HETM (3, 30 and 300 mg/kg), furosemide (25 mg/kg) or vehicle. All treatments were performed once a day for 4 weeks. On the first day of treatment (outset) and at weekly intervals for four weeks the animals were kept in individual metabolic cages for 8 hours for urine collection. Electrolyte concentrations (Na^+, K^+, Cl^-, $Ca^{++}$and HCO_3^-), pH, density, conductivity and creatinine levels were estimated from urine sample of each rat. The plasmatic concentration of total cholesterol (TC) and fractions (HDL-C and LDL-C), triglycerides, urea, creatinine and osteocalcin were also measured at the end of the experiment (4^{th} week). All experimental procedures were previously approved by the Institutional Ethics Committee of the Unipar (authorization 22833/2012). **Results:** The data revealed that the HETM was able to sustain its diuretic and natriuretic effect even after 4 weeks of treatment in OVX-rats. Moreover, their use has not affect the urinary excretion of calcium and potassium and reduced TC and LDL-C levels. Additionally, HETM-treatment maintained osteocalcin levels similar to those obtained in OVX-animals treated with vehicle alone. All others parameters showed no statistically significant differences between all experimental groups. **Discussion:** The results presented here demonstrate that HETM is able to sustain its diuretic activity after prolonged treatment in ovariectomized rats. Furthermore, we observed a significant natriuretic and potassium and calcium-sparing effect without adversely affecting serum lipids or osteocalcin levels. Although the flavonoid isoquercitrin was identified as the likely responsible for the diuretic effects of HETM (Gasparotto Junior *et al.*, 2011; 2012), further studies are needed to evaluate its role as a protective agent in this experimental model. Taken together, these findings support the potential of a preparation derived from *Tropaeolum majus* as a candidate to be a phytomedicine used as a diuretic in patients with low levels of estrogen. **Acknowledgments:** We are grateful to Diretoria Executiva de Gestão da Pesquisa e Pós-Graduação (DEGPP/UNIPAR-Brazil) for a financial support. **References:** Gasparotto Junior, A. *et al.* Natriuretic and diuretic effects of *Tropaeolum majus* (Tropaeolaceae) in rats. *J Ethnopharmacol* vol. 122, p. 517-22, 2009. Gasparotto Junior, A. *et al.* Diuretic and potassium-sparing effect of isoquercitrin-An active flavonoid of *Tropaeolum majus* L. *J Ethnopharmacol* vol. 34, p. 210-15, 2011. Gasparotto Junior, A. *et al.* Mechanisms underlying the diuretic effects of *Tropaeolum majus* L. extracts and its main component isoquercitrin, *J Ethnopharmacol* vol. 14, p. 501-09, 2012.

09.020

Effects of PhTx3-3 AND PhTx3-6 toxins obtained from the Brazilian spider *Phoneutria nigriventer* on glioma cells. Nicoletti NF[1,2], Erig TC[3], Campos MM[1,2,4], Gomez MV[5], Morrone FB[1,2,3] [1]INTOX-PUCRS, [2]PUCRS – Biologia Celular e Molecular, [3]PUCRS – Farmácia, [4]PUCRS – Odontologia, [5]UFMG – Neurociências

Introduction: Recent studies have suggested a possible implication of voltage-gated calcium channels (VGCC) in the mechanisms of proliferation, angiogenesis and invasion of gliomas (Wen, PY *et al*; *N Engl J Med* 359: 492, 2008; Pinheiro, AC *et al*; *Hippocampus* 19:1123, 2009). This study evaluated the effects of selective P/Q- and N-type VGCC blockers on glioma cells. **Methods:** Human glioma cells U251MG and U138MG were seeded in 96 or 24-well plates, at densities of $2x10^3$ to $5x10^3$ cells/well and treated for 24 h with the selective P/Q- and N-type VGCC inhibitors PhTx3-3 and PhTx3-6 (0.3 – 300 pM from *Phoneutria nigriventer*) or ω-conotoxin MVIIC and MVIIA (0.3 – 300 pM from *Conus magnus*). After 24 h, the cells were counted in hemocytometer and the viability was assessed by MTT assay. Cell death and proliferation were also analyzed by flow cytometry, using Annexin V/PI staining and Ki67 protein, a cellular marker associated with cell proliferation. The cells were treated with PhTx3-3 (10 pM) and PhTx3-6 (10 pM) toxins, or MVIIC (10 pM) and MVIIA (10 pM). Experiments were performed on FACSCanto II BD Biosciences. **Results:** PhTx3-3 and PhTx3-6 toxins displayed a significant inhibitory effect on proliferation and viability of U251MG and U138MG cells, in a concentration-dependent manner. The PhTx3-3 maximal anti-proliferative effects were 43 ± 11% (3 pM) and 52 ± 1% (3 pM) and it inhibited the viability in 20 ± 3% (3 pM) and 15 ± 3% (3 pM), respectively. PhTx3-6 showed maximal inhibitions of 43 ± 6% (30 pM) and 49 ± 6% (30 pM) on proliferation, and 23 ± 4% (10 pM) and 27 ± 4% (10 pM) on viability, respectively. The treatment with MVIIA diminished the proliferation by 34 ± 7% (10 pM) and 33 ± 2% (10 pM), and the viability by 21 ± 5% (3 pM) and 37 ± 6% (30 pM), respectively. Otherwise, MVIIC failed to affect the proliferation rates in either tested concentration. The flow cytometry analysis showed a marked cell death with apoptosis characteristics, according to assessment of AnnexinV/PI positivity. PhTx3-3 and PhTx3-6 evoked 82% and 84% of death in U251MG cells, and 86% and 52% in U138MG human cells, respectively. The ω-conotoxin MVIIA displayed a similar profile of apoptotic death, with inhibitions of 67% in U251MG and 77% in U138MG cells, while MVIIC induced death with necrosis features. Furthermore, PhTx3-3 and PhTx3-6 markedly reduced Ki67 labeling, by 69% and 55% in U251MG cells, and 69% and 79% in U138MG cells, respectively. The same inhibitory effect on proliferation was seen for MVIIA treatment with inhibitions of 74% for both U251MG and U138MG cells, while MVIIC failed to affect the proliferation of these cell lines. **Discussion:** Our data indicates a potential protective role for the Brazilian spider *P. nigriventer* toxins PhTx3-3 and PhTx3-6 on growth and invasiveness of glioma cells, pointing out P/Q-, and especially N-type VDCC, as therapeutic targets for this tumor type. Further studies are being carried out to assess the *in vivo* effects of this toxin on glioma progression. **Financial support:** PRPPG/PUCRS, Capes-AUX-PE Toxinologia, CNPq and FINEP/PUCRSINFRA #01.11.0014-00.

09.021

Healing potential of the ointment *Jacaranda decurrens* Cham. in skin injury in mice.

Serra MB, Abreu IC, TORRES NA, Sodré TP, Silva SN, Rocha AO, Ferreira FS, Lima NFM, Borges MOR, Borges ACR UFMA – Farmacologia.

Introduction: The species *Jacaranda decurrens* CHAM., popularly known as "carobinha" is from Brazilian cerrado, and presents prevalence in the state of Maranhão, popularly used as healing, blood depurative, and for treating skin disturbances caused by syphilis. **Methods:** To test the healing activity of the plant, it was obtained the leaves sprayed plant's hydroalcoholic extract (JHE) by the method of maceration in 70% ethanol and hydromodule of 1:10, concentrated in a rotary evaporator, lyophilized and used in ointment form. Thirty adults Swiss mice were divided into five groups (GI and GII – treated with saline solution and by bases of ointment, respectively); (GIII – treated with FIBRASE® ointment); (GIV and GV – treated with ointment of JHE at doses of 100 and 50 mg/kg, respectively). The trichotomy was held in the animals' dorsal region, and incision with assistance of a surgical punch (diameter of 1 cm^2), reporting daily developments. At the end of the 13th postoperative day (POD), the animals were euthanized and histological slides were prepared. **Results and discussion:** The results were obtained by the pachymetry of the wound, macroscopic and microscopic analysis. It has been found more than 30% regression of the initial area of the injury in the groups treated with JHE at doses of 100 and 50 mg/kg in the early 24 hours of application of the ointment, and lower than 20% in the other groups. In the microscopic analysis was observed increased re-epithelialization and collagen deposition and smaller neovascularization in JHE groups when compared to the others groups. On this way, it can be concluded than the JHE favored the cutaneous tissue healing of mices, accelerating the injury contraction, smudge formation, collagen deposition and re-epithelialization. Key-words: *Jacaranda decurrens* CHAM. Formulation. Healing. Number of the Ethics Committee: P07/06 **Support**: FAPEMA/CNPq

09.022

Cimicifuga racemosa attenuates inflammatory osteolysis in a rat model of periodontitis.

Medeiros AC[1], Oliveira JM[2], Bastos BM[1], Bastos JM[3], Parente ATPP[1], Filho SMP[1], Teixeira AH[2], Aguiar LMV[1], Pereira KMA[3], Benevides NBB[4], Filho GC[1], Pinto VPT[7], Silva AAR[3], Dutra PGP[3], Brito GAC[5], Chaves HV[3], Bezerra MM[1] [1]UFC-Sobral – Medicine, [2]Renorbio-UFC, [3]UFC-Sobral – Dentistry, [4]UFC – Biochemistry and Molecular Biology, [4]UFC – Morphology

Introduction: Periodontitis is a chronic inflammatory process that affects the supporting tissues of teeth characterized by extensive alveolar bone resorption. In periodontitis, increased inflammation and bone loss is modulated by proinflammatory cytokines such as TNF-α. *Cimicifuga racemosa* (Ranunculaceae) has been used in traditional medicine due to its analgesic and anti-inflammatory properties, and for the treatment of menopause symptoms. Aplause® is a commercial formulation that has as its active compound the dry extract from both rhizome and root of *Cimicifuga racemosa* (Cr). This study aimed to evaluate the effectiveness of *Cr* on periodontitis in rats, investigating the putative involvement of TNF-α, and the safety of this treatment. **Methods**: This study was conducted with the approval of the Ethics Committee of the Federal University of Ceará, Fortaleza, Brazil (CEPA no. 08/13). Periodontitis was induced by placing a nylon thread (3.0) in the upper molars of female Wistar rats (180-200 g). Rats (6 *per* group) were weighed and treated (*per os*) daily with *Cr* (Aplause® tablets of 20 mg from Marjan Pharmaceutical Industry, Brazil) (0.01, 0.1 or 1 mg/kg) or vehicle (saline) for 11 days. Following the treatment course, alveolar bone resorption (mm^2) was measured using the ImageJ® software. Periodontal tissues were analyzed by histopathology (H&E) and by ELISA assay to determine the levels (pg/ml) of TNF-α. Gastric mucosa was examined macro and microscopically (H&E). Peripheral blood was collected for biochemical analysis (alanine amino transferase – AST, aspartate amino transferase – ALT, creatinine, and total alkaline phosphatase – tAP). **Results and discussion:** *Cr* (0.01, 0.1 or 1 mg/kg) reduced ($p<0.05$) alveolar bone resorption (1.99 ± 0.46 mm^2; 1.55 ± 0.31 mm^2 and 1.64 ± 0.57 mm^2, respectively), compared to the vehicle group (3.60 ± 0.31). These data were confirmed by histopathology analysis of *Cr* (1 mg/kg) that showed discrete cell influx, reduction in osteoclast number, cementum and alveolar process well preserved [1(0-1)], compared to the vehicle group [2(1-3)]. Further, *Cr* (1 mg/kg) decreased ($P<0.05$) TNF-α levels in gingival tissues (17.99 ± 1.67 pg/ml), compared to the vehicle group (42.57 ± 2.8). *Cr* treatment did not change the macroscopic and histopathological analysis of gastric mucosa. Serum levels of ALT/AST, creatinine and tAP did not differ from respective controls. Experimental periodontitis in rats represents an attractive model to evaluate bone loss *in vivo*. *Cr* is safe and it reduces the alveolar bone resorption in periodontitis, at least in part, by reducing TNF-α levels. **Funding Sources:** FUNCAP, CNPq, Capes, and INCT-IBISAB.

09.023

Effects of McLTP1 from *Morinda citrifolia* (noni) in proteolytic activity by *Bothrops insularis*. Lopes PL[1], Marinho AD[1], Alves NTQ[1], Jorge RJB[1], Silveira JAM[1], Costa PHS[1], Silva PLB[1], Pimentel MD[1], Vasconcelos MJS[1], Oliveira HD[2], Campos DCO[2], Monteiro HSA[1]
[1]UFC – Farmacologia e Fisiologia, [2]UFC – Bioquímica e Biologia Molecular

Introduction: Snakebites are a public health problem in tropical regions in the world, especially in developing countries of South America, Africa and Asia, an important cause of morbidity and mortality and included by the World Health Organization (WHO) list of diseases neglected (Harrison, *PLoS Neglected Tropical Diseases*, v3, n12, p.569, 2009). *Bothrops* species venoms quickly develop severe local tissue damage, including swelling, hemorrhage, myonecrosis, skin ulceration and pain (Gutierrez, *Toxicon*, v54,p.976,2009). **Methods:** Proteolytic activity was tested on azocasein using 96 well plates as described by Rucavado *et al.* (2008). McLTP1 is a protein extract from the seeds of *Morinda citrifolia*. To obtain the extract, the macerated fruit seeds were suspended in extraction buffer pH 8.5. The suspension was filtered through fine mesh cloth. The filtrates were centrifuged at 10000 x *g* (30 min). For the fractionation of protein was added trichloroacetic acid (TCA) 20% to the total extract to achieve final concentrations of 0.5, 1.0, 2.0, 2.5 and 5.0% TCA. After addition of the acid, the extract was centrifuged at 10000 x *g* (30 min). The supernatant and the precipitate (separately) were subjected to extensive dialysis against distilled water (*cut off* 12kDa). The *B. insularis* venom (BiV) or mixtures containing BiV and the McLTP1 (1:1; w:w) were diluted in the reaction buffer (25mM Tris,150mM NaCl,5mM $CaCl_2$,pH7.4) at 1mg/mL. 10mL of this solution were incubated with 100µL of an azocasein solution (5mg/mL in the reaction buffer) at 37ºC for 60 min, after what the reaction was stopped by the addition of 200µL of 5% TCA, and centrifuged at 3500 rpm (5min). 100µL of the supernatant were transferred to another plate, to which were added 100µL of 0.5M NaOH. The absorbance was read at 450nm. The proteolytic activity was normalized considering the venom alone as 100% of activity. The results were submitted to analysis of variance (ANOVA), $p<0,05$. Etic committee protocol approval: 68/08. **Results:** The whole venom of *B. insularis* presented a percentage of proleolytic activity 16.95 ± 3.61, while when preincubated McLTP1, this activity was significantly reduced to 6.45 ± 0.58. **Discussion:** The proteolytic enzymes present in the snake venoms cause local hemorrhage and necrosis through the disruption of the protein arrangements in the basal membrane of capillaries and micro vessels. The damaged vessels enable the blood cells to come into the extravascular space causing an inflammatory reaction, also interrupting the oxygen supply. The inhibition of proteolytic activity by McLTP1 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* (Baldo, *PLoS Neglected Tropical Diseases*, v.4, n.6, p.727, 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:** *Fundação Cearense de Apoio ao Desenvolvimento Científico e Tecnológico*

09.024

Evaluation of acute toxicity of *Pedilanthus tithymaloides* (L.) *Poit.* in mice. Moura APG[1], Meireles DRP[1], Fernandes HMB[1], Xavier AL[1], González TL, López V de la P, Tavares JF[1], Silva MS[1], Sobral MV[1] DCF-UFPB

Introduction: *Pedilanthus tithymaloides* (L.) Poit. is considered a medicinal species of Cuban flora, belongs to the Euphorbiacea family, commonly known as Real díctamo and ipecacuana. Popularly, it is use for by people to treat oral thrush, stomatitis and gingivitis (Roig, JT Plant med Cuba, 458:59, 1974) . Phytochemical and pharmacological studies of ethanol extract of leaves showed antibacterial and antifungal properties (Vidotti, J G. *et al.* Fitoterapia, 43:46, 2006). The popular use for medicinal purposes justified the interest and importance of studies about their toxicological aspects. **Methods**: The experiment of acute toxicity was according to "Guide For The Studies Of Toxicity Pre-Clinical Herbal, which is regulated by the Specific Resolution (ER) No 90/2004 of "National Health Surveillance Agency" and It was approved by the Animal Studies Committee of the UFPB (n. 0508/ 13). Swiss albino mice, six males and six females per group, the animals were treated with a single dose of 2000 mg/kg of extract hydroalcoholic *Pedilanthus tithymaloides* (v.o), and the control group was treated only with the vehicle (tween 80, 5%). In order to map possible central and autonomic behavioral changes, observation to detect toxic signs was performed at the intervals: 0, 15, 30 and 60 minutes; after 4 hours and daily for 14 days (Almeida, R. N., *Rev Sci Farmacogn*, 80: 72, 1999). After of the observation period, hematological evaluations were performed. Furthermore, indexes of organs (organ mg/animal g), liver, heart, kidneys, spleen and thymus were evaluated. The results were expressed as mean ± standard error of the mean (SEM) and analyzed by Student's Test and were considered significant when $p<0.05$. **Results**: No behavioral changes were observed and no death occurred in animals treated with the extract, so it is estimated that the LD50 ≥ 2000 mg/kg In addition, no significant differences in water and food consumption. No significant differences in the indexes of heart, liver, kidneys, spleen and thymus were observed, both in males and females, when compared with the control group. There was only a slight change in the differential leukocyte in females treated on lymphocytes (78.67 ± 5.86%)* compared with control counts (69,60 ± 2,99%) and neutrophil whit (17.67 ± 5.00%)* and the control (24,60 ± 2,36%). **Discussion:** The preclinical toxicology study of a product is important for its safe use it aims to characterize the toxic effects produced from their administration. Metabolic parameters, such as drinking water and food, and weight assessment, should be analyzed in preclinical studies to investigate the toxicity of a sample study of the gastrointestinal system. Another important parameter to evaluate the toxicity of drugs are haematological changes such as altering the size and concentration of blood cells. The change on the observed in white blood cells of females does not possess clinical relevance because the values are within normal range for mice. So, the extract hydroalcoholic *Pedilanthus tithymaloides* has low toxicity in experimental models evaluated, providing important data to it safety for popular use and to conduct subsequent pharmacological studies (Evans, G.O*., Animal Hematotoxicology*, 121, 2008). **Financial support**: CNPq

09.025

Antioxidant activity of pequi leaves. Duavy SMP[1], Seeger RL[2], Ramos A[2], Costa JGM[1], Barbosa NBV[2] [1]URCA, [2]UFSM

Introduction: Due to a large number of diseases are associated with oxidative damage, the search for natural products with antioxidant potential has increased considerably in recent decades (HEMPEL, S.L. Free radic. biol. med. v. 27, p.146, 1999). In this context highlights the role of medicinal plants, which generally have a high amount of substances with antioxidant activity, such as phenolic compounds (ASCARI, *J Bol. Latinoam. Caribe Plantas Med. Aromat*. v. 9, p. 20, 2010). The plant *Caryocar coriaceum* (Caryocaraceae) is an endemic species of the Araripe, Ceará. Its fruit, popularly known as pequi, is used in local cooking and traditional medicine for treating infection and inflammation processes (ROESLER, R. Ci. *Tecnol Aliment*, v. 27, p. 53, 2007). Some studies have demonstrated the antioxidant and nutritional properties of pequi pulp, highlighting its chemical composition represented by oleic acid (QUIRINO, G da S. Phytochemistry letters. v. 2, p. 179, 2009). However, there is little research on the leaves of *Caryocar coriaceum*, a part also used in folk medicine. Thus, the present study evaluated the antioxidant of leaves aqueous extract (LAE) from *Caryocar coriaceum in vitro* and *in vivo* assays. **Methods:** The chemical composition of the LAE was identified by HPLC. The antioxidant potential of LAE was analyzed by its ability in reducing the formation of radical DPPH$^{\bullet}$, reactive oxygen species (ROS) (oxidation of dichlorofluorescein diacetate – DCFH2 assay) and lipid peroxidation (thiobarbituric acid reactive substances – TBARS levels test) as well as in reducing or chelating iron ions (1,10-phenanthroline-chelate test). This work was carried out in accordance with the Guideline of the Ethical Committee and Use of Animals of UFSM and approved by the institucional review board of UFSM (0089.0.243.000-07). **Results and discussion:** Quantitative analysis – HPLC of phenolic compounds determined the majority presence of chlorogenic acid (45.90 mg/g) and quercetin (28.61 mg/g). The LAE, at concentration of 25 µg/mL, reduced approximately 80% of DPPH$^{\bullet}$ formation when compared to the ascorbic acid. The LAE decreased significantly the lipid peroxidation iron-induced in rat liver homogenate. Indeed, the LAE, at concentrations of 25 and 50 µg/mL, reduced significantly the levels of ROS induced by Ca^{+2} 200µM in liver samples. The LAE (10 µg/mL) was also able to reduce Fe^{+3} to Fe^{+2}. The study of leaves from *C. coriaceum* possible the scientific knowledge of the pharmacological properties of the plant part unexplored. Our data indicate that the LAE exhibited antioxidant properties *in vitro* and *in vivo*, other further experiments are needed. **Financial support**: Capes, CNPq and FAPERGS

09.026

Evaluation of antidiarrheal and antispasmodic activities of total glycoalkaloids fraction from *Solanum crinitum* Lam. (Solanaceae). Ferreira SRD[1], Moreno GTA[1], Oliveira FRMB[1], Souza ILL[2], Silva TMS[3], Correia ACC[4], Cavalcante FA[5] [1]UFPB, [2]UFPB, [3]DCM-UFRPE, [4]ICBS-UFAL, [5]DFP-UFPB

Introduction: Many species of *Solanum* (Solanaceae) are used in folk medicine to treat several diseases, including gastric disorders (MESIA-VELA, Phytomedicine, v. 9, p. 508, 2002), muscle spasms and diarrhea (ABEBE, J. Ethnopharmacol., v. 18, p. 147, 1986). *S. crinitum* Lam., popularly known as "jurubeba" and "fruto-de-lobo", is a shrub or small tree (CORNELIUS, *J Braz Chem Soc*, v. 21, p. 2211, 2010). Thus, we investigated the antidiarrheal and antispasmodic activities of total glycoalkaloids fraction from *S. crinitum* (TGF-SC). **Methods:** In antidiarrheal activity model (n=6), mice were divided into negative control (saline 10mL/kg plus Cremophor®, p.o.), positive control (loperamide 10 mg/kg, p.o.) and TGF-SC (6.5–100 mg/kg, p.o.). After 30min, from respective treatment diarrhea was induced by oral administration of castor oil 10mL/kg. Total number of faecal output and wet faeces excreted by animals was recorded (4h). To assess the activity of TGF-SC in the intestinal transit, mice were divided into negative control (saline 10mL/kg plus Cremophor®, p.o.), positive control (atropine 2 mg/kg, p.o.) and TGF-SC (6.25-100 mg/kg, p.o.). Animals were euthanized 30 min after administration of activated charcoal 5% (0.01mL/animal g, p.o.), the intestine was isolated and determined the distance traveled by marker in absence or presence of castor oil. To investigate the antispasmodic activity (n=5), ileum was suspended in organ baths containing modified Krebs solution under appropriate conditions. Two similar phasic contractions were obtained with carbachol (CCh) or histamine 10^{-6}M and TGF-SC (9-729 µg/mL) was pre-incubated for 15 min. A new phasic contraction to contractile agents was obtained and inhibitory effect was measured. Relaxant effect of TGF-SC (1–729 µg/mL) also was evaluated on ileum pre-contracted with KCl 40mM, CCh 10^{-5}M or histamine 10^{-6}M. All the experimental protocols were approved by Ethical Committee in Animal Use of UFPB (Protocol 3206/13). **Results:** TGF-SC produced a dose-dependent and equipotent antidiarrheal effect on both defecation frequency (ED_{50}=27.4 ± 6.3 mg/kg) and wet faeces (ED_{50}=13.5 ± 2.5 mg/kg). TGF-SC inhibited in a dose-dependent manner both normal (ED_{50}=37.4 ± 2.9 mg/kg) and castor oil-induced intestinal transit (ED_{50}=15.1 ± 3.8 mg/kg). On guinea pig ileum, TGF-SC also antagonized phasic contractions induced by CCh (IC_{50}=61.7 ± 4.9 µg/mL) or histamine (IC_{50}=38.8 ± 4.1 µg/mL) and relaxed the ileum pre-contracted with KCl (EC_{50}=88.0 ± 15.3 µg/mL), CCh (EC_{50}=54.2 ± 4.7 µg/mL) and histamine (EC_{50}=16.1 ± 2.3 µg/mL). **Discussion:** Our results demonstrate that TGF-SC shows antidiarrheal activity, decreasing both defecation frequency and liquid stool. This activity of the TGF-SC may be explained by changes in motility since it inhibited intestinal transit induced by castor oil. Once TGF-SC alters intestinal motility, we decided to investigate a possible antispasmodic effect. The fraction showed high inhibitory potency in both phasic and tonic histamine-induced contractions, suggesting that TGF-SC may be acting in histaminergic receptor level. Moreover, TGF-SC could be acting by inhibiting Ca^{2+} influx through voltage-gated Ca^{2+} channels (Ca_V), since tonic component is maintained by the Ca_V opening. **Financial support:** PIBIC/CNPq/UFPB.

09.027

Protective effects of *Euterpe oleracea* Mart. (An Amazon Plant) on experimental metabolic syndrome in C57/BL6 mice. Costa CA[1], Oliveira PRB[1], Rocha APM[2], Carvalho LCRM[1], de Bem GF[1], Santos IB[1], Souza MAV[1], Cunha PJ[3], Soares de Moura R[1], Resende AC[1] [1]UERJ, [2]UNIRIO, [3]UFPA

Introduction: Metabolic syndrome (MS) is identified as an association of risk factors and a major cause of morbidity and mortality worldwide. It combines disturbances in glucose and insulin metabolism, excess predominantly abdominally distributed weight, dyslipidemia, a proinflammatory state, and hypertension, with the subsequent development of obesity, type 2 diabetes, and cardiovascular disease. The pathological effects of this syndrome are due largely to insulin resistance with excessive fatty acid flux. Many aspects of MS can be induced in experimental models such as C57BL/6 mice by manipulation of the diet given. *Euterpe oleracea* Mart., also known by the popular name of açaí, is widely diffused in Amazon region of Brazil, and rich in polyphenols has shown great therapeutic potential, since it has a potent vasodilator, antioxidant and antihypertensive action. Recent studies suggest that polyphenols reduce the incidence of cardiovascular diseases. The aim of study was designed to determine the protective effects of *E. oleracea* (açaí) stone extract (ASE, 300 mg/kg/day), rich in polyphenols, in C57BL/6J mice fed a high-fat diet (HFD) that delineate components of metabolic syndrome. All experiments on animals were reviewed and approved by the Animal Care and Use Committee of the Biology Institute Rio de Janeiro State University (protocol: CEA/025/2010). **Methods:** Male C57BL/6 mice at 6 weeks of age were housed in individual cases in a temperature-controlled room with 12-hour light/dark cycle and randomly allocated into a 4 groups. The control group was fed with a standard diet and received water (control group: 10% fat) or ASE (ASE group, 300 mg/kg/day). Two other groups were fed a HF diet with access to water (HF group: 60% fat) or ASE (HF + ASE group: 60% fat) for 12 weeks. The dose of ASE was based on pilot experiments that showed significant changes in the components of MS induced in HF group. The chronic treatment (12 weeks) with ASE was based on the period of time necessary to induce the metabolic alterations and vascular dysfunction in the C57BL/6 mice fed. The diets were elaborated by Rhoster (São Paulo, Brazil) in accordance with the standard recommendations for rodents in the maintenance state of American Institute of Nutrition (AIN-93M). Body weight, plasma and liver lipids, glucose and insulin plasma levels were determined and the insulin resistance measured by HOMA index. Lipid peroxidation, carbonyl protein, antioxidant activities were evaluated in liver homogenate by spectrophotometry. Expression of SOD-2, IRS1, phosphorylated IRS1 (pIRS1), PI3-K, phosphorylated AKT (pAkt) and tubulin protein were evaluated in liver homogenate by western blotting. **Results:** Increased body weight, plasma and liver lipids, glucose levels and insulin resistance, lipid peroxidation and carbonyl protein were observed in HFD and reduced by ASE. Antioxidant activities and expression of proteins were reduced in HFD and increased by ASE. **Discussion:** The results demonstrate a protective effect of an extract obtained from açaí stone in metabolic syndrome, since the obesity, hypercholesterolemia, hyperglicemia and insulin resistance induced by HFD were reduced by oral treatment with the extract. **Financial support**: CNPq and Faperj.

09.028

Antidepressant-like effect of *Ilex paraguariensis* in rats. Reis EM[1], Neto FWS[2], Cattani VB[2], Peroza LR[3], Busanello A[1], Leal CQ[4], Lehmen T[4], Libardoni M[4], Bressan GN[4], Boligon AA[5], Athayde ML[5], Fachinetto R[1,3] [1]UFSM – Farmacologia, [2]UNICRUZ – Farmácia, [3]UFSM – Ciências Biológicas / Bioquímica Toxicológica, [4]UFSM – Farmácia, [5]UFSM – Farmácia Industrial

Introduction: *Ilex paraguariensis* is a plant used as an infusion known as "chimarrão" or "mate", which is a stimulating beverage largely consumed in South America.. In this study, we investigated the possible antidepressant-like effect of *I. paraguariensis* in rats. **Methods:** Animals were maintained and used in accordance to the guidelines of the National Council for the Control of Animal Experimentation (Ethics Committee approval number A011-09). Rats were divided into two groups: control group received water *ad libitum*; *I. paraguariensis* group received during 24 hours or 4 weeks an aqueous extract of *I. paraguariensis* in the place of drinking water.. An additional group was injected with selegiline or saline 24 hours and 30 minutes before forced swimming test as positive control. An aliquot of the aqueous extract was used for phytochemical analysis by HPLC-DAD. After 24 hours or 4 weeks of treatment, behavioral (elevated plus-maze, open field test, and forced swimming test) and biochemical parameters (lipid peroxidation assay, thiol content, vitamin C levels, and monoamine oxidase activity) were evaluated. Data were analyzed by unpaired test t or one-way ANOVA, followed by Tukey's post-hoc test when appropriate. Significance was considered when $p<0.05$. **Results:** HPLC analysis of *I. paraguariensis* aqueous extract revealed the presence of gallic acid, catechin, chlorogenic acid, caffeine, quercetin, rutin, kaempferol, caffeic acid and theobromine. *I. paraguariensis* treatment did not cause any effect on locomotor activity. Also, any anxiolytic/anxiogenic effect of *I. paraguariensis* was observed in rats through the elevated plus-maze test. However, the *I. paraguariensis* extract was able to reduce the immobility time on forced swimming test in relation to control group either when administered during 24 hours ($F(2,11)=18.73$, $p<0.05$) or 4 weeks ($F(2,19)=25.45$, $p<0.05$) which was not accompanied by inhibitory effect on monoamine oxidase activity in brain tissue. Selegiline, caused a significant reduction of immobility time and a significant reduction in MAO-B activity ($F(2,11)=78.80$, $p<0.05$) when compared with control group. Any significant alteration was verified on lipid peroxidation, thiol levels, and ascorbic acid levels among the groups. **Discussion:** In conclusion, *Ilex paraguariensis* reduced immobility time on forced swimming test which could suggest an antidepressant-like effect. This effect does not seem to be associated with MAO inhibition or alterations in oxidative stress parameters. Furthermore, the aqueous extract of *I. paraguariensis* did not show anxiolytic or anxiogenic activity. However, additional studies are needed to elucidate the action mechanism of *I. paraguariensis*. **Financial support:** The financial support by PRONEM no. 11/2029-1, CNPq (473365/2009-0), and FINEP Research Grant from "Rede Instituto Brasileiro de Neurociência (IBN-Net)" (01.06.0842- 00) are gratefully acknowledged.

09.029

Effect of low level laser (LLL) in the expression of myod and myogenin in myoblast differentiation submitted to *Bothrops jararacussu* snake venom (BjssuV). Silva LMG[1], Silva CAA[2], Zamuner SF[1], Cogo JC[3], Zamuner SR[1] [1]Uninove – Medicina, [2]Uninove – Ciências da Reabilitação, [3]Univap – Fisiologia

Introduction: Local myonecrose is a common consequence in envenoming caused by snakes of the genus *Bothrops*. Antivenom therapy and other first-aid treatments do not reverse the local myonecrose. Thus, there is an urgent need to find therapies that can complement antivenoms in the neutralization of local tissue damage. The aim of this study is to analyses the effect of LLL on the expression of MyoD and myogenin in myoblast differentiation submitted to injury by Bothrops *jararacussu* Snake Venom (BjssuV). **Methods:** C2C12 myoblast were divided into tubes according to each experimental group and received the venom (12.5 mg/mL) or culture medium alone (control) and were centrifuged for 2 min. Cells were irradiated at the bottom of the tube for 13s with a laser at 635 and 830 nm, 4 J/cm^2 dose and power of 100 mW. Subsequently, the cells were plated on 13 mm coverslips in 24 well plates and incubated for 15, 30 and 60 min. Then, supernatant were removed and immediately adding to cells DMEM medium, containing 2% horse serum to induce differentiation, and incubated at 37°C with 5% CO2 for 4 days. The differentiation of myoblasts was determined by morphological analysis of the formation of multinucleated myotubes. MyoD and myogenin expression were evaluated by western blotting at 1 and 3 days after myoblast differentiation. **Results:** Untreated cells incubated with 2% horse serum exhibited an elongated and slim form, all cells incubated with venom died, it occurred in all periods of incubation. However, cells that received the venom and were laser irradiated exhibited a similar morphology to the control cell, showing elongated cells and slim form, which characterizes the differentiation of C2C12 muscle cells. The cells that were treated with venom and laser irradiated significant increased MyoD expression at 30 and 60 min and myogenin expression at 60 min of venom incubation compared to control cells. **Discussion:** BjssuV is toxic to muscle cells. The LLL protects against this effect and causes myoblast differentiation by increasing miogenic factors such as MyoD and myogenin. **Financial Support:** 2011/04660-0 and 2011/14376-7 São Paulo Research Foundation (Fapesp).

09.030

Essential oil from *Xylopia frutescens* Aubl. blocks Ca_V1 and inhibits Ca^{2+} mobilization to relax guinea pig ileum. Souza ILL, Araujo LCC, Vasconcelos LHC, Silva MCC, Ferreira PB, Costa VCO, Tavares JF, Cavalcante FA, Silva BA UFPB

Introduction: *Xylopia frutescens* Aubl. (Annonaceae) is popularly known as "embira", "semente-de-embira" or "embira-vermelha" (COSTA, *Acta Farm Bon*, v. 25, p. 184, 2006) and used in folk medicine as antidiarrheal agent (DUKE, *Amazon Ethnobotanic Dic*, v. 1, p. 205, 1994). In previous studies, results of essential oil obtained from leaves (XF-EO) showed inhibition of carbachol (CCh)- or histamine-induced phasic contractions on guinea pig ileum (CORREIA, 2013, IX Simpósio Brasileiro de Farmacognosia, Goiânia/GO). Thus, this study aimed to investigate the spasmolytic action of XF-EO on guinea pig ileum. **Methods:** Ileum was suspended in organ baths under appropriate conditions (n=5). Histamine (10^{-9}-10^{-4}M)- or $CaCl_2$ (10^{-5}-$3x10^{-1}$M)-induced cumulative contractions, histamine (10^{-5}M)- or CCh (10^{-6}M)-induced phasic contractions were obtained and the XF-EO (1-729 µg/mL) inhibitory effect was registered. Tonic contractions induced by KCl 40mM, histamine 10^{-6}M, S-(-)-Bay K8644 $3x10^{-7}$M were obtained and the XF-EO (0.1-729 µg/mL) relaxant effect was registered, in some experiments the CsCl 5 mM was added before the histamine tonic contraction. All experimental protocols were approved by Ethical Committee on Animal Use of UFPB (Protocol 0611/13). **Results:** XF-EO (9-81 µg/mL) antagonized histamine-induced cumulative contractions, shifting them to the right, in a non-parallel manner, with maximum effect (E_{max}) reduction that indicates a non-competitive antagonism profile. XF-EO (0.3-729 µg/mL) relaxed the pre-contracted ileum with both KCl (EC_{50}=13.9 ± 1.6 µg/mL) or histamine (EC_{50}=7.1 ± 0.6 µg/mL). In the presence of CsCl (non-selective K+ channels blocker), the relaxant potency of XF-EO (0.1-81 µg/mL) was not altered (EC_{50}=7.7 ± 1.2 µg/mL). In a depolarizing medium nominally without Ca^{2+}, XF-EO (9-81 µg/mL) inhibited $CaCl_2$-induced cumulative contractions and these were shifted to the right, in a non-parallel manner, with E_{max} reduction as well as XF-EO (0.1-729 µg/mL) relaxed the pre-contracted ileum with S-(-)-Bay K8644 (EC_{50}=74.5 ± 8.2 µg/mL), a Ca_V1 selective agonist. In experiments with ileum circular muscle layer, XF-EO (1-729 µg/mL) antagonized phasic contractions induced by both histamine (IC_{50}=2.8 ± 0.1 µg/mL) or CCh (IC_{50}=76.5 ± 10.1 µg/mL), being more potent to histamine. **Discussion:** As cited above, XF-EO antagonized the histamine-induced contractions and relaxed the guinea pig ileum. Since the relaxant potency was smaller on organ pre-contracted with KCl, it was hypothesized that XF-EO would be acting as a K+ channel positive modulator. However, a non-participation of K+ channels on the essential oil spasmolytic action was verified in CsCl presence. Moreover, XF-EO inhibited the $CaCl_2$-induced contractions and relaxed pre-contracted ileum with S-(-)-Bay K8644, demonstrating that XF-EO inhibits Ca^{2+} influx through Ca_V1. As the Ca^{2+} mobilization mechanism into circular and longitudinal muscle layers are different, it was showed that XF-EO antagonized phasic contractions on ileum circular layer, so, it indicates that the essential oil also modulates negatively the Ca^{2+} release to exert their spasmolytic effect. Thus, XF-EO showed to be a promising essential oil that can be used to gastrointestinal disorders treatment. **Financial support:** CNPq, Capes, PPgPNSB/CCS/UFPB.

09.031

Phytochemical characterization of green tea leaves (*Camellia sinensis* (L.) Kuntze).

Parente JM, Medeiros MAS UNIFOR

Introduction The green tea is a beverage originated from the infusion of the dried leaves of the plant *Camellia sinensis*, which belongs to the family Theaceae and it is native to Asia. Freshly collected leaves and immediately stabilized characterize themselves as green tea. This procedure preserves the polyphenol content of the leaves and makes them rich in catechins. The benefits of green tea include the reduction of cholesterol levels and anti-inflammatory and antioxidant activities, which helps in the prevention of chronic degenerative diseases, such as cancer or cardiovascular problems [1]. The objective of this study was to make a phytochemical characterization of green tea leaves, in order to identify its main components. **Methods** The green tea used was purchased in local trade (Fortaleza – CE, Brazil). It was leaves of *C. sinensis* produced in Brazil, dried, pounded until fine powder texture and packed in sachet. The sachets acquired were opened and their contents were used in the analysis. It was prepared four types of extract: Aqueous, aqueous acid, chloroformic and hydroalcoholic, all in the concentration of 10%. According to the methodology proposed by Matos [2], the phytochemical characterization was performed for the identification of the following classes of natural compounds: In the aqueous extract, saponins (triterpenoidals and steroidals), aurones, chalcones, phenols and tannins; In the aqueous acid extract, alkaloids and quaternary bases; In the hydroalcoholic extract, quinones, anthranols, anthocyanins, anthocyanidins, catechins, flavonols, flavones, flavononols, leucoanthocyanidins, flavanones, xanthones and digitalis; and in the chloroformic extract, coumarins, steroids and triterpenoids. It was also made the prospect of plant constituents, for lookup of cyanogenic heterosides. All protocols used were approved by UNIFOR Research Ethical Committee (number 327/1). **Results** The classes present in the sample of green tea were: Steroidal saponins, aurones, chalcones, tannins, phenols, flavonols, flavones, alkaloids, xanthones, catechins, flavononols, flavanones and steroids. **Discussion** There was a strong presence of polyphenol compounds in the sample. Such compounds are about 30% of the dry weight of leaves and presents, mostly, like flavonoids. Among them, the catechins predominate, being considered as potent antioxidants, acting as scavengers of free radicals, metal chelating and anti-peroxidation agents. Between the catechins contained in green tea, the 3-epigallocatechin gallate (EGCG) is the most abundant. The catechins has anti-inflammatory effects, such as the inactivation of the enzymes cyclooxygenase-2 and lipoxygenase and the inhibition of TNF-α gene expression. It was evidenced, also, the presence of alkaloids in the sample, especially caffeine, compound that has its action as a central nervous system stimulant and has concentrations of 2.5 to 5.5% in green tea leaves. It possesses, when isolated, a strong bitter taste, being responsible for the remarkable flavor of tea [3]. The results obtained in this work proved the presence of beneficial compounds in green tea, which makes it an alternative for the treatment of many diseases. **References** [1] Nishiyama MF, Ciênc. Tecnol. Aliment. v.30, p.191, 2010; [2] Matos FJ, Introduction of experimental phytochemical, 1997; [3] Prietsch RF, *Rev. Atomo*, v.11, 2011. **Acknowledgments:** UNIFOR.

09.032

Tocolytic effect of essential oil from *Lippia microphylla* Cham. involves positive modulation of K^+ channels on rat. Silva MCC, Vasconcelos LHC, Souza ILL, Araujo LCC, Ferreira PB, Sampaio RS, Tavares JF, Cavalcante FA, Silva BA UFPB

Introduction: *Lippia microphylla* Cham. (Verbenaceae) is popularly known as "alecrim-do-mato" or "alecrim-de-tabuleiro". It is used in folk medicine as decoct or macerated in alcohol to treat respiratory diseases such as colds, bronchitis, cough and asthma (AGRA, *Rev Bras Farmacogn*, v.18, p.472, 2008). The essential oil was extracted from *L. microphylla* aerial parts (LM-OE) and, in previous studies, showed tocolytic effect on rat (SILVA, 2013, 45º Congr Bras Farmacol Ter Exp, Ribeirão Preto/SP). This study aimed to investigate the involvement of K^+ and Ca^{2+} channels of LM-OE tocolytic effect on rat. **Methods:** Rat uterus was suspended in organ baths containing Locke Ringer solution under appropriate conditions. Isotonic and isometric contractions were monitored and recorded. All experimental protocols were previously approved by Ethical Committee in Animal Use of CBiotec/UFPB (CEUA Protocol 1005/13). Substances used in experimental protocols were KCl 60 mM, oxytoxin 10^{-2} IU/mL, CsCl 5 mM, S-(-)-Bay K8644 3 x 10^{-7} M, $CaCl_2$ 10^{-6}-3 x 10^{-1} M and LM-OE 0.003-243 µg/mL. **Results:** LM-OE (n = 5) (27-243 µg/mL) inhibited $CaCl_2$-induced cumulative contractions in a medium nominally without Ca^{2+} and depolarized organ by KCl 60 mM, shifting them to the right in a non-parallel manner, as well as LM-OE (0.03-243 µg/mL) relaxed the pre-contracted uterus with S-(-)-Bay K8644 3 x 10^{-7} M (EC_{50} = 58.2 ± 8.2 µg/mL), however about 12 times less potent than with pre-contracted organ by KCl 60 mM (EC_{50} = 5.0 ± 0.8 µg/mL). LM-OE (0.03-81 µg/mL) relaxed the pre-contracted uterus with oxytocin 10^{-2} IU/mL either in absence (EC_{50} = 2.7 ± 0.8 µg/mL) or presence (EC_{50} = 16.9 ± 3.4 µg/mL) of CsCl 5 mM, being about 6 times less potent. **Discussion:** Search for biologically active products has increased in recent years, stimulating the interest on chemical composition of essential oils, as well as its effects on smooth muscle (ASEKUN, *Food Chem*, v.101, p.995, 2007; MAGALHÃES, *Fundam Clin Pharmacol*, v.18, p.539, 2004). Smooth muscle contraction is triggered mainly by the increase the cytosolic Ca^{2+} levels, generated by membrane depolarization, which activates Ca_V (SOMLYO, *Nature*, v.372, p.231, 1994). So, the first question was whether LM-OE would inhibit Ca^{2+} influx through Ca_V. LM-OE inhibited cumulative concentration-response curves to $CaCl_2$ in previously depolarized preparations, suggesting blockade of Ca^{2+} influx through Ca_V. Furthermore, LM-OE relaxed the pre-contracted uterus with Bay K8644, Ca_V1 selective agonist, but with a relaxant potency about 12 times less, indicating that acts not directly blocking the Ca^{2+} influx through Ca_V. Since K^+ channels negatively modulate the Ca_V, it was investigated the role of K+ channels in LM-OE tocolytic effect using CsCl, non-selective K^+ channels blocker. Relaxant potency of LM-OE was reduced in CsCl presence, suggesting that positively modulates K^+ channels. Thus, LM-OE tocolytic effect on rat involves the positive modulation of K^+ channels to attenuate the Ca^{2+} influx through Ca_V. Therefore, this work shows that essential oils are interesting compounds to uterine contractility regulation, which can be used therapeutically to combat premature labor and uterine cramps. **Financial support:** CNPq, Capes, PPgPNSB/CCS/UFPB.

09.033

Further evidence on the protective effects of P/Q- and N-type voltage-gated calcium channel blockers in the mouse model of cyclophosphamide-induced hemorrhagic cystitis. Silva RBM[1], Sperotto NDM[2], Pereira TCB[1], Bogo MR[3], Morrone FB[2], Gomez MV[4], Campos MM[5] – [1]PUCRS – Medicine and Health Sciences, [2]PUCRS – Applied Pharmacology, [3]PUCRS – Genomics and Molecular Biology, [4]UFMG, [5]INTOX-PUCRS

Introduction: Voltage-gated calcium channels (VGCCs) have been recognized as potential targets to control inflammatory pain, manly by modulating calcium influx, and the consequent release of neurotransmitters from primary afferent neurons (Vink, *Br J Pharmacol*, 167, 970, 2012). Previous evidence from our group (unpublished data) demonstrated the ability of selective P/Q- and N-type VGCC blockers to prevent nociceptive, inflammatory and functional alterations related to the mouse model of cyclophosphamide (CPA)-induced hemorrhagic cystitis (HC). Herein, we investigated the effects of spinal administration of the P/Q- and N-type VGCC blockers Tx3-3 and Phα1β, respectively, isolated from the spider *P. nigriventer*, on TRPV1, TRPA1 and NK1 mRNA expression in CPA-evoked HC in mice. In addition, we evaluated the effects of co-injection of the selective NK1 receptor antagonist CP-96345 plus Phα1β, on symptomatic and inflammatory changes in this model. **Methods:** Male Swiss mice (n = 5-6 per group) were used. All the experimental procedures were approved by the Local Ethics Committee (CEUA-PUCRS, 12/00292). HC was induced by a single intraperitoneal (i.p.) injection of CPA (300 mg/kg). Tx3-3, Phα1β (50 pmol/site) or CP-96345 (50 µg/site) were injected intrathecally (i.t.), 2 h after CPA injection. The animals were euthanized 6 h following CPA administration, and bladders and spinal cords were dissected to evaluate TRPV1, TRPA1 and NK1 mRNA expression by qRT-PCR. The nociception parameters (4 h post-CPA) and the macroscopic inflammatory grade of bladders (6 h post-CPA) were also measured. The wet weight of bladders was also registered at this time-point. **Results:** CPA was associated to a slight increase of TRPV1 and TRPA1 mRNA receptor expression, in the urinary bladder. Interestingly, these results were partially reversed by Tx3-3 and Phα1β toxins. Additionally, the i.p. administration of CPA produced a marked increase of nociception scores, allied to decrease of mouse activity and high scores of macroscopic inflammation. The isolated i.t. treatment with either CP-96345 or Phα1β, reversed all the evaluated parameters. Curiously, the animals that received CP-96345 plus Phα1β displayed a remarkable reduction of nociception scores, which was significantly different from the inhibition obtained with Phα1β given alone. However, this combined strategy did not produce additive effects on locomotion, hemorrhage, edema or bladder wet weight, in comparison to the isolated treatment with Phα1β toxin. **Discussion:** The present results show, for the first time, that spinally-administered P/Q-type VGCC (Tx3-3) or N-type VGCC (Phα1β) blockers modulate TRPV1 and/or TRPA1 receptor expression in CPA-evoked HC in mice. Furthermore, the beneficial effects of Phα1β with CP-96345 on nociception it seems to be dependent to the modulation of NK1 receptor activity. **Financial Support:** PRPPG/PUCRS, Capes-AUX-PE Toxinologia and FINEP/PUCRSINFRA #01.11.0014-00.

09.034

Effects of garcinielliptona FC on the locomotor activity of mice: preclinical study for the development of new drugs. Silva APSCL[1], Oliveira GAL[1], Medeiros SC[1], Sousa GF[2], Silva Filho JCCL[3], Costa Júnior JS[2], David JM[4], Freitas RM[1] [1]UFPI, [2]IFPI, [3]UNOPAR, [4]UFBA

Introduction: The design of new drugs from medicinal plants has increased substantially each year. The identification of new molecules with pharmacological properties devoid of toxic effects has been an ongoing challenge for the pharmaceutical industry. Therefore the aim of the research was to evaluate the effects of Garcinielliptona FC (GFC) on motor coordination of adult mice, since, has not investigated the safety of using GFC in preclinical trials, in order to assess their safety in pharmaceutical formulations. **Method:** The substance has been isolated from the seeds of *Platonia insignis*, and confirmed by ^{1}H NMR and ^{13}C, after previously analyzed and purified by chromatographic methods and thin layer column. (25 – 30g) were used mice were kept in temperature controlled (26 ± 1 ° C) and 12 h light / dark cycle, the animals had free access to food and water. The GFC was emulsified in 0.05% Tween 80 in 0.9% saline (vehicle) and administered intraperitoneally (i.p.) into five groups of mice (n = 5) doses (1000, 2000, 3000, 4000 and 5000 mg/kg), G1, G2 and G3 groups, respectively, negative control received vehicle (saline) and positive control group was treated with diazepam (2 mg/kg) (i.p.). The test was performed 30 minutes after administration. All groups were treated acutely by a single 14 day dose period. All experiments were previously approved by the Ethics Committee of Experimental Animal, UFPI (078 / 2012). Results were expressed as mean ± Standard Error of Mean (SEM) using variance (ANOVA) followed by t – Student- Newman – Keuls test as post hoc test ($p<0.05$). **Results:** In any dose tested (G1: RT = 175.5 ± 1.6s; G2: RT = 179.4 ± 2.5s; G3: RT = 178.2 ± 1.4s) no change in the Residence Time in seconds (s) on the rotating bar suggesting that does not induce changes in locomotors activity of animals when compared to controls (RT = 179.1 ± 0.8 sp > 0.05) . Was not observed change in the number of falls (G1: 0.8 ± 0.2, G2: 0.9 ± 0.4 and G3: 1.05 ± 0.4) compared to the control group (p > 0, 05), suggesting that this polyisoprenylated benzophenone does not produce muscle relaxant effect and does not alter motor coordination. **Discussion:** In this test, the difference in length of stay and number of falls between groups treated with vehicle and GFC was taken as an index of muscle relaxation. Thus, the GFC at the doses tested (1.000, 2.000 and 3.000 mg/kg) showed no psychomotor alterations, suggesting possible neurological safety. According to a survey conducted, to our knowledge, this study is the first on the safety assessment of Garcinielliptona FC isolated from *Platonia insignis* Mart. species. The GFC no showed muscle relaxant activity and represents an innovative and promising compounds for research and drug development. **Acknowledgements**: CNPq, Capes and FAPEPI

09.035

Strength training changes aorta reactivity by modulating oxide nitric pathway on Wistar rat. Ferreira PB, Brito AF, Silva AS, Souza AA, Félix GS, Sampaio RS, Tavares RL, Souza ILL, Pereira RA, Araujo LCC, Miranda Neto M, Silva BA UFPB

Introduction: strength training (ST) is a modality that has been target of several investigations, particularly with respect to the numerous benefits, as improves on age-associated endothelial dysfunction in femoral arteries (HARRIS, Eur. J. Appl. Physiol., v. 108, p. 533, 2010) and rat aorta (FIGARD, *Appl. Physiol. Nutr. Metab* v. 3, p. 621, 2006). Nevertheless, number of research on vascular muscle is still limited and response can change according to mechanical characteristic of exercise. Thus, we aimed to evaluate the changes induced by strength training on rat aorta reactivity. **Methods**: after one week of adaptation, Wistar rats (250-300 g, n = 10) were undergone for 8 weeks of progressive strength training (STG) (MARCHETT, Am. J. Sports Med., v. 34, p. 1274, 2006). The control group CG (n = 10) was only acclimatized (non-exercised). 48 hours after exercise, rats were euthanized and the aorta removed and suspended in organ baths with Krebs solution, under rest tension of 1 g at 37 °C and bubbled with carbogen mixture. After a stabilization period of 1 h, isometric contractions were recorded. All experimental protocols were previously approved by Ethical Committee on Animal Use of CBiotec/UFPB (Protocol 1101/11). **Results:** all experimental groups exhibited maximum effect (E_{max}) of relaxation equal to 100%. In aortic rings with intact endothelium, ACh (10^{-10} to 10^{-4} M) relaxant potency was increased on the STG (pD_2 = 7.3 ± 0.02), compared with the CG (pD_2 = 6.4 ± 0.06). FEN contractile potency was not altered in STG (pD_2 = 6.9 ± 0.06), compared with the CG (pD_2 = 7.0 ± 0.10) in endothelium absence. Contrary, in the endothelium presence, FEN contractile potency was reduced in STG (pD_2 = 5.6 ± 0.07), compared with the CG (pD_2 = 6.3 ± 0.23). In L-NAME presence, nitric oxide (NO) sintase inhibitor, FEN contractile potency was increased in both CG (pD_2 = 7.1 ± 0.08) and STG (pD_2 = 7.4 ± 0.10). **Discussion:** the results demonstrate that strength training decreases the contractile response and increases the relaxation in rat aorta. These results could be explained by a possible higher participation of NO pathway since, in L-NAME presence, the contractile potency to FEN was increased in STG, in endothelium presence. These results are in accordance with the guidelines for exercise prescription, which indicates strength training as a new alternative for people with circulation system pathologies, where this disturbance on vasomotor response might be related to a decreased on NO bioavailability (BECHARA, J. Smooth Muscle Res., v. 44, p. 101, 2008). Thus, the strength training promotes alterations in contractile and relaxation on rat aorta and NO pathway influences this phenomenon. However other studies are required to better characterize the relation between strength training and NO signaling. **Financial Support:** CNPq, Capes, PPgPNSB/CCS/UFPB.

09.036

Study of neurotoxic effects of intrahippocampal injection of three isolated toxins from venom of the scorpion *Tityus bahiensi.* Freitas LA[1], Kuniyoshi AK[2], Carvalho DC[2], Paulo MEFV[1], Sobral ACM[1], Portaro FCV[2], Dorce VAC[1], Nencioni ALA[1] [1]IBu – Farmacologia, [2]IBu – Imunoquímica

Introduction: In Brazil, *Tityus* scorpions from Buthidae family are the main responsible for accidents in humans and are considered the scorpions of greater medical importance. The scorpion venoms consist of a complex mixture of active components, being the neurotoxins the main toxic elements. Few studies are devoted to evaluate the actions of the venom of *T. bahiensis* mainly on the central nervous system. Thus, this study aimed to assess the possible neurotoxic effects of three toxins isolated from *T. bahiensis* scorpion venom after intrahippocampal injection in male Wistar rats. **Methods:** The procedures were approved by the Ethics Committee on Animal Use (CEUA/IBU protocol 870/11). Male Wistar rats, weighing 240-260g were used. To obtain isolated toxins, *T. bahiensis* crude venom was fractionated by gel filtration, and five pools were obtained. The activities of these pools were tested. Pool 2, which showed the greatest potential to promote changes, was chromatographed on HPLC equipped with a C18 analytical column and the three most abundant peaks obtained were used in the following tests. The animals were submitted to stereotaxic surgery and cannulas and electrodes were implanted in the CA1 hippocampal area. The animals were divided into 4 groups (n=6) which received intrahippocampal injection of the 1µL Ringer's solution (control group) or toxins TbII-I, TbII-II or TbII-III 2µg/µl (experimental groups), respectively. After electrographic recording (EBA) and behavioral observation, the animals had their brain removed and processed for histopathological analysis of CA1, CA3 and CA4 hippocampal areas, ipsilateral (i) and contralateral (c) to injection. One group of animals (n = 4) were injected with toxin Tb II-II (2µg/µl) by intrahippocampal via and submitted to the microdialysis to evaluate the levels of neurotransmitters (glutamate, GABA and glycine). Data were statistically analyzed by Fisher exact test (behavioral activity and AEC), by analysis of variance (one way ANOVA) followed by Tukey (histological evaluation) and ANOVA for repeated measures followed by Tukey's test (measurement of neurotransmitters). **Results and discussion:** The animals of groups injected with toxins TbII-I and TbII-II, respectively, showed significant changes in behavioral parameters: respiratory distress (66.6%); myoclonus (66.6% and 83.3%); WDS (66.6 % and 83.3%) and electroencephalographic parameters emergence of spicules (66.6% and 83.3%) and discharge (66.6% and 83.3%). Histological analysis demonstrated that the three toxins decreased the number of viable cells in CA1 and CA3 (i) and (c) areas. The toxins TbII-I and TbII-II also decreased the number of viable cells in CA4 area. Regarding the levels of neurotransmitters, the injection of toxin TbII-II increased significantly the levels of glutamate (C 0.0123 ± 0.0007; TbII-II 0.023 ± 0.002) and GABA (C 0.030 ± 0.010; TbII-II 0.055 ± 0.008). The three toxins used caused behavioral, electroencephalographic and histopathological alterations, specifically the TbII-II change the levels of the neurotransmitters glutamate and GABA. **Financial Support:** Capes.

09.037

Antiulcerogenic effect of riparin II on ethanol-induced gastric lesions in mice: role of prostaglandins, nitric oxide and KATP+ channels. Carvalho AMR[1], Vasconcelos LF[1], Rocha NFM, Rios ERV, Dias ML[1], Bastos MVR[1], Vidal LMT[1], Barbosa Filho JM[2], Gutierrez SJC[3], Sousa FCF[1] [1]UFC – Physiology and Pharmacology, [2]UFPB – Pharmaceutics Technology, [3]UFPI – Biochemistry and Pharmacology

Introduction: The ethanol-induced gastric lesions model is considered to be a reliable tool to study the pathogenesis of acute gastric mucosal ulceration. Riparin II (RipII) is an alkamide compound isolated from *Aniba riparia*, collected from the Amazonas's forest. This substance presents anxiolytic, antidepressant-like and antiinflamatory effects in animal models. In this study, we decide to evaluate the gastroprotective effect of Riparin II (RipII) against ethanol-induced lesions and verify the role of nitric oxide, ATP-dependent K+ channel and prostaglandins in this action. **Methods:** Male Swiss mice (25-35g) were divided in groups: vehicle (3% Tween 80 in distilled water; p.o.), RipII was used at the doses of 25 and 50 mg/kg, by gavage and CIPRO (Ciproheptadyne 10 mg/kg; used as a reference drug). *One hour later*, absolute ethanol (0.2 mL/animal) was administrated orally to all groups. Thirty minutes after the administration of ethanol, the mice were killed and their stomachs were removed and opened along the greater curvature for examination. The total and injured stomach areas were measured and expressed in terms of percent (%) of ulcerated gastric area. To evaluate the possible Rip II action mechanism, separate experiments were conducted using the following drugs: L-NAME, an inhibitor of the NO synthase activity (10 mg/kg; i.p.); glibenclamide, an antagonist of KATP + channels (10 mg/kg; i.p.) and indomethacin a nonselective cyclooxygenase inhibitor (10 mg/kg; p.o.). L-NAME and glibenclamide were administered 30 min before animals receiving RipII-50 mg/kg, while indomethacin was administrated 2 h before the drug test. One hour later, absolute ethanol 0.2 mL was applied in each animal. Thirty minutes after the administration of ethanol, the mice were killed and their stomachs were removed for examination as previously described. Data are presented by mean ± S.E.M and analyzed by ANOVA followed by Student Newman Keuls as the *post hoc* test. This work was submitted to local Ethics Committee on Animal Research (protocol 40/10). **Results and discussion:** The administration of ethanol produced lesions in the gastric mucosa (17.84 ± 0.4818), which were reduced in the animals pretreated with RipII-25 mg/kg (8.675 ± 0.8159) RipII-50 mg/kg (11.48 ± 0.6790) and CIPRO (4.100 ± 0.6097). Previous administration of L-NAME and glibenclamide reverted the gastroprotection offered by RipII-50 (L-NAME + RipII-50: 22.15 ± 1,541; GLIB + RipII-50: 17.06 ± 2.657), compared to RipII-50 alone. On the other hand, the pretreatment with indomethacine failed to block effectively the gastroprotective action of RipII-50. The results provide evidence that RipII reduces the gastric damage induced by ethanol and this gastroprotective effect appears to be mediated, at least in part, by endogenous NO and KATP+ channels opening. **Financial Support:** CNPq/ Capes and FUNCAP

09.038

Antinociceptive effect of Riparin III: Role of glutamate and mechanical hypernociception induced carrageenan. Vasconcelos LF[1], Rocha NFM, Carvalho AMR[1], Rios ERV, Dias ML[1], Vidal LMT[1], Costa FL[1], Gutierrez SJC[2], Barbosa Filho JM[3], Sousa FCF[1] [1]UFC – Physiology and Pharmacology, [2]UFPI – Biochemistry and Pharmacology, [3]UFPB

Introduction: Riparin III (RipIII) is an alkamid compound that was firstly isolated from unripe fruit of *Aniba riparia*. This substance presents antimicrobial, anxiolytic and antidepressant-like effects in different animal models. The objective of this work was to investigate the antinociceptive effect of RipIII. **Methods:** This work was submitted to local Ethics Committee on Animal Research (protocol 22/12).Mice *Swiss*, male weighing 25-30g, were used (6-8 animals/group). RipIII was used at the doses of 25 and 50 mg/kg, by gavage. Data were analyzed using One-Way ANOVA and Student Newman-Keuls test *post hoc*. Firstly, the acetic acid-induced abdominal writhing (Koster *et al.*, 1959) and formalin test (Hunskaar; Hole, 1987) were performed. Indomethacin 10 mg/kg (p.o.) or morphine 7.5 mg/kg (i.p.) were used as standard drugs. In the glutamate test, animals were treated with RipIII (25 and 50 mg/kg, p.o.), and after 1 hour, each animal received 20 µL of glutamate solution (10 µmol/paw), injected in the intraplantar region of the right hind paw. The amount of time spent licking the injected paw was recorded. In the test of mechanical hypernociception induced by carragenann (Cg), animals were pre-treated with RipIII at both doses (25 and 50 mg/kg) 1h before Cg (0.1%) application (20µl/paw) and assessed 30, 60 and 180min after intraplantar injection of Cg. The mechanical hypernociception was evaluated by increasing pressure method of rat paw (von Frey electronic). **Results and discussion:** RipIII 25 e 50 mg/kg decreased significantly the number of writhes (50.78% and 69.53%, respectively), when compared to the vehicle group. In the formalin test, RipIII decreased paw licking time at both doses, only at the second phase of the test (25 mg/kg: 75.46%; 50 mg/kg: 60%) and morphine reduced paw licking time at both phases. In the glutamate test, RipIII at both doses (25 and 50 mg/kg, p.o.) significantly reduced the reaction time of the nociceptive response compared to the vehicle (25 mg/kg: 44.65% and 50 mg/kg: 43.57%). Pre-treatment with RipIII-25 and 50 mg/kg was able to decrease the intensity of hypernociception observed at 30, 60 and 180min after intraplantar injection of Cg, when compared to the animals treated with vehicle. **Conclusion:** The results indicate that RipIII presents an antinociceptive activity, probably due to prevention of nociceptors sensibilization. **Financial support:** CNPq/Capes/FUNCAP

09.039

Relevance of C-terminal amidation of Ts1 on its modulation of voltage-gated sodium channels. Cremonez CM[1], Peigneur S[2], Waelkens E[3], Tytgat J[2], Arantes EC[1] [1]FCFRP-USP – Física e Química, [2]KU Leuven – Toxicology and Pharmacology, [3]KU Leuven – Protein Phosphorylation and Proteomics

Introduction: The main neurotoxin from *Tityus serrulatus* venom, mature Ts1, is a β-toxin which acts on Sodium channels, and has its C-terminal Cys amidated. The generation of Ts1 requires, the removal of the signal peptide, followed by a post-translational cleavage of the peptide bonds involving Lys residues by a carboxypeptidase, after which the remaining Gly residue has its NH_3^+ linked to the C-terminal residue Cys by the action of the α-amidating enzyme on its precursor [1,2]. Using a combination of cation exchange and reverse fase chromatograpy, it was possible to isolate a unique isoform of Ts1 preceding the last step of maturation, thus still has the Gly residue intact, named Ts1-G [3]. The aim of this study was to use Ts1-G, not amidated isoform, to study the functional implications of the C-terminal amidation and hence compare the mature Ts1 and its isoform with a Gly residue tail (Ts1-G). **Methods:** We combined cation exchange chromatography using CM-Cellulose-52 column, followed by C-18 reverse phase FPLC to obtain both toxins, Ts1 and Ts1-G. The identity was confirmed by MALDI/TOF. The electrophysiological study comparing Ts1 with its isoform Ts1-G was conducted using Two Microelectrode Voltage Clamp technique. Both toxins were screened against a panel of 9 voltage gated sodium channels (Nav): the mamallian isoforms Nav1.1, Nav1.2, Nav1.3, Nav1.4, Nav1.5, Nav1.6, Nav1.8, and the insect isoform *Dm*Nav1. **Results and discussion:** Our results show loss of activity on the channels tested by the isoform not amidated Ts1-G at 100nM, confirming the important structure-function role played by the C-terminal amidation in determining Ts1 activity. As such, the obtained data show that the C-terminal region may be involved in subtype selectivity and potency of this peptide toxin. **References:** [1]Martin-Eauclaire *et al*., *Febs Lett*., 342, p.181, 1994. [2]Martin-Eauclaire *et al*., *Febs Lett*., 302, p. 220, 1992. [3]Coelho, V. A., Cremonez, C.M. *et al*., *Toxicon*, 83, p.15, 2014. **Financial** Agencies and acknowledgments: CNPq, Science without Borders Program.

09.040

Antiproliferative activity of a new compound isolated from *Bauhinia acuruana*. Silva PBN[1], Silva TG[2], Gois RWS[3], Santiago GMP[3], Militão GCG[1] [1]UFPE – Fisiologia e Farmacologia, [2]UFPE – Antibióticos, [3]UFC – Farmácia

Introduction: *Bauhinia acuruana* (Caesalpiniodeae) is one of 300 species of the genus *Bauhinia*, is usually found in the states of Bahia, Ceará (CE), Pernambuco and Piauí. A new compound, 2-hydroxy-2,3,5-trimetoxibibenzila (TM) was isolated from roots of *Bauhinia acuruana* collected at Tianguá-CE, and its cytotoxic activity was accessed in HL60. **Methods:** Firstly, the cytotoxic effect of the compound TM was evaluated by MTT on HL-60 (leukemia pro – myelocytic) from human cell line after 24 hours treatment in order to determine the IC50 value. Then, the effect of the compound at the IC50 concentration and 1/2 x IC50 values was observed on the viability of HL-60 cells by trypan blue exclusion assay and morphological changes was observed after 24h determined using May-Grunwald-Giemsa dye. **Results and discussion:** TM showed moderate cytotoxic activity against promyelocytic leukemia (HL-60), with IC50 value of 5.6 µg/mL and confidence interval of 4.5 to 6.9 µg/mL. Trypan blue exclusion test showed a reduction in the number of viable HL60 cells after 24 hours treatment with 3 µg/mL of doxorubicin as positive control (63% inhibition), TM 2.9 µg/mL (46% inhibition) and TM 5.8 µg/mL (69% inhibition). Morphological alteration as cell shrinkage, DNA fragmentation and pyknotic nuclei was observed. **Conclusion**: The new compound presented cytotoxic effects on HL60 cells and further studies must be done to indicate the mode of action. **Financial Support**: CNPq, UFPE.

09.041

Effects of polyanions on some activities of *Bothrops leucurus* venom. Cons BL[1], Tomaz MA[1], Ricardo HD[1], Strauch MA[2], Monteiro-Machado M[1], Tavares-Henriques MS[1], Cruz JMT[1], Saturnino-Oliveira J[3], Melo PA[1] [1]UFRJ – Farmacologia das Toxinas e Substâncias Antagonistas, [2]IVB, [3]UFS – Morfologia

Snakebites are common in Brazil, manly in the rural area, specifically in cacao plantations of the northeast of Bahia, where humans are frequently bitten by *Bothops leucurus*. This snake genus is well adapted to humidity forest. In this envenomation are observed edema, hemorrhage and myonecrosis. We investigated the polyanions ability to antagonize the *in vitro* and *in vivo B. leucurus* venom effects. The venom enzyme activities were assessed by using 10 µg/mL of *B. leucurus* crude venom and we tested phospholipase, proteolytic, hyaluronidase and collagenase activities, with each respective substrate. Hemorrhagic lesions were induced by 1.0 mg/kg intradermic injection of the venom or the venom incubated with the polyanions compounds. The *in vivo* myotoxicity were performed by i.m. injection of 1.0 mg/kg of *B. leucurus* venom and the plasma CK activity analyzed two hours after the injection. Myotoxic experiments were also performed *in vitro*, with isolated mouse *extensor digitorum longus* muscle (EDL) and assessed by the increase of creatine kinase (CK) release following the exposure of muscle to the venom (25 µg/mL). Suramin 30 µM inhibited circa of 100% of the phospholipase, hyaluronidase activities and proteolytic activity only circa of 30%. Fucosylated chondroitin sulfate inhibited circa of 40%, 70% and 100%, the phospholipase, and proteolytic and hyaluronidase activities, respectively. Dextran sulfate inhibited about 60% of phospholipase activity. On the *in vivo* pre-incubated protocols experiments, Suramin (30 mg/kg) reduced the increase of plasma CK activity induced by venom injection from 3128.4 ± 277.4 U/L to 854.8 ± 55.2 U/L (n=4). In the same animals the thigh edema was reduced from 47,2 ± 4.3 mm² to 32.8 ± 5.2 mm² (n=4). In the same injection protocol the hemorrhagic effect expressed in arbitrary units was decreased from 972.4 ± 25.9 to 649.6 ± 31.9 (n=4). Suramin was not able to protect significantly the tail bleeding. Our results are showing that polyanions were able to inhibit some important activities of *B. leucurus* venom which are involved in the tissue damage. All the animal procedures were approved by the CEUA-CCS-UFRJ nº DFBCICB072-04/16. **Financial support**: Capes, CNPq, PRONEX and Faperj

09.042

Trypanocidal effect of *Bothropoides insularis* venom. Canuto JA[1], Bezerra GF[1], Lima DB[1], Mello CP[1], Bandeira ICJ[1], Pereira TP[1], Tessarolo LD[1], Menezes RRPPB[2], Sampaio TL[2], Toyama MH[3], Martins AMC[1] [1]UFC – Clinical and Toxicological Analysis, [2]UFC – Physiology and Pharmacology, [3]UFC

Introduction: Snake venoms are investigated in many biomedical laboratories around the world, focusing on the discovery of new components that can be used in the treatment of several diseases [1]. According to estimates by the World Health Organization,7.7-10 million people are infected with *Trypanosoma cruzi* worldwide. Drug therapy for Chagas disease remains unsatisfactory. In addition to low efficiency at some stages of the disease, the toxicity of current treatments limits tolerability and hinders compliance, which may prevent treatment success [2]. *Bothropoides insularis* snake is a native from the Island of Queimada Grande, Brazil, of which venom has shown strong toxic effects in several experimental models [3]. The aim of this work was to evaluate the trypanocidal potential of *Bothropoides insularis* venom (Biv). **Methods:** Different concentrations (25, 12.5, 6.25, 3.12, 1.56, 0.78, 0.39 µg/mL) of Biv were incubated with cultures of epimastigotes of the Y strain of *Trypanosoma cruzi* (grown in LIT medium at 28 º C) for 48 hours [4] and different concentrations (1.56, 0.78, 0.39, 0.16 µg/mL) of Biv were incubated with cultures of trypomastigotes of the Y strain of *Trypanosoma cruzi* (obtained by infection of LLCMK2 cells and cultured in MEM at 37 ˚C and 5% CO_2) [5] for 24 hours. The cell viability was determined by counting in a Neubauer chamber. **Results:** Biv showed a dose-dependent cytotoxic effect after 48 hours of incubation to epimastigote forms (IC_{50} = 1.20 µg/mL) and 24 hours of incubation to tripomastigote forms (IC_{50} = 0.47 µg/mL). **Discussion:** Similar trypanocidal effect was observed to that of *Bothropoides lutzi* [6] and *B. leucurus* venoms [7]. Other Bothrops venoms and their isolated fractions also showed similar effects as the one observed with *B. jararaca* venom and its LAAO, which were able to induce apoptosis in *T. cruzi* epimastigotes [8]. In conclusion, snake venoms have showed trypanocidal potential. **Financial Agencies:** CNPq. **References:** [1] HYNES, RO. *Cell*, v69, p11, 2002. [2] RASSI JR, A. *Infect Dis Clin North Am*, v26, p275, 2012. [3] VALENTE, RH. *J Proteomics*, v72, p241, 2009. [4] CAMARGO, EP. *Rev Inst Med Trop São Paulo*, v6, p93, 1964. [5] GONÇALVES, AR. *Parasitol Res*, v88, p598, 2002. [6] DE MENEZES, RR. *Nat Prod Commun*, v7, p71, 2012. [7] TORRES, AFC. *JVAT*, v16, p614, 2010. [8] DEOLINDO, P. *Toxicon*, v56, p944, 2010.

09.043

Antiobesity effect of hancornia speciosa gomes (Apocynaceae) in mice. Pereira AC[1], Silva JF[2], Pereira ABD[3], Barbosa LCO[1], Braga FC[3], Lemos VS[2], Côrtes SF[1] [1]ICB-UFMG – Farmacologia, [2]ICB-UFMG – Fisiologia e Biofísica, [3]UFMG – Farmácia

Introduction: It is well known that obesity increases the risk of cardiovascular diseases and diabetes. Recently, obesity has been evolved into a global epidemic and many efforts have been done to treat it. Since antiquity, plants represent an important source of drugs. *Hancornia speciosa* Gomes (Apocynaceae), commonly known as mangabeira, is popularly used to treat hypertension, diabetes and obesity. Despite the large use of this plant, there is no scientific report supporting it and we aimed to evaluate the potential of *H. speciosa* to treat obesity. **Methods:** Dried leaves from *H. speciosa* (voucher specimen BHCB 49895) were percolated with 96% ethanol at room temperature. The solvent was removed under reduced pressure furnishing a residue named HE, which contain 7.1 % of cyclitols. Experimental protocols conformed local ethics committee (protocol 163/2010, UFMG). Male Swiss mice (8 weeks age) were divided in three groups: standard diet (control), high-fat diet and high-fat diet plus HE (treatment). The obesity was induced using high-fat diet for 8 weeks. During the fourth until eighth week, treated group received HE (50 mg/kg) in drinking water. Acute citotoxicity assays with *Artemia salina* showed that HE is safe. In addition, previous studies with mice showed that 50 mg/kg of HE by oral route had a minor effect on the systolic blood pressure. Body weight and blood glucose levels were recorded once weekly. After 4 weeks of HE treatment, oral glucose tolerance test were performed. At the end of the experiment, blood and tissues were collected and stored at –80 ˚C until use. Biochemical parameters were measured using commercial kits. Western blot analyses were performed to evaluate RAGE, COX-2, NF-κB, p22phox and SOD-2 expression in liver. One-way ANOVA analyses followed by Bonferroni pos-test were employed and significance was accepted at $p<0.05$. **Results and discussion:** Animals receiving high-fat diet showed increased body weight (58.4 ± 2.0 versus 47.5 ± 2.16 g; $p<0.05$), blood glucose (178.7 ± 5.0 versus 107.8 ± 7.1 mg/dL, $p<0.01$), glycosylated hemoglobin (HbA_{1c}; 11.0 ± 0.68 versus 7.42 ± 0.54 %; $p<0.01$), cholesterol (289.3 ± 10.4 versus 145.0 ± 9.0 mg/dL; $p<0.001$) and triglycerides levels (233.0 ± 7.4 versus 113.6 ± 2.4 mg/dL; $p<0.001$). Treatment with HE significantly reduced most of this parameters to the control levels (body weight: 48.8 ± 3.0 g; blood glucose: 139.0 ± 15.0 mg/dL; HbA_{1c}: 8.0 ± 0.53 %; cholesterol: 220.0 ± 26.5 mg/dL; triglycerides: 138.1 ± 6.5 mg/dL). In addition, western blot analyses showed that high-fat diet increased the expression of RAGE, NF-κB, p22phox and of the enzymes COX-2 and SOD-2 in liver. Interesting, HE treatment normalized the expression levels of RAGE and of all the pro-inflammatory and pro-oxidants enzymes evaluated in liver, indicating the beneficial effect of this plant in the treatment of obesity. Therefore, the present study supports the use of HE by the traditional medicine for the treatment of obesity and may have a potential use for the treatment of individuals with metabolic syndrome. **Financial Support:** We thank Capes (PNPD), CNPq and FAPEMIG.

09.044

Anti-inflamatory potencial of crotoxin: A natural inhibitor of functions and signaling pathways in bone marrow neutrophils. Neves CL[1], Lima TS[1], Sampaio SC[1], Zambelli VO[2], Cirillo MC[1] [1]IBu – Pathophysiology, [2]IBu – Pain and Signaling

Introducion: Crotoxin (CTX), a toxin isolated from *Crotalus durissus terrificus* snake venom, modulates the inflammatory response. A single administration of CTX inhibits, for 7 days, cell migration, leukocyte-endothelium interactions and secretion of proinflammatory cytokines. The same inhibitory effect was observed in phagocytosis by macrophages and neutrophils. Despite these evidences, until now it was difficult to explain how a single dose of CTX remains for a long time inhibiting the inflammatory response, particularly neutrophil functions. Thus, the aim of this study was to investigate the effect of *in vitro* and *in vivo* CTX on the functionality of bone marrow neutrophils and on the molecular mechanisms involved in these functions. **Methods:** For *in vitro* assays, bone marrow neutrophils from C57black/6 mice (protocol number 880/12 approved by CEUAIB) were incubated with CTX (0.08 µg/mL) for 1 h. For *in vivo* studies, mice were pretreated with CTX (44 µg/kg, subcutaneous) or saline, 1 day before bone marrow neutrophil isolation. The concentration and dose of CTX used were based on previous studies and did not show cell toxicity as assessed by trypan blue exclusion and the MTT test. Furthermore, the subcutaneous administration of CTX did not cause signs of envenomation in the animals (Lima *et al.*, 2012). The following functional parameters were assessed: phagocytosis, chemotaxis and adhesion. Phagocytosis of opsonized zymosan particules was performed after stimulation with fMLP (N-Formyl-Met-Leu-Phe) 10 µM for 30 min. Chemotaxis was done in a transwell chamber using fMLP 10 µM as a chemeoattractant for 4 h. Adhesion to matrix proteins was realized in a plate covered with fibronectin and PMA (Phorbol 12-myristate 13-acetate) 100 ng/mL was used as a stimuli for 1 h. To investigate signaling pathways involved in these functions, neutrophils were incubated with CTX (0.08 µg/mL) for 24 h, stimulated with fMLP for 3 min and then, the total expression and the activity of Vav1, RhoA, Rac1 and Cdc42 were evaluated by Western blotting assays. **Results:** ***in vitro*** CTX inhibited phagocytosis by bone marrow neutrophils in 47% (control: 78.50 ± 6.53, CTX: 41.5 ± 3.70, $P<0.001$), chemotaxis in 55% (control: 3.99 ± 0.68, CTX: 1.80 ± 0.20, $P<0.05$) and adhesion in 39% (control: 82.84 ± 7.07, CTX: 51.05 ± 0.59, $P<0.05$). The pretreatment of animals with CTX inhibited phagocytosis by neutrophils in 40% (saline: 134.33 ± 6.17, CTX: 80.00 ± 7.50, $P<0.01$), chemotaxis in 55% (saline: 2.135 ± 0.435, CTX: 0.965 ± 0.060; $P<0.05$) and adhesion to fibronectin in 51% (saline: 64.58 ± 4.937, CTX: 31.605 ± 2.930, $P<0.05$). The activity of pVav1 was inhibited in 97% (saline: 100 ± 13.767, CTX: 2.546 ± 1.127, $P<0.001$), Cdc42 in 97% (saline: 100 ± 32.715, CTX: 2.683 ± 0.515, $P<0.0001$), RhoA in 66% (saline: 100 ± 20.483, CTX: 34.050 ± 5.716, $P<0.01$) and Rac1 in 62% (saline: 100 ± 18.423, CTX: 38.433 ± 1.411, $P<0.01$). No differences were observed in the total expression of Vav1 or GTPases. **Discussion:** The results presented herein evidenced that CTX down-regulates functions of bone marrow neutrophils, particularly phagocytosis, chemotaxis and adhesion. Furthermore, CTX inhibits the activity of Vav1, RhoA, Cdc42 e Rac1. Thereby, these results contribute to explain the long-lasting anti-inflammatory effect of CTX, particularly on cellular events of the inflammatory response. These data reinforce that CTX represents a potential natural product in controlling inflammatory diseases. **References:** Lima TS *et al.*, Exp Biol Med, 237, 1219, 2012. **Financial Support:** Fapesp e INCTTOX

09.045

Crotoxin, a toxin from rattlesnake venom, inhibits the angiogenic function of macrophages in co-culture model. Pimenta LA, Pereira JF, Kato EE, Cirillo MC, Sampaio SC IBu – Pathophysiology

Introduction: Several lines of evidence demonstrate that substances released of the macrophages induce angiogenesis. Crotoxin (CTX), the main component of *Crotalus durissus terrificus* snake venom, stimulates the secretory activity of macrophages and decrease the tumor neovascularization. Besides this, was not investigated the CTX effect on macrophage modulation on angiogenic process yet. **Objectives**: To evaluate the secretory activity of macrophages treated with CTX on migration and proliferation of endothelial cells. **Methods**: *Proliferation assay*: Macrophages were obtained of the peritoneal cavity of male Wistar rats and incubated ($1x10^6$/100µL) in a 6 well plate for 1 hour. Then, they were washed with PBS and incubated in the presence or not of CTX (0.3 µg/ml of RPMI-1640 culture medium) for 2 hours. This concentration was the same as that used in previous research (Sampaio *et al.*,2003, 2006a,b; Costa *et al.*, 2013), which did not exhibit cytotoxicity as assessed by Trypan blue exclusion and byflow cytometry for the exclusion of propidium iodide. The murine endothelial cells derived from thymus haemangioma- t.End.1 ($5x10^4$ cells/well) were adhered cells in 6 well plates for 2 hours. After this period, the wells were washed and added to the supernatants obtained from macrophages and were maintained for 24 hours at 37ºC in a 5% CO_2 humidified atmosphere. After 24 hours of culture, supernatants are removed and the endothelial cells were trypsinized, resuspended in PBS and trypan, and the cell number was determined in Neubauer chamber. *Wound Healing Assay*: Macrophages ($1x10^6$/100µL) were incubated in a 6 well plate for 1 hour. Then, they were washed with PBS and incubated in the presence or not of CTX (0.3 µg/ml of RPMI-1640 culture medium) for 2 hours and washed and incubated with culture medium until the next day. In next day when t.End.1 ($1x10^6$/100µL) it became confluent in a 24 well plate a wound was made with a sterile tip. Cells were washed with PBS, then the wells were incubated in the presence of macrophages or only macrophages supernatants for 24 hours, at 37ºC in a 5% CO_2 humidified atmosphere. After this migration was determined by counting cells in five distinct fields. To analyse the data from the other assays, a one-way analysis of variance was used, followed by Bonferroni's test for multiple comparisons against a single control or by an unpaired Student t-test to compare two groups. Differences with $P<0.05$ were considered significally significant. The results are presented as the mean values/standard error of means. **Results and discussion:** The results showed that CTX decreased cell proliferation [cell-cell contact: 32% (Control: 10.9 ± 0.7, CTX: 7.4 ± 0.7, $P<0.05$); supernatant: 33% (Control: 9.8 ± 0.6, CTX: 6.5 ± 0.5, $P<0.05$)] and cell migration [cell-cell contact: 49% (Control: 190.2 ± 3.9, CTX: 96.5 ± 2.5, $P<0.05$); supernatant: 48% (Control: 99.8 ± 1.4, CTX: 52.1 ± 1.0, $P<0.05$)], when compared to control macrophage. Taken together, the results showed that CTX modulates the angiogenic function of macrophages, suggesting that CTX potencial on control to angiogenesis process such as tumoral process which these cells are modulators. **References:** Sampaio *et al.*, *Toxicon*, 47(8)-909, 2006a Sampaio *et al.*, *Toxicon*, 47(3)-313, 2006b, Costa *et al.*, *Toxicon*, 74- 167, 2013. **Supported by:** Fapesp (2012/51241-5), INCTTOX program (2008/57898-0) and CNPq AND PIBIC-CNPq

09.046

Effects of prolonged administration of *Echinaceae purpurea* against acute and chronic infection with different strains (RH and ME-49) of *Toxoplasma gondii* in mice. Cosmo ML[1], Lima DA[2], Santos PS[2], Lourenço ELB[1], Gasparotto Junior A[3] – [1]Unipar – Ciência Animal, [2]Unipar – Curso de Farmácia, [3]UFGD – Ciência Animal

Introduction: The knowledge of pharmacological effects of different preparations of *Echinacea purpurea* (L.) Moench (Asteraceae) has been used for the medical treatment of several complaints for centuries now. Historical traces are going back to the North-American Indians. Recent studies have proved the stimulation of the non-specific immune system as the main action of this species. In fact, none of the identified compounds of the polar and lipophilic fraction could have been determined to be solely responsible for this activity. Several compounds as alkamides, polysaccharides and glycoproteins are discussed to be potentially part of the "active principle". Since immunomodulation is a pharmacological effect, most of the clinical studies have focused on treatment and prevention of common cold and infections of the upper respiratory and the urinary tract (Hudson, 2012). So, the aim of this study was to evaluate the influence of prolonged administration of *E. purpurea* (EP) extract in acute and chronic infection with different strains (RH and ME-49) of *Toxoplasma gondii* in mice. **Methods**: The standardized aqueous extract of *E. purpurea* (AEEP) was obtained from Quimer herbs and spices Co. (São Paulo, SP, Brazil). The parts used for obtaining of the extract were the roots, stems and leaves (technical report no. 3623). The bioactive compounds in the plant material were standardized in sulfated ash (7.97%), phenolic compounds (1.78%), and tannins (1.74%). For experimental protocol, different groups of male Swiss mice (n=5) were treated with vehicle or different doses (30, 100 and 300 mg/kg) of AEEP per oral route for four weeks. After this period, for the evaluation of acute infection, the animals received intraperitoneally 0.5 mL of a solution containing tachyzoites of *T. gondii* (RH strain; 10^3 units/0,1 mL). Seven days after infection, the animals were euthanized and blood collected to hemogram. Moreover, were measured the total number of tachyzoites present in the peritoneal fluid and liver imprint's. In the other hand, for the evaluation of prolonged infection, different groups of mice received orally 1 mL of a solution containing tissue cysts of *T. gondii* (ME-49 strain; 300 units/mL). 20 days after infection were collected both brain hemispheres for counting the total number of cysts. Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test. A p value less than 0.05 was considered statistically significant. All experimental procedures were previously approved by the Institutional Ethics Committee of the Unipar (authorization no. 22961/2012). **Results**: All mice treated with AEEP at doses of 300 mg/kg there was a significant reduction in the number of tachyzoites in the peritoneal fluid (control: 75 ± 5; AAEP: 33 ± 7* tachyzoites/mL; $p<0.05$) and liver imprint's (control: 28 ± 3; AAEP: 7 ± 2* tachyzoites/mm^2; $p<0.05$) after acute infection with RH strain. Moreover, the treatment with the AEEP (300 mg/kg) significantly accelerated the encystment of the parasite (ME-49 strain) in the brain (control: 643 ± 39; AAEP: 939 ± 52* cysts; $p<0.05$). All other parameters showed no statistically significant differences between all experimental groups. **Discussion:** and conclusion: The results obtained in this study allow us to suggest that the crude extract from *E. purpurea* has protective effects against acute and prolonged infection by different strains of *T. gondii*. Additional studies should be conducted to investigate the mechanisms involved in this protective effects and the effectiveness of this extract in other forms of the life cycle of *T. gondii*. **Acknowledgments**: The authors are grateful to Diretoria Executiva de Gestão da Pesquisa e Pós-Graduação (DEGPP/UNIPAR) for

financial support. **References:** Hudson, J. B. Applications of the Phytomedicine *Echinacea purpurea* (Purple Coneflower) in Infectious Diseases. Journal of Biomedicine and Biotechnology. Vol. 2012, Article ID 769896, 16 pages, 2012. doi:10.1155/2012/769896.

09.047

Crude venom from scorpion *Tityus bahiensis* is able to change offspring development when injected in female rats during lactation. Martins AN, Nencioni ALA, Fusco CBP, Frare EO, De Paulo MEFV, Dorce VAC IBu – Farmacologia

Introduction: *T. serrulatus* and *T. bahiensis* (Perty, 1834) scorpions are the main responsible for human envenomation in Brazil. There are no studies that report its effects on postnatal development of offspring of mothers who received the venom during the lactation period. Simulating an accident with a human we chose to inject the venom in a single day of lactation period. Our objective was to study the effects on the physical, behavioral and reflexological development of the offspring from mothers injected with the venom of the scorpion *T. bahiensis* at 2nd(2 control(C) or experimental(E)), 10th(10C or E) or 16th(16C or E) day of lactation. **Methods:** Females and their litters were divided into 3 control groups injected with 1ml/kg of saline and 3 experimental groups injected with venom in a dose of 2.5 mg/kg (this dose was determined in previous studies being able to promote a moderate envenomation without death). The rat pups, in the neonatal period, were evaluated on postnatal days (PN) according to their neurobehavioral development. Palmar grasp (PN4,6,8), negative geotaxis (PN6,8,10,12) and general activity (PN10,14,18,22-general activity and locomotion) were studied. The pups were evaluated to neuronal integrity in the CA1, CA3 and CA4 of the hippocampus areas. For determination of cytokine levels (IL-1α,IL-1β,IL-6,IL-10,INF-γ,TNF-α) the rats and their pups were treated on the 10th or 16th day of lactation 1ml/kg saline or 100µg/kg LPS or 2.5 mg/kg venom. The "t" Student test was used to determine differences of behavioral and histological analysis. For the evaluation of cytokine levels one way ANOVA followed by Tukey test was used. Data were presented as mean ± standard deviations mean and the significance level was 0.05. **Results and discussion:**On our results on the reflex development parameters of the offspring, female and male animals presented alterations related to the gender on the parameters observed, such as in righting reflex of PN10(♂2C 1.1 ± 0.3;2E 1.5 ± 1.0), negative geotaxis of PN6(♀2C 44.7 ± 36.3;2E 57.5 ± 33.4) and general activity in PN10(♂2C 259.5 ± 114.0;2E 196.3 ± 95.7), PN14(♀10C 242.9 ± 100.2;10E 192.9 ± 106.9), PN18(♀16C 276.3 ± 156.8;16E 353.7 ± 145.5/♂16C 281.4 ± 144.4;16E 359.7 ± 143.9) and PN22(♀2C 337.4 ± 131.6;2E 443.1 ± 130.3), locomotion in PN10 (♂2C 105.2 ± 58.3;2E 71.7 ± 44.6) PN18(♀16C 184.1 ± 122.6;16E 246.8 ± 109.2/♂16C 185.7 ± 106.8;16E 244.9 ± 116.8). These alterations could indicate changes in the maturity of the structures in the central nervous system (CNS) involved with the animal's motor and spatial abilities. We observed an increase in the number of viable cells in the CA1(control(C)64 ± 5;experimental(E)89.7 ± 12.1) and CA4(C 38 ± 2.7;E 52.9 ± 5.1) region, especially in puppies the 2nd day of lactation. We observed an increase in the concentration of IFN-γ in group day 10 of lactation (E 414.7 ± 26.8;C 358.5 ± 27.5), and the 16th day (E 534.8 ± 57.4;LPS 415.5 ± 44.5). With these results, we conclude that the venom, directly or indirectly, is able to cause physical, reflexology and behavior changes in offspring of mothers injected during the lactation period. These changes may be related to interference in hippocampal neurogenesis mediated by immune factors present in this microenvironment. These changes may be due to interference during critical periods of maturation of the CNS. Supported by Fapesp

09.048

Effect of lectin from *Abelmoschus esculentus* (Malvaceae) on free radicals and oxidative stress on ethanol-induced gastropathy in mice: involvement of alpha-2 adrenoceptor.

Matos SO[1], Ribeiro KA[2], Maciel LM[3], Pereira Filho SM[3], Pinto IR[2], Monteiro DAM[1], Lacerda JTJG[4], Gadelha TS[4], Gadelha CAA[4], Chaves HV[1,5], Aguiar LMV[3,2,5], Pereira KMA[1,5], Benevides NMB[6], Pinto VPT[3,2,5], Cristino Filho G[3,2,5], Bezerra MM[3,2,5], Silva AAR[1,2] [1]UFC – Dentistry, [2]UFC – Biotechnology, [3]UFC – Medicine, [4]UFPB – Molecular Biology, [5]UFC – Health Sciences, [6]UFC – Biochemistry and Molecular Biology

Introduction: *Abelmoschus esculentus*, known as okra, is used in folk medicine in the management of diabetes, diarrhea and inflammation. In this study, we sought to investigate the protective effect of a newly-discovered lectin isolated from seeds of *A. esculentus* on ethanol-induced gastropathy. The antioxidant activities were also assessed. **Methods**: The seeds were collected at Conde-Paraíba-Brazil, the lectin of *A. esculentus* (AEL) was isolated by precipitation with ammonium sulfate and purified by ion exchange chromatography(Sephacel-DEAE). Fasted mice treated with ethanol 99.9% (0.2 ml/animal, p.o., n=6 animals/group) were pre-treated with AEL (0.01, 0.1 or 1 mg/kg; i.v.), ranitidine (80 mg/kg; p.o.) or saline (0.3 ml/kg; i.v.). In different experimental sets L-NAME (NO synthesis inhibitor) or yohimbine (alpha-2 adrenoceptor antagonist) were added in order to clarify the possible action mechanism of AEL. Mice were sacrificed 30 min after ethanol and lesions were measured using a planimetry program (ImageJÒ). AEL effect was checked on gastric tissue evaluating hemoglobin levels (Hb), iron-induced lipid peroxidation (shown by suppress TBARS concentration), glutathione content (GSH), and histological assessment (H&E). Data are shown as means ± S.E.M. **Results and discussion:** AEL (1 mg/kg) reduced the percent area (%) of gastric lesions compared to the ethanol-challenged group (7.2 ± 1.9 *versus* 49 ± 5.4 injured area %, respectively), as did ranitidine (6.3 ± 1.9). Gastroprotective effects of AEL were counteracted by treatment with yohimbine (56.1 ± 8.3), but not with L-NAME (6.96 ± 1.5). AEL reduced both Hb tissue levels (6.0 ± 0.56 Hb µg/100 mg) and lipid peroxidation (169.6 ± 17.99 TBARS tissue mcg/g), and enhanced GSH (1843 ± 264.8 GSH tissue mcg/g), compared to ethanol-challenged group (14.3 ± 1.82; 299.3 ± 46.24; 1357 ± 380.7, respectively). AEL histological (H&E) characteristics were compatible with the protective effects. AEL possesses gastroprotective effects in ethanol-induced gastropathy model in mice. This activity is mediated, at least in part, by alpha-2 adrenoceptors activation, but not by NO release. AEL also has antioxidant activity that is thought to either play a role in this biological activity or to be a byproduct of alpha-2 adrenergic complex activation. **Funding Sources:** FUNCAP, CNPq, Capes, and INCT-IBISAB.License number of the Animal Ethics Committees of Federal University Pernambuco : 230760009313/2003-04.

09.049

Cinnamaldehyde reduces the production of virulence factors in Pseudomonas aeruginosa. Ferro TAF[1], dos Santos JS[1], Pinto BLS[1], Arauj JMM[1], Souza EB[1], Mendes SJ[1], Figueiredo P de MS[1], Neto VM[1], Fernades ES[1,2] [1]CEUMA, [2]King's College – Cardiovascular Division

Introduction: the production of virulence factors by pathogenic bacteria is directly related to quorum-sensing, a mechanism of communication between bacterial cells. Quorum-sensing thus, coordinates the synthesis and release of virulence factors. Molecules that inhibit quorum-sensing may represent an attractive strategy for the discovery of novel antimicrobials and also to overcome antibiotic resistance. Here, we investigated the effects of cinnamaldehyde on Pseudomonas aeruginosa, frequently associated with infection in immune compromised patients (Sousa *et al* 2013) **Methods:** Cinnamaldehyde (62.5-2000 mg/ml) effects on P. aeruginosa (ATCC 27853) growth and production of virulence factors were investigated *in vitro*. Bacteria was incubated with cinnamaldehyde for 3, 24 e 48 h in a 96-well plate containing Muller-Hinton broth and absorbance was taken as growth index (Smânia *et al* 1995). The minimal inhibitory concentration (MIC) values were then determined and used to all subsequent analysis. Cinnamaldehyde effects on bacteria adhesion to inert surfaces (latex and plate well) were evaluated in Luria-Bertani and BHI broth, respectively (Stepanovic *et al* 2004). Also, cinnamaldehyde was investigated on bacteria-induced haemolysis of human erythrocytes (Geoffroy *et al* 1987). Results: Cinnamaldehyde exhibited bacteriostatic and bactericide activity at 1000 and 2000 mg/ml, respectively. At concentrations ranging from 62.5 to 500 mg/ml, cinnamaldehyde reduced P. aeruginosa's growth in a concentration-dependent manner with an IC50 of 73.5 µg/mL. A similar effect was observed for haemolysis (IC50=43.9 µg/mL). Cinnamaldehyde also diminished P. aeruginosa adhesion to latex and the plate well, indicating a reduced biofilm formation occurs in the presence of cinnamaldehyde (IC50 of 281.5 µg/mL and 54.9 µg/mL, respectively). **Discussion:** Recent studies have investigated the antibacterial effects of cinnamaldehyde on P. aeruginosa. Indeed, evidence has demonstrated that cinnamaldehyde inhibits biofilm formation and also the production of some virulence factors released from Gram-negative pathogens (Brackman *et al* 2008). Here, we present newer and additional mechanisms by which cinnamaldehyde affects bacteria function and its interaction with the environment. This drug may be valuable to treat P. aeruginosa-related infection in immune compromised patients. This research was funded by FAPEMA, CNPq and Capes. Brackman, G., *et al*., *BMC Micro*, 8: 1, 2008. Geoffroy, C., *et al*., *Infect Immun*,55: 1641, 1987. Smânia, A., *et al*., *J Ethnopharmacol* 45: 177, 1995. Sousa, D., *et al. Clin Micro and Infect*, 19: 187, 2013. Stepanovic, S., *et al*., *Lett Appl Microbiol*, 38: 428, 2004.

09.050

A novel cytotoxic *ent*-kaurane diterpene from the stem bark of *Annona vepretorum* (Annonaceae). Dutra LM[1], Bomfim LM[2], Rocha SLA[2], Nepel A[3], Soares MBP[2], Barison A[3], Costa EV[1], Bezerra DP[2] [1]UFS – Chemistry, [2]Fiocruz-Bahia – Tissue Engineering and Immunopharmacology, [3]UFPR – Chemistry

Annona vepretorum Mart. (Annonaceae), popularly known as "bruteira", has both nutritional and medicinal uses. This work describes a novel *ent*-kaurane diterpene, *ent*-3β-hydroxy-kaur-16-en-19-al (**1**), along with 10 known compounds, *ent*-3β,19-dihydroxy-kaur-16-eno (**2**), *ent*-3β-hydroxy-kaur-16-eno (**3**), *ent*-3β-acetoxy-kaur-16-eno (**4**), *ent*-3β-hydroxy-kaurenoic acid (**5**), kaurenoic acid (**6**), caryophyllene oxide (**7**), humulene epoxide II (**8**), β-sitosterol (**9**), stigmasterol (**10**) and campesterol (**11**) from the stem bark of *A. vepretorum*. Cytotoxic activities towards tumor cell lines B16-F10 (murine melanoma), HepG2 (human hepatocellular carcinoma), K562 (human erythromyeloblastoid leukemia) and HL60 (human promyelocytic leukemia) and non-tumor cells PBMC (human peripheral blood mononuclear cells, the Research Ethics Committee of the Oswaldo Cruz Foundation [Salvador, Bahia, Brazil] approved the experimental protocol # 031019/2013) were evaluated over **1**-**6** compounds. Compound **1** was the most active showing IC_{50} (half maximal inhibitory concentration) values ranging from 2.49 to 21.02 µg/mL in K562 and B16-F10 cell lines, respectively, while presented IC_{50} value of 7.20 µg/mL in PBMC. In summary, the stem bark of *A. vepretorum* is an important source of cytotoxic *ent*-kaurane diterpenes. Supported by: Capes, CNPq, FINEP, FAPITEC/SE, FAPESB and UFPR.

09.051

Gastric ulcer healing promoted by hydroalcoholic extract and ethyl acetate fraction of green tea (*Camellia sinensis* (L.) Kuntze) in rats. Borato DG[1], Scoparo CT[2], Maria-Ferreira D[1], da Silva LM[1], Souza LM[2], Iacomini M[2], Werner MFP[1], Baggio CH[1] [1]UFPR – Pharmacology, [2]UFPR – Biochemistry and Molecular Biology

Introduction: *Camellia sinensis* (L.) Kuntze (Theaceae) is traditionally used for treatment of obesity, hypercholesterolemia and dyspepsia. Besides, the high and worldwide consumption of its leaves infusions (tea) is also due to the pleasant flavor and aroma. Our previous data demonstrated the gastroprotective action of hydroalcoholic extract from green tea (GEt) (*C. sinensis*) and its isolated compound, epigallocatechin gallate (EGCG). Taking into account, this study aimed to investigate the gastric ulcer healing effect of GEt and its ethyl acetate fraction (GEAc) using a model of chronic gastric ulcer. **Methods:** The chronic gastric ulcer was induced by application of 80% acetic acid on serosal mucosa of fasted Wistar rats (~ 200 g). After 7 days of oral treatment twice a day with vehicle (water – 1 ml/kg), omeprazole (40 mg/kg), GEt (1, 3, 10 and 30 mg/kg) and GEAc (1.8 mg/kg), the ulcer area, mucin content, inflammatory parameters [myeloperoxidase (MPO) and N-acetilglucosamidase (NAG)] and antioxidant system [reduced glutathione (GSH), lipid hydroperoxides (LOOH), superoxide dismutase (SOD) and glutathione S-transferase (GST) activities] were evaluated. *in vitro*, the scavenging activity of GEt (1-1000 µg/ml) and GEAc (0.18-180 µg/ml) were also measured. The antisecretory action was studied on the pylorus ligature method in rats (CEUA/BIO-UFPR number 689). **Results and discussion:** Oral treatment with GEt (3, 10 and 30 mg/kg) and GEAc (1.8 mg/kg) reduced significantly the gastric ulcer area induced by acetic acid in 32, 63, 71 and 70%, respectively, when compared to control group. The gastric ulcer healing promoted by GEt (10 mg/kg) and GEAc (1.8 mg/kg) was accompanied by increase of mucin content (94 and 120%) and reduction of MPO (73 and 44%). Furthermore, GEt and GEAc also prevented the depletion of GSH levels and restored the SOD activity but did not alter the GST activity. However, only GEt prevented the increasing of LOOH content compared to control group. In addition, GEt (10, 100 and 1000 µg/ml) and GEAc (18 and 180 µg/ml) reduced the DPPH free radicals in 25, 62, 80, 55 and 62%, respectively. The administration of GEt (1, 3 and 10 mg/kg, i.d. or 10 mg/kg, p.o. or 1 mg/kg, i.p.) did not alter the volume and total acidity of gastric secretion. Furthermore, the treatment of animals with GEt and GEAc for 7 days did not cause signs of toxicity. **Conclusions:** Taken together, GEt and GEAc showed pronounced antiulcer effects, possibly through maintenance of mucin content and reduction of inflammation and oxidative stress. In addition, the reduction of gastric acid secretion is not involved in the ulcer healing acceleration promoted by GEt and GEAc. However, further studies are necessary to elucidate additional mechanisms underlying this action. **Financial support:** Capes, Fundação Araucária

09.052

Antinociceptive effect of beta-caryophyllene in paclitaxel-induced peripheral neuropathy.

Segat GC[1], Costa R[2], Manjavachi MN[1], Setim C[1], Calixto JB[1] [1]UFSC – Farmacologia, [2]UFRJ – Farmácia

Introduction: Peripheral neuropathy is an important side effect that often occurs in oncologic patients treated with chemotherapeutic agents, generally presenting as pain and hyperalgesia, which impairs the patients' quality of life and hampers the treatment adhesion. The peripheral neuropathy affect up to 67% of the patients using taxanes, such as paclitaxel (PTX). Recent data showed the potential of cannabinoid agonists for the treatment of chemotherapy-induced peripheral neuropathy. In this study, we investigated the role of a selective agonist of cannabinoid receptor type 2, the sesquiterpene beta-caryophyllene (BCP), in the peripheral neuropathy induced by PTX. **Methods:** Male Swiss mice (± 30 g, n= 5-10 per group) were treated with PTX (2 mg/kg, i.p.) or vehicle (NaCl 0.9%, i.p.) in days 0, 2, 4 and 6 of experimental protocol. The mechanical hyperalgesia was evaluated using von Frey filaments (up-down method). To evaluate the acute effect of BCP on established hyperalgesia, mice were treated with the drug (12.5, 25 or 50 mg/kg, v.o.) on day 10. The chronic effect of BCP (25 mg/kg, v.o, twice a day) on established hyperalgesia was evaluated among days 10 and 16 (therapeutic treatment), while its preventive effect was evaluated among days 0 and 9 (preventive treatment). BCP effect was compared with gabapentin (50 mg/kg, v.o.). After preventive treatment, mouse spinal cord was collected in different time points for analysis by immunohistochemistry and real-time PCR (CEUA protocol number: PP00624 and PP00811). Results of *in vivo* experiments are expressed as Area Under the Curve (AUC) of defined time intervals, while Immunohistochemistry results are expressed as Optical Density (OD). **Results:** Animals treated with PTX showed a significant decrease in the withdrawal threshold starting from day 10 to at least day 28 of experimental protocol (AUC of day 0-28: 30,9 ± 2,3 for control and 11,1 ± 1,1 for PTX, $p<0,05$). Notably, the three doses of BCP were able to significantly increase the withdrawal threshold up to 5 h after acute treatment (AUC of 0-7 hours: 1,6 ± 0,3 for PTX and 5,1 ± 0,7 for BCP, $p<0,05$). When given in therapeutic treatment protocol, BCP had a more subtle acute effect than gabapentin, but its chronic antinociceptive effect was significant (AUC of day 0-28: 1,6 ± 0,5 for PTX and 4,4 ± 0,5 for PTX + BCP, $p<0,05$) . Nonetheless, when given preemptively, BCP significantly prevented the development of mechanical hyperalgesia at least for 28 days (AUC of day 0-28: 11,1 ± 1,1 for PTX and 22,7 ± 1,4 for PTX + BCP, $p<0,05$) . Interestingly, the preventive treatment with BCP produced a significant increase in the levels of CB2 receptor in the mouse spinal cord (OD: 0,2 ± 0,1 for control 7d and 17,3 ± 2,0 for PTX + BCP 7d, $p<0,05$). In addition, BCP also prevented the increase of microglial activation marker (Iba-1) levels induced by PTX in the spinal cord (OD: 7,3 ± 2,2 for PTX 10d and 3,6 ± 1,0 for PTX + BCP 10d, $p<0,05$). **Discussion:** Present data suggest that BCP seems to act by reducing the neuroinflammation and mechanical hyperalgesia caused by PTX, probably via activation of the CB2 receptor in the spinal cord. These data shows a potential role of BCP on the prevention and treatment of the peripheral neuropathy induced by PTX. **Financial support:** Capes, CNPq, FAPESC

09.053

Action of clathrodin and analogues: small molecule modulators of voltage-gated ion channels. Peigneur S[1], Zula A[2], Zidar N[2], Chan-Porter F[3], Kirby R[3], Rogers M[3], Madge D[3], Ilas J[2], Kikelj D[2], Tytgat J[1] [1]KULeuven – Toxicology & Pharmacology, [2]UL – Pharmacy, [3]Xention Ltd

It has been well recognized that voltage-gated ion channels play a crucial role in inherited diseases, such as cardiovascular arrhythmias, central nervous system disorders and pain syndromes. This knowledge highlights these channels as targets of novel compounds that will hopefully fulfil the unmet therapeutic need to successfully treat these disorders. Therefore, small molecules capable of selective targeting and modulation of voltage-gated ion channels represent attractive pharmacological tools, either to identify the specific isoform involved in different channelopathies or as potential therapeutics. Over the last few decades, a number of compounds with promising pharmacological activity have been characterized from marine organisms. Clathrodin is a marine alkaloid and believed to be a modulator of voltage-gated sodium (Na_V) channels. Therefore, clathrodin could represent an interesting lead compound. In this aim clathrodin has been chemically synthesised and a library of 120 synthetic analogues was constructed. Clathrodin and its synthetic analogues were subjected to screening on a broad range of ion channels, both in voltage clamp and patch clamp conditions. Clathrodin was reinvestigated for its potency and Na_V channel subtype selectivity against a panel of 8 Na_V channel isoforms. Even though clathrodin was not found to exert any activity on Na_V channel isoforms, some analogues were capable of modulating the Na_V channels (EC_{50} = 3 µM), hereby validating the pyrrole-2-aminoimidazole alkaloid structure as a core structure for future design of small molecule-based Na_V channel modulators. Interestingly, when these small molecule compounds were tested for activity against voltage-gated potassium channels (K_V), it was found that several compounds were capable of potently targeting Kv channels. The most promising compounds displayed IC_{50} values ranging from 200 nM to 2 µM. In order to obtain a selectivity profile, the selected lead compound were subjected to a screening against 20 different K+ channels among wich 18 K_V channel isoforms (K_V1.1–K_V1.6, K_V2.1, K_V3.1, K_V4.2, K_V4.3, K_V7.1-K_V7.5, K_V10.1, hERG, the insect channel Shaker IR) and 2 cloned hyperpolarization activated cyclic nucleotide-sensitive cation non-selective channels (HCN1 and HCN2). Furthermore, we performed in depth structure-function studies, allowing us to pinpoint the structural moieties contributing to subtype selectivity or potency of these alkaloid structures. In conclusion, it was found that the 2-aminoimidazole alkaloid structure represents an interesting pharmacological template for the development of novel lead compounds in the frame of drug discovery focussing on ion channel involved disorders.

09.054

High-mobility group box-1 protein in adenine-induced chronic renal failure and the influence of gum arabic thereon. Ali B H[1], Al Za'abi M[1], Al Shukaili A[2], nemmar A – [1]Sultan Qaboos – Pharmacology, [2]Sultan Qaboos University – Microbiology and Immunology

Introduction: Pathogenesis of adenine–induced chronic renal failure may involve inflammatory, immunological and/or oxidant mechanisms. Gum acacia (GA) is complex polysaccharide that acts as an anti-oxidant, and to modulate inflammatory and/or immunological processes. Therefore, we tested here the effect of GA treatment (15% in the drinking water for 4 weeks) in plasma and urine of rats, on a novel cytokine that has been shown to be pro-inflammatory, viz, DNA-binding high-mobility group box-1 protein (HMGB1). **Methods**: Male Wistar rats (n =24), aged 8 weeks and initially weighing about 200g were used. They were kept at standard conditions, and randomly divided into four equal groups as follows: **Group 1**: Fed normal diet, and given normal saline (control, 0.25 mL/rat), orally daily for 28 days. **Group 2**: Fed normal diet, and given GA (15%) in the drinking water daily for 28 days. **Group 3**: Given adenine (0.75% w/w) in the feed, and given normal saline (control, 0.25 mL/rat), orally daily for 28 days. **Group 4**: Given adenine (0.75% w/w) in the feed, and given GA (15% w/v) in the drinking water daily for 28 days. The research was approved by our Animal Research Ethics Committee of our college (# SQU/COMHS/ Pharma, 2012/010). High-mobility group box-1 protein was measured using commercial ELISA kit bought from Shino-Test Corporation (Japan) **Results & Discussion**: Adenine (0.75% in the feed, 4 weeks) significantly increased indoxyl sulphate, urea and creatinine concentrations in plasma, and significantly decreased the creatinine clearance as reopted before [1,2]. GA significantly abated these effects. The concentrations of HMGB1 in urine before the start of the experiment were similar in all four groups. However, 24-h after the last treatment, adenine treatment increased significantly the concentration of HMGB1 when compared to the control. GA treatment insignificantly decreased the HMGB1 concentration in urine. Moreover, the concentration of HMGB1 in plasma obtained 24h after the last treatment in rats treated with adenine was drastically reduced compared to the control group. This may explain its significant rise in urine. In conclusion, the HMGB1 can be considered a potentially useful biomarker in adenine induced CRF and its treatment. **Acknowledgments**: This work was funded by The Reserach Council (SQU/ Pharm/ 2011/01). **References:** 1. Ali BH, Al-Salam S, Al Za'abi M, Waly MI, Ramkumar A, Beegam S, Al-Lawati I, Adham SA, Nemmar A. New model for adenine-induced chronic renal failure in mice, and the effect of gum acacia treatment thereon: comparison with rats. *J Pharmacol Toxicol Methods*: 2013 ;68:384-93. 2. Ali BH, Al-Husseni I, Beegam S, Al-Shukaili A, Nemmar A, Schierling S, Queisser N, Schupp N. Effect of gum arabic on oxidative stress and inflammation in adenine-induced chronic renal failure in rats. *PLoS One.* 2013;8(2):e55242.

09.055

Effects of (-)- myrtenol on human gastric epithelial cell viability and migration. Viana AFSC[1], Santos VG[2], Silva ACA[2], Lopes MTP[2], Sousa DP[3], Oliveira RCM[4], Santos FA[1] [1]UFC – Pharmacology, [2]UFMG – Pharmacology, [3]UFPB, [4]UFPI – Medicinal Plants

Introduction: The (-)-myrtenol (MYRT) is a natural monoterpene found in the essential oils of some aromatic plants, such as the *Rhodiolarosea* L.(Crassulaceae). It is mostly used as a flavouring agent. Previous studies revealed the gastroprotective effect of MYRT in different experimental models of the gastric ulcer. Cell migration and/or proliferation plays a role in the repair process of gastric ulcer, so this study investigated the effect of MYRT on cell migration and viability using human epithelial gastric cell AGS. **Methods:** AGS cells (ATCC CRL-1739) were cultured at 37°C in 5% CO_2 in Ham F-12 medium supplemented with 2 mM L-glutamine, 10% FBS, 100 mg/mL penicillin and 100 mg/mL streptomycin. The cytotoxicity assay was performed using AGS cells ($5x10^3$cells/mL). Briefly, confluent cultures AGS cells were treated with MYRT (0.001-10000 nM) for 48 hours. Untreated cells served as controls. After 48 h, 10 µL of MTT solution (0.5 mg/ml in PBS) was added. Cell viability was determined with a 570 nm filter. In the cell migration scratch assay the AGS cells were grown to the confluence of $2x10^5$ cells/mL on 24-wells tissue culture plate. P200 pipette tip was used to perform a linear scratch across the cell monolayer. The cells were treated with negative control (FBS 0.5%); positive control (FBS 10%) or MYRT (0.1 – 100 nM) in the presence of a proliferation inhibitor, hydroxiurea (5 mM). At 6 and 24 hours of treatment, the scratched area images were performed, using an inverted microscope with acoupled camera. The migration rate of AGS cells was calculated using Image Analyzer Software (TScratch) and results reported as the percentage scratch closure. The results are presented as the mean ± SEM. ANOVA one way or two way followed by the Tukey or Bonferroni *post hoc* test, respectively. Values of $*p<0.05$, $**p<0,01$,$***p<0,001$ were considered to be significant. **Results:** Higher concentration of MYRT (10000 nM) demonstrated a reduction in cell viability by 14.6% (44 ± 0.4%***) compared to untreated cells (58 ± 1%), suggesting a likely toxic response at higher concentrations. In contrast, low concentrations of MYRT0.01; 0.1; 1; 10 or 100 nM demonstrated an increase in cell viability (64 ± 1%*; 64 ± 1%*; 64 ± 1%*, 65 ± 1%** and 66 ± 1%***, respectively). To determine the effect of MYRT treatment on cell migration capacity, a scratch assay was performed. Cells treated with MYRT (0.1; 1; 10 or 100 nM), showed an approximately 30 %** or 70 %***(FBS 10%) increase in scratch closure, compared to negative control 25 %(FBS 0.5%), after 6 hours of treatment. A similar closure injury profile was observed for 24 hours treatment also (aproximately 60%*** MYRT, 100%*** – FBS 10%, and 29% – FBS 0.5%). The results suggest that MYRT has the ability to heal scratch wounds or stomach lesions, which may result from an increase in gastric epithelial cell migration. Further investigations are needed to elucidate the molecular mechanism(s) involved in these effects. **Financial Support:** UFC/UFPI/Capes/CNPq.

09.056

Efficacy of lectin from *Abelmoschus esculentus* on zymosan-induced temporomandibular joint inflammatory hypernociception in rats. Freitas RS[1], Fernandes MEF[2], Vieira FTA[3], Lacerda JTJG[4], Gadelha TS[4], Pereira KMA[5], Brito GAC[6], Cristino Filho G[1], Pinto VPT[1], Bezerra MM[1], Pinto IR[1], Gadelha CAA[4], Chaves HV[3] [1]UFC – Biotecnologia, [2]UFC – Odontologia, [3]UFC – Medicina, [4]UFPB – Biologia Celular e Molecular, [5]UFC – Ciências da Saúde, [6]UFC – Ciências Morfofuncionais

Introduction: The antinociceptive and anti-inflammatory effects of a newly-discovered lectin, isolated from organic Okra seeds (Abelmoschus esculentus L Moench) (AEL), were investigated in the zymosan-induced temporomandibular joint (TMJ) inflammatory hypernociception in rats. **Methods:** Experiments were approved by the Institutional Animal Care and Use Committee of the Federal University of Ceará, Fortaleza, Brazil (74/2013). Rats were pretreated i.v. with Ae (0.01, 0.1 or 1 mg/kg) or saline (non-treated group) 30 min before the intra-articular injection of zymosan (2 mg, 40 µL) in the left TMJ. Von Frey test was used to evaluate hypernociception (g) at 4 h after Zy. 6 h after Zy injection it was collected synovial lavage for leukocyte counting and myeloperoxidase (MPO) measurement, and joint tissue and trigeminal ganglion for histopathological analysis (H&E) and IL-1β dosage (ELISA). Vascular permeability was evaluated by Evans Blue extravasation measurement. **Results**: AEL (0.01, 0.1 or 1 mg/kg) increased ($P<0.05$) the nociceptive threshold (56.7 ± 1.1; 69.1 ± 2.1 or 81 ± 1.7, respectively), when compared to non-treated group (43.8 ± 2.2). AEL (1 mg/kg) reduced ($P<0.05$) cell influx (1433 ± 609), MPO activity (22.1 ± 10.7), inflammatory cell influx in the synovial membrane (0.5 ± 0.2), and Evans Blue extravasation measurement (131.7 ± 3.1), compared to non-treated group (37844 ± 6203; 127.5 ± 27.6; 3 ± 0.4; 166.3 ± 6.7, respectively). AEL (1 mg/kg) also reduced IL-1β levels in both joint tissue (3.11 ± 0.49) and trigeminal ganglion (1.55 ± 0.34), when compared to non-treated group (11.01 ± 0.83 and 4.72 ± 0.47, respectively). **Conclusions**: AEL is effective in zymosan-induced TMJ inflammatory hypernociception in rats, and its efficacy at least in part, depends on IL-1β inhibition. AEL may represent a potential therapeutic to ameliorate the inflammatory TMJ painful condition. **Funding Sources**: FUNCAP, CNPq, Capes, and INCT-IBISAB.

09.057

Analysis of phenolic contents and harpagoside of crude extract and ethyl acetate fraction of *Harpagophytum procumbens* by HPLC/DAD and its inhibitory activity on monoamine oxidase. Schaffer LF[1], Busanello A[1], Peroza LR[2], Boligon AA[3], Dotto MM[4], Athayde ML[3], Sudati JH[5], Fachinetto R[1], Wagner C[5] – [1]UFSM – Farmacologia, [2]UFSM – Bioquímica Toxicológica, [3]UFSM – Ciências Farmacêuticas, [4]UFSM – Farmácia, [5]Unipampa

Introduction: *Harpagophytum procumbens*, popularly known as devil's claw, is a plant widely used in the treatment of diseases of inflammatory origin. Many of the pharmacological actions of *H. procumbens* have been attributed to the presence of iridoid glycoside, Harpagoside. However, other studies showed the effects of *H. procumbens* are not exclusively due to the presence of harpagoside and, instead of this; their effects have been associated with the presence of other compounds naturally present in plants, such as flavonoids. However, little is known about the effects of *H. procumbens* on central nervous system (van Wyk, 2008; Mncwangi *et al.*, 2012).Objective: This study analyzed the effects of crude extract and ethyl acetate fraction of *H. procumbens* on the activity of monoamine oxidase (MAO) in rat brain homogenate, as well as the content of phenolic compounds and harpagoside present in the same. **Methods:** The inhibitory activity of MAO isoforms (A and B) in rat brain was evaluated as previously described (Villarinho *et al.*, 2012). Furthermore, the phenolic contents and harpagoside present in crude extract and ethyl acetate fraction were quantified by high performance liquid chromatography (HPLC/DAD) (Schaffer *et al.*, 2013). The experimental protocol was approved by internal ethical commission of UFSM under the number 025/2011. Results were expressed as mean ± SEM. **Results and discussion:** In the present study, it was showed that *H. procumbens* crude extract and ethyl acetate fraction had the ability of significantly inhibit both isoforms of MAO ($p<0.001$) in a concentration dependent. However, the ethyl acetate fraction showed higher inhibitory potency on MAO activity (MAO A- IC_{50}: 137.47 ± 15.35 µg/ml, MAO-B IC_{50}: 130.86 ± 7.41 µg/ml) than the crude extract (MAO A IC_{50}: 386.21 ± 19.19 µg/ml, MAO-B IC_{50}: 342.34 ± 21.02 µg/ml), besides showing higher concentration of phenolic compounds and harpagoside. However, more studies should be conducted to investigate other effects of this medicinal plant relating its constituents, with the aim of investigating the whole therapeutic potential of this plant as well as identify some possible effects of its indiscriminate use. **References**: Mncwangi, N. *J Ethnopharmacol* v. 142, p. 756, 2012. Schaffer, L.F. Neurochem Res. V. 38, p. 2257, 2013. van Wyk, B.E. *J Ethnopharmacol* v.119, p.342, 2008. Villarinho J.G. Prog Neuropsychopharmacol *Biol Psychiatry*. V. 39, p. 32, 2012. **Sources of Financial support**: CNPq/Capes/FAPERGS/DECIT/SCTIE-MS/PRONEM #11/2029-1

09.058

Effects of long-term administration of *Senna occidentalis* seeds on the hematopoietic tissue of rats. Teles AVFF, Górniak SL FMVZ-USP – Pathology

Introduction: During the development of new drug or therapeutic agent, an important aspect is to predict whether the substance in question is toxic to the bone marrow and if the toxicity will be specific to one or more of its cell lines. Due to the large proliferative capacity of hematopoietic tissue, the bone marrow cells may be targets of the toxic action of various substances, including herbal products. *Senna occidentalis* (*S. occidentalis*) is a toxic leguminous plant found ubiquitously as a contaminant of crops. All parts of the plant are toxic, but most of the *S. occidentalis* toxicity is found in the seeds. *S. occidentalis* has been shown to be toxic to several animal species, causing degenerative lesions mainly in muscles. This is the first report describing alterations in rats' hematopoietic tissues caused by *S. occidentalis* seeds. The aim of the present work was to investigate the effects caused by the chronic use of *S. occidentalis* on the hematopoietic organs. **Materials and Methods:** For this study 40 male Wistar rats with approximately 60 days old were used and divided in the following groups: 1 (control), 2 (0.5%), 3 (1%) and 4 (2%). The effects of 0.5%, 1.0 % and 2.0% w/w concentrations of *S. occidentalis* seeds mixed with commercial ration were studied. The rats in the experimental groups received daily for a period of 90 days diets containing 0.5%, 1%, and 2% of *S. occidentalis*. The rats in the control group received a diet without added seeds of the plant throughout the experimental period. At the end of this period, the animals were euthanized with a solution of ketamine (50 mg/kg) and xylazine (5 mg/kg). After deep anesthesia and euthanasia of animals, bone marrow was collected for the determination of cellularity and mielogram. We also performed the blood count as well as the pathological examination of the thymus and spleen. All experimental procedures were performed in compliance with the Committee on Care and Use of Laboratory Animal Resources at Faculty of Veterinary Medicine and Animal Science, University of São Paulo, Brazil (n° 152/FMVZ/2012 – project 2692/2012). **Results and discussion:** The blood toxicity may be manifested by changes in the number of mature cells in blood or bone marrow. This study showed that rats from group 4 presented a significant decrease ($p<0.05$) in the number of total leukocytes ($4.57x10^9$/L) in comparison with control group ($6.59x10^9$/L). Additionally, we observed reduction in hemoglobin values on group 4 (15.98 g/dL) when compared with control animals (17.21 g/dL). The blood test data corroborates the changes observed in bone marrow of these animals since it was observed a significant decrease of the Myeloid:Erythroid (M/E) ratio which reduced from 3.20 to 2.80. The significant reduction in the M / E ratio in animals belonging to different experimental groups occurred due to increase of polychromatic erythroblasts in the bone marrow of these animals. Chronic treatment for 90 days with *S. occidentalis* in di*et al*so promoted a significant reduction in spleen cellularity (from $3.80x10^6$ cells to $2.32x10^6$ cells) and histopathological changes. Based on the data presented we could suggest that the chronic treatment for 90 days with *S. occidentalis* in diet causes blood toxicity. **Support:** National Council for Scientific and Technological Development (CNPq)

09.059

Antimicrobial activity of *Nectandra lanceolata* essential oil. Pires LC[1], Garlet QI[2], Spall S[1], Gressler LT[3], Júnior GB[3], Silva DT[4], Vargas APC[5], Heinzmann BM[6] [1]UFSM – Farmácia, [2]UFSM – Farmacologia, [3]UFSM – Medicina Veterinária, [4]UFSM – Engenharia Florestal, [5]UFSM – Medicina Veterinária Preventiva, [6]UFSM – Farmácia Industrial

Introduction: Bacterial contamination of fish and fish products goes to humans through feeding and for this reason, microbiological control in aquaculture proceeding is relevant. *Plesiomonas shigelloides* and *Aeromonas hydrophila* are Gram-negative bacillus linked with fish infection (JOH, S.J., Vet Microbiol, 163: 190, 2013). Classic antibiotics have been used on fish for treatment bacterial infections, which can promote microbial resistance (JUN, J. W., Afr J Microbiol Res, 5: 5019, 2011). Nowadays, natural products, mainly essential oils (EO), are target for studies of new drugs with antibiotic properties that can be suitable in aquaculture. *Nectandra lanceolata* (Lauraceae), known as "canela-amarela", widely occurs in Brazil (FORZZA, R.C., *Angiospermas* in Lista de Espécies da Flora do Brasil, 2014). There are no literature data about the evaluation of antimicrobial activity of its EO. **Methods:** Leaves of *N. lanceolata* were colleted in November, 2013, in Silveira Martins, RS, Brazil. EO extraction was performed by hidrodistillation in Clevenger type apparatus for 3h (FARMACOPÉIA BRASILEIRA, 5th. ed., 2010). The yield was calculated on dried weight basis, and EO constituents were identified by GC-MS. The antimicrobial assay were performed by microdillution method according to *Clinical Laboratory Standards Institute* guidelines (2008), using two clinical isolated strains of *P. shigelloide s*and a single strain of *A. hydrophila* from infected silver catfish (*Rhamdia quelen*). The applied concentrations ranged from 6400 to3.12 µg/mL for determination of minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MCB). **Results:** EO yield was 0.16% (w/w). From the 43 constituents identified, bicyclogermacrene (16%), β-caryophyllene (14%), spathulenol (11%) and dilapiol (10%) are the major ones. The two strains of *P. shigelloides* showed distinct susceptibility profile to the EO. For one of the strains a MIC at 200 µg/mL and MCB at 6400 µg/mLwere observed. For another stain of *P. shigelloides* as well as for a strain of *A .hydrophila* assayed, no antibacterial activity could be detected at the tested concentrations. **Discussion:** There are no reports in the literature on the antimicrobial activity of EO *N. lanceolata*. The sesquiterpene β-caryophyllene identified in this study, has been reported in the literature for EO of this species (SILVA, G.T., XXIII SIC, UFRGS, 2011). On the other hand, the other detected components are not described for this EO yet. Regarding literature data for natural products, MIC values bellow 600 µg/mL are considered strong inhibitors (ALIGIANIS, N., J Agr Food Chem, 49: 4168, 2001). Additionally, previous studies showed antimicrobial action for the major constituents identified in this work against of *Aeromonas* spp. (STARLIPER, C.E., J Adv Res., in press, 2014). **Financial Support:** FAPERGS/ PRONEX; Ministério da Pesca e Aquicultura/ MCT/ FINEP; INCT ADAPTA; CNPq and FAPEAM

09.060

Resin from *Protium heptaphyllum* inhibits adipogenesis through the PPAR-γ signaling Pathway in 3T3-L1 cells. Melo KM, Marinho Filho JDB, Araujo AJ, Rao VS, Santos FA
UFC – Physiology and Pharmacology

Introduction: *Protium heptaphyllum* is a plant popularly known as "almecegueira" and "breu-branco-verdadeiro" and grows all over the Brazil, largely in Amazon region. The *P. heptaphyllum* resin (RPH) has gastroprotective and anti-inflammatory effects in mice and rats and is used in folk medicine as analgesic, anti-inflammatory, antiulcer and wound healing actions. **Objective:** The aim of this study was to investigate the antiadipogenic effect of the resin obtained from *P. heptaphyllum* (RPH) in 3T3-L1 cells. **Methods:** The cytotoxicity of the RPH was tested against the preadipocyte cell line 3T3-L1 by MTT assay, after 72h of incubation. The differentiation of the 3T3-L1 cells to adipocytes was induced using prodifferentiative agents. Simultaneously, the cells were treated with the RPH at concentrations of 6.25; 12.5; 25 and 50μg/ml. The results were observed by Oil-red-O stain. The proteins expression (PPARγ, C/EBPα, C/EBPβ, β-actin) were analyzed by Western Blot test. **Results and discussion:** The RPH showed no cytotoxic effects (IC_{50}> 50μg/ml) although a reduction in the adipogenesis process was observed in the 3T3-L1 cells treated with RPH. In addition, it was observed a down-regulation of the several proteins levels (PPARγ, C/EBPα and C/EBP-β) dose dependent manner. **Conclusions**: Our findings suggest that the resin obtained from *P. heptaphyllum* down-regulates the expression of C/EBP-β and subsequently inhibits the activation of PPARγ and C/EBPα with no significant cytotoxicity and therefore may be a promising candidate in the development of newer anti obesity drugs. **Supported by**: CNPq, Capes, FUNCAP.

09.061

Heparin antagonize the cytotoxic and some enzymatic activities of *Apis mellifera* bee venom. El-kik CZ[1], Gaban GA[1], Fonseca TF[1], Tavares-Henriques MS[1], Calil-Elias S[2], Melo PA[1] [1]UFRJ –Farmacologia, [2]UFF – Farmacologia

Apis mellifera venom comprises a mixture of components such as proteins, peptides and small organic molecules (Habermann, Science, 1972). Toxic effects of bee venom are attributed to mellitin, apamin and phospolipase A2. Mellitin and phospolipase A2 interact with lipid membranes and consequently have a hemolytic action and apamin is an antagonist of calcium-activated potassium channels. Victims of multiple Africanized bee stings present allergic reactions and systemic alterations such as renal acute failure, cardiac failure, hemorrhage and shock (El-Kik *et al*., *Toxicon*, 2013). There is no specific bee anti-venom described, so we investigate the ability of heparin, a sulphated glycosaminoglycan, to antagonize the cytotoxic and some enzymatic activities of A. mellifera venom in different experimental conditions. Both heparin and the fractionated heparin (0.1-10.0 µg/mL) inhibited the phospholipase and hyaluronidase venom activities. The edema induced by 0.3 mg/kg of bee venom injection in mice was also inhibited by 1.0-10.0 mg/kg of heparin or fractionated heparin. The venom myotoxicity induced by perimuscular venom injection in mice induced increase of plasma creatine kinase activity from the basal level of 69.66 ± 9.28 (n=5) to 2101.32 ± 161.03 U/L (n=5), two hours after the injection. This effect was inhibited by both heparins (10.0 mg/kg), in three different protocols, pre-incubation, pretreatment and post treatment and the inhibition were in the range of 25 to 70%. Microscope examination of mouse EDL muscle exposed to the venom after perimuscular injection showed cell edema and myofibrillar disorganization, myonecrosis and inflammatory cells. These microscopy findings also showed that heparin protected and antagonized the venom effect in three different protocols, pre-incubation, pretreatment and post treatment. They also indicated a strong anticytotoxic effect of heparin against the A. mellifera venom protecting the muscle tissue from the myonecrosis and enzymes effects. All the animal procedures were in accordance with international guidelines for the use of animals and were approved by the CEUA-CCS, UFRJ No DFBCICB072-04/16. (Gaban, G.A **in memorian*) Faperj; CNPq; Capes

09.062

Ex vivo **effect of** ***Terminalia tanibouca*** **ethanolic extract on the gastric wall mucus of mice**. Nunes PHM[1], Sousa PMB[2], Barros RM[3], Brito KS[1], Martins MCC[1,3] [1]UFPI, [2]UESPI, [3]UNINOVAFAPI

Introduction: *Terminalia tanibouca* Smith (*Combretaceae*) is commonly called "pau d'água", "cuiarana" or "tanibouca" and has been used for the treatment of diseases involving the gastrointestinal system such as diarrhea and ulcers. The ethanolic extract from the stem bark of this plant (TtEE) showed significant antiulcerogenic activity against acute gastric ulcer induced by ethanol in rats (CARVALHO, XX Congresso Ítalo-Latinoamericano de Etnomedicina, 2011, Fortaleza-CE, Resumos, 137, 2011). The ethanolic extract from the stem bark of *Terminalia fagifolia* Mart. & Zucc, another *Combretaceae*, showed similar antiulcerogenic activity but reduced the *in vivo* gastric wall mucus content of rats (NUNES, BioM Res Int, 2014, 1, 2014) and the *ex vivo* of mice (data not published). This study investigated the effect of TtEE on the *ex vivo* gastric wall mucus content of mice. **Methods:** The project was approved by the Ethics Committee for Animal Experimentation (CEEA) of UFPI (Nº-042/09). Groups of female mice (23.9 ± 0.9 g, n=3) held in fasting for 18 h were euthanized with thiopental (100 mg/kg) and have had their stomachs removed, opened by the lesser curvature and washed. Fragments of the body of the stomach were removed, weighed and immersed in 2 mL of distilled water (control), suspension of TtEE (5 mg/mL) or N-acetyl cystein (NAC, 5 mg/mL). After 30 minutes the gastric wall mucus content was quantified by the alcian blue method (CORNE, J Phys, 242, 116P, 1974). Data were expressed as mean ± standard error of mean (SEM) and subjected to analysis of variance followed by Tukey's post-test for comparison between the groups. The significance level was set at 5% ($p<0.05$). **Results:** The gastric wall mucus content (µg/g) of the stomachs of the mice treated with TtEE (43.3 ± 4.5) or NAC (40.3 ± 15.5) was similar but significantly lower ($p<0.001$) than that observed in the stomachs of the animals of the control group (304.0 ± 26.9). **Discussion**: The ethanolic extract of *T. tanibouca* showed similar effect to that observed with *T. fagifolia*, reducing the *ex vivo* gastric wall mucus content of mice. Complementary studies are required in order to clarify the mechanism of this "mucolytic" activity of these plants.

09.063

Fish oil reduces cfa inflammatory pain by modulating PGE2, TNF-α and resolvins levels.

Lobo BWL[1], Teixeira MS[1], Silva NLC[2], Silva LL[2], Lima CKF[2], Miranda ALP[2], Ramos MFS[1], Dellamora-Ortiz GM[1] [1]DEFARMED-FF-UFRJ, [2]BioTecFar-UFRJ-FF

Introduction: Omega-3 fatty acids (ω-3 FA) from fish oil (FO) has been widely studied as a complementary therapy in the treatment of inflammatory diseases. However knowledge is lacking as to the effective dose and treatment duration for each inflammatory disease (NOBRE *et al.*, 2013; CALDER, 2011; VEIGAS *et al.*, 2011). Currently available drugs used to treat inflammatory and painful diseases are, in most cases, inadequate and ineffective (MEOTTI *et al.*, 2006; SILVA *et al.*, 2012). We studied the effects of oral doses of FO in a model of sub-chronic inflammatory pain induced by Complete Freund Adjuvant (CFA), considering the amount of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) administered per day. **Methods:** Protocols were approved by UFRJ ethical animal care committee (FARMACIA01). Inflammatory pain (IP) was induced in Wistar rats of both sexes by intraplantar injection of CFA in the right hind paw (KITAGAWA *et al.*, 2005; BORTALANZA *et al.*, 2002) in 6 different groups (6-8 animals/group). Three groups were orally treated with ω-3 FA (commercial fish oil concentrate – FOC and a natural FO) during 7 days before and 5 days after IP induction: FOC 460 mg, FOC 690 mg and FO 460 mg. DEXA group was pre-treated with saline solution and received dexamethasone 1 mg/kg/day. SALINE and CFA + SALINE control groups were treated with saline during 12 days and the IP induced on the 7th day (CFA_SALINE). Edema and pain were evaluated on the day of induction and on the 4 following days. Pain sensivity was assessed by mechanical stimulation (Von Frey method) and edema by measuring paw thickness (MEERT and VERMEIRSCH, 2005). Subplantar tissue of inflamed paws was removed after 96h to evaluate mieloperoxidase activity (MPO), prostaglandin E_2 (PGE_2), tumor necrosis factor-alpha (TNF-α) and resolvins (RvD2) levels (. Results were expressed in mean ± SEM and % inhibition compared to CFA + SALINE group. Data were statistically analyzed by Student's *t* test and ANOVA (Tukey post-test and Bonferroni post-test) (*p<0.05; **p<0.01; ***p<0.001). **Results:** The edema was only slightly reduced at 48 hs e 96 hs after all treatments (by 20-30% of inhibition). Mechanical pain sensitivity was inhibited in DEXA (61%* – 48h) and FOC 690 (50%* – 48h and 100%* – 96h) groups compared to CFA + SALINE group. The MPO, PGE_2, TNF-α levels were reduced, respectively, by FOC 690 (56.7%*; 71.5%***; 67.9%**), FOC 460 (62.60%**; 61.7%**; 57.7%**), FO 460 (69.8%**; 52.7%*; 26.3%) and DEXA (74.3%**; 61.7%**; 87.7%***) compared to the CFA + SALINE. In contrast, RvD2 levels were significantly increased in treated groups (FOC690 x SALINE=336%*; FOC690 x CFA + SALINE=3196%**; FOC460 x SALINE=352%**; FOC460 x CFA + SALINE=3347%**; FO x SALINE=74%; FO x CFA + SALINE=1653%). **Discussion and conclusion:** The mechanism of action of ω-3 FA seem to be related to 3-series PGs synthesis from EPA, which are less inflammatory than 2-series PGs, and to resolvins formation originated from EPA and DHA. An effective dose and treatment duration have not yet been established for each inflammatory/painful disease (CALDER, 2011). Our results corroborate previously suggested mechanism of action of EPA and DHA, leading to the onset of resolvins, and indicate that the used doses were effective to reduce inflammatory pain in a short period of pre-treatment indicating potential use of ω-3 FA from FO as an adjuvant treatment for painful and inflammatory diseases. FOC presented better results than FO, probably due to the presence of other fatty acids in FO composition. Capes, CNPq, Faperj, PIBIC.

09.064

Evaluation of the antispasmodic effect of *Cardiospermum corindum* L. (Sapindaceae) on rat ileum. Silva VA[1], Freire IS[1], Rigoni VLS[2], Costa AEA[2], Silva FL[3], Barbosa-Filho JM[3], Nouailhetas VLA[2], Silva JLV[1] [1]Uninove – Farmácia-Bioquímica, [2]Unifesp –Biofísica, [3]UFPB

Introduction: *Cardiospermum corindum* L. (Sapindaceae), popularly named "balãozinho" and autoctone in Brazil, showed antispasmodic effect on rat ileum, when used an ethanol crude extract from its aerial parts (Cc-EtOH). The aim of this study was the evaluation of extract on tonic contraction of rat ileum. **Methods:** ileum was isolated from rats (250-300g), after 24h fasting period, was prepared on glass baths containing Krebs modified solution, at 37˚C, 1g/force resting tension and bubbled O_2. Tissue contractile response was recorded by acquisition analogy system AQCD (AVS Projetos, Brazil). The contractions were stimulated by addition of KCl (40 mM), a depolarizing agent, or carbachol (1 µM), a muscarinic agonist. After sustented contraction, about 15 minutes, the Cc-EtOH (1, 3, 9, 27, 81, 243 or 730 µg/mL) was incubated in cumulative manner. The results were expressed by the inhibition of maximum response to contractile agents. Values EC_{50} were calculated by non-linear regression from extract response. These procedures were approved for ethics committee in animal use of Unifesp (CEUA 4295060514/14). The data were expressed as mean ± SEM and analyzed by GraphPad Prism 5.0 software, tested for significance by T-test, where results were regarded as significant when $p<0.05$. **Results and discussion:** the extract Cc-EtOH (1-730 µg/mL) relaxed the ileum rat (n = 4) in a concentration-dependent and equipotent manner, when pre-contracted with KCl (EC_{50} = 63.1 ± 18.9 µg/mL) or carbachol (EC_{50} = 65.2 ± 15.5 µg/mL). As extract Cc-EtOH presented EC_{50} with similar values, it is indicative that extract may act through the same signaling pathway used by those contractile agents, probably the voltage-dependent calcium channels (VOCCs). These results demonstrate that the aerial parts from Cardiospermum corindum have antispasmodic effect on rat ileum, probably due to VOCCs blocking. Support: CNPq/ Capes

09.065

Cytotoxic activity of BTAHF, a new hemorrhagic P-I class SVMP isolated FROM *Bothriopsis taeniata* snake venom, on C2C12 myoblasts and myotubes cell lines.

Torres-Huaco FD[1,2], Bustillo S[3], Leiva LC[3], Marangoni S[1] [1]IB-Unicamp – Bioquímica, [2]FCM-Unicamp – Farmacologia, [3]UNNE – Bioquímica

Introduction: Snake Venom Metalloproteinases (SVMP) are one of the main constituents of botropic venoms. This protein family has an important role on local effects following botropic envenomation. Cytotoxicity is among the main mechanisms involved in local lesions, which, is produced by PLA_2, L-amino oxidases, SVMP and others. However, the role and mechanisms by which SVMP induce cytotoxicity still are under controversy. In this work we showed the purification of BtaHF, from *Bothriopsis taeniata* crude venom, and its cytotoxic effects on C2C12 myoblast and myotubes cell line. **Methods:** BtaHF was purified by combination of molecular size exclusion (Sephadex G-75, 1x100 cm) and cation exchange high pressure liquid chromatography (DEAE 8HR AP-Minicolumn, 5x50 mm, Waters). The molecular mass was determinate using Polyacrilamide gel electrophoresis. The proteolytic activity and minimum hemorrhagic dose of BtaHF were determinate using azocasein as substrate and mouse skin model (CEEA/Unicamp protocol 1836-1), respectively. Myoblast C2C12 cell line was cultivated using Dulbecco's minimum essential medium supplemented with 10% bovine fetal serum, penicillin and streptomycin. C2C12 myotubes were obtaining from differentiation of myoblasts cell line after three days of bovine fetal serum deprivation and 5% CO_2 exposure. BtaHF cytotoxic effects (31-500 µg/mL) were determinate qualitatively with Tripan Blue coloration and quantitatively with Lactate Desidrogenase determination kit (LDH-UV kit, Wiener, Argentine). The effect of BtaHF (30 and 60 µg/mL) on cellular adhesion was determinate by Cristal Violet coloration method measuring the absorbance of suspended cells at 620nm. **Results:** BtaHF, purified from two chromatographic steps, is a single chain protein with a molecular mass of 25kDa, a specific caseinolytic activity of 189.89 U/µg and a minimum hemorrhage dose of 20.32 µg. Tripan blue coloration showed cytotoxic dose-dependent effect of BtaHF on C2C12 myoblasts cells but non effect in myotubes. Despite that BtaHF showed a cytotoxic activity in myoblasts, levels of LDH were comparative low with 30 (40.2 ± 5.3%) and 60 µg/mL (55.15 ± 3.25 %) when compare with positive control (Triton-X, 100%). Absorbance measurements of Crystal Violet coloration at 620nm showed dose dependent cellular adhesion reduction of myoblasts cell line. With 30 µg/mL and 60 µg/mL doses, 59.65 ± 4.94% and 39.43 ± 6.15% of cells remain adhered, respectively, when compared with control (culture without enzyme). **Discussion:** The chromatographyc and electrophoresis data, along with the proteolytic and hemorrhagic activity, strongly suggest that BtaHF is a new P-I class SVMP. This protein showed an indirect cytotoxic effect on C2C12 myoblast cell line, mainly through impairment of cellular adhesion, as described for others P-I class SVMP, like BaP1 (*Bothrops asper*). Thus, this effect must be related to interactions with specific protein membrane on C2C12 myoblats cell surface, since C2C12 myotubes did not suffer any cytotoxic effect, therefore, suggesting of key features in the molecular architecture of this protein. **Financial support:** Capes

09.066

Neuropharmacological characterization of hexanic extract isolated from *Prasiola crispa* antarctic algae. Lorensi GH[1], Oliveira RS[1], Almeida CGM[1], Correa MS[1], Pereira AB[1], Ramos BCJ[2], Teixeira VL[2], Dal Belo CA[1] [1]Lanetox-Unipampa, [2]ALGAMAR-UFF

Introduction: *Prasiola crispa* is an alga from ice-free areas of Antarctica. In this work we investigated the pharmacology of *P. crispa* at central and peripheral nervous system of cockroaches (*Phoetalia pallida*). **Methods:** Male *P. pallida* were reared with water and food *ad libitum* (22-25ºC). The hexane-extract of *P. crispa* (HPC) was prepared using the conventional phytochemical technique. The biological activity of HPC was investigated using the *in vivo* muscle coxal-metathoracic abductor preparation of cockroaches (Martinelli *et al*., BBA Gen. Sub. 1840, 935p, 2014) and by the recordings of grooming activity (Gal and Libersat, Plos One, 5, 1p, 2010). To the assays, HPC were dissolved in dimethyl sulfoxide (0.1% DMSO) and was injected (20 µL) in the second abdominal segment of the insect. The lethal dose (LD_{50}) was determined in 24 hours by injecting 10 animals with HPC. **Results:** The LD_{50} of HPC was (400 µg/g of animal weight, n=3). The neuromuscular assays of HPC (100, 200, 400, 800 µg/g of animal weight) showed a dose-dependent blockade of twitch tension in 120min ($p<0.05$, n=6). The dose of 800 µg/kg induced complete neuromuscular paralysis in 10min ($p<0.01$). Pretreatment of animals with chloral hydrate (10 µg/g of animal weight) 15min previously to HPC (400 µg/g) inhibited (by 80 ± 5%, n=6) the neuromuscular blockade. The recordings of grooming activity showed a time-dependent increase in the grooming activity that was significative ($p<0.05$) for the antennal-grooming (average of 13 ± 1.5s/30min) but not for the leg counterparts (7 ± 1.2s/30min), when compared to the control DMSO (6 ± 1s/30min and 7 ± 1.5s/30min), respectively (n=20). In all experiments DMSO alone did not induce alterations. **Discussion:** The results confirm the entomotoxic activity of *P. crispa* hexane-extract. The blockade of the cockroach neuromuscular junctions by HPC suggests that at least part of the insecticidal activity is related to a direct activity at this site. The prevention of neuromuscular blockade by chloral hydrate suggests that the n-methyl-d-aspartate receptors (NMDAr) are involved (Osborne, Pharmacol Ther, 62, 117p, 1996). The effect of HPC on grooming activity showed an interaction with the insect central nervous system, and suggests that the octopaminergic system might be involved (Gal and Libersat, PLOS One, 5, 1p, 2010). **References:** GAL, R., LIBERSAT, F. A. (2010). A wasp manipulates neuronal activity in the sub-esophageal ganglion to decrease the drive for walking in Its cockroach prey. PLoS ONE 5, 1-10. MARTINELLI, A.M *et al.* (2014). Structure–function studies on jaburetox, a recombinant insecticidal peptide derived from jack bean (Canavalia ensiformis) urease. Biochimica et Biophysica Acta 1840, 935–944. OSBORNE, R.H. (1996). Insect neurotransmission-neurotransmitters and their receptors. Pharmacol. Therap. 69, 117–142. **Financial Support:** Brazilian Antarctic Program, CNPq (process 574018/2008, Faperj (process E-26/170.023/2008) Ministry of Science and Technology – MCT, Ministry of Environment – MMA and CIRM INCT-APA Notice. 063/2010 Toxinology Capes. G.H. Lorensi has a FAPERGS master's fellowship.

09.067

Mucoadhesive formulation containing *Bidens pilosa* L. (Asteraceae) reduces intestinal injury against 5-fluorouracil-induced mucositis in mice. Ávila RI[1], Ávila PHM[1], Santos Filho EX[1], Bastos CCC[1], Batista AC[2], Serpa RC[3], Marreto RN[1], Lima EM[1], Mendonça EF[2], Valadares MC[1] [1]FARMATEC-UFG – Farmacologia e Toxicologia Celular, [2]FO-UFG – Patologia Bucal

Introduction: *Bidens pilosa* L. (Asteraceae) (BPL) is a plant from South America used in medicine folk, especially as an anti-inflammatory agent. In this context, this plant seems to be a relevant therapeutic option for treatment of inflammatory diseases/process, in particular intestinal mucositis. In this regard, the present study investigated the action of a mucoadhesive formulation containing BPL in mice bearing mucositis induced by 5-fluorouracil (5-FU). **Methods:** Mucoadhesive formulation containing BPL was obtained using Ecobidens® and vehicle prepared with polyethylene glycol 400 (17.5%, w/w) and poloxamer 407 (15%, w/w). The total polyphenol content (88.2 ppm) of this formulation was performed by Folin Ciocalteau Method: The experiments were carried out on Swiss male mice (age: 7-8 weeks; weight: 35-40 g) obtained from the Bioterium at the UFG. The experimental protocol (FUG no. 036/2012) was approved by the Research Ethics Committee of this University. BPL (75, 100 or 125 mg/kg) was administered in mice with or without 5-FU-induced intestinal mucositis during 6 days. At 7th day, small intestine of each animal was removed for histomorphometry, immunohistochemistry for detection of Bcl2, Bax, p53 and Ki-67, myeloperoxidase and malondialdehyde assays. **Results and Discussion:** Controls groups gained body weight throughout the experimental period and showed no changes on the intestinal morphometry and histopathological analysis. In contrast, animals only treated with 5-FU had a significant loss of weight in parallel with histomorphometry damage. On the other hand, the treatment with BPL formulation prevents these findings, especially at the dose of 100 mg/kg. Additionally, this dose promoted a decline on the imbalance in the rate of expression between Bax and Bcl2 and reduced the expression of p53, inhibiting the apoptotic response promoted by 5-FU. Moreover, BPL promoted intestinal proliferative activity by restoring of the Ki-67 levels, decreased inflammatory infiltrate and, consequently, prevented lipid peroxidation. Taken together, this work demonstrated that the mucoadhesive formulation of BPL presents safety and efficacy on the treatment of 5-FU-induced intestinal mucositis in mice. These results can be correlated with the presence of phytochemicals related with anti-inflammatory, immunomodulatory and antioxidant activities. More studies are necessary to further characterize the mechanisms involved in the protective effects of BPL in order to make it a clinical tool to prevent and treat the intestinal injury in patients undergoing chemotherapy. **Financial agencies:** FAPEG, FUNAPE, CNPq, FINEP and Capes.

09.068

Effect of monoterpene 1.8-cineole in the gastric ulcer healing. Caldas GFR[1], Silva ELV[1], Araújo AV[2], Neto JCS[3], Wanderley AG[1,4] [1]UFPE – Pharmaceutical Sciences, [2]CAV-UFPE – Nutrition, [3]UFPE – Histology and Embryology, [4]UFPE – Physiology and Pharmacology

Introduction: Recently our research group identified the presence of monoterpene 1,8-cineole (CIN) as the main constituent in the essential oil of leaves of *Hyptis martiusii* (EOHM). This oil showed antiulcer activity in different models of acute gastric lesions in rats and also speeded up healing of chronic ulcers. The aim of this study was to investigate the healing activity of CIN on model of chronic ulcer induced by acetic acid to confirm its correlation with the gastroprotective effect of oil. **Methods:** Male and female Wistar rats (200-300 g) after restricted solid food diet for 24h were anesthetized in order to perform surgery to expose the stomach. The chronic ulcer induction was based on method described by Takagi *et al.* (1969) by injection of 0.05 mL of 30% acetic acid into the subserosal layer of the external wall of the stomach. One day after administration of acid, daily treatment began, the animals were treated orally once daily for 14 days with 1% Tween-80 aqueous solution (control), pantoprazole (40 mg/kg) or CIN (100 mg/kg). On day 15th, the groups were euthanized, the stomachs removed, photographed and the area of gastric lesion determined by computerized planimetry (ImageJ Software®) and the date expressed in mm² (ANOVA and Tukey's test, $p<0.05$). Samples of stomach were processed for histological and immunohistochemical analysis (HE, PAS and PCNA). The experiments were approved by the Animal Experimentation Ethics Committee of the UFPE, license nº. 037544. **Results and discussion:** The oral administration of CIN for 14 consecutive days decreased (43.1%) the area of chronic ulcer to 27.3 ± 3.2 mm^2 when compared to the control group (48.0 ± 7.5 mm^2). Pantoprazole (40 mg/kg) speeded up the healing of gastric ulcers in rats, significantly reducing the area of the injury to 20.1 ± 6.2 mm² (58.1%). CIN promoted significant regeneration of gastric mucosa as confirmed by HE staining and restore mucus production in glandular cells by PAS staining, as evidenced by the accumulation of pink in the layer of mucus cells. The immunohistochemical analysis for PCNA, a cell proliferating marker, confirm that there was a significant increase in cell proliferation in the region of healing of gastric mucosa of animals subjected to treatment with CIN. Our results demonstrated for the first time, the role of 1.8-cineole as an important healing agent. **Financial support:** FACEPE (project no.: APQ-0591-4.03/10) and to CNPq (process no.: 554207/2010-9). **References:** Takagi, K., Okabe, S., Saziki R. A new method for the production of chronic gastric ulcer in rats and effects of several drugs on its healing. Jpn. J. Pharmacol, v. 19, p. 418, 1969.

09.069

Fixed oil from the pulp of *Caryocar coriaceum* Wittm. (Pequi) delays the onset of convulsive behavior induced by pentylenetetrazol in mice. Ribeiro LR[1], de Oliveira CC[2], de Oliveira CV[1], Grigoletto J[1], de Souza TL[1], Grauncke ACB[1], Furian AF[3], de Menezes IRA[4], Oliveira MS[1] [1]UFSM – Physiology and Pharmacology, [2]URCA – Nursing, [3]UFSM – Food Science and Technology, [4]URCA – Biological Chemistry

Introduction: Epilepsy consists of a large group of neurological diseases with incidence of 0.5–1% in the general population, characterized by behavioral and electroencephalographic changes (1, 3). Although single-drug therapies provide optimal seizure control in approximately 80% of all patients, seizure activity remains uncontrolled in a significant number of individuals, regardless of the type of therapy (3). Considering the historical perspective, natural products have played an important role in drug discovery and, for this reason, we decided to investigate the influence of fixed oil from the pulp of *Caryocar coriaceum* Wittm. (Pequi) on behavioral and pentylenetetrazol (PTZ)-induced seizure in mice. **Methods:** To evaluate the effect of Pequi on seizures induced by PTZ, male C57BL/6 mice (20-40g) were treated with Pequi fixed oil (25, 50 or 100 mg/kg) or saline (0.9% NaCl containing Tween 20) by intragastric gavage (i.g.) and, after 1 hour, animals were administered with PTZ (30 mg/kg; i.p.) and observed for the appearance of generalized tonic-clonic convulsive episodes for 15 min. Other groups of animals received Pequi fixed oil (100 mg/kg; i.g.) and were evaluated in object recognition test (2); and beam walk test (4). These were conducted with the approval of the Ethics Committee for Animal Research of the UFSM (process 048/2013). Statistical analysis was carried out by Kruskal-Wallis test, unpaired t test or two-way analysis of variance (ANOVA) when appropriate. **Results:** Only the pretreatment with Pequi fixed oil at 100 mg/kg (i.g.) increased the latency to first myoclonic jerk [$H(4) = 8.608$, $P<0.05$], but not alters the latency to the tonic-clonic [$H(4) = 6.794$] or duration of generalized seizures [$H(4) = 2.889$]. Furthermore, Pequi fixed oil (100 mg/kg, i.g.) did not alter the recognition index in object recognition test at 4 or 24 hours after its administration [$t = 0.9645$ and 0.3707, respectively], or affect the locomotor condition in beam walk test [$F(1,4) = 1.602$]. **Conclusions**: The systemic administration of Pequi fixed oil at 100 mg/kg in mice delays the onset of convulsive behavior induced by pentylenetetrazol, without influencing in short or long term memory and motor skills. Therefore, Pequi could be a new therapeutic approach for preventing seizures without adverse effects. **References:** 1. Andrade and Minassian, Expert Rev. Neurother., v7, p727, 2007. 2. Bevins and Besheer, Nat Protoc., v1, p1306, 2006. 3. Dichter *et al.*, In: Engel *et al.* (Eds.), Epilepsy: a Comprehensive Textbook. Lippincott Williams & Wilkins, 2007. 4. Irintchev *et al.*, Eur J *Neurosci*, v22, p802, 2005. Work supported by CNPq and Capes.

09.070

Protective effect of lectin from *Mucuna pruriens* (*Mp*) seeds on ethanol-induced gastropathy depends on alpha-2 adrenoceptors and prostaglandins. Pinto IR[1], Maciel LM[2], Monteiro DAM[3], Matos SO[3], Ribeiro KA[1], Freitas RS[1], Gadelha TS[4], Lacerda JTJG[4], Gadelha CAA[4], Benevide NMB[5], Brito GAC[6], Pinto VPT[1,2,8], Bezerra MM[2,1,8], Pereira Filho SM[2], Cristino Filho G[2,1,7], Silva AAR[3,1] [1]UFC – Biotechnology, [2]UFC – Medicine, [3]UFC – Dentistry, [4]UFPB – Molecular Biology, [5]UFC – Biochemistry and Molecular Biology, [6]UFC – Morphology, [7]UFC – Health Sciences

Introduction: The traditional uses of *Mucuna Pruriens* Linn. (*Mp*) includes: arthritis, dysentery, and cardiovascular diseases. We sought to investigate the putative effect of lectin from *Mp* seeds on ethanol-induced gastropathy. **Methods**: Fasted mice treated with ethanol 99.9% (0.2 ml/animal, p.o.) were pre-treated with *Mp* (0.001, 0.01 or 0.1 mg/kg; i.v.), ranitidine (80 mg/kg; p.o.) or saline (0.3 ml/30g; i.v.). In other experiments yohimbine (alpha-2 adrenoceptor antagonist), indomethacin (dual inhibitor of COX-1 and COX-2), naloxone (non-selective opioid receptor antagonist) or L-NAME (NO synthesis inhibitor) were added in order to clarify the possible action mechanism of *Mp*. Mice were sacrificed 30 min after ethanol and lesions were measured using a planimetry program (ImageJÒ). *Mp* effect was checked on tissue hemoglobin levels (Hb) and histological assessment (H&E). **Results and discussion:** *Mp* (0.1 mg/kg) reduced the percent area (%) of gastric lesions compared to the ethanol-challenged group (2.4 ± 0.8 *versus* 24.5 ± 3.0 injured area %, respectively), as did ranitidine (6.3 ± 1.9). Histological assessment (H&E) revealed that ethanol induced edema as well as hemorrhagic patch formation which was reduced by *Mp*. *Mp* reduced the gastric mucosal Hb (9.4 ± 1.2 tissue Hb µg/100 mg), compared to ethanol-challenged group (15.8 ± 2). *Mp* effect was reversed by both yohimbine (33.5 ± 3.3) and indomethacin (36.5 ± 5.1), suggesting the alpha-2 adrenoceptors and prostaglandin participation on *Mp*-gastro-protection. Nevertheless, *Mp* was able to protect mucosa against ethanol gastropathy (2.4 ± 0.8 injured area %) even in presence of both naloxone (8.8 ± 1.7) and L-NAME (9.8 ± 1.5). *Mp* effect in ethanol-induced gastropathy is mediated by, at least in part, alpha-2 receptors activation and prostaglandin synthesis, but not by NO release and opioid receptors. Since any substance stimulating alpha-2 receptors and prostaglandin synthesis may be assumed to possess antiulcer activity, these proteins could be suggested as an alternative tool for ethanol-mediated gastric mucosal damages. **Funding Sources:** FUNCAP, CNPq, Capes, and INCT-IBISAB. License number of the Animal Ethics Committees of Federal University Pernambuco: 230760009313/2003-04.

09.071

Gastroprotective effect of ethanolic extract chloroform fraction of *Croton argyrophyllus* Kunth. Oliveira DF, Estevam CS, Araújo SA, Batista JS UFS – Fisiologia

Introduction: *Croton argyrophyllus* Kunth is a shrub found in the semi-arid of the Brazil Northeast (GOMES, AP *et al*., *Acta Bot Bras*, v. 24, p. 905, 2010). This plant is known popularly as "marmeleiro" and "alecrim-de-vaqueiro" and it is used to digestive system disorders. Several species of the genus *Croton* present gastroprotective effect, including *Croton cajuara* Benth (PAULA, ACB *et al*., *Phytomedicine*, v. 15, p. 815, 2008) and *Croton urucurana* Baillon (CORDEIRO, KW *et al*., *J Ethnopharmacol* v. 143, p. 331, 2012). In addition, we have demonstrated in previous studies that ethanolic extract of *C. argyrophyllus* also shows antiulcerogenic effect. Therefore, the present study was performed with chloroform fraction obtained from ethanolic extract of *C. argyrophyllus* (CFEET) in order to investigate if this fraction presents gastroprotective action. This way, this study will guide isolation of active principles. **Methods:** The experimental protocols were approved by Ethics Committee for Animal Research of the UFS (protocol 37/13). The rats were orally treated with CFEET at doses of 30, 100 and 200 mg/kg (ethanol-induced ulcers protocol), CFEET at dose of 100 mg/kg (indomethacin-induced ulcers protocol), ranitidine (50 mg/kg) and 5% Tween 80 (control). One hour after the above treatments, the animals received ethanol (0,4 ml/100g) or indomethacin (100 mg/kg) by route oral. After 30 and 360 min of ethanol and indomethacin administration, respectively, the animals were sacrificed, their stomachs were removed and ulcers were quantified. **Results:** The CFEET presented gastroprotective effect against ethanol-induced ulcers at all doses tested. The percentages of gastric lesions induced by ethanol (m $\pm$ epm) were 21.1 $\pm$ 4.6, 8.0 $\pm$ 1.5 and 4.6 $\pm$ 2.1% in the animals treated with CFEET at the doses of 30, 100 and 200 mg/kg, respectively, 38.4 $\pm$ 7.3% in the group treated with Tween 80 and 0.8 $\pm$ 0,3% in the group treated with ranitidine 50 mg/kg. However, CFEET did not present effect against indomethacin-induced ulcers at tested dose. The percentages of gastric lesions induced by indomethacin in animals treated with Tween 80, ranitidine and CFEET (100 mg/kg) were 1.0 $\pm$ 0.2, 0.1 $\pm$ 0 and 0.6 $\pm$ 0.1%, respectively. **Discussion:** The present results indicate that CFEET presents antiulcer effect against ethanol-induced ulcers, but does not present antiulcer effect against indomethacin-induced ulcer. As only one dose of CFEET was tested, it is not possible to conclude that CFEET is active against indomethacin-induced ulcer. Thus, the experiments are being carried out with larger doses of CFEET. In conclusion, the present results suggest that the antiulcerogenic constituents of ethanol extract of *C. argyrophyllus* are present in the CFEET. **Support financial**: CNPq.

09.072

Antiophidic activity of solanidane and iminosolanidane alkaloids from *Solanum campaniforme.* Jorge RJB[1], Torres MCM[2], Ximenes RM[3], Alves NTQ[1], Santos JVA[1], Marinho AD[1], Toyama MH[4], Braz-Filho R[5], Silveira ER[2], Silveira JAM[1], Costa PHS[1], Pessoa ODL[2], Monteiro HSA[1] [1]UFC – Fisiologia e Farmacologia, [2]UFC – Química Orgânica e Inorgânica, [3]UFPE – Antibióticos, [4]Unesp – Química de Macromoléculas, [5]Faperj/UENF/UFRRJ

Introducion: Snake envenoming is an important health problem widespread in tropical countries. Among the most dangerous species in South America is the *Bothrops* genus. Snakebites accidents caused by *Bothrops* species quickly develop severe local tissue damage, including swelling, hemorrhage, myonecrosis, skin ulceration and pain (Baldo, *PLoS Neglected Tropical Diseases*, v.4, n.6, p. e727, 2010). The traditional serum therapy has limited effectiveness against these effects. Natural compounds isolated from plants, mainly from plants used in folk medicine to treat snakebite, are a good choice to find new lead compounds to improve the snakebite treatment and minimize the sequelae of the victims (Rocha, Invest. New Drugs, v.30, 959-966, 2012) **Methods:** Antiophidic activity of the eight new solanidane alkaloids were isolated from the leaves of *Solanum campaniforme* (Solanaceae), four of which containing a *p*-hydroxyphenylethylamine unit. Their structures were established as: 22β,23β-epoxy-solanida-1,4-dien-3-one (**1**); 22α,23α-epoxy-10-epi-solanida-1,4,9-trien-3-one (**2**); 22α,23α-epoxy-solanida-4-en-3-one (**3**); 22β,23β-epoxy-solanida-4-en-3-one (**4**); (*E*)-*N*-[8'(4-hydroxyphenyl)ethyl]-22α,23α-epoxy-solanida-1,4,9-trien-3-imine (**5**); (*E*)-*N*-[8'(4-hydroxyphenyl)ethyl]-22α,23α-epoxy-solanida-1,4-dien-3-imine (**6**); (*Z*)-*N*-[8'(4-hydroxyphenyl)ethyl]-22α,23α-epoxy-solanida-1,4,9-trien-3-imine (**7**) and (*Z*)-*N*-[8'(4-hydroxyphenyl)ethyl]-22α,23α-epoxy-solanida-1,4-dien-3-imine (**8**). The antiophidic tests were performed to evaluate the ethanol extract of *S. campaniforme* (EESc), the dichloromethane fraction (EESc-D) and the alkaloids **1**, **3** – **5**, and **7** – **8** isolated from that fraction. was tested through inhibition of phospholipasic activity, proteolytic activity, myotoxicity, hemorrhage and necrosis induced by *Bothrops pauloensis* venom. The results were submitted to analysis of variance (ANOVA), $p<0,05$. Ethic committee protocol approval: CEPA/UFC N° 68/08. **Results:** Both, crude extract and the CH_2Cl_2 fraction inhibited the proteolytic activity of azocasein, and the most important related toxic actions of the bothropic venoms in general, including the hemorrhagic and skin necrotizing activities. When the alkaloids were assayed individually, no inhibition of the proteolytic activity *in vitro* was observed, except for compound 8 which showed 15% of inhibition. However, the *in vivo* tests showed a significant decrease in the hemorrhagic area when the venom was incubated with 1, 3 and 8. The necrotic areas were also smaller after the treatment with 3 – 5, and 8. Alkaloid 1 – 4 also inhibited the increase of CK activity. **Discussion**: These results suggest a synergistic or additive action among the alkaloids, and the involvement of the three major alkaloids isolated from the AcOEt fraction (Torres, J. Nat. Prod. v.74, 2168-2173, 2011). It drive to the conclusion that the alkaloids acting together are responsible for the activity of the crude extract since none of them, separately was able to neutralize the toxic actions as much as the crude extract did. **Financial support**: FUNCAP and CNPq.

09.073

A rapid method to isolate bufadienolides from toad *Rhinella schneideri* venom by flash column chromatography. Cunha-Filho GA[1], Costa PRR[2], Texeira Jr E[2], Barcellos J[2], Martins-Ferreira J[1], Quintas LE[1], Nöel F[1] [1]ICB-UFRJ, [2]IPPN-UFRJ

Introduction: Cutaneous secretions of toad species are an important source of bufadienolides, steroids characterized by a six-membered lactone ring located at position C-17β. Bufadienolides can act as endogenous steroidal hormones (Schoner and Scheiner-Bobis, *Semin. Nephrol.*, **25**, 343, 2005) and display a range of pharmacological properties related to their Na+ /K+ -ATPase enzyme interaction/inhibition. Our research group has demonstrated that some bufadienolides have proliferative or antiproliferative activity and induce morphological changes in porcine renal LLC-PK1 cells; they also exhibit a cytotoxic profile in tumor cells (Cunha-Filho and cols., *Toxicon*, **56**, 339, 2010). Here we describe the isolation of bufadienolides from the Brazilian toad *R. schneideri* venom by a flash column chromatography (CC) method and production of 15-hidroxy-telocinobufagin. **Methods:** The CC method was adapted from Still and cols. (*J. Org. Chem.*, **43**, 2923, 1978). Brown residue (2.5 g) extracted (four times with one week intervals) with 50% methanol:ethyl acetate from 19 g of powdered dried *R. schneideri* parotoid glandular secretions was fractionated by CC on silica-gel (100 g, 220-400 mesh, Aldrich; column diameter 4.1 cm), eluting by a solvent system consisting of 50% ethyl acetate (EtAc):*n*-hexane (Hex) (fractions of 60 mL). Fractions containing mixture of compounds were submitted to a second separation by CC on silica-gel 60 (7.5 g, 220–400 mesh, Aldrich; column diameter 1.4 cm), eluting by 65% EtAc:Hex (fractions of 20 mL) or 1 to 5% methanol (MeOH):dichloromethane [DCM (fractions of 20 mL)]. Fractions were monitored by TLC (silica gel 60), eluting by 5 to 10% MeOH:DCM. The production of 15-hidroxi-telocinobufagin was carried out according to Kamano and cols. (*Tetrahedron*, **31**, 2359, 1975). One- and two-dimensional NMR were used for structural characterization. **Results:** The first chromatographic separation of the organic extract on silica gel column afforded four major compounds: *β*-sitosterol (86 mg), bufalin (57 mg), marinobufagin (1230 mg), and telocinobufagin (147 mg). Mixtures of compounds were subjected to further separation using silica gel, providing 32 mg of bufalin and 14 mg of cinobufagin (1 to 2% MeOH:DCM) or 220 mg of marinobufagin and 22 mg of 20,21-epoxymarinobufagin (65% EtAc:Hex). Analyses of the reaction mixture obtained by the treatment of marinobufagin (200 mg) with 70% perchloric acid (0.2 mL) suggest the formation of 15-hidroxi-telocinobufagin (^{1}H-NMR 500 MHz, δ 2.75 ppm; 1H, m, H-15). **Discussion:** The CC method described here has proved to be an alternative for rapid isolation of bufadienolides (1 to 2 hours for each CC), when compared to the step by step solvent gradient of other CC methods (4 to 12 hours for each CC; Cunha-Filho and cols., *Toxicon*, **56**, 339, 2010). The solvent system consisting of MeOH:DCM improved the resolution of bufalin and cinobufagin separation. The purity of the isolated compounds, isolation and structural characterization of unknown compounds, as well as their effects on Na+ /K+ -ATPase activity, proliferation of neuroblastoma SH-SY5Y cells and related intracellular signaling pathways are being provided. **Financial Support/Acknowledgement**: Capes, Faperj, Licence IBAMA/RAN n. 097/06 (process 02010.000832/04-74).

09.074

Isolation and biochemical characterization of A γ-type phospholipase A2 inhibitor from *Crotalus durissus* collilineatus snake serum. Gimenes SNC[1], Ferreira FB[1], Silveira ACP[1], Rodrigues R S[1], Yoneyama KAG[1], dos Santos JI[2], Fontes MRM[2], Brites VLC[3], Santos ALQ[4], Borges MH[5], Lopes DS[1], Rodrigues VM[1] [1]UFU – Genética e Bioquímica, [2]Unesp – Física e Biofísica, [3]UFU – Biologia, [4]UFU – Medicina Veterinária, [5]FUNED

Introduction: There are many compounds capable of neutralizing snake venom toxins, such as antibodies, chemical substances and plants compounds (Lizano *et al.*, 2003). Inhibitors of PLA_2s from animal plasma/serum, especially snakes, are able to protect the animal against their own toxins that eventually reach the circulatory system (Kinkawa *et al.*, 2010; Lima *et al.*, 2011). In the present work, we describe the isolation and partial structural and biochemical characterization of the first phospholipase A_2 inhibitor (γPLI) from *Crotalus durissus collilineatus* (*Cdc*) snake serum. **Methods and Results**: Initially, the Cdc serum was subjected to a Q-Sepharose ion exchange column, producing six peaks at 280 nm absorbance (Q1–Q6). Subsequently, Q4 fraction was submitted to affinity chromatography with immobilized PLA_2 BnSP-7, a step that resulted in two fractions (NHS-1 and NHS-2). The latter contained the inhibitor, denominated γCdcPLI. The molecular mass of gCdcPLI, determined by Matrix-Assisted Laser Desorption Ionization Time-of-Flight (MALDI-TOF), was 22,340 Da. Partial sequences obtained by Edman degradation and by mass spectrometry (MALDI-TOF/TOF), showed similarity, as expected, to other related inhibitors. Circular Dichroism (CD) analysis showed the presence of approximately 22% alpha helices and 29% beta sheets in the protein secondary structure. **Discussion:** Additionally, CD studies also indicated no significant changes in the secondary structure of γCdcPLI when it is complexed to $BpPLA_2$-TXI. On the other hand, dynamic light scattering (DLS) assays showed a temperature-dependent oligomerization behavior for this inhibitor. Biochemical analyses showed γCdcPLI was able to inhibit the enzymatic, cytotoxic and myotoxic activities of PLA_2s. Structural and functional studies performed on this inhibitor may elucidate the action mechanisms of PLA_2 inhibitors. In addition, we hope this study may contribute to investigating the potential use of these inhibitors for the treatment of snakebite or inflammatory diseases in which PLA_2s may be involved. **Reference:** Lizano, S. *Toxicon*, 42, 963, 2003. Kinkawa, K., Biochem. Biophys. Res. Commun. 395, 377, 2010. Lima, R.M. *Toxicon* 57, 172, 2011. **Financial support:** FAPEMIG, CNPq, Capes, INCT Nano-biofar, UFU, INGEB

09.075

Neuroprotective efficacy of eugenol in Huntington's disease: Behavioral alterations and oxidative stress. Nobrega RF[1], Teixeira HAP[1], Linard-Medeiros CFB[2], Silva JL[2], Sereniki A[2], Sales VAW[1], Melo VCS[1], Melo THC[1], Silva JBR[2], Lagranha C[3], Wanderley AG[1], Lafayette SSL[1] [1]UFPE – Fisiologia e Farmacologia, [2]UFPE – Ciências Farmacêuticas, [3]CAV-UFPE

Introduction: Research has found that the developments of neurodegenerative diseases are associated with high levels of oxidative stress, mitochondrial alterations and apoptosis. Huntington's disease (HD) is a neurodegenerative disorder characterized by motor impairment, cognitive decline and psychiatric symptoms. Systemic administration of 3-nitropropionic acid (3-NP) irreversibly inhibits the enzyme succinate dehydrogenase thus causing oxidative stress and neuronal death, similar to that of HD. Eugenol (4-allyl-2-methoxy phenol) is a natural product found in clove, basil, cinnamon and nutmeg that contains high amounts of polyphenols. It is known as a potent anti-inflammatory and antioxidant.The present study evaluated the possible activity of eugenol on the behavioral and biochemical alterations induced by the administration of 3-NP to rats. **Methods**: Male Wistar rats, weighing 280-350g, divided into six groups (n=8), were used in these experiments. Rats received, intraperitoneally, vehicle (NaCl 0.9%) or 3-NP (20 mg/kg) and were treated, one hour before, orally, with water or eugenol (12.5, 25, 37.5 and 50 mg/kg), for seven days. Twenty four hours after the last day of treatment, the rats were evaluated with behavioral tests. The following behavioral tests were performed: open field test, rotarod test and elevated plus maze test. After behavioral assessments, the rats were decapitated, the brains removed, placed on ice, the striatum, cortex and substantia nigra dissected and prepared for biochemical analyzes. Data are expressed as mean ± S.E.M. Differences between groups were analyzed by ANOVA and Tukey's test ($p<0.05$). **Results and discussion:** The administration of 3-NP caused significant body weight loss, hypolocomotion, muscle incoordination and memory deficit when compared to control animals. Daily treatment with eugenol at all doses analyzed, one hour before to 3-NP administration for a total of 7 days, improved the 3-NP-induced motor and cognitive impairment. Biochemical analysis in brain (substantia nigra, striatum and cortex) revealed a significantly increase in lipid peroxidation in rats treated with 3-NP. Furthermore, the treatment with eugenol attenuated lipid peroxidation and oxidative damage. These results suggest that the eugenol has neuroprotective effect against the degeneration induced by the neurotoxin 3-NP and significantly reversed 3-NP-induced alterations in various behavioral and biochemical parameter, probably because it is a phenolic compound having antioxidant activity and it could be a therapeutic agent for the treatment of HD. **Financial Support**: FACEPE and Capes.

09.076

Evaluation of the antifungal activity of the essential oil of *Tagetes minuta* L. Against *Candida albicans*. Cunha JA[1], Bolzan LP[2], Oliveira AM[3], Fausto V[2], Vaucher RA[2], Silva DT[4], Baldisserotto B[1], Heinzmann B[1] [1]CCS-UFSM – Farmacologia, [2]UNIFRA, [3]UFSM – Farmácia, [4]UFSM – Engenharia Florestal

Introduction: Preparations obtained from *Tagetes minuta* L. (Asteraceae) have shown photosensitizing and antiviral activities due to the presence of thiophenes. On the other hand, its antimicrobial activity is assigned to acyclic monoterpenoids and ketones (CHAMORRO, E. R., J. Argent. Chem. Soc., 9: 1-2, 2008; LETIZIA, C.S., TheToxicologist, 54: 397, 2000). The objective of this work is to evaluate the antifungal activity *in vitro* of the essential oil (EO) of the inflorescences of *T. minuta* against *Candida albicans* ATCC 14053 by disc diffusion, determination of the Minimal Inhibitory Concentration (MIC) and by the germination tube test. **Methods:** EO of the inflorescences of *T. minuta* collected in Itaara- RS were extracted by hydrodistillation for 3h (Farmacopéia Brasileira, 5th ed., 2010). Before the assays, the yeast was peaked into Sabouraud Agar and incubated at 37˚C for 24h. For the disc diffusion technic (CLSI M44-A2, 2010), the paper discs were transferred to the surface of the medium, and 10 µL of EO was placed on them. The plates were incubated at 37˚C for 24h and the zone of inhibition was determined. The microdillution technic (CLSI M27-A3, 2008), the medium was distributed in the wells, and the EO was added at concentrations of 411.5, 205.75, 102.8, 51.4, 25.71, 1.28 and 0.32 µg mL^{-1}. After the addition of the inoculum (10 µL), the plate was incubated for 24h and the evaluation of the results occurred by microscopy. The technic of tube germination test consisted in the preparation of an inoculum in saline to achieve a 0.5 McFarland scale. Next, a dilution of minimum inhibitory concentration of EO and citrated human plasma was prepared and 200 µL of inoculum was added, following incubation for 2h at 37˚C. The observation of the results occurred by microscopy in a Neubauer chamber. Positive and negative controls were inserted into all the trials. **Results:** In the disk diffusion test the EO showed an inhibition zone of 21 mm and the MIC was detected at0.32 µg mL^{-1}. In the test of germination tube formation, the EO at concentrations of 1, 5 and 10% led to an inhibition in the proportions of 80, 90 and 100%, respectively. **Discussion**: The results indicate that the EO of *T. minuta* has potential to be used in the control of fungal infections caused by *C. albicans*. In general, the antimicrobial effect of EO is attributed to its interaction with the structural components of microbial cell (BELLETTI, N. J. Agric. Food Chem. 52(23):6932-6938, 2004) as the phospholipid layer of the cell membrane, increasing the permeability and bonding to constituents of vital importance for the microorganism (SINGH N., Wiss. u. Technol. 35:720-729, 2002). Although the results of *in vitro* tests are positive and *T. minuta* is a medicinal plant used for many years, further studies are needed to assess efficacy and safety of EO as an antimicrobial. **Financial Support:** Capes, FAPERGS/PRONEX, Document No. 10/0016-8; Ministério da Pesca e Aquicultura/MCT/FINEP; INCT ADAPTA; CNPq and FAPEAM.

09.077

Antiophidic properties of a polysaccharide isolated from brazilian marine algae.

Rodrigues-Silva AC[1], Fuly AL[1], Duarte MER[2], Noseda MD[2], Ferreira LG[2], Sanchez EF[3] – [1]UFF – Biologia Molecular e Bioquímica, [2]UFPR, [3]FUNED

Introduction In Brazil, snakebites are considered a public health problem and those caused by *Lachesis muta* are low when compared to *Bothrops* genus; however the mortality rate of the former is three fold higher. Envenomation by *L. muta* is characterized by systemic (neurotoxicity, bleeding disorders) and local effects (hemorrhage, necrosis and edema, and to neutralize these symptoms and to treat victims, antivenom is injected intravenously into humans. However, this treatment does not neutralize tissue necrosis, and therefore, alternative methods are suggested. So, the objective of this work was to evaluate the effect of a polysaccharide, called FHS isolated from a Brazilian alga *Chondrophycus flagelliferus* to neutralize some toxic activities of *L. muta* venom. **Material and Methods**: The polysaccharide was incubated with *L. muta* venom for 30 minutes at room temperature, and then proteolytic, hemolysis, hemorrhagic and coagulation activities were performed. The hemolytic activity was performed by incubating *L. muta* with hen's egg yolk, and then the hemolytic activity was measured by the release of hemoglobin caused by lysolecithin formed enzymatically. The proteolytic activity was performed using azocasein as substrate and coagulation upon plasma from healthy volunteers. Hemorrhage was evaluated after injecting intradermally samples into the abdomen of mice. Two hours after injection, the animals(C.E nº 217) were euthanized, skin removed, stretched and injection sites were measured using a caliper rule. **Results and discussion:** Our results showed that FHS inhibited hemorrhage, hemolysis, proteolysis and coagulation induced by *L. muta*, but with different potencies. FHS inhibit 50 % hemolysis and 95% proteolysis, and protected mice ca. 55 % from hemorrhage. **Conclusions**: These results suggest that FHS from *C. flagelliferus* algae may be effective against some *L. muta*'s toxic activities, thus providing a promising strategy to treat envenomation by this snake. **Acknowledgements**: Capes, CNPq, Faperj, PROPPi-UFF and IFS

09.078

Antidepressant-like effect of the lectin from the marine red alga *Solieria filiformis* (Kützing) P.W. Gabrielson. Abreu TM[1], Martins AB[1], Monteiro VS[1], Rivanor RLC[1], Teles FB[1], Souza RB[1], Danziato PM[1], Dantas RC[1], Vasconcelos SMM[2], Júnior JERH[2], Benevides NMB[1] [1]UFC – Biochemistry and Molecular Biology, [2]UFC – Physiology and Pharmacology

Introduction: The marine algae are sources of many bioactive compounds. Among these, there are the lectins, which are proteins that bind to specific mono-or oligosaccharides. These protein-carbohydrate interactions make of lectins valuable tools in many biological processes, being of great interest for pharmacological applications. In the literature, there are reports of lectins from marine algae with mitogenic, anti-inflammatory and antinociceptive activities, which may involve the central or peripheral nervous system. However, there are still several aspects to be explored in relation to pharmacological potential of these lectins in the neurobehavioral disorders, such as depression. Thus, this study aimed to evaluate the antidepressant action of the lectin from the red marine alga *Solieria filiformis* (SfL) in classical neurobehavioral models of depression in mice. **Methods:** SfL was purified by extraction with Tris-HCl buffer 25 mM (pH 7.5), precipitation with ammonium sulfate (70%) and chromatographies in DEAE-cellulose and Sephadex G-100 columns. To evaluate the antidepressant effect of the SfL, tail suspension (TST) and forced swimming (FST) tests were performed, using male *Swiss* mice (25-30 g; n=10). The use of animals in this study was approved by the Ethics Committee on Animal Research of the UFC (nº 45/13). For the tests, the animals were pretreated with the SfL (1, 3 or 9 mg/kg), intravenously (iv.). As control, one group of animals received only sterile saline (NaCl 0.9%; iv.). In the TST, 30 min after the pretreatment, the animals were suspended by the tail on the edge of a shelf 58 cm above a table top by adhesive tape placed approximately 1 cm from the tip of the tail and watched for 5 min. In the FST, 30 min after the pretreatment, each animal was placed in a tank (22 cm in diameter and 40 cm in height) containing freshwater at 25°C to a depth of 20 cm, and watched for 5 min. In both tests, the observed parameter was the immobility time, which was registered in seconds. Data were analyzed by ANOVA, followed by Student–Newman–Keuls *post hoc* test. *P*-values<0.05 were considered statistically significant. **Results and discussion:** In the TST, the SfL, at doses of 3 and 9 mg/kg, reduced, significantly, the immobility time of the mice by 48.4 and 47.1%, respectively, when compared with saline control. In the FST, this protein, at doses of 3 and 9 mg/kg, reduced, significantly, the immobility time of the mice by 58.3 and 54.9%, respectively, when compared with saline control. In both assays, the SfL, at a dose of 1 mg/kg, did not differ significantly from the control and there was no significant difference between the doses of 3 and 9 mg/kg. This work showed that the *S. filiformis* lectin decreased the immobility time of the animals in the tests performed, suggesting, therefore, an antidepressant-like effect of this lectin.

Financial Agencies: UFC, CNPq, Capes and FUNCAP. The use of animals in this study was approved by the Ethics Committee on Animal Research of the UFC (nº 45/13).

09.079

Evaluation of synthetics derivatives in neutralization some toxic activities of *Bothrops jararacussu* venom. Pires AMG[1], Rodrigues- Silva AC[1], Fuly AL[1], Ferreira SB[2], Sanchez EF[3] [1]UFF – Biologia Molecular, Celular e Bioquímica, [2]UFRJ – Química, [3]FUNED

Introduction: In Brazil, the genus *Bothrops* are responsible for 94% of snake bites. Snake venoms are composed of a mixture of proteins that display several pharmacological effects, as, neurotoxicity, hemorrhage, coagulation disorders, necrosis, edema and in some cases lead victims to death. According to the Health of Ministry, the injection of antivenom is performed to treat snake bites. However, there are drawbacks for such therapy, since local effects are not well blocked (leading to limb amputation or deformation), and eventually, patients experience side effects (allergic reactions, fever and anaphylaxis). Because of these disadvantages, alternative methods are suggested. Therefore, in this study we evaluate the ability of six 1,2,3-triazol derivatives to neutralize some toxic activities of *B. jararacussu* venom. **Material and Methods**: *Coagulation Activity*: The derivatives were preincubated with venom for 30 min. at room temperature, and after, the clotting time was initiated by addition of human plasma. *Proteolytic Activity*: Aliquots of venom were incubated with azocasein in a buffer solution for 90 min. to 37ºC. After this time, the enzymatic reaction was stopped and quantified by spectrophotometer at 420 nm. *Hemorrhagic activity*: Samples of venom containing derivatives were injected intradermally (i.d.) in belly of mice (ethics comittee nº 297). Two hours after the injection, the animals were sacrificed and their skins removed. The hemorrhagic activity was measured by hemorrhagic halo. *Edematogenic activity*: Groups of mice were injected subcutaneously (s.c.) in the right foot pad with samples containing venom or derivatives, whereas the left pad received saline. One hour after injection, edema was evaluated and expressed as the percentage of increase in the weight of the right foot pad compared to the left one. **Results and discussion:** The derivative AM054 inhibited more efficiently all the tested activities, except the edematogenic activity that none of derivatives was able to counteract them. **Conclusions**: Our results show that Compounds could be a promising source of inhibitors of enzymes involved in the main biological activities of *B. jararacussu* venom. **Acknowledgment**s: Faperj, CNPq and Capes, PROPPi-UFF

09.080

Acetone extract of NP01 stimulates the healing activity in skin pressure ulcer and acts as a potent anti-inflammatory. Gomes MF, Santos GCQ, Castro LD, Bastos AC, Mota AS, Nascimento JLM, Bastos GNT UFPA

Introduction: Many plant extracts from the Amazonian flora are used by traditional populations in the treatment of pathologies. The ethnopharmacology research is a very important tool in the prospection of molecules with desirable pharmacological activities. But many popular medicinal plants have been used indiscriminately without a solid scientific basis for their efficacy, as is the case of acetone extract of NP01.Throw an ethnopharmacolgy research in a local community of Pará state the Laboratory of Neuroinflamation from the UFPA, acquired information about a seed extract used as wound healer. The lab denominated these extract as NP01. In this work we examined the effects of NP01 in the inflammatory process and the healing of a skin pressure ulcer induced in Wistar rats. **Methods and results:** The ulcer was induced by cycles of ischemia-reperfusion by applying and removing a rectangular permanent magnet (40 x 25 x 10 mm) in dorsal region of rat skin under which a ferromagnetic steel plate was implanted. Twelve male Wistar rats (6-8 weeks) were randomly allocated in four groups: control, NP01 (4mg), Diethylamine Diclofenac (0.04mg) and Dersani® treatment. To clarify the mechanism of the NP01 effects, area of the lesion, exudates volume, number of migratory cells, nitrite level and collagen of formation were investigated. The ischemia-reperfusion promoted a lesion area of 87 ± 0.155%, while the lesion area of the ulcers treated with Diethylamine Diclofenac, Dersani® and NP01 were decreased in 79 ± 0.028%, 66.50 ± 0.063% and 65 ± 0.007% respectively. The volume of exudates (mL) decreased (4.33 ± 1.52; 1.33 ± 0.5; 2 ± 0.57; 1.75 ± 0.35 to control, Diethylamine Diclofenac, Dersani® and NP01, respectively). The acetone extract of NP01 markedly inhibited the cellular migration (Nºx10^6/mL) to lesion area (127 ± 47.03; 11.33 ± 9.86; 72 ± 21.2, 32.6 ± 17.44 respectively to control, Diethylamine Diclofenac, Dersani® and NP01). Therefore, the level of nitrite (μM) was also significantly decreased by NP01 (10.45 ± 0.91; 2.41 ± 1.98; 2.54 ± 1.32, 1.11 ± 1.26 respectively to control, Diethylamine Diclofenac, Dersani® and NP01). The staining of the tissue samples showed that acetone extract of NP01 stimulates the formation of collagen types I and III. All procedures involving animal care and experimentation were performed in accordance with guidelines of the Ethical Committee for Research with Experimental Animals of the Federal University of Pará (123-13). **Conclusion:** These data suggest that NP01 is a powerful healing to skin pressure ulcer methods due to inhibit the first inflammatory phase and accelerating the process of tissue reparation. **Sources of research support:** CNPq, Capes, UFPA.

09.081

Nanoemulsion-based formulation increases antiedematogenic activity for hidroalcoholic extract from *Casearia sylvestris*. Lenfers B[1], Batisti AP[2], MArtins TC[1], Benevides MLACS[3], Custodio KM[4], Kanis LA[5], Santos ARS[1], Martins DF[5], Piovezan AP[5] [1]UFSC – Neurobiology of Pain and Inflammation, [2]UNISUL – Naturology, [3]UNISUL – Medicine, [4]UNISUL – Pharmacy, [5]UNISUL – Health Sciences.

Introduction: *Casearia sylvestris* Sw (CS) is widely used in Brazil in the folk medicine for treating different disorders of inflammatory origin, such as gastric ulcers and wounds, as well as being used as an analgesic and antipyretic. We have recently demonstrated the anti-inflammatory action for its hidroalcoholic extract (HCE) in different models of inflammation, as well its antioxidant activity linked to protection against lipid peroxidation and damage to proteins. In the present work we have investigated if a formulation using nanotechnology to incorporate this plant extract (HCEnano) could influence its anti-inflammatory action. **Method:** Paw edema was induced by intraplantar (i.pl.) injection of λ-carrageenan (CAR 300 µg/paw,in 30 µL of sterile saline=vehice) in the right hind paws of mice (day 0). Prior to this, the thickness of the hind paws were registered (µm) with the use of a digital micrometer and recorded as baseline edema values. One hour after CAR injection, different groups of animals were orally treated with vehicle (VEH, 0.1 ml/10g weight), dexamethasone (DEXA 0.5 mg/kg), nanoemulsion without HCE of CS (EMU, 0.1 ml/10g weight), HCE of CS (30 mg/kg) or nanoemulsion with HCE of CS (HCEnano, 30 mg/kg). After this oral treatment, individual right hind paw thickness was further measured at 1h, 2h, 3h, 4h, 5h and 6h post injection. Edema was expressed as the difference between the thickness of treated paws after (tested post administration times) and before CAR administration (µm) for each animal. Treatments (same doses) were repeated up to 2nd day after initial experiment and observation for edema continued for more 24 h (day 3). Protocols were approved by Ethics Committee on Animal Use -UNISUL (Register number 13.021.4.03.IV). **Results and discussion:** Administration of CAR into the right hind paw of the mice induced an important edema with a peak at 4 h after its injection (852.9 ± 71.8 µm); at this same period, this effect was inhibited by DEX (287.4 ± 86.3 µm), HCE (367.9 ± 86.4 µm) and HCE nano (501.5 ± 54.8 µm). Repeated treatment of the animals with HCEnano (same dose) produced, on day 3, an expressive augment in antiedematogenic activity (7.7 ± 52.2 µm) in relation to HCE (223.3 ± 56.0 µm), in a similar extent of that promoted by the steroidal anti-inflammatory DEXA (58.5 ± 45.2 µm). **Conclusion**: Incorporation of HCE from CS in nanoemulsion-base formulation increases its anti-inflammatory action, observed as a higher antiedematogenic activity in CAR-paw induced model in mice. **Support**: FAPESC and UNISUL.

09.082

The extract MS2AP improves the anti-inflammatory process and the tissue repair in pressure ulcer model. Santos GCQ, Gomes MF, Castro LD, Mota AS, Nascimento GS, Bastos AC, Nascimento JLM, Bastos GNT UFPA

Introduction: The Amazon flora is the subject of many bioprospection researches due the wide quantity of unknown substances. The ethnopharmacology research is a very important tool in the prospection of molecules with desirable pharmacological activities. These studies bring to the scientific community the knowledge and experience of traditional societies about the biodiversity. Throw an ethnopharmacolgy research in a local community of Pará state the Laboratory of Neuroinflamation from the UFPA, acquired information about a seed extract used as wound healer. The lab denominated these extract as MS2AP. These work investigated the anti-inflammatory activity and the influence of MS2AP in the tissue repair using the model of skin ulcer induced by ischemia and reperfusion. **Methods:** Twelve male Wistar rats were divided equally in four groups: Sun flower oil,Diethylamine Diclofenac, MS2AP and Saline. All animals were submitted to surgery to placement of a steel plate (40 x25x 1 mm) under the subcutaneous layer of their skin, after one day of rest, a magnet (20 x10x 10 mm) was used to produce ischemia in the contact with the steel plate beneath the skin (2h) after, the removal of the magnet produced reperfusion (30min) duration) these procedure was repeated four times a day in a period of three days. After the period of lesion a skin ulcer was formed, that were treated topically with Sun flower oil or Diethlamine Diclofenac or MS2AP extract, the saline group was used as negative control. Past the treatment period the animas were scarified and the exudates present withdraw for volume analysis, nitrite concentration and quantity of migration cells. Remain tissue of the ulcer were photographed to dimension analysis and after that dissected to histological investigation by picrosirius stain and hematoxilin and eosin. All experimental procedures were reviewed and approved by the Ethics Committee on Research with Animal Experimentation from the Universidade federal do Pará (No. 123-13).**Results:** The exudates volume removed from the saline group were (5ml ± 1.52) the treated groups Sun flower oil, Diethylamine Diclofenac and MS2AP presented respectively (2ml ± 0.73; 1.37ml ± 0.57 and 2.5ml ± 0.63). The numbers of migration cells decreased (120x10^6/ml ± 46.3; 80x10^6/ml ± 11.33; 20x10^6/ml ± 1.7; 19x10^6/ml ± 2.3) to saline, Sun flower oil, Diethylamine Diclofenac and MS2AP. Therefore, the level of nitrite was also significantly decreased by MS2AP (10.45 ± 0.91; 2.41 ± 1.98; 2.54 ± 1.32, 1.02 ± 1.26 μM) respectively to Saline, Diethylamine Diclofenac, Sunflower oil and MS2AP). The histological analysis using hematoxilin and eosin showed that the tissues treated with MS2AP has skin layers well defined and organized different from the tree other groups, this demonstrated a powerful influence on the process of wound repair. The picrosirius stain showed a bigger concentration of collagen type one on the tissues treated by MS2AP, a slight concentration on the group Sun flower oil. In the groups Diethylamine Diclofenac and Saline a few traces of collagen type one were found. **Conclusion**: All these data suggest that MS2AP extract has powerful anti-inflammatory activity and remarkable positive influence on the tissue repair, demonstrating closer activities to anti-inflammatory drugs and wound healers. **Sources of research support:** CNPq, Capes, UFPA.

09.083

The effect of Syzygium cumini (L.) Skeels (jambolão) in a model of polycystic ovaries induced in rats. Soares CR, Sena GM, Silva SN, Benevides ROA, Silveira WF, Mendes EG, Abreu IC, Cartágenes MSS, Borges MOR, Pedrosa MCG, Borges ACR UFMA – Ciências Fisiológicas

Background: The Polycystic Ovary Syndrome (SOP) is a complex endocrine disorder that is very common among women of reproductive age, its main elements are hyperandrogenism, chronic anovulation, obesity, insulin resistance, among others. The drugs used to treat SOP have shown many side effects, which led to a search for effective alternative treatments to maintain the syndrome with fewer side effects. *Syzygium cumini* (L.) has broad popular use because of its antioxidant and hypoglycemic effects, a fact that led to the choice of this plant to the acquisition of a possible therapeutic agent in the management and minimizing of SOP symptoms. **Methods:** The Project was aproved by the Ethics Committee on Animal Use-UFMA (Nº 23115.004018/2012-31). Thirty adult female rats (60 days old) of Wistar lineage were used. The free-radical scavenging capacity (2, 2 diphenyl-1-picrylhydrazyl, DPPH) and the detection of secondary metabolite were evaluated in the extract. The rats were divided into three groups: 2 groups (SOP-IND e SOP-HE) were subjected to SOP (Letrozole, 2 mg/kg, orally for 21 days); one group was not subjected to SOP (CTRL group) and received saline solution (0.1 ml/100g) for 21 days. From the 22th day, the animals were subjected to a 30-day treatment by 2 different therapies, one for each treatment group: 1) saline solution (0.1ml/100g); 2) oral treatment with hydroalcoholic extract (HE) of *S. cumini* (500 mg/kg); the CTRL group continued to receive the saline solution. The fresh wet mount method was conducted during all the experiment period. The specimens were subjected to arterial blood collection, euthanasia and dissection of organs for biochemical and histopathological analyzes. **Results and discussion:** The extract showed satisfactory effects such as decrease of abdominal fat deposition with a reduction of 56.1%; decrease in total cholesterol with SOP-HE group, with an average lower than CTRL (68.77 ± 3.92 and 116.6 ± 15.9, respectively) and SOP-IND (92.79 ± 2.78) group which simulates an untreated individual; there was a gradual resumption of ovulatory cycles with increase in proliferative phases (estrus + proestrus) approximately 44.26% for the SOP-HE group and 3.36% for the SOP-IND. In histomorphometric analysis, the number of corpora lutea in the group SOP-HE was significantly higher than in SOP-IND group, while the number of cystic follicles was lower. HE was effective in removing free radicals at all concentrations tested, with maximum antioxidant activity (AA% max) 92.50%, and high amount of phenols and flavonoids. This study indicates that the HE *S. cumini* has high potential in the prevention and maintenance of SOP and that the flavonoid contained in the plant leaves can be directly related to the beneficial effects of the extract in rats with SOP, with regard to metabolism glucose, lipid and ovarian folliculogenesis, and it may become an effective herbal treatment in aid of women with SOP, with considerable reduction of side effects. **Financial Support:** FAPEMA and CNPq

09.084

Effect of Glycine max(L.) (soy isoflavones) in model in rats induced ovarian polycystic.

Sena GM, Soares CR, Silveira WF, Mendes EG, Borges ACR, Borges MOR, Freire SMF, Cartágenes MSS, Abreu IC, Silva SN UFMA – Farmacologia

Background: Polycystic ovary syndrome (PCOS) is an endocrine disorder that affects approximately 10% of women during reproductive life, and it is responsible for 90% of infertility cases by anovulation. Insulin resistance and compensatory hyperinsulinemia are the most important elements in the etiopathogenesis of PCOS. Current therapy for such a syndrome is use of insulin sensitizers. Large randomized clinical trials of metformin as the insulin-sensitizing drug, however, suggested that it produces many side effects after prolonged usage.*Glycinemax*(L.), popularly known as soy, has hypoglycemic action, and is rich in isoflavones, which have similar chemical structure to estradiol. Because of this, this study aims to evaluate the effects of *G.max* (L) in the treatment of PCOS. **Methods**: The Project was aproved by the Ethics Committee on Animal Use-UFMA (Nº 23115.007284/2014-87). Adult female rats (21 days old) of Wistar lineage were used.The SOP was induced in rats that were days of life by subcutaneous administration of testosterone (10 mg/kg). The rats were subdivided into three groups: control (CTRL: non-induced), SOP-IND (SOP: treated with saline) and SOP-ISO (SOP:treated with isoflavones *G. max*; 10mg/100g body weight). It was evaluated by the uterotrophic assay and the estrous cycle. **Results and discussion:** The CTRL group showed uterine weight of 0.227 ± 0.01. The induction was a decrease of uterine weight, SOP-IND group (0.165 ± 0.01) compared to CTRL. The treatment with G. *max* promoted uterotrophic (0.233 ± 0.01) effectwhen compared to the experimental group. In the estrous cycle was observed that the CTRL group showed regular cycles**:** proestrus – 14.28%; estrus – 21.00%; metaestrus – 24.37%; diestrus – 40.34%. The SOP-IND group presented a significant increase in the frequency of diestrus phase (67.97%) and a marked reduction in the proestrus phase (0.00%) and estrus (0.00%). The SOP-ISO group showed an increase in proestrus phase (5.30%) and estrus (6.62%) compared to SOP-IND. In the biochemicalparameters analyses were observed that induction increased glucose levels (176.0 ± 14.79) and the treatment led to a regression (126.1 ± 10,52). The same occurred with triglyceride levels: SOP-IND (119.1 ± 2.88); SOP-ISO (107.0 ± 3.65). It was also observed that the administration of isoflavones caused an increase in HDL (49.50 ± 5.417) compared to the SOP-IND (43.72 ± 3.91) group. **Conclusion**: *Glycine max* exerts a protective effect in against the PCOS phenotype by restoring the estrus cyclicity, glucose sensitivity and altering the lipidprofile. This can be attributed to phytoestrogens components present in the extract.These findings may provide subsidies to validate natural products in ovarian regulation what can be used in the treatment of polycystic ovary syndrome. **Support:** Maranhão Foundation for the Protection of Research and Scientific and Technological Development (FAPEMA).

09.085

Lectin from the green seaweed *Caulerpa cupressoides* down regulates inflammatory hypernociception in the temporomandibular joint arthritis in rats. Rivanor RLC[1], Fernandes MEF[2], Val DR[3], Freitas AR[4], Lemos JC[5], Pereira KMA[4], Rodrigues JAG[1], Araújo IWF[6], Bezerra MM[5], Chaves HV[4], Benevides NMB[1] [1]UFC – Bioquímica e Biologia Molecular, [2]UFC – Odontologia, [3]RENORBIO-UFPE, [4]UFC-Sobral – Odontologia, [5]UFC-Sobral – Medicina, [6]UFC – Engenharia de Pesca

Introduction: Seaweed lectins have been widely investigated as anti-nociceptive and anti-inflammatory agents. Herein, we analyzed the anti-nociceptive and anti-inflammatory responses of a lectin from the green seaweed Caulerpa cupressoides (CcL) on zymosan-induced arthritis of the rat temporomandibular joint (TMJ). **Methods**: Rats received i.v. CcL 30 min prior to injection of zymosan (2 mg/art.) or 0.9% saline into the left TMJ. Mechanical hypernociception was measured by the electronic von Frey method at baseline and 4 h after zymosan injection. Animals were euthanized 6 h after zymosan injection and the synovial fluid was collected for leukocyte counting and myeloperoxidase activity assessment. Other animals were treated with ZnPP-IX (3 mg/kg; s.c.), a specific heme oxygenase-1 pathway inhibitor, and naloxone (10 μg/art.), a nonselective opioid receptor antagonist. TMJ tissues were excised to perform histopathological and immunohistochemistry analyses. **Results and discussion:** CcL (0.1, 1 and 10 mg/kg) significantly reduced zymosan-induced hypernociception (81, 83 and 89.5%, respectively) and inhibited the leukocyte influx (77.3, 80.7 and 98.5%, respectively) compared with the zymosan-only group, as confirmed by myeloperoxidase activity; however, treatment with naloxone or ZnPP-IX did not revert the effects of CcL (10 mg/kg), suggesting that the naloxone-sensitive opioid and heme oxygenase-1 pathways are not involved. CcL also reduced the leukocyte influx and the expression of IL-1β and TNF-α in the TMJ, based on histopathological and immunohistochemistry analyses, respectively. CcL reduces TMJ hypernociception and inflammation with a mechanism that is partially dependent on TNF-α and IL-1β inhibition. CcL reveals a potentially valuable alternative tool for future studies of TMJ disorders.
Funding Sources: FUNCAP, CNPq, Capes, and INCT-IBISAB.

09.086

Antispasmodic activity of crude ethanolic extract from *Xylopia frutescens* Aubl. aerial parts on guinea pig ileum. Moreno GTA[1], Ferreira SRD[1], Oliveira FRMB[1], Vasconcelos LHC[2], Correia ACC[3], Silva MS[2,4], Tavares JF[2,4], Cavalcante FA[2,5] [1]UFPB, [2]UFPB, [3]ICBS-UFAL, [4]DCF-UFPB, [5]DFP-UFPB

Introduction: *Xylopia frutescens* Aubl. (Annonaceae) is popularly known as "embira", "envira", "pindaíba" and "pau-de-embira" (COSTA, Acta Farm. Bonaerense, v. 25, n. 2, p. 184, 2006) and is popularly used for diarrhea treatment (DUKE, Amazonian Ethnobotanical Dictionary, 1994). Recently, we showed that the ethanolic extract from *Xylopia frutescens* aerial parts (XF-EtOH$_{AP}$) presents antidiarrheal activity in mice (MORENO, COIFFA, 2013). Thus, this study aimed to investigate a possible antispasmodic activity of XF-EtOH$_{AP}$ on guinea pig ileum. **Methods:** all experimental protocols were previously approved by Ethical Committee on Animal Use of CBiotec/UFPB (protocol 3206/13). Guinea pig ileum was suspended in organ baths containing modified Krebs solution at 37 °C, bubbled with carbogen mixture under a resting tension of 1 g. Isotonic and isometric contractions were monitored and recorded. **Results:** XF-EtOH$_{AP}$ (81, 243 and 729 µg/mL, n = 5) antagonized phasic contractions induced both by carbachol (CCh) 10^{-6} M (IC_{50} = 270.9 ± 38.9 µg/mL) and histamine 10^{-6} M (IC_{50} = 371.0 ± 69.3 µg/mL) in an equipotent and concentration-dependent manner. XF-EtOH$_{AP}$ (1-729 µg/mL, n = 5) also relaxed, equipotently, the pre-contracted ileum with KCl 40 mM (EC_{50} = 114.0 ± 17.5 µg/mL) or CCh 10^{-5} M (EC_{50} = 141.4 ± 6.2 µg/mL). Furthermore, XF-EtOH$_{AP}$ (81, 243 and 729 µg/mL, n = 5) shifted to the right, in a non-parallel manner, the cumulative concentration-response curves to $CaCl_2$, with reduction of its maximum effect (E_{max}). Moreover, the relaxation curve of XF-EtOH$_{AP}$ (EC_{50} = 141.4 ± 16.2 µg/mL) was not displaced in the presence of CsCl 5 mM, non-selective K+ channel blocker (EC_{50} = 102.3 ± 24.1 µg/mL, n = 5). **Discussion:** once *Xylopia frutescens* is used in folk medicine as antidiarrheal, and diarrhea is caused by gut disorders in smooth muscle contractions, we decided to verify whether XF-EtOH$_{AP}$ presents antispasmodic activity. The absence of significant differences between the IC_{50} values front histamine and CCh is suggestive that XF-EtOH$_{AP}$ may be acting in a simillar signaling pathway to both agonists, and not in a level of plasma membrane receptor, since that each agonist tested has its own receptor system. Moreover, XF-EtOH$_{AP}$ relaxed equipotently pre-contracted ileum with KCl or CCh, suggesting that XF-EtOH$_{AP}$ could be acting by inhibiting Ca^{2+} influx through voltage-gated Ca^{2+} channels (Ca_V). This hypothesis was confirmed by the observation that XF-EtOH$_{AP}$ inhibited, in a concentration-dependent manner, $CaCl_2$-induced contractions in depolarizing medium. Since K+ channels negatively modulate the Ca_V (THORNELOE, *J Physiol Pharm*, v. 83, p. 215, 2005), it was investigated the role of these channels in XF-EtOH$_{AP}$ antispasmodic effect using CsCl, non-selective K^+ channel blocker. The XF-EtOH$_{AP}$ relaxant potency was not changed in the blocker presence, indicating that K^+ channels are not modulated by this extract. Therefore, XF-EtOH$_{AP}$ have antispasmodic activity on guinea pig ileum by inhibiting Ca^{2+} influx through Ca_V supporting the *Xylopia frutescens* use in traditional medicine. **Financial support:** PIBIC/CNPq, Capes, CCS/UFPB.

09.087

***Hypericum perforatum* inhibits inflammatory pain in mice.** Mizokami SS[1], Staurengo-Ferrari L[1], Fattori V[1], Colombo BB[1], Casagrande R[2], Verri WA[1] – [1]UEL – Ciências Patológicas, [2]UEL – Ciências Farmacêuticas

Introduction: *Hypericum perforatum* is popularly known as St. John's Wort and is largely used in traditional and regular medicine. There is evidence that *Hypericum perforatum* possesses skin wounds, eczema, burn, antimicrobial, antinociceptive, anti-inflammatory, and antioxidant activities. However, the clinical use of the extract is restricted to its antidepressant effect. Therefore, the analgesic effects and mechanism of *Hypericum perforatum* were evaluated. **Methods:** Male Swiss mice (25-30 g) were treated orally (p.o., 10-300 mg/kg) with *Hypericum perforatum* or vehicle (20% Tween 80 in saline) 30 min before after inflammatory stimulus. The writhing response was evaluated for 20 min after intraperitonealy (i.p.) injection of phenyl-*p*-benzoquinone (PBQ, 1890 µg/kg) or acetic acid (0.8%). The paw flinching and licking nociceptive responses were quantified during 30 min after formalin 1.5% (25 µL/paw) or CFA (10 µL/paw) injection. Mechanical hyperalgesia was evaluated 1-5 h after carrageenan (300 mg/paw), and IL-1β cytokine level was determined 3 h after carrageenan (300 mg/paw) injection. This study was approved by the Ethics Committee on Animal Studies of the UEL. The license number of The Research and Ethics Committee of UEL: 132080.2011.64. **Results:** *Hypericum perforatum* (30, 100 and 300 mg/kg) dose-dependently inhibited PBQ-induced writhing (up to 48.0%, 55.2% and 74.2%, respectively), and the dose of 300 mg/kg inhibited acetic acid-induced writhing (up to 57.6%). *Hypericum perforatum* also inhibited both phases of the formalin test (up to 56.5% and 37.2% for paw flinching and licking in the first and up to 96.0% and 58.1% for paw flinching and licking in second phase, respectively) and CFA (up to 83.7% and 88.1% for paw flinching and licking, respectively) induced nociceptive behavior. *Hypericum perforatum* inhibited the carrageenan-induced mechanical hyperalgesia (up to 75.8%) and the production of pro-hyperalgesic cytokine, IL-1β (64.1%). **Discussion:** These results demonstrate that *Hypericum perforatum* presents analgesic effect in a variety of inflammatory pain models and this effect may depend on inhibition of IL-1β production. **Financial Support:** Capes, CNPq, MCTI, SETI / Fundação Araucária, and Governo do Estado do Paraná (Brazil).

09.088

Hepatoprotective effect of the aqueous extract of *Simarouba amara* Aublet (Simaroubaceae) stem bark against carbon tetrachloride (CCL4) – induced hepatic damage in rats. Maranhão HML[1], Martins AOBPB[1], Vasconcelos CFB[1], Rolim LA[1], Silva-Neto JC[2], Silva Filho RC[3], Costa-Silva JH[3], Fernandes MP[3], Araújo AV[4], Wanderley AG[1,5]
[1]UFPE –Pharmaceutical Sciences, [2]UFPE – Histology and Embryology, [3]CAV-UFPE – Physical Education and Sports, [4]CAV-UFPE – Nutrition, [5]UFPE – Physiology and Pharmacology

Introduction: *Simarouba amara* Aublet, popularly known as "praíba", "marupá" and "pau-paraíba", is a large tree that reaches up to 40 m height and 0.5 to 0.9 m diameter. *S. amara* stem bark decoction has been traditionally used in Brazil to treat malaria, inflammation, fever, abdominal pain, diarrhea, wound and as a tonic[1]. **Objectives:** This study aimed to investigate the hepatoprotective effects of the *S. amara* stem bark (SAAE) against CCl_4-induced hepatic damage in Wistar rats. **Methods:** The main tannins present in SAAE were quantified by HPLC. Liver injury was induced according to Simile *et al.*[2] with slight modifications. The animals were randomly divided into six groups (n = 6/group). Groups I (vehicle – corn oil), II (control – CCl_4), III, IV, V and VI were pretreated during 10 consecutive days, once a day p.o, with corn oil (vehicle), Legalon® 50 mg/kg b.w. or SAAE at doses 100, 250 and 500 mg/kg b.w, respectively. The hepatotoxicity was induced on 11th day by administering 2 mL/kg of CCl_4 (20%, diluted in corn oil). 24 h after injury, blood samples were collected. Serum analyzes of liver enzymes such as alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AF), gamma-glutamyltranspeptidase (GGT), lactate dehydrogenase (LDH) and total bilirubin (TB), direct bilirubin (DB) and indirect bilirubin (IB) were estimated by using standard Boehringer Ingelheim® diagnostic kits. The livers were removed to evaluate lipid peroxidation level[3] and superoxide dismutase[4] and catalase[5] activities. Immunoreactivity to Proliferating Cell Nuclear Antigen (PCNA) was also performed. All protocols were approved by the Animal Experimentation Ethics Committee of the UFPE (license no. 026449). **Results:** Catechins were the main tannins presents in the extract. SAAE decreased the levels of AST, ALT, TB, IB, LDH and lipid peroxidation in all doses (Control: 5.94 ± 0.88 vs SAAE 100: 3.42 ± 0.37*, SAAE 250: 2.69 ± 0.42* and SAAE 500: 3.56 ± 0.55* nmol/mg protein, n=4) and increased the catalase activity (Control: 0.33 ± 0.03 mU/mg protein, n=4) at the doses of 250 (0.92 ± 0.12* mU/mg protein) and 500 mg/kg (0.83 ± 0.02* mU/mg protein, n=4). The PCNA immunoreactivity revealed a great number of proliferating cells in the livers treated with SAAE in all doses (over 50% reactive nuclei, intense immunoreactivity) when compared to control. The dose of 500 mg/kg of SAAE had an immunoreactivity similar to Legalon® (over 75%). The intense immunoreactivity to PCNA suggested hepatocyte proliferation in all doses of the treatment. **Conclusion:** The SAAE prevented the oxidative damage as well as increased regenerative and reparative capacities of the hepatocytes. **References:** 1- Bonté *et al.*, J. Ethnopharmacol. 53: 65, 1996. 2- Simile *et al.*, J. Hepatol. 34: 386, 2001. 3- Buege and Aust, Met. Enzymol. 52: 302, 1978. 4- Misra and Fridovich, J. Biol. Chem. 247: 3170, 1972. 5- Aebi, Met. Enzymol.105: 121, 1984. **Financial support:** FACEPE.

09.089

***In vitro* antioxidant activity of a semisynthetic derivative of the natural riparins.** Araújo EJF[1], Oliveira JS[1], Lima FCA[2], Freitas RM[1], Gutierrez SJC[1] [1]UFPI, [2]UESPI

Introduction: The phenomenon of oxidation is a process that can lead, when exacerbated, to cell damage and result in the development or aggravation of diseases (ALAM *et al.*, 2013). The elucidation of a new antioxidant compound, derivative of the natural riparins, pharmacologically active compounds extracted from the unripe fruit of *Aniba riparia* (Nees) Mez, Lauraceae family, can be an alternative for the sustainable development of a new drug (GUTIERREZ, 2007). Based on the above, this study aims to characterize the *in vitro* antioxidant activity of riparin A (rip A), *N*-fentenilbenzamide, semisynthetic derivative of the natural riparins, through chemical reactions that evaluate the potential of the substance to reduce the formation of $DPPH^{\cdot}$ and $ABTS^{+}$. **Method:** Using the Schotten-Bauman reaction of the riparin A was obtained starting from the mixture of 0.41 mL of acid chloride and 0.89 mL of 2-phenylethylamine with triethylamine (GUTIERREZ, 2007). The stock solution of rip A (7.2 mg/mL), $DPPH^{\cdot}$ (40 mg/mL), $ABTS^{\cdot+}$ (7 mM) and Trolox standard (140 mg/mL) was prepared (RE *et al.*, 1999; SILVA *et al.*, 2005). The absorbance values converted to percent inhibition of radical (% I) and the 50% effective concentration (EC_{50}) of rip A was determined spectrophotometrically at 517 nm for $DPPH^{\cdot}$ and 734 nm for $ABTS^{+}$ and data were analyzed with *GraphPad Prism*® version 6.04 using Analysis of Variance (ANOVA) followed by *t*-Student-Newman-Keuls test as a *post hoc* test with $p<0.001$. **Results:** All tested concentrations of rip A (0.003, 0.008, 0.016, 0.023 and 0.032 nM) significantly reduced the concentration of free radical in relation to the production medium with decreased of 28.2, 31.0, 32.5, 41.3 and 45.3% respectively. In the test it was determined that EC_{50} of rip A was 8.8 nM. In the assay of radical $ABTS^{\cdot+}$ was determined that there was a reduction of 54.5, 58.9, 61.7, 66.5 and 67.3% respectively with EC_{50} calculated as 0.46 nM. **Discussion:** Can see the dependent-dose relationship in riparin A activity, since all concentrations of substance, led to a gradual increase in the percentage inhibition of radicals. This fact becomes more relevant if one takes into consideration the fact of riparin A molecule has in its structure free electrons associated with atoms of nitrogen and oxygen of the central amide group. This is because the mechanism of the tests used is based on oxidoreduction reactions (BADARINATH, 2010). **Acknowledgment:** This study was supported by National Council of Technological and Scientific Development (CNPq/Brazil – Process No. 153996/2013-7 and 407575/2013-8) **References:** ALAM, M. N. *et al.* Review on *in vivo* and *in vitro* methods evaluation of antioxidant activity. *Saudi Pharm J.*, v. 21, p.143, 2013. BADARINATH, A.V. *et al.* A review on in-vitro antioxidant methods: comparisions, correlations and considerations. *AJPTR*, v. 2 p. 1276, 2010. GUTIERREZ, S.T.C. Synthesis of Bowdenol, an dihidrobenzofuranoide isolated of *Bowdichia virgilioides* and preparation of derivatives of Riparin isolated from *Aniba riparia* with potential biological activity. Thesis (Ph.D.) UFPB/CCS. João Pessoa, 2007. RE, R., *et al.* Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.*, v. 26, p. 1231, 1999. SILVA, C.G. *et al.* Evaluation of antioxidant activity of Brazilian plants. *Pharmacol Res*, v. 52, p. 229, 2005.

09.090

Role of the ethyl acetate fraction of *Platonia insignis* Mart. in NO-synthase and lipid peroxidation in gastric lesions induced by absolute ethanol. Lima GS[1], Oliveira IS[1], Silva-Freitas FV[1], Lira KL[1], Nicolau LAD[1], Nunes PHM[2], Chaves MH[3], Medeiros JVR[1], Oliveira RCM[1,2] [1]NPPM-UFPI, [2]UFPI – Biophysics and Physiology, [3]UFPI – Department of Chemistry

Introduction: *Platonia insignis* Mart. (Clusiaceae) is a plant found in the state of Piauí. The fruit pulp of bacuri is used as a aliments. Previous studies have shown that ethyl acetate fraction (Pi-AcOEt) obtained from the fruit peel of *Platonia insignis* Mart., exhibit gastroprotective activity in models of gastric lesions induced by ethanol and ischemia and reperfusion. This study evaluated the participation of the NO-synthase pathway and the possible antioxidant potential involved in gastroprotection. **Methods:** To evaluate the role of NO-synthase, mice Swiss (25-30 g, n = 7), females, fasted for 18 h were pretreated with saline or nitro L-NG-nitro arginine (L-NOARG, 70 mg/kg, i.p.). After 30 min received vehicle (p.o.), Pi-AcOEt (50 mg/kg, p.o.) and L-arginine (600 mg/kg, i.p.). After 60 min treatment with the vehicle and Pi-AcOEt and 30 min later administration of L-arginine, each animal was given orally 0.2 mL of absolute ethanol. They were euthanized 30 min later and the area of injury was calculated by planimetry ($mm^{2)}$. The level of malondialdehyde (MDA) in the homogenates stomachs of rats subjected to the protocol of absolute ethanol was measured by reaction with thiobarbituric acid. Fragments of gastric mucosa weighing between 100 mg were homogenized with cold KCl (1.15%) to prepare a 10% solution of homogenate. In brief, 250 µl of this homogenate was added to 1.5 ml of 1% H_3PO_4 and 0.5 ml of 0.6% thiobarbituric acid (aqueous solution). Then, the mixture was stirred and heated in a boiling water bath for 45 min. Next, the reaction mixture was cooled immediately in an ice water bath, followed by addition of 4 ml of n-butanol. This mixture was shaken for 1 min, and the butanol layer was separated by centrifugation at 1200g for 10 min. Optical density was determined at 535 and 520 nm, and the optical density difference between the 2 determinations was calculated and considered as thiobarbituric acid value. MDA concentrations were expressed as nanomoles per gram of tissue. All animal experiments protocols were approved by Ethics Committee on Animal Experiments of the Federal University of Piauí (CEEA/UFPI 008/12). The significance level was evaluated for values of $*p<0.05$. **Results and Discussion:** L-NOARG, a blocker of the enzyme NO-synthase, reversed the protection of gastric mucosa presented by Pi-AcOEt (10.25 ± 1.86 to 29.67 ± 2.93 mm^2) and L-arginine (5.64 ± 0.90 to 13.83 ± 2.34 mm^2) suggesting the involvement of nitric oxide synthase in the gastroprotective effect produced by these fraction. Administration of absolute ethanol in the control group (195.66 ± 13.45), resulted in increased MDA (end product of lipid peroxidation) concentration when compared with the SHAM group (124.41 ± 11.51). Pi-AcOEt and carbenoxolone (122.98 ± 11.21 and 109.88 ± 5.16, respectivily) were able to reduce the levels of MDA in homogenates of stomach, showing a possible reduction of lipid peroxidation. However, new protocols are required to isolate the gastroprotective compounds and to elucidate their mechanisms. **Financial Support:** UFPI/Capes/FAPEPI/CNPq

09.091

In vitro **antioxidant capacity of a new ester phenylpropanoid.** Machado KC[1], Oliveira GLS[1], Sousa EBVS[2], Sousa EBVS[2], Machado KC[1], Sousa DP[2], Freitas RM[1] [1]UFPI, [2]UFPB

Introduction Essential oils have played a prominent role in research on natural products, due to the high level of bioactive constituents; with include those derived from phenylpropanoids or terpenoids (AGUIAR, U. N., *Quím. Nova*, v. 15, p.1, 2013). This study aimed to evaluate the antioxidant capacity of isopentanoyl ferulate. Methods We used experimental *in vitro* models such as elimination of the radical 1,1 diphenyl-2-picrylhydrazyl (DPPH·), 2,2 'azinobis-3-ethylbenzothiazoline-6-sulfonic acid (ABTS· +), hydroxyl (OH·) and nitric oxide (NO·), ability to inhibit lipid peroxidation by thiobarbituric acid reactive substances (TBARS) method as well as its reduction potential. Results **and discussion** In all *in vitro* antioxidants results, isopentanoyl ferulate showed to be potent in a concentration of 14.4 µg/ml, presenting a percentage inhibition of 91.29 ± 0.57, 92.63 ± 0.28, 16.37 ± 1.40, 20.30 ± 1.62 and 18.8 ± 1.57% for the DPPH· radical, ABTS+, hydroxyl, nitric oxide and lipid peroxidation, respectively. In relation to its reducing power was no increase in absorbance at 700 nm at all concentrations. Similar results were obtained with Trolox (559 nM), a hydrophilic synthetic analogue of α-tocopherol, which is widely used as a standard antioxidant. The present study demonstrated that isopentanoyl ferulate has an antioxidant activity *in vitro* experimental models, suggesting that this compound could enhance the development of a new product with antioxidant potential. However, further *in vivo* studies are needed to assign possible implications in the treatment of diseases related to stress. **Financial support**: CNPq and FAPEPI.

09.092

Parameters of quality and identity of geoprópolis of *Melipona fasciculata* Smith. Rocha AO, Ferreira FS, Serra MB, Mesquita LSS, Mesquita JWC, Cunha MS, Batista MC, Dutra RP, Ribeiro MNS UFMA

Introduction: The stingless bees, the meliponines, are diversifieds groups of social insects, with high diversity and abundance in tropical regions, with great importance in pollination and agriculture. *MeliponafasciculataSmith* (*tiúba*) is grown in the state of Maranhão, produces honey, wax, geopropolis and accumulates pollen, and this are the main attractions for the rational creation and management. Geopropolis is formed by collecting resinous material of plants, mixed with wax and salivary secretions and added land. Antibacterial biological actions, anti-inflammatory, antioxidant, antitumor and leishmanicide were described for the geopropolis of *Melipona fasciculata* Smith and substances of classes of phenolic acids, flavonoids, tannins etriterpenos are involved in the actions. **Methodology:** The four samples of geopropolis were collected in meliponários in the county of Barreirinhas and Belagua in the state of Maranhao, Brazil. The geopropolis, separately, have been crushed, macerated in 70% ethanol for 48 h at room temperature and concentrated on a rotary evaporator, obtaining hydroalcoholic extracts. These extracts were determined the levels of total contents of polyphenols and flavonoids by spectrophotometry, using reagent FolinCicoaulteau and aluminum chloride, using concentrations of gallic acid and quercetin as standards respectively; The antioxidant activity by the method of *in vitro* fotocolorimetric of the stable radical 2,2 – diphenyl-1-picrylhydrazyl (DPPH) and the chemical profiles obtained by absorption spectrophotometry in the ultraviolet region and visible (UV-Vis) and by liquid chromatography of high performance (HPLC-UV-Vis). **Results and discussion:** The extracts showed polyphenols and flavonoids total levels range of 20,38 to 77.1% and from 0.81 to 2.02% respectively, antioxidant activity CE_{50} 20,76 µg/mL, 24,59 µg/mL, 30,49 µg/mL and 45,66 µg/mL. The chemical profiles of extracts in the absorption spectra in the UV region showed absorption maxima at 270nm, 272nm, 277nm e 280nm, compatible with chromophores of phenolic compounds and the chromatograms showed a complex chemical composition with substances of medium and high polarity, with a predominant substance with time of retention in 28 minutes in the extracts, probably a chemical marker of geopropolis in the cities. The hydroalcoholic extracts of geopropolis have antioxidant activity and phenolic compounds are related to this activity. The results allow us to establish chemical parameters for determiningthe profile of the quality and identity of the geopropolis in the cities of Barreirinhas and Belagua profile in the state of Maranhao and add value to the product of meliponiculture in the state of Maranhao, Brazil

09.093

Antioxidant potential of *Passiflora edulis.* Ferreira FS, Mesquita LSS, Rocha AO, Mesquita JWC, Serra MB, Cunha MS, Batista MC, Dutra RP, Ribeiro MNS UFMA

Introduction: *Passiflora edulis* Sims (Passifloraceae), known as passion fruit, plant species is of great economic importance and medicinal value, commonly used in the treatment of hypertension, insomnia and irritability. Some pharmacological activities have been demonstrated for the species, such as anti-inflammatory, antibacterial, antioxidant, giardicidal, hypoglycemic and anxiolytic, and found the presence of pharmacologically active substances, such as alkaloids and flavonoids; soon, with the potential to obtain bioproduct. Aims to assess the hydroalcoholic extracts of leaves of *Passiflora edulis* using chemical assays to contribute to the development of herbal antioxidants. **Methodology**: The leaves were collected in the Paço of Lumiar / Maranhão / Brazil in January/2013, subjected to drying and grinding, followed by maceration and extraction hydromodule (drug / solvent ratio) 1:8, 1:10 and 1:12 (w / v) using ethanol 70% (v / v) as solvent. The extraction solutions were filtered and concentrated on a rotary evaporator, obtaining hydroalcoholic extracts. The level of antioxidant activity was performed in UV-Vis spectrophotometer (Lambda 35, PerkinElmer) at 517nm with stable employment free radical 2,2-diphenyl-1-picrilidrazila (DPPH) and the quantification of total flavonoids fotocolorimétrico method with the methanol solution of aluminum chloride (AlCl3) chloride to 5% by spectrophotometry (UV-Vis spectrophotometer Lambda 35, PerkinElmer) at 425 nm using concentrations of quercetin (Merck) as standard. **Results and discussion:** The hydroalcoholic extracts of the leaves of *P. edulis* obtained by maceration and different hidromódulos showed total flavonoids from 2.5 to 3.9% and antioxidant activity with EC50 between 84.23 and 99.92 $\mu g.mL^{-1}$. Logo are considered moderately active. The major contribution of the antioxidant activity may be due to the presence of flavonoids, which have generally ideal for the kidnapping of radical structure. Many biological functions have been attributed to antioxidant activity, including anti-inflammatory, antitumor and antimicrobial. The chemical integrity of plant extracts and antioxidant activity is critical in ensuring achievement of quality derived preparations, aiming at effective contribution in access to effective and safe herbal remedies that can fight free radicals and associated diseases.

09.094

Effects of synthetic natriuretic peptide of *Crotalus durissus cascavella* venom in isolated perfused rat kidney system. Silveira JAM[1], Marinho AD[1], Jorge ARC[1], Costa PPC[1], Jorge RJB[1], Morais GB[2], Evangelista JSAM[2], Monteiro HSA[1] – [1]LAFAVET-UFC – Physiology and Pharmacology, [2]UFC-HISTOVESP – Veterinary

Introduction: The snake *Crotalus durissus cascavella* is characteristic of the caatinga of northeastern Brazil. In its venom, as well as other snakes, there are several biologically active substances with a wide pharmacological activity, among them, the natriuretic peptides (Evangelista JSAM *et al.*, v.52, p.737, 2008). This study aimed to evaluate the effects of a synthetic natriuretic peptide of *C. d. cascavella* venom (NPCdc) in isolated perfused rat kidney system. **Methods:** Adult male Wistar rats weighing between 250 and 300g were used, whose kidneys were surgically excised and perfused separately with Krebs-Henseleit solution containing 6% w/v bovine serum albumin previously dialyzed. The effects of synthetic NPCdc (0.03mg/mL, n=6) were evaluated for Perfusion Pressure (PP), Renal Vascular Resistance (RVR), Urinary Flow (FU), Glomerular Filtration Rate (GFR), Percentage of Total Tubular Transport of Sodium (%TNa^+), Potassium (%TK^+) and Chloride (%TCl^-) and Percentage of Proximal Tubular Transport of Sodium (%$pTNa^+$). Statistical analysis was performed with two-way ANOVA followed by Bonferroni post-test, considering α=0.05. This study was approved by the Ethics Committee on Animal Research of the Federal University of Ceará, with protocol number 79/08. **Results:** NPCdc 0.03mg/mL promoted a statistically significant increase in UF at 120min and a reduction in GFR at 60, 90 and 120min, while the PP and RVR were not changed. There was a reduction in the times of 60, 90 and 120min of all transports evaluated (%TNa^+, %$pTNa^+$, %TK^+ and %TCl^-). **Discussion:** These effects on the transport of Na^+, K^+ and Cl^-, as well on RFG, are suggestive of renal tubular epithelial injury, which affects the transport function of renal epithelial cells. Thus, it is believed that the NPCdc have nephrotoxic potential at this tested concentration. The findings suggest further investigation of the effects of this substance to the histological level in order to clarify in more detail the mechanism of action of this peptide. **Acknowledgments:** CNPq for the Financial support.

09.095

Vasodilatory activity and mechanism of action of the ethanolic extract and fractions of aerial parts of *Stachytarpheta schottiana* (Verbenaceae). Moreira AP, Moura GR, Muzitano MF, Barros CM, Carmo PL, Raimundo JM UFRJ-Macaé

Introduction: Systemic hypertension, the major risk factor for cardiovascular diseases, is associated with the death of 7.4 million people per year in the world[1]. Species of the *Stachytarpheta* genus, as *S. jamaicensis* and *S. cayennensis*, are popularly used for the treatment of hypertension[2,3]. We have previously shown that the ethanolic extract of aerial parts of *S. schottiana* causes intense vasodilation and has antioxidant activity[4]. Thus, the objective of this study was to investigate the vasodilatory effect and mechanism of action of different fractions from *S. schottiana* ethanolic extract. **Materials and methods**: The ethanolic extract of *S. schottiana* aerial parts was submitted to a multi-step liquid–liquid fractionation with solvents of increasing polarity leading to four fractions: hexanic (HSS), dichloromethane (DSS), ethyl acetate (EASS) and butanolic (BSS). Vascular effects of the different fractions were assessed in endothelium-intact aortic rings from male Wistar rats (220–280 g), which 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 (Phe, 10 μM) was measured before (control) and after exposure to increasing concentrations of fractions (1-300 μg/mL). To investigate the mechanism of action, EASS was tested in aortas without endothelium and in aortas with endothelium pretreated with L-NAME (100 μM), an inhibitor of nitric oxide (NO) synthase. Effects on NO production will be determined using 4,5-methylamin-2 ',7'-difluorescein (DAF-FM) in transverse arteriolar cryostat sections and images will be collected on a fluorescence microscope. One-way analysis of variance (ANOVA) followed by Dunnett's test was used to compare the experimental and control groups (GraphPad Prism 5.0). All protocols were approved by the Animal Care and Use Committee under license Macaé01. **Results**: EASS relaxed pre-contracted endothelium-intact aortic rings in a concentration-dependent manner. At 300 μg/mL, EASS-induced vasodilation was 47.2 ± 3.1 % ($P<0.05$ compared to control; n=5). HSS, DSS and BSS had no significant effect on pre-contracted aortic rings. EASS relaxant effect was completely inhibited in denuded aorta and in aorta with endothelium pre-treated with L-NAME. **Discussion**: Our findings suggest that bioactive compounds present in EASS seem to be the main responsible for the vasodilatory activity of the ethanolic extract of *S. schottiana* aerial parts, which is dependent on endothelium. Effects on NO production are still being investigated. **References:** [1]Cipriano Jr. G. *et al*, *Progressin Cardiovascular Diseases* 56:493-500, 2014; [2]Garcia-Gonzales M. *et al* Rev Cubana *Plant Med* 7:100, 2002; [3]Idu M. *et al* Int *J Pharmacol* 2:163, 2006; [4]Moreira, AP *et al* 45° Brazilian Congress on Pharmacology and Experimental Therapeutics, p. 46, 2013. **Financial support:** Faperj, FUNEMAC, UFRJ.

09.096

Snake venom PLA_2 activity inhibition by different protocols on neuromuscular junction.

Schezaro-Ramos R[1], Collaço RCO[1], Randazzo-Moura P[2], Cogo JC[3], Rodrigues-Simioni L[1] – [1]FCM-Unicamp – Farmacologia, [2]PUC-SP – Farmacologia, [3]UniVaP – Estudos da Natureza

Introduction: Snake venoms phospholipase A_2 (PLA_2) acts in neuromuscular junction cleaving the sn-2 ester glycerophospholips linkage, causing a complete neuromuscular blockade. Some usual procedures are used to inhibits the PLA_2 activity: low temperature bath, substituting the Ca^{2+} by Sr^{2+} of nutritive solution (for Asp 49 PLA_2 inhibition), and chemical modification with *p*-bromophenacyl bromide (*p*-BPB). *Bothrops fonsecai* (*B. fonsecai*), an endemic pitviper restrict to high altitude forests on southwestern Brazil, has a venom primary compounded by PLA_2 (~30% of venom proteins) and metalloproteases P-I and P-III (~42%). Studies showed a great similarity between the amino acid sequence of an fraction isolated from *B. fonsecai* and others bothropic PLA_2, as those present in *B. jararaca* and *B. insularis* venoms, which has potent myotoxic action. **Methods:** In this study, we used *Bothrops fonsecai* (*B. fonsecai*) venom as the research tool. Extensor digitorum longus of mice preparations (EDL) were treated with *B. fonsecai* (100 µg/ml) at different bath temperatures (24ºC or 37˚C) or nutritive solution composition (Tyrode solution with Ca^{2+} 1,8 mM or Sr^{2+} 4 mM), or had its phospholipasic activity inhibited by *p*-BPB (0.6 µM, 24 h, 23 ˚C). Aliquots (100 µl) of the solution were collected at different times after venom addition (0, 15, 30, 60, 90 and 120 minutes) for creation kinase activity dosage. All muscles were histologically analyzed. This work was approved by the Animal Ethics Committee (CEUA/Unicamp protocol No. 3311-1). **Results:** *B. fonsecai* venom (100 ug/ml) caused total neuromuscular blockade after 86 ± 4 min incubation at 37˚C, in presence of Ca^{2+} 1,8 mM. The replacement of Ca^{2+} 1.8 mM by Sr^{2} 4.0 mM partially inhibited the venom blocking effect, resulting in 82,9 ± 7% blockade 120 min after venom addition. Reducing the organ bath temperature from 37˚C to 24˚C, it was observed only 61,2 ± 7,6% blockade after 120 min, instead of the characteristic neuromuscular blocking effect. **Discussion:** All the PLA_2 activity inhibition protocols reduced the blocking activity of the *B. fonsecai* venom, showing different efficiency, what is in accordance with their different specificities for venom protein inhibition. **Financial agencies:** Unicamp, CNPq, Fapesp

09.097

Involvement of monoaminergic system in the antidepressive-like effect of chloroformic fraction of *Lafoensia pacari* A. ST.-HIL. ethanolic extract. Galdino PM[1,2], Carvalho AAV[1], Florentino IF[1], Martins JLR[1], Rodrigues ORL[1], Gazola AC[3], Reginatto FH[3], Paula JR[4], Torres LMB[5], Costa EA[1], de Lima TCM[2] [1]ICB-UFG – Farmacologia de Produtos Naturais, [2]CCB-UFSC – Farmacologia, [3]CCS-UFCS – Ciências Farmacêuticas, [4]FF-UFG – Produtos Naturais, [5]IBot

Introduction: *Lafoensia pacari* A. St.-Hil. (Lythraceae), known popularly as "pacari" or "mangaba-brava" is among the plants used in the state of Goiás, Brazil. The stem bark and leaves are used in popular medicine to treat cancer, gastric disturbs, inflammation and as tonic to treat despondency. Previous results suggest that the ethanol extract of the stem bark of pacari (EP) has antidepressant-like activity in mice (Galdino *et al.*, 2009). Our aim was to perform the bioassay-guided fractionation of the EP, and to evaluate the possible involvement of monoaminergic system in the effect of the active fraction. **Methods:** The stem barks of *L. pacari* were authenticated by Prof. Dr. José Realino de Paula and collected in the savannah region of Bela Vista-GO. The voucher specimen was deposited at the Herbarium of the UFG (27031/UFG). The EP was obtained by maceration in 70% hydro-alcoholic solution, followed by filtration and evaporation (yield = 16.1% w/w). EP (20 g) was dissolved in 300 mL of methanol/water (1:9), and partitioned successively with chloroform, ethyl acetate and *n*-butanol. Lupeol was detected in the chloroform fraction by nuclear magnetic resonance. The *Swiss* male mice (groups of 9), weighing 30-35g, were provided by Central Animal House/UFG. All experimental protocols were approved by the Ethic Commission of UFG (104/08). Antidepressant-like activity was evaluated by the forced swimming test (FST). The animals were treated (p.o.) with vehicle (2% Tween, 10 ml/kg), chloroform (ChloF – 70 mg/kg), ethyl acetate (180 mg/kg), n-butanol (370 mg/kg) and aqueous (1g/kg) fractions, 24, 5 and 1 h before the behavioral tests. To assess the involvement of serotoninergic and catecholaminergic systems in the ChloF effects, the animals were pre-treated (i.p.) with PCPA 100 mg/kg (4 days) and AMPT 100 mg/kg (4 h). In another series of experiments, the animals were treated (p.o.) with vehicle (2% Tween, 10 ml/kg) and Lupeol (9, 17 and 35 mg/kg), 24, 5 and 1 h before the behavioral tests. A possible inhibition on MAO-A activity by ChloF and Lupeol was assessed *in vitro*. Results were expressed as mean ± SEM and analyzed using one way-ANOVA followed by Dunnett test, two way-ANOVA followed by Student-Newman-Keuls test or nonlinear regression. **Results:** Phytochemical screening in TLC showed the presence of saponins, tannins and triterpene in the EP, and the presence of triterpene in the ChloF. After the fractionation, the ChloF was the most active fraction, reducing the immobility time by 22 % (from 209.8 ± 6.51 s to 163.1 ± 11.71 s), this result suggest that this fraction better retained the active constituents. Both pre-treatment with PCPA and AMPT, abolished the reduction in immobility time produced by ChloF: PCPA + ChloF = 209.4 ± 11.54 s, AMPT + ChloF = 210.6 ± 9.72 s) suggesting that ChloF antidepressant-like effect is dependent on serotonergic and catecholaminergic systems. Lupeol 35 mg/kg reduced the immobility time by 25 % (from 210.8 ± 6.43 s to 158.7 ± 11.07 s) suggesting that this compound may be one of the active constituents presents in the ChloF and EP. Neither ChloF nor Lupeol inhibit MAO-A activity, excluding this as mechanism of action. In conclusion, ChloF presents antidepressant-like effect that may involve both serotonergic and catecholaminergic systems without inhibition of MAO-A enzymatic activity, and Lupeol may be one of the active constituents responsible for *L. pacari* antidepressant-like effect. **Financial support**: Capes and CNPq.

09.098

Effects of Morus nigra (mulberry) leaves tea on arterial blood pressure in adult rats

Rodrigues BB[1], Oliveira RE[1], Araujo-Alves MA[1], Silva MTB[2], de Andrade CR[1] – [1]INTA-NUBEM – Bioprospecção e Experimentação Molecular Aplicada, [2]CCS-UFPI – Educação Física

Aim: In folk medicine, it has been widely used the infusion of *Morus nigra* (Mulberry) leaves to prevent hypertension in humans, however, there were no conclusive studies confirming this anti-hypertensive effect. Thus, our aim was to verify the effects of *M. nigra* leaves infusion in adult rats blood pressure. **Methods:** Male Wistar rats (280–320 g) were kept under standard conditions (temperature at 22 ± 2C; 12h light/12h dark cycle and food and water *ad libitum*). Proceddings were performed according to guidance and approval to the ethics committee (Protocol 2013-06.001). Rats were treated with an infusion of triturated leaves from *M. nigra* 1,5 mg/kg/day in the drinking water, control rats received water, during 7 days. For arterial blood pressure analysis, rats were anesthetized and catheters were implanted in the femoral artery. Baseline mean arterial pressure (MAP – mmHg) and heart rate (HR – bpm) values were recorded using a Powerlab system. In order to observe the infusion effect on MAP and HR, before each experiment, a period of 15–20min was allowed to obtain a stable MAP and HR tracing from control and treated groups. Values (mean ± SEM / n=6) were compared using Unpaired Student t test ($p<0.05$). **Results and Conclusion:** Despite the fact that treated rats intake more liquid (infusion) than control rats (water) (35 ± 6 mL vs. 11 ± 4 mL*, p=0.008), there were no alterations on body weight (control: 257 ± 15 vs. treated: 273 ± 14 g). MAP were not altered between control and treated groups (116 ± 8 *vs.* 108 ± 7 mmHg) neither HR, control 329 ± 12 bpm compared with treated 342 ± 1 bpm. These preliminary results indicate that, under normal conditions of arterial blood pressure, infusion of *M. nigra* leaves does not influence these cardiovascular parameters. **Financial support:** Faculdades INTA

09.099

Biochemical characterization of a PLA_2 Btae TX-I isolated from *Bothriopsis taeniata* snake venom: A pharmacological and morphological study. Romero-Vargas FF[1], Rocha T[2], Cruz-Höfling MA[3], Rodrigues-Simioni L[4], Marangoni S[1] [1]Unicamp – Biochemistry, [2]San Francisco University – Multidisciplinary Research Laboratory, [3]Unicamp – Histology and Embryology, [4]Unicamp – Pharmacology

Introduction: In this research a preliminary identification and biochemical and biological characterization of a PLA_2 (Btae TX-I) from the venom of a viperid snake, *Bothriopsis taeniata* (Speckled forest pit viper) were obtained. **Methods:** Btae TX-I was purified by two chromatographic steps, molecular exclusion chromatography followed by analytical chromatography reverse phase HPLC. Enzymatic activity was measured using a synthetic chromogenic substrate. Molecular mass and identification of tryptic peptides from Btae TX-I PLA_2 were evaluated by mass spectrometry. The study protocol was approved by the Ethics Committee of the State University of Campinas under the protocol number 1492-1. **Results and discussion:** Molecular mass of Btae TX-I behaved as a homogeneous single chain protein on SDS–PAGE, confirmed by MALDI-TOF spectrometry, indicating a molecular mass of 13889.98 Da. Tryptic peptides were determined *in tandem* mass spectrometry and showed similarity with other myotoxic PLA_2s. Btae TX-I belongs to the Asp49 PLA_2 class, is enzymatically active in presence of a synthetic substrate and shows a minimum sigmoidal behavior, reaching its maximal activity at pH 8.0 and 35–45°C. PLA_2 activity in presence of $Mn^{2+,}$ $Mg^{2+,}$ Cd^{2+} and Zn^{2+} was reduced either in presence or absence of $Ca^{2+,}$ suggesting that the arrangement of the catalytic site presents an exclusive structure for Ca^{2+} . Crotalic crotapotins from rattlesnake venom has significantly inhibited ($p<0.05$) the enzymatic activity of Btae TX-I. In ex vivo experiment, Btae TX-I caused partial blockade of the neuromuscular transmission in chick biventer cervicis preparations in a similar way to other *Bothrops* species. Btae TX-I also inhibited contractures in the upper concentration (50 µg) to exogenous KCl (20 mM). Histological analysis of the biventer cervicis incubated with Btae TX-I showed that just the highest Btae TX-I PLA_2 dose (50 µg) caused almost 27.4 ± 0.3% damaged fibers. The results give evidence that the main effect of type Asp49 Btae TX-I PLA_2 from *Bothriopsis taeniata* is at the post-synaptic site. **Financial Support:** Capes.

09.100

Proteolytic fraction FROM *Vasconcellea cundinamarcensis* latex stimulates the bone neoformation probably involving the proliferation and differentiation of osteoblasts.

Santos VG[1], Braga AD[1], Reis IDG[2], Freitas KM[1], Salas CE[3], Silva GAB[2], Lopes MTP[1] – [1]UFMG – Pharmacology, [2]UFMG – Morphology, [3]UFMG – Biochemistry and Immunology

Introduction: P1G10 is a proteolytic fraction obtained from *Vasconcellea cundinamarcensis* latex, a native plant of South America, which shows skin and gastric healing activities in lesions of different etiologies. These effects are attributed to its angiogenic and cell proliferative activities. Thus, the aim of this study was investigated bone repair activity of this fraction, using a model of intraoral bone defect. Furthermore, we also evaluate the involvement of proliferation and differentiation of osteoblasts by the treatment with sub fraction CMS2 and protease CMS2MS3, purified from P1G10. **Methods:** Male Wistar rats (n=36) were anesthetized and submitted to extraction of the maxillary first molar (right and left), and then, on the local of extractions, bone defects were performed with a drill (2.5 mm in diameter and depth). The treatment with P1G10 (0.01-0.1% w/v) was done and maintained for up to 14 days. After sacrifice, the maxillaries were processed for histological and morphometrical analysis. Osteoblasts were obtained from calvaria of newborn rats by enzymatic digestion. The cell proliferation (BrdU incorporation) and cell differentiation (alkaline phosphatase activity – ALP and mineralization of the extracellular matrix -alizarin staining) assays were evaluated in the presence of CMS2 or CMS2MS3 (0.1-100 ng/ml) (CETEA nº 107/2013). Differences between groups were performed using the one-way ANOVA test followed by Student-Newman-Keuls post-test. **Results and discussion** Regarding the inflammatory response, there was a mild inflammatory infiltrates in groups, P1G10 and control, after 3 and 7 days of treatment. We also observed that the number of blood vessels was similar between groups after 7 and 14 days of treatment. The bone neoformation was visualized within 7 days and the treatment with P1G10 (0.01 and 0.05% w/v) increased the percentage of formation from 7.6% in control (7.56 ± 1.93 %) to 20.5% (20.50 ± 4.43 %, $p<0.05$) and 26.0% (26.00 ± 4.25 %, $p<0.01$), respectively. On the 14th day, the defects were predominantly filled by immature bone tissue but no differences between groups were observed. *in vitro*, CMS2 or CMS2MS3 stimulated the osteoblasts proliferation between 13 ($p<0.05$) and 32% ($p<0.01$), after 48 hours of treatment. As regards the cell differentiation, the levels of ALP in the presence of CMS2 or CMS2MS3 increased about 12% ($p<0.05$), after 9 or 14 days of treatment. The extracellular matrix mineralization also was increased in cells exposed to CMS2 or CMS2MS3 at 14 days (≅ 50 and 60%, respectively, $p<0.05$). **Conclusion:** The proteases here studied promoted the bone neoformation and this one is probably linked to proliferation and differentiation of osteoblasts, cells that are intimately involved in bone formation. **Financial support:** Capes, CNPq and FAPEMIG.

09.101

Effect of the extract of *Euterpe oleracea* Mart. (Açai) on cardiovascular and renal disorders in animals with spontaneous hypertension (SHR) associated with Diabetes Mellitus Type 1. Cordeiro VSC[1], Carvalho LCRM[1], Costa CA[1], Bem GF[1], Santos IB[1], Oliveira PRB[1], Okinga A[1], Souza MAV[1], Sousa JP[2], Soares de Moura R[1], Resende AC[1]
[1]UERJ – Cardiovascular Pharmacology and Medicinal Plants, [2]UFPA – Pharmacy

Introduction: Epidemiological studies suggest that individuals with hypertension associated with DM are at greater risk for cardiovascular events than individuals with each disease alone. Studies by our group showed that the hydro-alcoholic extract of the seed of açai (ASE), induces an endothelium-dependent vasodilator response, antihypertensive and anti-hyperglycemic effects. However, we do not know the effect of treatment with ASE on renal and cardiovascular changes that may be amplified in hypertension associated with DM. **Objective:** This study aims to evaluate beneficial effects of chronic treatment with ASE in preventing the development of physiological, molecular and morphological changes in an experimental model of hypertension associated with diabetes. **Methods:** The experiments were approved by the Ethics Committee Rio de Janeiro State University (CEUA/059/2012). Wistar normotensive and hypertensive rats (SHR) received intraperitoneal injection of 50 mg kg^{-1} streptozotocin (STZ) (groups D and SHRD) or vehicle (groups W and SHR). The ASE treatment (200 mg/kg / day) was performed for 45 days in animals D (D + ASE) and SHRD (SHRD + ASE). Systolic blood pressure (SBP) were determined; the vasodilator effects of acetylcholine (ACh) and nitroglycerin (NG); serum albumin, urea, creatinine, glucose, insulin, glycated hemoglobin (GH) and renal excretion of albumin, creatinine and urea. The number of glomeruli, oxidative damage, the activity and expression of antioxidant enzymes (SOD, catalase and GPx), the expression of eNOS, iNOS, p47 and Nox4 in kidney were evaluated as well as the levels of nitrite. **Results:** SBP was higher in SHR vs W, D vs W and SHRD vs SHR. ASE prevented the increase in SBP in D and reduced in SHRD. The vasodilator response to ACh was reduced in D, SHR and SHRD vs W and ASE prevented endothelial dysfunction in all groups. The effect of NG was not different between groups. Serum levels of glucose, insulin, GH, creatinine and urea were higher in D and SHRD groups and ASE reduced levels of GH and creatinine. There was no difference in the levels of albumin. In samples of the urine, urea and microalbuminuria were higher in SHRD vs SHR and reduced by ASE. The increased levels of malondialdehyde and carbonyl protein in kidney of SHRD and D were associated with increased expressions of the pro-oxidant enzyme Nox4 and its subunit p47 that were reduced by ASE. The antioxidant enzyme activity was reduced in SHRD and enhanced by ASE, except that of SOD that remained high in SHRD + ASE. ASE increased nitrite levels and reduced the iNOS expression in the kidney of SHRD. Finally, the reduction of the number of glomeruli in SHRD was prevented by ASE. **Discussion:** The results demonstrate that diabetic hypertensive animals develop kidney injury associated with reduced renal function, endothelial dysfunction, increased blood pressure and oxidative damage. The improvement of vascular function and the antioxidant effect of ASE may contribute to the reduction of blood pressure and renal injury. **Financial Support:** Capes and Faperj.

09.102

Evaluation of the acute toxicity of essential oil from the leaves of *Tagetes minuta* L. in silver catfish (*Rhamdia quelen*). Oliveira AM[1], Cunha JA[2], Sutili FJ[2], Pinheiro CG[3], Garlet QI[2], Baldisserotto B[2], Heinzmann B[2] [1]CCS-UFSM – Farmácia, [2]CCS-UFSM – Farmacologia, [3]UFSM – Engenharia Florestal

Introduction: The use of plants for the treatment of illnesses has been widespread by the population since the earliest times. Several species used in folk medicine have shown biological activities which may be useful in fish farming. Therefore, studies are being conducted aiming to evaluate plant extracts as alternatives for anesthetic, antibacterial, antiparasitic and larvicide in aquaculture (EDRIS, A. E. *et al.* Phythoter Res, 21:308, 2007; GARCIA, M. V. *et al.* Rev. Bras. Parasitol. Vet., 21(4):405, 2012). *Tagetes minuta* L. is an aromatic medicinal plant native to South America, known as marigold, which is used as sedative in popular medicine (CHAMORRO, E. R. J. Argent. Chem. Soc., 96(1):80, 2008). Considering the use of essential oil (EO) in fish farming and the lack of scientific information concerning the use of EO from *Tagetes minuta* L. in aquaculture, this study aims to evaluate its acute toxicity in silver catfish (*Rhamdia quelen*). **Methods:** The methodologies of the experiments were approved by the Ethical and Animal Welfare Committee of the UFSM (Process nº. 46/2010). Leaves of *T. minuta* were collected in Itaara, RS, in autumn 2013. The EO was extracted by hydrodistillation for 3h (Farmacopéia Brasileira, 5th ed, 2010). The identification of the components of the EO was performed by Gas Chromatography (GC) coupled to Mass Spectrometry (Silva *et al.*, Aquaculture, 350:91, 2012), and the quantification was determined by GC with flame ionization detection. The EO was diluted in ehanol (1:10) and added to tanks with 1.5 L water at concentrations of 50, 100, 200 mg L^{-1}. Water and ethanol control groups were also included. To evaluate the toxicity, 50 animals (4.56 ± 0.5g, N=10) were previously kept in continuously aerated 250 L tanks with controled parameters of dissolved oxygen (7.85 ± 0.2), pH (6.55 ± 0.09) and temperature (21.3 ± 0.06). Two animals were placed in each tank, and the number of individuals living and dead within 24, 48 and 96h after application of the EO was observed. The percentage of mortality was calculated by considering the total number of animals exposed and killed by each concentration tested. **Results:** The EO of the leaves of *T. minuta* presented dihydrotagetone, *E*-tagetone, *Z*-tagetone, hexenyl acetate, limonene and β-ocymene as major compounds. The concentration of 50 mg L^{-1} did not provoke mortality in 24 and 48h, but within 96h 1% of mortality was observed. At 100 mg L^{-1} 70% of mortality was observed, whereas after 48 and 96h, 80% of the animals died. The concentration of 200 mg L^{-1} presented a mortality rate of 80% at 24h, 90% at 48h, and 100% at 96h. Mortality was also observed in both control groups (20%) in 96h. **Discussion**: The results indicate that *T. minuta* is potentially toxic for *R. quelen*, and this toxicity was concentration dependent therefore, the use of its EO is not recommended for aquaculture practices. **Financial Support:** FAPERGS/PRONEX, Document Nº. 10/0016-8; Ministério da Pesca e Aquicultura/MCT/FINEP; INCT ADAPTA; CNPq and FAPEAM.

09.103

Safety and efficacy of the lectin from *Mucuna pruriens* (*Mp*) seeds on free radicals and oxidative stress on ethanol-induced gastropathy in mice. Maciel LM[1], Pinto IR[2], Assis EL[3], Matos SO[3], Pereira Filho SM[1], Monteiro DAM[3], Ribeiro KA[2], Chaves HV[3,4], Gadelha TS[5], Gadelha CAA[5], Lacerda JTJG[5], Viana AFSC[6], Vasconcelos AS[6], Sousa FCF[6], Pinto VP[1,2,4], Cristino Filho G[1], Aguiar LMV[1,2,7], Bezerra MM[1,2,4], Silva AAR[3,2] [1]UFC-Sobral – Medicine, [2]UFC – Biotechnology, [3]UFC-Sobral – Dentistry, [4]UFC – Health Sciences, [5]UFPB – Molecular Biology, [6]UFC – Pharmacology, [7]UFC

Introduction: Our group has recently demonstrated that lectin from *Mucuna pruriens* (*Mp*) seeds prevents ethanol-induced gastric ulcers in mice. Gastric ulcer is an oxidative state where acid and pepsin contribute to the development of the condition, and therefore antioxidant enzyme activities and of glutathione content (GSH) as well as TBARS concentration explain the gastric oxidative–antioxidant imbalance. **Methods**: Fasted mice treated with ethanol 99.9% (0.2 ml/animal, p.o.) were pre-treated with *Mp* (0.1 mg/kg; i.v.) or saline (0.3 ml/30g; i.v.). Animals were sacrificed 30 min after ethanol and lesions were measured using a planimetry program (ImageJÒ). The effect of *Mp* was checked on iron-induced lipid peroxidation (shown by suppress TBARS concentration) and oxidation of GSH. Further, fasted mice received *Mp* (5 or 10 mg/kg; i.v.) and were observed over 48 h for toxicity signs and mortality. Blood samples were collected to evaluate biochemical parameters glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT) and urea were evaluated. The hemoglobin concentration and leukocytes counting were also evaluated. **Results and discussion:** *Mp* reduced the percent area (%) of gastric lesions. *Mp* increased GSH (1147 ± 107.2 GSH tissue mcg/g), compared to ethanol-challenged group (883.7 ± 38.5). *Mp* reduced lipid peroxidation (134.8 ± 20.1 TBARS tissue mcg/g), compared to ethanol-challenged group (318.4 ± 46.5). Biochemical parameters (GOT, GPT and urea) was also unchanged, as well as, hemoglobin blood concentration and leukocytes counting. *Mp* has beneficial antioxidant properties against ethanol-induced gastric damage in mice. Additionally, the lectin did not show visible signs of toxicity in the animals tested. Therefore, it seems that a combination regimen including both antioxidant and antisecretory drugs may be beneficial in prevention of ethanol-mediated gastric mucosal damages. **Funding Sources:** FUNCAP, CNPq, Capes, and INCT-IBISAB. License number of the Animal Ethics Committees of Federal University Pernambuco: 230760009313/2003-04.

09.104

Effect of polysaccharides plant extracts against epimastigote forms of *Trypanossoma cruzi*. Souza ROS[1], Sousa PL[1], Menezes RRPPB[1], Pereira MG[2], Martins AMC[1] [1]FF-UFC – Cultivo Celular, [2]ISCB-UECE

Introduction: Chagas disease is a major health problem in Latin America (McKERROW, *Mem. Inst. Oswaldo Cruz*, v.104, p.263, 2009). Unfortunately, the drugs used so far to treat this disease have not been successful. In Brazil, only benznidazole (BZ) have been on the market, and this drug present low efficacy and many side effects. In this way, the development of new safe and effective therapeutic agents is urgently needed, and medicinal species are potentially sources of bioactive substances (TASDEMIR, *Antimicrob. Agents Chemother*, v.50, p.1352, 2006; SALEM, *Curr. Med. Chem*, v.13, p.2571, 2006). The aim of this study was evaluate the *in vitro* effects of polysaccharides extracts from teguments of *Azadirachta indica* (AiPL), *Geoffroea spinosa* (GsPL) and *Ximenia americana* (XaPL) barks against epimastigote forms of *T. cruzi*. **Methods:** Teguments and barks were collected in Quixadá, Ceará, Brazil. The dry powder (5 g) of teguments and barks were depigmented with methanol and extracted in 0.1 M NaOH to obtain the Total Polysaccharides (TPL) (YOON, *Thromb Research*, v.106, p.51, 2002). The trypanocidal effects polysaccharides extracts were evaluated on epimastigote forms of *T. cruzi* (CAMARGO, *Rev. Inst. Med. Trop*, v.6, p.93, 1964). Seven-day-old cultures of epimastigotes, cultured in LIT (Liver Infusion Tryptose) medium, were collected, centrifuged (3.000 rpm for 7 minutes) and quantified. The cells (1×10^6 cell/mL) were incubated with the extracts in several concentrations (0.37, 0.75, 1.5, 3.0 and 6.0 mg/mL) in a 96-well plate. Phosphate-Buffered Saline (PBS, pH 7.4) was used as negative control and BZ used as positive control. After 24, 48 and 72 hours of incubation at 28°C, aliquots were collected to quantification of cell density in Neubauer Chamber. Viability percentage data were analyzed by non-linear regression to determine IC_{50} (concentration able to inhibit 50% of cell growth). The results were expressed as mean ± SEM and analyzed by ANOVA and Dunnett post-test. Significance was considered for $p<0.05$. **Results:** All compounds were active against epimastigote forms at all times. The positive control BZ exhibited IC_{50}= 19,18 ug/mL after 48 hours; and IC_{50}= 8,23 ug/mL after 72 hours. AiPL exhibited IC_{50}=0.86 mg/mL after 24 hours; IC_{50}=1.09 mg/mL after 48 hours; and IC_{50}=1.44 mg/mL after 72 hours. GsPL also presented inhibitory effect, with IC_{50}=0.77 mg/mL after 24 hours; IC_{50}=4.76 mg/mL after 48 hours; and IC_{50}=2.97 mg/mL after 72 hours. Finally, the values of IC_{50} observed for XaPL over *T. cruzi* were 4.77mg/mL after 24 hours; 2.71 mg/mL after 48 hours; and 1.44 mg/mL after 72 hours. **Discussion:** The polysaccharide extracts from *A. indica*, *G. spinosa* and *X. americana* presented accentuated trypanocidal effect over epimastigote forms. TPL from *A. indica* were the most active against this parasite, reaching high levels of growth inhibition (over 80%) at 6.0 mg/mL. These results indicate that these plants are potential sources of bioactive substances to development of new trypanocidal drugs. **Conclusion:** Polysaccharide extracts from *Azadirachta indica*, *Geoffroea spinosa* and *Ximenia americana* present high inhibitory effect against epimastigote forms of *Trypanosoma cruzi*. Financial agencies: Capes

09.105

Wound repair, inflammatory and oxidative stress aspects, promoted by proteolytic fraction from *Vasconcellea cundinamarcensis* latex on UVB-induced model. Freitas KM, Teixeira LCR, Lage FDO, Lopes MTP UFMG – Farmacologia

Introduction: UV radiation-induced skin damages may result in acceleration of skin aging, pre-cancerous and cancerous skin lesions. It involves an imbalance of the endogenous antioxidant system that leads to the increase of free radical levels and inflammation (CASAGRANDE *et al*,. *Eur. J. Photochemistry and Photobiology*, 84; 21, 2006). The P1G10 fraction, from *Vasconcellea cundinamarcensis* latex, rich in cysteine proteases, has angiogenic, mitogenic, antiinflammatory activities and wound healing on different cutaneous lesion models. In the present study, we investigated the possible antioxidant and antiinflammatory effects of topical formulations containing P1G10 against UVB-induce damages in mice. **Methods:** Hairless mice (n=49) were irradiated by UVB light (dose 2.4J/cm^2 – Coler-Parmer ®, 15W, maximum length of 312 nm) on dorsal area (protocol CETEA 174/2010). P1G10 (0.1, 0.5, 1.0% or vehicle – Natrosol®) was administered on the damage area, immediately after irradiation. The mice were killed 24 hs after the UVB exposure and the full thickness of the dorsal skins were removed for further analysis. The levels of inflammatory infiltrate (macrophages, N-acetyl-β-glucosaminidase – NAG and neutrophils, myeloperoxidade – MPO), superoxide dismutase (SOD) activity and lipid peroxidation (LPO) levels were determined by kinetic-colorimetric assay. The catalase activity was measured spectrophotometrically and GSH (Glutathione) determined by fluorescence assay. In addition, the production of cytokines was measured by ELISA and metalloproteinases by enzymography. **Results and discussion:** The treatment with 0.1% or 1.0% of P1G10 increased MPO levels (0.002 ± 0.000 *vs* 0.002 ± 0.000 D.O/mg of skin- control, $p<0.05$, ANOVA, Newman-Keuls *post-test*) and did not alter the NAG levels (0.016 ± 0.001 *vs* 0.011 ± 0.001 D.O/mg of skin – control, $p<0.05$, ANOVA, Bonferroni *post-test*). At the dose 0.1% was observed a significantly increase of SOD activity (0.011 ± 0.004 *vs* 0.060 ± 0.0007 µg/mg of skin – control, $p<0.01$, ANOVA, Newman-Keuls *post-test*). However, LPO levels were did not change when compared to control. The catalase activity was significantly enhanced in the presence of P1G10 0.5% (0.019 ± 0.003 *vs* 0.008 ± 0.001 ΔABS/µg of protein/min – control, $p<0.05$, ANOVA, Newman-Keuls *post-test*). Furthermore, 0.1% of P1G10 inhibited the depletion of GSH (0.184 ± 0,034 *vs* 0.118 ± 0,025 mM of skin-control, $p<0.01$, ANOVA, Newman-Keuls *post-test*). All doses of P1G10 prevented the decrease of VEGF (Vascular Endothelial Growth Factor) levels, however at doses 0.1 or 0.5% showed significantly decrease of TNF-α levels (0.915 ± 0.2393 *vs* 1.411 ± 0.262 ρg/mg – control, $p<0.05$, ANOVA, Newman-Keuls *post-test*) and increase of IL-10 levels at 1% (3.45 ± 0.973 *vs* 2.07 ± 0.322 ρg/mg, $p<0.05$). The fraction was effective to inhibit secretion/activity of metalloproteinases (61.95 ± 15.17 *vs* 108.64 ± 26.75 %, $p<0.01$, ANOVA, Newman-Keuls *post-test*). For the above, we suggest that the fraction prevents the lesion chronicity by modulating the levels of pro and anti-inflammatory cytokines and activates the enzymatic antioxidant system. Thus, we show the effectiveness of topical formulation containing P1G10 on UVB-induced to tissue repair. **Financial support:** CNPq, FAPEMIG and Capes.

09.106

Cellular mechanisms in antimetastatic action of protease from *V. cundinamarcensis* latex on murine melanoma cells. Dittz D[1], Tatsumi G[1], Salas CE[2], Lopes MTP[1] – [1]UFMG – Farmacologia, [2]UFMG – Bioquímica

Introduction: Our research group has demonstrated that proteolytic fractions from *Vasconcellea cundinamarcensis'* latex (P1G10 and CMS2) have antitumor/antimetastatic activity on murine melanoma by reduction of cell adhesion and migration, and induction of apoptosis. From CMS2, five proteases (CMS2MS1-5) are isolated when submitted to chromatography on MonoS Sepharose resin (FPLC). We aim to identify a possible protease and the cellular mechanisms that contribute to the effects described previously. **Methods**: For determination of IC-50, B16F10 cells were exposed to proteases (0.1 to 500 ug/mL) for 72 hs. The cellular viability was assessed by resazurin (0.1 mg/mL) metabolite, quantified at 570 and 600 nm. The effect on cell adhesion was determined by exposing B16F10 to proteases (1, 10, 30 or 50 ug/mL) for 2-24 hs. Then, cells were washed with PBS and that remaining quantified considering their capacity to metabolize resazurin. In B16F10 cells, exposed to CMS2MS3 (10 ug/mL) by 2-24 hs were evaluated the α5β1 integrin levels (flow cytometry), number of focal adhesion by vinculin measurement (immunohistochemistry), sub-diploid content (*flow cytometry*), activation of caspase-3, caspase-9 and BAX (Western Blot) and calcium transient (confocal microscopy). The cell death was analyzed in a time-lapse imaging experiment, in a Nikon BioStation microscopy. B16F10 cells (pre-incubated or not with a pan-caspase inhibitor, ZVAD-FMK) were treated with CMS2MS3 (10 ug/mL). Then, cells were stained with Hoechst 33242 (0.5 mg/mL) and monitored for 24 hs. **Results and discussion:** Among the five analyzed proteases, CMS2MS3 showed the lowest IC-50 (7.81 ug/mL) compared to others (34.5 to 303.5 ug/mL). All proteases promoted loss of adhesion in B16F10, especially CMS2MS3 at 10 – 50 ug/mL. The level of α5β1 integrin was reduced by CMS2MS3 10 ug/mL in all analyzed time showing that this integrin contribute, at least partially, with the loss of adhesion. The number of vinculin/cell reduced 65 – 85% from 2hs exposure to CMS2MS3, showing that focal adhesion may be linked to this integrin. After 2 – 24 hs of exposure to CMS2MS3, sub-diploid DNA increased only at a concentration of 50 ug/mL. Although DNA fragmentation was only observed at 50 ug/mL of CMS2MS3, it is possible that the cell death events start at lower concentrations. When exposed to 10 ug/mL of CMS2MS3, a reduction in intracellular levels of caspases 3 and 9 was observed from 2 hs of exposure, and an increase in BAX levels after 24 hs, showing that the cell death occurs by apoptosis. A rapid increase in nuclear calcium was observed after when B16F10 were exposure to CMS2MS3 10 ug/mL for 10 s and this may be linked to the activation of endonucleases, leading to DNA fragmentation. In lapse imaging experiment we noticed that CMS2MS3 promotes cell death mediated by loss of adhesion, since cells pre-incubated with ZVAD loses the adhesion without changes in cell viability. Thus, among the five proteases from CMS2, CMS2MS3 showed the best cytotoxic effect and ability to reduce the adhesion, mediated by α5β1 integrin in B16F10 cells. The cell death, by apoptosis, occurs after loss of adhesion, an event known as Anoikis. **Financial Support**: CNPq, FAPEMIG and Capes.

09.107

Pharmacological analysis of the hemodynamic response to *Bothrops atrox* snake venom in anesthetized rats. Rodrigues MAP, Dias L, Stroka A, Rennó AL, Inoue BR, Panunto PC, Hyslop S Unicamp – Farmacologia

Introduction: We have previously shown that *B. atrox* venom causes hypotension in anesthetized rats. In this work, we investigated the possible mediators involved in this venom-induced hypotension. **Methods and Results:** Male Wistar rats (300-400 g) were anesthetized with isoflurane (2% in 98% air); the left carotid artery was cannulated for blood pressure measurement and a femoral vein was cannulated for venom injection (0.4 mg/kg) and arterial blood sampling. Heart rate and ECG were monitored electronically and respiratory rate was determined manually. Changes in blood pressure were monitored for 120 min after which the rats were killed with an overdose of anesthetic. The experiments were approved by an institutional Committee for Ethics in Animal Use (CEUA/Unicamp, protocol no. 2181-1). The results (mean ± SEM) were analyzed using ANOVA followed by the Tukey-Kramer test, with $p<0.05$ indicating significance. Venom caused immediate hypotension that was maximal after 5 min (mean arterial blood pressure fell from 110 ± 6 to 56 ± 3 mmHg; n=8; $p<0.05$) but gradually returned to baseline over 20 min, decreasing again at 120 min. There were no significant changes in heart rate, respiratory rate or ECG. All rats injected with venom survived until the end of the experiment. Pretreatment with atropine (non-selective muscarinic receptor antagonist; 2 mg/kg, i.v., 20 min before venom (BV); n=6), sildenafil (inhibitor of phosphodiesterase 5; 4 mg/kg, i.v., 20 min BV; n=6) or antibody to tumor necrosis factor (TNF)-a (Infliximab; 1 mg/kg, i.v., 15 min BV; n=6) did not attenuate the venom-induced hypotension. In contrast, pretreatment with the nitric oxide synthase (NOS) inhibitors L-N^G-monomethyl-L-arginine (L-NMMA; 30 mg/kg, i.v., 20 min BV; n=6) or N^w-nitro-L-arginine methyl ester (L-NAME; 20 mg/kg, i.v., 20 min BV; n=6) potentiated the hypotension and lethality of the venom, with death occurring in 6 ± 0.3 min and 10 ± 4 min after treatment with L-NAME and L-NMMA, respectively ($p<0.05$ compared to rats injected with venom alone). Pretreatment with HOE-140 (bradykinin B_2 receptor antagonist; 0.6 mg/kg, i.v., 20 min BV; n=3) significantly reduced the venom-induced hypotension at 1, 5, 10 and 20 min; at 5 min (corresponding to the peak hypotensive response) the decrease in mean arterial blood pressure was 30% (from 125 ± 3 to 87 ± 12 mmHg; n=3) compared to a decrease of ~50% with venom alone ($p<0.05$), whereas pretreatment with indomethacin (non-selective cyclooxygenase inhibitor; 5 mg/kg, i.v., 20 min BV; n=6) caused only slight attenuation of the hypotension at 1 min post-venom. **Conclusion**: In rats, *B. atrox* venom causes hypotension primarily by affecting the vasculature, with little direct cardiac involvement (no changes in heart rate or ECG). The finding that L-NAME and L-NMMA potentiated the hypotension and hastened death suggests that NO has a protective role in venom-induced hypotension. Bradykinin is also involved in the venom-mediated hypotension, whereas arachidonic acid metabolites such as prostaglandins have a minimal role. **Financial support:** Capes, CNPq, Fapesp.

09.108

Calcium mobilization induced by *Bothriopsis bilineata smaragdina* venom (Forest viper) and its toxin Bbil-TX (Asp49 PLA_2) on neuroblastoma cell line and mouse triangularis sterni nerve-muscle preparation. Floriano RS[1], Carregari VC[2], Marangoni S[2], Hyslop S[3], Rowan EG[4], Rodrigues-Simioni L[3] – [1]UNIP-Sorocaba – Health Sciences / FCM-Unicamp, – Pharmacology, [2]IB-Unicamp – Department of Biochemistry, [3]FCM-Unicamp – Pharmacology, [4]SIBS-University of Strathclyde

In this study, we used calcium fluorescence to extend our investigation of the mechanism of Bbil-TX action in mouse neuromuscular preparations *in vitro* and cell culture in order to improve our understanding of how this toxin works. To examine whether *B. b. smaragdina* venom and Bbil-TX were able to increase intracellular Ca^{2+}, calcium mobilization was monitored in neuroblastoma cell line and in muscle fibers from mouse triangularis sterni nerve-muscle preparations (TSn-m) using Ca^{2+} sensitive fluorescent indicators (Fluo-3 AM and Fluo-4 AM, respectively). There was no change in calcium fluorescence amplitude (Ca^{2+} -FA) following exposure to either venom (3 µg/ml) or Bbil-TX (210 nM) when cells were incubated in Ca^{2+} -free bath solution [FA: 33 ± 8.4% (control), 34 ± 6.6% (venom) and 26 ± 4.6% (toxin) above basal values after 10 min incubation; n = 15-20 cells; $p > 0.05$]. However, both venom and toxin caused a progressive increase in Ca^{2+} -FA in cells maintained in low Ca^{2+} bath solution [FA: 30 ± 6.5% (control), 76 ± 5.3% (venom) and 48 ± 7.5% (toxin) above basal values after 10 min incubation; n = 15-20 cells; $p<0.05$]. In cells maintained in normal Ca^{2+}, the increase in Ca^{2+} -FA was accompanied by marked, irregular and frequent calcium transients. After treatment with *p*-bromophenacyl bromide (PLA_2-inhibitor), the venom- or toxin-induced calcium transients were less frequent and showed lower amplitude compared to those seen with untreated venom or toxin. Bbil-TX (210 nM) did not alter the Ca^{2+} -FA of muscle fibers in TSn-m preparations [FA: 4.8 ± 0.6% (control) and 2.5 ± 0.3% (toxin) below basal values after 10 min incubation; n = 3; $p > 0.05$]. In contrast, *B. b. smaragdina* venom (10 µg/ml) caused an immediate increase in Ca^{2+} -FA in the muscle fibers followed by frequent oscillations in fluorescence and an accompanying transient muscle contracture. In Ca^{2+} -free experiments the initial increase in intracellular calcium was still present but reduced in magnitude. *B. b. smaragdina* venom causes presynaptic neuromuscular blockade modulated by its major toxin Bbil-TX[1-3] which reduces the perineural waveform associated with outward K^+ currents measured in TSn-m preparations. The blockade of nerve terminal potassium channels will prolong the action potential resulting in delayed membrane repolarization. A prolonged action potential would be predicted to prolong the opening of voltage-dependent calcium channels and consequently increase neurotransmitter release[4,5]. However, the evidence that β-neurotoxins operate in this manner is still controversial since these toxins do not act directly on cloned potassium channels[5]. We hypothesize that the ability of *B. b. smaragdina* venom and Bbil-TX to increase synaptic calcium concentrations could account for the increase in quantal content via a PLA_2-dependent mechanism and a fusogenic mechanism of action, as proposed for other PLA_2 neurotoxins[6], could be involved here. **Financial** Agencies: CNPq (Brazil) and SIBS (UK). **References:** 1. Rodrigues-Simioni, L. *et al. Toxicon* 58: 140, 2011. 2. Floriano, R.S. *et al. Toxicon* 69: 191, 2013. 3. Carregari, V.C. *et al.* BioMed. Res. Int. ID 612649. 4. Penner, R. and Dreyer, F. Pflügers Arch. 406: 190, 1986. 5. Fathi, B. *et al. Toxicon* 39:1871, 2001. 6. Tedesco, E. *et al. Toxicon* 54: 138, 2009.

09.109

Effect of *Euterpe oleracea* Mart. (Açaí) extract and exercise training on the changes caused by Type 2 Diabetes. Bem GF, Costa CA, Santos IB, Oliveira RB, Cordeiro VSC, Carvalho LCRM, Souza MAV, Ribeiro JH, Okinga A, Rocha APM, Ognibene DT, Resende AC, Moura RS UERJ – Farmacologia e Psicobiologia

Introduction: Type 2 diabetes (DM2) is characterized by metabolic defects, such as, insulin resistance. Polyphenols possess anti-inflammatory, antioxidant and vasodilator activities. Thus, the aim of this study was to evaluate the effect of treatment with açaí stone extract (ASE), rich in polyphenols, and exercise training, on metabolic disorders and hepatic morphological changes observed in an experimental model of DM2. **Methods:** The experiments were approved by the Ethics Committee of Animal Experiments Rio de Janeiro State University (protocol: CEUA/058/2012). Two groups of Wistar rats were fed experimental diets: control (10% fat) and high fat (HF) diet (55% fat) for 5 weeks. In the third week, HF group received an intraperitoneal injection of streptozotocin (35 mg kg-1), that increased blood glucose levels to more than 250/100 ml. The animals received ASE (200 mg/kg^{-1}) by intragastric gavage and training on a treadmill for a period of four weeks, and were divided into eight groups: sedentary control and training (SC and TC) sedentary control and training treated with ASE (SCA and TCA), sedentary diabetic and training (SD and TD) and sedentary diabetic and training treated with ASE (SDA and TDA). Glycemia was measured with a glucometer. Serum cholesterol, triglycerides, HDL, LDL, VLDL and insulin levels were determined by kit. Insulin resistance and beta cells function were calculated by HOMA index and HOMA β, respectively. Oxidative damage, nitrite levels and antioxidant enzymatic activity were measured in liver homogenates. Glycogen, triglycerides and cholesterol hepatic levels were determinated by kit. The expression of ABCG8, AMPK, pAMPK and pNFkB were determined by western blotting. Hepatic steatosis was examined histologically. **Results:** The increased glucose levels in diabetic animals were reduced by treatment with ASE alone or associated with exercise. Insulin and HOMA index were increased and HOMA β was decreased in SD group. These results were reversed by treatment with ASE and exercise training. SD group showed increased serum levels of cholesterol, triglycerides and VLDL, and decreased levels of HDL, which were reversed by ASE and exercise. LDL levels were not different among groups. Malondialdehyde and protein carbonyl levels were increased in SD group and reduced by treatment with ASE and exercise. Antioxidant enzyme activities and nitrite levels were decreased in SD group and recovered by ASE and exercise. Hepatic Cholesterol and triglycerides levels were increased and glycogen content was reduced in SD animals. These results were reversed by treatment with ASE and exercise. In SD group, the reduced expression of ABCG8 and pAMPK, and increased expression of AMPK and pNFkB were reversed by treatment with ASE and exercise. SD group showed hepatic steatosis which was reduced by treatment with ASE associated with exercise training. **Discussion:** this study demonstrates the importance of a balanced diet and a less sedentary life style to minimize the metabolic changes associated with DM2, demonstrating for the first time the beneficial effect of oral administration of ASE in the treatment of diabetes. **Financial Support:** CNPq and Faperj.

09.110

Hypotensive effect and vascular reactivity induced by (-)-borneol/β-ciclodextrin in L-NAME hypertensive rats. Silva-Filho JC[1], Souza FM[1], Azevedo PSS[1], Santos MEP[1], Mendes MB[1], Arcanjo DDR[1], Rocha MS[2], Lima SG[2], Oliveira AP[1], Santos MRV[3] – [1]NPPM-UFPI, [2]UFPI – Química, [3]UFS – Fisiologia

Introduction: The monoterpene (-)-borneol ($C_{10}H_{18}O$) is present in essential oils of various medicinal plants. In previous studies, (-)-borneol showed anti-thrombotic activity and antiplatelet (Li, Y.H., *Am J Chin Med*. 36: 719, 2008), antioxidant (KORDALI, S., J *Sci Food Agric*., 53: 9452, 2005) and vasorelaxant properties (SILVA-FILHO, J.C., Basic *Clin Pharmacol*. *Toxicol* 37: 2, 2011). However, low solubility in water reduces their technological application. **Aim:** This study aimed to evaluate the effects the inclusion complex between (-)-borneol and β-cyclodextrin on the cardiovascular system of hypertensive L-NAME rats. **Methods**: All experimental protocols and procedures were approved by UFPI Ethics Committee on Animal Experimentation (CEEA/UFPI nº 008/12). For *in vivo* studies, hypertensive L-NAME Wistar rats (250 – 300g, n=5) were anesthetized with sodium pentobarbital (45 mg/kg, i.p.) for implantation of catheters made of polyethylene (PE-10) in the abdominal aorta. The values of blood pressure were obtained by a pressure transducer coupled to an amplifier (AVS projects, SP, Brazil). The results were expressed as mean ± SEM and considered significant when $p<0.05$. For *in vitro* studies, mesenteric artery rings (1-3 mm) without functional endothelium were kept in Tyrode at 37˚C, aerated with (95% O_2 and 5% CO_2), suspended by cotton threads and coupled to force transducers linked to a data acquisition system (AVS Projects, SP, Brazil) for registration of isometric tension. After 1 h of stabilization (basal tension of 0.75 g), cumulative concentration-response curves for Phe (10^{-9} to 10^{-5} M) were obtained before and after pre-incubation separately with (-)-borneol (10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} M) for 30 min. **Results and discussion:** The oral administration of (-)-borneol/β-ciclodextrin (50 and 100 mg/kg) significantly reduces the main arterial blood pressure of the rats for a period 300 minutes. *in vitro* experiments in which vascular reactivity was checked has been found that after (-)-borneol pretreatment (10^{-2}, 10^{-3}, 10^{-4} and 10^{-5} M), the phenylephrine-induced vasoconstriction was markedly inhibited in endothelium-denuded rings by a concentration-dependent manner, and the maximal response was significantly reduced. Hence, (-)-borneol/β-ciclodextrin induces its hypotensive effect probably due to a decrease in vascular resistance.

09.111

Local and systemic effects of BdipTX-I, a new phospholipase A_2 Lys-49 isolated from *Bothrops diporus* snake venom. Teixeira LF[1], Carvalho LH[1], Castro OB[1], Bastos JSF[1], Néry NM[1], Oliveira GA[2], Kayano AM[2], Calderon LA[2], Soares AM[2], Zuliani JP[1,2] [1]Fiocruz-RO – Imunologia Celular, [2]CEBio-UNIR – Medicina / Fiocruz-RO

Introduction: Phospholipases A_2 (PLA_2) are enzymes that catalyze the hydrolysis of the acyl ester in the *Sn*-2 position of membrane phospholipids producing free fatty acids and lysophospholipids. This study aimed to isolate basic phospholipase A_2 (PLA_2) from *Bothrops diporus* venom (VBd) and to evaluate and compare the edema and myotoxic activities, as well as the systemic effects caused by both the PLA_2 isolated and VBd in *Swiss* mice. **Methods:** 50 mg of the VBd was submit to an ion exchange and reverse phase chromatographic steps. The fractions obtained were analyzed by SDS-PAGE on 12,5% polyacrylamide gel and by indirect hemolysis and activity on the 4-nitro-(3-octanoyloxy) benzoic acid (4N3OAB) for enzymatic activity. In this process, two PLA_2 were obtained, an Asp-49 (BdipTX-II) and a Lys-49 (BdipTX-I). BdipTX-I and VBd were then evaluated for biological activity: local (edema and myotoxicity) and systemic effects on the liver and kidney functions. In the tests male *Swiss* mice were used, the project was approved by the Ethics Committee on the Use of Animals (number 2012/08). For assessment of edema activity the doses 5 and 20 µg / paw of BdipTX-I and VBd were utilized. To evaluate the systemic effects, the mices have received intramuscular injection of 50 µg of BdipTX-I or VBd. After three hours, blood samples were collect to obtain plasma, which was subject to laboratory dosage of specific markers: AST, ALT, GGT and FAL to assess liver function; serum and urine levels of urea and creatinine, dosage levels of urinary protein and calcium to assess kidney damage. **Results and discussion:** The results obtained showed that the edema activity was significant at all doses tested for BdipTX-I and for both VBd, observing an increase above 30% of the volume of tested paws from 0,5 hour. Regarding myotoxicity, BdipTX-I and VBd caused an increase in serum creatine kinase (CK) and lactate dehydrogenase (LDH), and VBd caused an increase of 284% and BdipTX-I 127% in CK-total activity. While the activity of LDH increased by 165,9% induced VBd and 348,3% induced BdipTX-I. There were changes in serum levels of AST (VBd > 216,6% and BdipTX-I > 304%). VBd caused elevation in serum levels (104%) and urine (46%) of urea. While the levels of creatinine and urine protein were altered by both VBd (creatinine > 262,5%; protein > 101,8%) and by BdipTX-I (creatinine > 143,7%, protein > 80,3%) . Cardiac and hepatic lesions were not confirmed, although there has been elevated levels of LDH and AST. Therefore, BdipTX-I and VBd were able to induce renal changes in experimental models tested, resulting in proteinuria (induced by BdipTX-I and VBd) and uremia (effect present in VBd, but not BdipTX-I). Thus, it is established that the systemic actions of the venom and protein occur differently. This fact is probably related to other components present in VBd. **Financial support:** Fiocruz, FINEP and CNPq.

09.112

Protective effect of *p*-cymene against NSAIDs and stress-induced gastric ulcers in mice.

Paulo LL[1], Formiga RO[2], Lima GRM[1], Sales IRP[1], Castello Branco MVS[1], Batista LM[1] – [1]UFPB – Produtos Naturais e Sintéticos Bioativos, [2]CCS-UFPB

Introduction: *p*-cymene (*p*-isopropyltoluene) is a naturally-occurring aromatic organic compound. It is classified as a hydrocarbon related to a monoterpene and it is a precursor of carvacrol, also being one of the main constituents of the essential oil from *Protium* species, with more than 80% of these species found in the Amazon. *p*-cymene is present in volatile oils from over 100 plants and occurs naturally in more than 200 types of food. The objective of this study was to evaluate the gastroprotective activity of *p*-cymene in two models of induced acute ulcer: stress (immobilization and cold) and non-steroidal anti-inflammatory drugs (NSAIDs). **Methods:** The animals used were male mice *Mus musculus*, Swiss strain (n = 5-7) weighting 25-35 g, fasted for 24 hours and pre-treated with negative control (Tween 80 solution – 5%), positive control (carbenoxolone 100 mg/kg) and *p*-cymene (25, 50, 100 and 200 mg/kg). Morever, an induction of ulcers by immobilization and cold stress (Levine R, Munksg., 92, 97, 1971 – with modifications), followed by administration of piroxicam (30 mg/kg) subcutaneously (Puscas I, Arzneim., 47, 568, 1997 – with modifications) was performed. The results were analyzed using ANOVA followed by Dunmett's test; experimental protocols were approved by the Ethics Committee on Animal Use (CEUA/CBIOTEC/UFPB) with number 0110/13. **Results and discussion:** In NSAIDs-induced gastric ulcer approach the results show a reduction in the ulcer index (UI) at all doses evaluated of *p*-cymene (25, 50, 100 and 200 mg/kg) at 57.29 ± 11.71, 32.00 ± 6.59, 34.80 ± 16.71 and 28.67 ± 6.34 ($p<0.0001$), respectively, when compared to the negative control (154.5 ± 22.88). For the model of stress (immobilization and cold) the doses evaluated of *p*-cymene (25, 50, 100 and 200 mg/kg), the UI decreased by 59.83 ± 10.53, 57.83 ± 15.68, 53.71 ± 15.57 and 50.00 ± 13.81 ($p<0.0001$), respectively, when compared with the negative control group (91.60 ± 24.20). By analysis of the data collected, it can be inferred that gastroprotective activity of *p*-cymene in two models of acute ulcer induction was properly evaluated and further research is necessary in order to elucidate the mechanisms of this protective activity of gastric mucosa. **Acknowledgments:** CNPq/Capes/PgPNSB /UFPB.

09.113

Anthelmintic potential of a natural compound against *Schistosoma mansoni*. Coelho ML, Costa LM, Moraes J, Freitas RM. UFPI

Introduction: Schistosomiasis is a neglected parasitic disease of great social impact. Recent studies by our research group with natural products have shown promising activity against schistosomiasis. The objective of this study was to explore the use of STRGL, a phenylpropene, and its potential use in formulation of pharmaceutical products for prevention and/or treatment of parasitic diseases such as schistosomiasis, by evaluating the effects *in vitro* of STRGL against *Schistosoma mansoni*. **Methods**: Study *in vitro* of STRGL effect on viability and on motor activity of *S. Mansoni* was conducted. The cycle of parasite was maintained in hamsters and *Biomphalaria glabrata* snails. Antischistosomal tests were carried out with incubation of the worms with STRGL (31.25, 62.5, 125, 250 and 500 µg/mL) and monitored from 1 to 5 days by microscopy and stereomicroscopy. Control wells with couples of *S. mansoni* in 0.85% saline, 1% DMSO and growth medium were also monitored. To assess the toxicity of compounds on *S. mansoni*, muscle activity and mortality rate were observed. The mortality of the worms were detected by loss of movement for 2 min. **Results and discussion:** STRGL reduced motility and caused the death of all adult nematodes directly dependent on the concentration and time of incubation. The lethal effect of the natural compound at every adults forms after 24 hours was the concentration 250 µg/ml and 500 µg/ml. Overall, there was no difference in mortality rate seen between males and females helminths. Incubation of adult forms of *S. Mansoni* with STRGL kept the male and female parasites separate, which prevented the mating process and oviposition. Thus, the studied substance has a potential anthelmintic activity, especially against *S. mansoni*, being interesting to pharmaceutical industry. **Financial Support**: FAPEPI – Fundação de Amparo à pesquisa do estado do Piauí.

09.114

Potential anti-allergic action of three medicinal plants used in Amazon region: Effects on histamine release. de Oliveira DM[1,2], Di Stasi LC[3], Paracampo NENP[4], Lameira OA[5], Crespo-Lopez ME[1] [1]ICB-UFPA – Farmacologia Molecular, [2]ISPA-UFRA – Farmacologia, [3]IBB-Unesp-Botucatu – Fitoterápicos, [4]EMBRAPA – Agroindústria, [5]EMBRAPA – Biotecnologia

Introduction: In the Amazon region, *Morinda citrifolia* Linn (noni), *Mansoa alliacea* (Lam.) A.H. Gentry (cipó ďalho) and *Luehea speciosa* Willd (açoita-cavalo) are used in traditional medicine to treat diseases of inflammatory or allergic origin. Mast cells are key cells of allergic responses and they release proinflammatory mediators, especially histamine, with spasmogenic and vasoactive actions. **Aim:** To analyze effects on histamine release of mast cells exposed to three medicinal plants used in Amazon region. **Methods:** Plants were dried at 45°C for 120h and posteriorly submitted to extraction (1h at 80ºC) with 70% ethanol. Final total volume was concentrated in rotary evaporator to eliminate ethanol and diluted in 0.2% dimethylsulfoxide. Mast cells of rat peritoneum were *in vitro* exposed to 0–100 µg/mL of each extract for 15 minutes and histamine release was measured by an automatic fluorometric method (Shore, J.Pharmacol.Exp. Ther., 127;182; 1959). Also, effects of the extracts on histamine release induced with compound 48/80 (0,5 µg/mL) and A23187 ionophore (1,6 µg/mL) were assayed. ANOVA followed by Tukey test were used to analyze data. Additionally, a qualitative phytochemical screening (Gonzalez, Phytomedicine., 9,125, 2002) of each extract was performed. **Results and discussion:** No extract interfered in the spontaneous release of histamine. Lower concentrations (3 and 0,3 µg/mL) of *M. citrifolia* L. fruits significantly inhibited histamine release (about 95% and 96%, respectively) induced by compound 48/80 and A23187 ionophore. Three and ten µg/mL of extract of *M.citrifolia* L. leaves also inhibited histamine release induced by compound 48/80 in 81% and 83 %, respectively. Extract of *M.alliacea* leaves (100 µg/mL) was able to inhibit about 89% of histamine release induced by compound 48/80. No changes on histamine release induced by ionophore were detected after incubation with *L. speciosa* W. However, when histamine release was stimulated by compound 48/80, 10 and 100 µg/mL of this extract inhibited about 97% of histamine release. Catechins, steroids, alkaloids and coumarin-related compounds in *M. alliacea*; steroids, triterpenoids, tannins in *M. citrifolia* L. and catechins, flavonoids, steroids and tannins in *L. speciosa* W. were identified as possible compounds responsible for the effects. **Conclusion:** The present study demonstrates for the first time the inhibitory action of three species of medicinal plants on histamine release. Flavonoids, tannins, catechins, terpenoids and coumarin are highlighted as possible compounds responsible for these actions. **Animal Ethics Committees** n° 42/04/unesp. **Acknowledgments:** CNPq, Fundação Amazônia Paraense de Amparo à Pesquisa -FapespA.

09.115

Evaluation of antioxidant potential *in vitro* and quantification of phenolic compounds found in extracts of pequis pulp. Lima LAR, Ferreira JAN, Sousa ACP, Calou IBF, Portela JVF, Cerqueira GS, Lima A – CSHNB-UFPI – Nutrição

Introduction: The pulp of Pequi (Caryocar brasiliense Camb) presents like a good source of antioxidants such as vitamin C, carotenoids, vitamin E and phenolic compounds (Cardoso *et al.*, 2013).

The purpose of this research was to quantify the content of the phenolic compounds and establish the antioxidant potential by of extracts of the defatted bran pulp.

Materials and Methods: The pequi fruits was collected in the city of Exu-PE. The fruit was extracted and later submitted to drying. After dehydrating, the sample was triturated and bran homogenized. After processing, the pulp was degreased using ether. For the synthesis of the extracts was used 5g of defatted bran pulp pequis and 50 mL of solvent (distilled water, acetone and 95% ethanol), the mixture was homogenized for 1 minute and then subjected to agitation by ultrasound for 1 hour. The solutions were centrifuged for 5 minutes at 3000 rpm and the supernatant removed with the aid of the Pasteur pipette. The quantification of the phenolic compounds following the methodology described by Hills and Swain (1959) adapted to Sousa *et al* (2011). For determination of antioxidant activity using the $ABTS^{\cdot+}$ the method used was described by Re *et al.* (1999), adapted by Sousa *et al.* (2011). For the determination of the antioxidant activity using de DPPH method was describe for Sousa *et al* (2011). The results of antioxidant activity of pequis were expressed as TEAC in mM de Trolox.100 mL^{-1} pequi. Data were reported as mean ± standard deviation and evaluated by ANOVA One-Way ANOVA followed by Newman kells test was considered statistically significant * $P < 0.05$ and ** $P < 0.001$ by Graph Pad Prism 4.0 software. All experiments were performed in triplicate (n = 3). **Results and Discussion:** The determination of total phenolics of the extracts was measured by the Folin-Ciocalteau method, expressed as gallic acid equivalents (GAE) per gram of extract. The amount of total phenols from aqueous (EACB) was 9.040 ± 0.3700 mgGAE/g sample; the ethanol extract (EECB) was 4.260 ± 0.1756 mgGAE/g sample and acetone extract (EAceCB), 5.017 ± 0.4013 mgGAE/g sample. There was no significant difference between the EAceCB and EECB extracts, however, the EACB extract differed significantly from the other, which was the best result obtained. The findings of this study show total phenolic content higher than those reported by Lima (2007). The results for the antioxidant properties of the samples by the method of ABTS show that gave better success in EACB (9.74 mM Trolox / g) corroborates this point with the work of Lima (2008), however, presents greater than this value. For antioxidant activity by DPPH, the best effect was obtained in EECB extract (2.204 mM Trolox / g), figure 3, diverging the results found by Ribeiro (2011) which the aqueous extract showed higher potential compared to ether and ethanol extracts. **Financial agencies:** UFPI. **References:** 1. CARDOSO, LM *et al.* Fruits, vol. 68, p. 3, 2013. 2. LIMA, A. Rev. Bras. Fruticultura, v.29, p.695, 2007. 3. LIMA, A.Tese, p.186, 2008. 4. RE, R. Free Radic Biol Med, v.26, n.9-10, p.1231, 1999. 5. SOUSA, J. Sci. Food Agr., v. 14, n. 3, p. 1, 2011. 6. SWAIN,T. J. Sci. Food Agr. v.19, p. 63, 1959.

09.116

Study of anticholinesterasic activity of a mixture of CIS and trans nerolidol. Carvalho RBF[1], Almeida AAC[1], Mata AMOF[2], Freitas RM[1], Nunes LCC[1] – [1]UFPI, [2]UNINOVAFAPI

Introduction: The plants are used for medicinal purposes since the beginning of human civilization for many different finalities, from prevention to cure of several diseases (CARVALHO, R.B.F., *Quim. Nova*., v. 36, p. 1375, 2013). Among the compounds of natural origin, the sesquiterpenes have revealed a variety of biological activities such as insecticidal, anorectic, antineoplastic and antimicrobial. (NOGUEIRA NETO, J.D., *Journal of Basic and Applied Pharmaceutical Sciences*, v. 34, p. 125, 2013). Many of these actions are related to Alzheimer's disease, a neurodegenerative disease that affects memory and reasoning ability. A promising treatment for this disease is increasing the level of acetylcholine in the brain using inhibitors of acetylcholinesterase (AChE), justifying the research for new agents more effectives and with less cost to human health (CARVALHO, R.B.F., *Quim. Nova*., v. 36, p. 1375, 2013). **Methods:** The sample containing the mixture of geometric isomers cis- and trans- nerolidol was dissolved in methanol to a concentration of 1 mg/mL, then applied to CCD (DC Alufolien, Silicagel 60 F_{254} 0.2 mm, Merck) and eluted in chloroform: methanol 9:1. After the plate be developed, the inhibitory activity was detected using a developer based on the method of Ellman (ELLMAN, G.E., *Biochem. Pharmacol*., v. 7, p. 88, 1961) modified by Rhee and cols (RHEE, I.K., *J. Chromatogr. A*, v. 915, p. 217, 2001). The plate was sprayed with DTNB (5,5'-dithiobis-[2-nitrobenzoic acid)/ATCI (acetylthiocholine iodide) (1 mM DTNB and 1 mM ATCI in buffer A). After dry (5 min.) was sprayed with 3 units/mL of the enzyme acetylcholinesterase, type VI-s, lyophilized, 292 U/mg solid, 394 U/mg protein (Sigma Chemical Co.) and after 10 min, the yellow color was observed. Visualization of inhibition was accomplished by observation of white halos. Coloration disappears in approximately 15 to 30 min. Caffeine was used as a standard substance (KARADSHEH, N., *Toxicol Lett*., v. 55, p. 335, 1991). **Results and discussion:** The sample containing a mixture of geometric isomers cis- and trans- nerolidol showed a positive result against qualitative inhibition of the acetylcholinesterase. The result was observed through the thin layer chromatography plate, which showed yellow color with white halos, suggesting that the fraction has an inhibitory effect on the enzyme AChE. Comparing the results with other studies, our data corroborate the hypothesis that sesquiterpenes present a potential anticholinesterase (ZANARDI, L.M., *New Chemistry*, v. 35 p 2233, 2012; ALCÂNTARA, J.M., *Acta Amaz*, v. 40, p. 567, 2010). **Acknowledgments:** For Coordination of Personnel Improvement of Higher Education (Capes) and the Foundation Support for Research of the State of Piauí (FAPEPI) for financial support.

09.117

Effects of *Myracrodruon urundeuva* All. essential oil against *Leishmania amazonensis* and its cytotoxicity in macrophages. Carvalho CES, Junior EPCS, Brito ML, Oliveira JMG, Silva RM, Citó AMGL, Carvalho FAA – UFPI

Introduction: Leishmaniasis is currently spread worldwide. The disease is transmitted by small biting sandflies (Diptera, Phlebotominae). The treatment consist in the use of pentavalent antimonial and Amphotericin B, however, these drugs are toxic and generally expensive. Many natural products have been reported topossess leishmanicidal activities. *Myracrodruon urundeuva* Allemão is a plant utilized in the northeast region of Brazil as an antiinflammatory, wound healing and in gynecological illnesses. The main aid of this study was evaluate the anti-leishmania, cytotoxic and hemolytic activities of *Myracrodruon urundeuva* All. essential oil (EOMu). **Methods**: Promastigotes in the logarithmic growth phase were seeded in 96-well cell culture plates at 1×10^6 leishmania per well. Then, essential oil was added to the wells in serial dilutions ranging from 800, 400, 200, 100, 50, 25, 12.5 and 6.25$\mu g \cdot mL^{-1}$. The plate was kept at 26 °C in a biological oxygen demand (BOD) incubator. Leishmania was observed and counted by using a Neubauer's hemocytometer after 24, 48, and 72 h to monitor growth and viability. Assays were performed in triplicate and were repeated 3 times on different days. Cytotoxicity of was assessed using the MTT test. In a 96-well plate, 100μL of supplemented RPMI 1640 medium and about 1×10^5 peritoneal murine macrophages were added per well. They were then incubated at 37 °C in 5% of CO_2 for 48 h, for each well at the tested concentrations (800, 400, 200, 100, 50, 25, 12.5, and 6.25$\cdot mL^{-1}$). Cells were then incubated for 48 h. At the end of the incubation, 10μL of MTT diluted in phosphate-buffered saline (PBS) was added at a final concentration of 5 $mg \cdot mL^{-1}$ (10% of volume, i.e., 10μL for each 100μL well) and was incubated for an additional 4 h at 37 °C in 5% CO_2. The supernatant was then discarded, and 100μL of DMSO was added to all wells. The plate was then stirred for about 30 min at room temperature to complete formazan dissolution. **Results and discussion:** The OEMu in 24 hours inhibited the growth of the parasite in the concentrations of 800, 400, 200 and 100 $mg.mL^{-1}$. The IC_{50} value in 48 hours was 206,236 $\mu g.mL^{-1}$. Concentration Cytotoxy media is 5 $mg.ml^{-1}$ in macrophages murine. Cytotoxicity Based in this results the OEMu can be used against *L. amazonensis* in the precise concentration and not be cytotoxic to macrophages. **Acknowledgement:** Capes, CNPq, UFPI

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Effects of a grape skin extract of *Vitis vinifera* (ACH09) on hepatic metabolic disorders in C57BL/6 mice fed a high-fat diet. Santos IB, da Costa GF, de Bem GF, Costa CA, Carvalho LCRM, Okinga A, Rocha APM, Cordeiro VSC, Oliveira RB, Moura RS, Resende AC UERJ – Farmacologia e Psicobiologia

Introduction: The prevalence of obesity over the past decades has shown rapid rise worldwide and is considered an important public health problem in most developed and developing countries. Hepatic steatosis, characterized by increased levels of lipids (triglycerides) in the liver is frequently associated with obesity and is the early stage of nonalcoholic fat liver disease (NAFLD). Recent studies from our group have shown that the hydro-alcoholic grape skin extract (ACH09) rich in polyphenols lowers blood glucose in experimental model of diabetes induced by alloxan, and increases the expression of the insulin signaling cascade proteins in skeletal muscle. Thus, the aim of this study was to evaluate the beneficial effects of preventive treatment with ACH09 on metabolic disorders observed in an experimental model of obesity. **Methods:** The Ethics Committee of Animal Experiments Rio de Janeiro State University approved the experiments (protocol: CEA/025/2010). Male C57BL / 6 mice were separated in four groups. The control group was fed a standard diet and received water (control group: 10% fat) or ACH09 (ACH09 group, 200 mg/kg/day). Two other groups were fed a high fat diet (HF) with access to water (HF group: 60% fat) or ACH09 (HF + ACH09 group: 60% fat) for 12 weeks. Lipid peroxidation, carbonyl protein, and antioxidant activities of superoxide dismutase (SOD), catalase (CAT) and gluthatione peroxidase (GPx) were evaluated in liver homogenates by spectrophotometry. We also determined the body weight, plasma levels of total cholesterol, triglycerides, glucose and insulin, and hepatic levels of cholesterol, triglycerides and glycogen. Expression of insulin receptor (IR), PI3-K, phosphorylated AKT (pAKT), glucose transporter 2 (Glut 2), proteins involved in the synthesis of fatty acids and cholesterol (phosforilated AMP-activated protein kinase – pAMPK, HMG-CoA reductase, fatty acid synthase – FAS), as well as the expression of proteins involved in the excretion of cholesterol (ABCG5 and ABCG8 transporters) were evaluated in liver homogenates by western blotting. **Conclusions:** In HF group the increased body weight, plasma and liver cholesterol and triglycerides were reduced by treatment with ACH09. Expression of AMPK/pAMPK and ABCG5 and ABCG8 transporters were increased in HF group and reduced by ACH09. Increased plasma levels of glucose and insulin resistance in HF group were associated with reduction of glycogen and expression of the insulin signaling cascade proteins. Treatment with ACH09 prevented the insulin resistance and increased the expression of the proteins. Finally, the reduced antioxidant enzyme activity in HF group was recovered by ACH09 decreasing oxidative damage. In conclusion, treatment with ACH09 improved insulin resistance by increasing expression of insulin signaling cascade proteins, as well as the lipid profile by decreasing lipogenesis and normalizing the excretion of cholesterol. These effects associated with the antioxidant action of ACH09 may protect against the phenotypic and metabolic characteristics of obesity. **Financial Support:** CNPq and Faperj.

09.119

Evaluation of the anti-leishmania and anti-trypanosoma activity of ethanol extract of leaves from *Anonna squamosal.* Cesário FRAS[1], Monteiro AB[1], Rodrigues LB[1], Rodrigues CKS[1], Sales VS[1], Figueiredo FRSDN[1], Amaro EN[1], Delmondes GA[1], Cruz LP[1], Cunha FB[1], Nascimento EP[1], Costa JGM[1], Kerntopf MR[1], Coutinho HDM[1], Menezes IRA[1], Felipe CFB[2], Tintino SR[1], Gomes MCV[4], Coronel C[4] [1]URCA – Biological Chemistry, [2]UFPB – Molecular Biology, [4]FMB – Desarrollo de La Investigación Científica

Introduction: Leishmaniasis is a disease that can occur in men, women and children in many countries around the world. The disease may evidence various clinical symptoms, and is classified as cutaneous, mucocutaneous or visceral. Leishmaniasis is caused by parasites of the genus *Leishmania*, has a heteroxenic lifecycle and two morphological forms, promastigote and amastigote. In the case of Chagas disease, It is caused by the protozoan parasite *Trypanosoma cruzi*. This disease affects millions of people in the southern United States to Patagonia. The Chagas disease is also a great social and economic problem for Latin America. (MICHALICK, 2003). **Objective:** This study aims to evaluate antileishmanial and trypanocidal activity of ethanol extract of leaves of *Annona squamosa* L. in promastigote forms of *Leishmania braziliensis* and *Leishmania infantum* and epimastigote forms of *Trypanosoma cruzi*, besides analyzing the cytotoxic activity in fibroblasts. **Methodology**: *in vitro* tests for the evaluation of the activity antipromastigota and antiepimastigota, as well as cytotoxicity, were carried out in Microplates with 96 cavities, with exponential phase cultures, in which each concentration was tested in triplicate, following the methodology described by Le Senne *et al.* (2002). For the evaluation of leishmanicida activity were used the performance lineages of l. braziliensis (MHOM/CO/88/UA301) and l. infantum (MHOM/ES/92/BCN83), grown at 22° C in Schneider's Drosophila medium supplemented with fetal bovine serum to 20%. Regarding the evaluation of the activity performed to assess their tripanocidal *in vitro* tests of t. cruzi using clone CL-B5 (ROLÓN; *et al.*, 2006). Cytotoxicity assays utilize the fibroblast lineage NCTC929, grown in Minimal Essential Medium (Sigma). **Results:** The data show that the mortality *Leishmania* ranged from 39.44 to 83.30% at concentrations of 125-500 µg/mL. However, in the same concentrations, for *T. cruzi* activity ranged from 8.81 to 19.21%. The high toxicity to fibroblasts that was presented at concentrations 250-500 µg/mL ranged from 48.66 to 84.88% is a limiting factor for the use of this extract. **Discussion:** The results demonstrated that the ethanol extract of *Annona squamosa* L. (EEAS) tested, showed better activity against the promastigote form of *Leishmania* when compared to the results for *T. cruzi*. The best effects of the extract showed an inhibition rate ≥ 50% at concentrations of 250 and 500 µg/mL for *L. infantum* and *L. brasiliensis*, this is an important finding, considering that this level inhibition at a concentration ≤ 500 µg/mL clinically relevant. The toxicity to fibroblasts is a limiting factor to the use of this extract. In this case, it is necessary to conduct tests with components in an isolated form to identify the antiparasitic activity, whether it is caused by any of the constituents or by synergistic action these components. **Conclusion**: the present study revealed that the extract tested feature clinically relevant leishmanicida activities and low activity to assess their tripanocida. However, due to the high cytotoxicity revealed, the Annona squamosa. opens space for new biological *in vivo* studies with a view to reduce the cytotoxicity and increase the pharmacological effect. **References:** 1- LE SENNE, et. al. Mem Inst Oswaldo Cruz. 1101, 2002. 2- MICHALICK, Parasitologia humana. 10ª ed. São Paulo: Atheneu; p. 31-35, 2003. 3- ROLÓN, et. al. J Antimicrob. Agents. 104, 2006. **Financial support**: Capes, CNPq.

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Silymarin protects against irinotecan-induced non-alcoholic steatohepatitis through the inibition of protein nitrosylation and toll-like receptor 4 immunoexpression. Assis-Júnior EM[1], Sousa NRP[1], Moreira LS[1], Malveira LRC[1], Wong DVT[1], Melo AT[1], Pereira VBM[1], Wanderley CWS[1], Soares BM[1], Almeida PRC[2], Ribeiro RA[1], Lima-Júnior RCP[1] [1]UFC – Physiology and Pharmacology, [2]UFC – Pathology and Forensic Medicine

Introduction: Colorectal Cancer (CRC) is the 3th most prevalent cancer disease in the world. Irinotecan (IRI), a first line drug for CRC and its liver metastasis, has improved patients' survival. However, its side-effects, including non-alcoholic steatohepatitis (NASH), may limit the course of treatment. IRI-based anticancer regimens have been associated with a 3.4-fold increased risk of NASH. However, NASH pathogenesis is unknown, one reason why no effective therapy is available. Silymarin (SIL) has shown to prevent fatty liver diseases, drug- and chemical-induced hepatic toxicity in animal models. Then, we aimed to study the effect of SIL on IRI-induced NASH, and the likely mechanisms involved. **Methods:** Swiss male mice (n=8), divided into 6 groups, were injected with saline (SAL, 5ml/kg, i.p.), IRI (50 mg/kg, i.p.), SIL (150 mg/kg p.o.) or IRI (50 mg/kg i.p) + SIL (SIL1.5, 15 and 150 mg/kg p.o) 3x/week/7 weeks. Blood samples were collected at week 7 to determine serum concentration of the hepatic enzymes ALT and AST (U/L). Animals were killed and the livers were removed to assess tissue damage (Kleiner's scores), total lipid (mg/g tissue), IL-1β (pg/mg tissue) dosages, Inducible Nitric Oxide Synthase (iNOS), 3-Nitrotyrosine (NTyr) and Toll-Like Receptor 4 (TLR4) immunoexpression. ANOVA/Bonferroni's test or Kruskal Wallis/Dunn was used for statistical analysis. $P<0.05$ was accepted. Ethics committee approval CARE: 21/12. **Results**: IRI induced a significant ($P<0.05$) increase in serum ALT and AST, hepatic lipids accumulation, histopathological injury, neutrophil infiltration/field, IL-1β tissue level, iNOS and TRL4 immunostained cells/field (94.8 ± 21.7, 103.7 ± 6.1, 27.3 ± 6.6, 6.5[5-7], 3.90 ± 0.55, 18.4 ± 4.5, 15.5 ± 0.7, 27 ± 2.21, respectively) and NTyr marked area *vs.* SAL (ALT: 48.2 ± 3.2, AST: 41.5 ± 2.7, Total lipids: 7.7 ± 0.9, Tissue damage: 1[0-3], neutrophil infiltration: 0.32 ± 0.10; IL-1β: 10.8 ± 0.6; iNOS: 0.0 ± 0.2; TRL4: 2 ± 0.78). SIL (1.5 mg/kg) prevented the increase in these parameters (ALT: 34.2 ± 12.2, AST: 33.8 ± 6.4, Total lipids: 11.45 ± 1.795, Tissue damage: 3[2-6], TLR4: 11 ± 1.23) versus IRI group ($P<0.05$). Neutrophil infiltration was only prevented by SIL 15 mg/kg (2.3 ± 0.4), but TLR4 immunostaining was magnified in that group. Besides, SIL reduced the NTyr immunoexpression *vs.* IRI in both doses. However, IL-1β and iNOS expression were not affected by SIL pre-treatment ($P>0.05$) and even the higher dose of SIL was more deleterious. **Conclusions:** SIL prevented IRI-induced liver injury likely through the reduction of protein nitrosylation. The damage observed in the group of animals treated of higher doses of SIL seems to be dependent on bacterial translocations from the gut which is associated with TLR4 activation. **Financial Support**: CNPq/Capes/FUNCAP.

09.121

Hypotensive effect of ethanol extract from *Lippia origanoides* H.B.K. in normotensive rats. Carvalho GD[1], Miranda VC[1], Coelho AG[2], Mendes MB[1], Arcanjo DDR[1], Citó AMGL[2], Oliveira AP[1] [1]NPPM-UFPI, [2]UFPI – Química

Introduction: The *Lippia origanoides* H.B.K (Verbenaceae) is an aromatic shrub native of Central America and northern South America commonly known as "Oregano-del-Monte", "Salva-de-Marajó" or "Alecrim-d'Angola". It is also an apiarian plant used as a spice in cooking and in the folk medicine to treat gastrointestinal, respiratory and skin disorders as a topical lotion (Pascual, M.E., *J. Ethnopharmacol.*, 76, 201, 2001). This study concerns the investigation of the hypotensive effect of ethanol extract obtained from the leaves of the species *L. origanoides* (*Lo*-EtOH) in normotensive rats. **Methods:** Male Wistar rats (250-350 g) were used for all experiments (Animal Research Ethics Committee/UFPI no. 008/2012). For the direct measurement of MAP and HR, the abdominal aorta and inferior vena cava were cannulated using a polyethylene catheter; when oral administration was performed, a catheter was implanted in the abdominal aorta only. Blood pressure was measured after 24 hours by connecting the arterial catheter to a pre-calibrated pressure transducer (Statham P23 ID, Gould, Cleveland, OH, USA) coupled to a computer equipped with AQCAD 2.3.9 software (AVS Project, São Paulo, SP, Brazil). After stabilization of basal parameters, *Lo*-EtOH was intravenously administered at doses of 12.5, 25 and 50 mg/kg. In another group of animals, a pre-treatment with L-NAME (2.0 mg/kg, i.v.) was realized 30 min before the administration of *Lo*-EtOH (12.5, 25.0 and 50 mg/kg, i.v.), aiming to obtain a dose-response curve. The *Lo*-EtOH-induced hypotensive effect was also evaluated after oral administration. The animals were divided into 02 groups: The first group received saline (1 ml/100 g, b.w.), and the second one received *Lo*-EtOH (100 mg/kg, p.o.). The values of mean arterial pressure (MAP) and heart rate (HR) were registered ranging to 0 to 360 minutes, at intervals of 30 min, where the time-response ratio was observed. All values were expressed as mean ± S.E.M. Values were considered significant at $p<0.05$ by unpaired Student's t-test application using GraphPad Prism 5.0. **Results and discussion:** The intravenous administration at doses of 12.5, 25 and 50 mg/kg (n=4) promoted a hypotensive effect (in mmHg: -15.44 ± 2.14, -47.66 ± 6.64, -65 68 ± 9.07, respectively), followed by tachycardic response at doses of 12.5 and 50 mg/kg. Pre-treatment with L-NAME (n=4) attenuated the hypotensive effect (in mmHg: -17.67 ± 2.90*, -29.21 ± 3.86* and -35.26 ± 4.04*, respectively; *$p<0.05$ vs control, t-Student test) and tachycardic response at dose of 50 mg/kg. When orally administered, *Lo*-EtOH promoted a reduction in MAP from 90 min without significantly alteration in heart rate, returning to baseline after 360 min. The *Lo*-EtOH induces hypotensive effect followed by tachycardia when administered intravenously, and this response was attenuated after treatment with L-NAME, possibly suggesting the participation of the NO synthase enzyme in this effect. Oral administration of the *Lo*-EtOH decreases blood pressure during 360 minutes with no alteration in heart rate. **Keywords:** *Lippia origanoides*, hypotension, blood pressure. **Financial Support**: This work was supported by UFPI/FAPEPI/Capes/CNPq.

09.122

Antinociceptive and toxicological analysis of an Amazon oil: Pp-oil. Mota AS[1], De Lima AB[1], Gomes MF[1], Dias DCR[1], Santos GCQ[1], Nascimento GS[1], Silveira TS[2], Albuquerque TLF[1], Do Nascimento JLM[3], Da Silva JKR[4], Maia JGS[4], Ribeiro AF[5], Bastos GNT[1] [1]UFPA – Neuroinflamação, [2]UEPA, [3]UFPA – Neuroquímica Molecular e Celular, [4]UFPA, [5]UNIFESSPA

Introduction: Pp001 is an Amazon plant and its seed oil (Pp-oil) is used in folk medicine in order to fight against inflammatory processes such as rheumatism. Since Pp-oil mechanism is not well known the aim of this study was to investigate the acute, sub chronical toxicity and antinociceptive effect of Pp-oil. **Methods:** Male Swiss albino mice were used in accordance to the CEPAE-UFPA (124-13) and observed for toxic symptoms and mortality daily for 14 days and were also submitted to thermal and chemical stimulation at the hot plate test, acetic acid -induced abdominal writhing test and formalin test to evaluate the analgesic activity. Results were expressed as mean ± S.E.M. Statistical evaluation and were made using ANOVA followed Dunnett's *t*-test and Tukey test and values were considered significantly different when $P \leq 0.05$. **Results and discussion:** As a result no toxicological problems were observed between the doses 2000 mg/kg and 5000 mg/kg as well in 100 mg/kg and 200 mg/kg body weight in both acute and sub chronical tests respectively. In the hot plate (55 ± 0.5° C) dosed in 200 mg/kg presented no alterations in latency time when compared to control. However in the writhing test animals presented a significant decrease in abdominal writhes at the doses 25, 50 and 100 mg/kg. Furthermore the formalin test dosed at 50 and 100 mg/kg reduced significantly the second phase of the algic stimulus. Moreover its nociception was reversed by naloxone in order to evaluate its mechanism of action. Therefore the results suggest that Pp-oil has analgesic activity which tests demonstrated to be putative of peripheral origin. The mechanisms are not completely understood even though these results suggest that opioid receptors are involved in the antinociceptive action of Pp-oil. **Financial Agencies:** CNPq ; Fapespa, UFPA. **Number of ethics committee:** CEPAE-UFPA: 124-13
Keywords: *P.p -oil* ; *Antinociception*, *Toxicology.*

09.123

Effect of low level laser on tend endothelial cells subjected to injury by *Bothrops jararaca* venom. Zamuner SF[1], Franco ATB[1], Silva LM[1], Teixeira CFP[2], Zamuner SR[1]
[1]Uninove, [2]IBu

Introduction: Local hemorrhage is part of the complex pathological alterations at the bite site characteristic of Bothrops envenoming, and contributes to tissue damage and impaired muscle regeneration. Antivenom treatment reverses systemic effects induced by Bothrops snake venoms but is ineffective in neutralizing the local effects. Thus, there is an urgent need to find therapies that can complement antivenoms in the neutralization of local and systemic damage. The low level laser (LLL) therapy is being considered as an alternative treatment for endothelial injury situations because its bioestimulation effect. Accordingly, the present study was designed to investigate the effect of LLL on tEnd endothelial cells submitted to injury by *Bothrops jararaca* venom (BjV). **Methods:** tEnd cell line was used. We analyzed cell viability by MTT assay and lactate dehydrogenase (LDH) activity. The cells were grown in culture medium DMEM supplemented with 10% fetal bovine serum, incubated at 37˚C with 5% CO^2 for 48 hours for cell attachment, after that, the cells received BjV venom in the concentration of 10 µg/mL. Cells were irradiated for 10 s immediately after the venom administration with a semiconductor laser at red and infrared laser at 660 and 780 nm, respectively, dose of 4 J/cm^2 and power of 100 mW and were incubated for 30, 60 and 120 minutes. The cells that did not receive venom served as control. **Results:** BjV caused a decrease in cellular viability at 60 and 120 min after venom incubation by 42 and 65%, respectively. LLL irradiation increases cell viability by 40 and 31% of tEnd cell at wavelengths 660 and 780 nm, respectively, within 60 min of BjV incubation. Moreover, at 120 min after the venom incubation LLL causes an increase of cell viability that was 22% and 24% higher than BjV by 660 and 780 nm, respectively. BjV caused a release of LDH at 60 and 120 min after venom incubation by 53 and 50%, respectively. LLL irradiation at 660 nm was able to promote a reduction in LDH release by 33% and 49% at 60 and 120 min, respectively. The LLL irradiation at 780 nm was able to promote a reduction in LDH release by 28% and 54% at 60 and 120 min, respectively. **Conclusion:** The results of this study clearly indicate that irradiation with red and infrared laser caused a protection on endothelial cell after venom incubation. **Financial support**: Uninove

09.124

Effect of diosgenin on cardiac oxidative stress in female ovariectomized rats. Morais ICPS[1], Moura IJL[2], Nicolau LAD[1], Arcanjo DDR[2], Carvalho CES[1], Piauilino CA[1], Santos MEP[1], Carvalho EF[1], Medeiros JR[3], Oliveira AP[1] [1]NPPM-UFPI, [2]UFPI, [3]UFPI-Parnaíba

Introduction: Menopause is a period of intense hormonal changes, characterized by ovarian atrophy and regression of hormone estrogen. The female hormone deficiency at this stage plays an important role in cardiovascular pathogenesis. (Dowling, M *et al*, PLos. vol17, p11, 2013; Ying – hui, G *et al*, Chin Med J. vol 17, p 125, 2012). Diosgenin (3β-hydroxy-5-spirostene), a phytoestrogen, structurally similar to estrogen and progesterone. Multiple beneficial effects of diosgenin in the scientific community in several areas and even in the evaluation of cardiovascular system. The objective of search was analyze effects of phytoestrogen Diosgenin on cardiac oxidative stress ovariectomized rats. **Methods:** 5 Wistar rats (Rattus norvegicus) adult females of gruop over the age of 7 weeks (190 – 250g) was used. The protocols were approved by the Ethics Committee on Animal Experimentation of UFPI No. 008/12). Hormone regression was obtained by surgical procedure dorsal bilateral ovariectomy (Aydin, A *et al*, Arc Ira Med. vol 16, p 12, 2013; Ribeiro, G *et al*, Cad Pesq. vol 18, p 11, 2011). After 60 days of surgery, began treatment groups: Group 1: Sham/ Salina,. Group 2: ovariectomized (OVX) group, 3: OVX + Diosgenin (25 mg/kg); Group 4: OVX + Diosgenin (50 mg/kg PO) ; Grupo 5: OVX + 17β – estradiol (2.5 mg/kg), once daily for 4 week. The reduced glutathione (GSH) content of the cardiac tissue was estimated according to the method described in Sedlak and Lindsay (1968). The absorbance was measured at 412 nm on a spectrophotometer. The level of Malondialdeidio (MDA) in the homogenate cardiac tissue was measured using the method described in Mihara and Uchiyama (1978), which is based on a thiobarbituric acid reaction. Mieolperoxidade (MPO) activity was assayed by measuring the change in absorbance at 450 nm using o-dianisidine dihydrochloride and 1% hydrogen peroxide. Results were expressed as mean ± SEM and significant when * $p<0.05$ – "Student-t" test – PRISM 5.03. **Results:** Diosgenin 50 mg/kg increased concentrations GSH in relation sham group (7.77 ± 2.63 *; 2.72 ± 1.03, diosgenin 50 mg/kg and sham group, respectively) and decreased MDA concentrations (124.05 ± 24.09* ; 199.48 ± 30.53, diosgenin 50 mg/kg and sham group, respectively). MPO concentration (17.05 ± 1.19; 16.91 ± 2.92, diosgenin 50 mg/kg and sham group, respectively) was unchanged in cardiac tissue *versus* others groups. **Discussion:** These findings suggest that diosgenin could have a beneficial role against miocardial muscle damage induced by oxidative stress in menopause, which was evidenced by the propensity of diosgenin to modulate the antioxidant defense and to decrease the lipid peroxidation in the heart. **Financial agencies and acknowledgements**: FAPEPI/CNPq

09.125

Canavalia brasiliensis **lectin relaxes resistance and capacitance vessels in normoglycemic and diabetic rats.** Laranjeira EPP[1], da Silva DHM[1], Bringel PHSF[1], Cajazeiras JB[2], Cavada BS[2], Soares PMG[3], Assreuy AMS[1], Pires AF[1] [1]ISCB-UECE, [2]UFC – Bioquímica e Biologia Molecular, [3]UFC – Morfologia

Introduction: Hyperglycemia leads to endothelial dysfunction in both resistance and capacitance vessels due to reduction in vasodilation (RAJENDRAN, **Int J Biol Sci**. v. 9, p. 1057, 2013). Vasodilator effects in aorta of normoglicemic rats have been described for homologous lectins isolated from seeds of the *Canavalia* genus (Diocleinae subtribe) (ASSREUY, *Naunyn-Schmiedeberg's Arc. Pharmac*, v. 380, p. 509, 2009). Besides, there are reports that *Canavalia* lectins modulate diabetes development and its complications (KOLB, *Diabetes Res*. v. 3, n. 4, p. 183, 1986). This study investigated the endothelium-dependent vasorelaxant effects of *Canavalia brasiliensis* lectin (ConBr) in isolated aorta and mesenteric vascular bed (MVB) of normoglycemic and diabetic rats. **Methods**: Wistar rats (200–300 g), manipulated according to the principles established by the Ethics Committee of UECE (CEUA N° 12776260-4), were sacrificed for removal of thoracic aorta and MVB after 8 weeks of i.p. injection of STZ (65 mg/kg) (diabetic group) or citrate buffer (normoglicemic group). Aortic rings were mounted in an organ chamber containing Tyrode solution and MVB mounted under a perfusion flow of 4 mL/min of Krebs–Henseleit solution (PIRES, ***Can. J. Physiol. Pharmacol***. v. 90, p. 1380, 2012). Both tissues were gassed with 95% O_2 and 5% CO_2 at 37 ˚C (pH 7.4) and set up to equilibrium for 40 min before tests. ConBr (10-100 µg/mL) was cumulatively added at the contraction plateau induced by phenylephrine- in aorta or the noradrenaline-induced plateau in MVB with endothelium preserved (relaxant responses to acetylcholine greater than 50%). **Results**: ConBr elicited relaxation in aorta of the phenylephrine-induced contractions at 30 µg/mL (normoglicemic: 37.6 ± 6.47; diabetic: 32.1 ± 12.45%) and 100 µg (normoglicemic: 73.8 ± 10.49; diabetic: 76.9 ± 12.36%), with no statistical differences between groups. In LMV, addition of increasing doses of ConBr (10, 30 and 100 ng) did not affect the contraction induced by noradrenaline. However, in normoglicemic animals ConBr induced relaxation at 10, 30 and 100 µg by 68.1 ± 2.26; 73.76 ± 3.75; 50.18 ± 10.21%, respectively, an effect that was absent in diabetic animals. **Conclusion**: ConBr exerts dose-dependent vasodilator response in the capacitance vessel of aorta in either normoglicemic and diabetic rats. However, in the mesenteric vascular bed, a resistance blood vessel, ConBr elicited relaxation only in normoglicemic rats. These data highlight the major alteration of hyperglycemia in microvasculature. **Financial** Agencies: Capes, FUNCAP, CNPq.

09.126

Evaluation of antinociceptive activity by central mechanisms of protein extract of the green seaweed Caulerpa racemosa (Forsskål) J. Agardh in mice. Ribeiro NA, Servente P, Maia CY, Benevides NMB UFC – Biochemistry and Molecular Biology

Introduction: Natural products in general, play an invaluable role in drug discovery. Preliminary tests performed by the group of Carbohydrates and Lectins from UFC revealed the antinociceptive potential of protein extract of the green seaweed *Caulerpa racemosa*. Tests with analgesic drugs using animals are very common to evaluate if nociceptive action occurs by peripheral or central mechanisms. This study aims to evaluate if the antinociceptive action of the protein extract of the green seaweed *C. racemosa* occurs by central mechanisms. **Methods:** Protein extraction (CrEB) was performed with 25 mM Tris-HCl, pH 7.5 at room temperature. All of the tests followed the required standards for ethics and biosafety, and the experiments were only initiated after receiving approval from the ethics committee on animal research of the UFC (Fortaleza, Brazil; CEPA n°. 80/10). Central analgesic activity was evaluated using the formalin and hot plate test. In the formalin test mice were injected with CrEB (50 mg/kg; i.v.), naloxona, an opioid antagonist (2 mg/kg; s.c.) + CrEB or sterile saline (0.9%, w/v, NaCl). After 30 min, 10 µL of 2% formalin solution was injected into the right paw of male mice, and the duration of licking was recorded during the first 5 min (1st phase, which corresponds to the direct application of a chemical stimulus to the nociceptors – the initial phase) and for an additional 5 min after a 20 min time period (2nd phase, which involves inflammation – late phase). Hot plate test involves recording the time(s) that animals require to manifest a response when in contact with a heated metal plate (51 ± 1°C), which corresponds to the act of removing or licking the hind paw and/or jumping. This specific test is used to verify central nociception. The animals were first acquainted with the hot plate to observe the control reaction time. Animals with a reaction time exceeding 10 s were discarded from the test. Immediately after the trial (control reaction time), the mice were divided into groups of six. The mice then received an injection of sterile saline (0.9%, w/v), CrEB (1, 10 or 50 mg/kg; i.v.), morphine (5 mg/kg; s.c.) or indomethacin (5 mg/kg; s.c.), and the reaction times were measured at 0, 30, 60 and 90 min after drug administration. A cut-off time of 40 s was used to avoid paw lesions. **Results and discussion:** It was observed that the CrEB and CrEB + naloxona reduced significantly the 1st phase (neurogenic) and the 2nd phase (inflammatory) observed after administration of the formalin (50.57; 95.58% and 54.97; 97.12%, respectively) demonstrating that CREB does not act through opioid mechanisms. However, the CrEB (1; 10 and 50 mg/kg) was not capable to reduce the nociception evaluated by Hot Plate test, compared to morphine. Therefore, it is suggested that the antinociceptive activity of the CrEB can be predominant by inhibition of peripheric mechanisms. **Financial** Agencies: CNPq and Capes. **Acknowledgements**: This work was supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) and Coordenacão de Aperfeiçoamento de Pessoal de Nível Superior (Capes). Benevides, N.M.B. is senior investigator of CNPq/Brazil.

09.127

Monoterpene nerol induces vasorelaxant effect by inhibiting calcium influx in rat aorta.

Branco NC[1], Carvalho EF[1], Nunes AF[1], Santos RF[1], Sousa DP[2], Oliveira AP[1], Oliveira RCM[1] [1]NPPM-UFPI, [2]UFPB – Pharmaceutical Science

Introduction: Nerol is monoterpene originally isolated from the neroli oil is also found in many essential oils such as lavender and rose (Kumar *et al*; MSAADA *et al*, 2012). Previous studies show that the nerol shows antinociceptive effect in rodents. **Aim:** To investigate the vasorelaxant effect of monoterpene nerol in the isolated rat aorta rings. **Methods:** The protocols were approved by the Ethics Committee on Animal Experimentation (CEEA/UFPI 008/2012). Wistar rats were used (250-300g), male, originating from the Vivarium Sectorial NPPM/UFPI, kept under controlled temperature conditions (24 ± 1˚C) and light-dark cycle of 12h, with free access to food and water. After euthanasia, the artery thoracic aorta was sectioned into rings (3-4 mm), which were incubated at 37˚C in normal Krebs solution (pH 7.4) aerated with carbogen (95% O_2, 5% CO_2), suspended by cotton threads and attached to force transducers coupled to a data acquisition system (AQCAD/Projects AVS, SP-Brazil) for recording of isometric tension. After stabilization (1.0 gf, 1h), was verified the integrity of the vascular endothelium in the aortic rings by addition of acetylcholine (ACh, 1 µM) on the component and sustained tonic contraction with phenylephrine (PHE, 1 µM) considering its absence (E-) of less than 10% and presence (E +) when the relaxation exceeded 50%. During the tonic phase of a second response to FEN, nerol was cumulatively added to the bath chambers (0.1 to 750 µg/mL). In a second step, the aortic rings without endothelium were contracted with 80 mM KCl and the tonic phase of a second response nerol cumulatively was added (0.1 to 750 µg/mL). It was also reported the inhibition of the contractile response induced by cumulative concentrations of KCl (10, 20, 40, 60, 80 and 100 mM) in the presence of nerol in the individual concentrations (9, 27, 243 and 750 µg/mL). The relaxation was expressed as percentage reverse or phenylephrine-induced contraction by 80 mM KCl. The observed inhibition of contraction was expressed as a percentage of direct contraction characteristic for each concentration. Results were expressed as mean ± SEM pD_2 obtained by non-linear regression. For comparisons of means, was used the Student's t unpaired test, considering significant *$p<0.05$ values (GraphPad Prism, 5:03). **Results**: The vasorelaxant effect nerol presented in pre-contracted aortic rings with phenylephrine in a concentration-dependent manner in the presence (+ E: pD_2 = 1.78 ± 0.02, n = 6) and in the absence of the endothelium (E: pD_2 = 1.74 ± 0.07, n = 6 ;). The nerol also promoted a concentration-dependent vasorelaxant effect in aortic rings precontracted with 80 mM KCl (E: pD_2 = 1.46 ± 0.01, n = 6 ;). The nerol inhibited those induced by the cumulative addition of KCl (n=6), Control contractions: E_{max} (%) = 91.8 ± 8.16, nerol 9 µg/mL: E_{max} (%) = 94.6 ± 3.61; 27 µg/mL: E_{max} (%) = 59.9 ± 10.3; 243 µg/mL, E_{max} (%) = 11.8 ± 2.60*; 750 µg/mL: E_{max} (%) = 6.0 ± 3.28*. **Conclusion:** Nerol present vasorelaxant effect independent of the endothelium in isolated thoracic aorta of rats rings precontracted and suggesting the involvement of blocking the influx of calcium confirmed by inhibition of contraction induced by KCl cumulatively. **Financial Support:** UFPI/Capes/CNPq

09.128

***Platonia insignis* Mart. ethanolic extract as a potential anti-leishmania agent: Effects on *Leishmania amazonensis* promastigotes and cytotoxicity in macrophages.** Sobrinho Junior EPC, Carvalho CES, Brito LM, Costa ICG, Chaves MH, Carvalho FAA UFPI

Introduction: The Medicinal plants represent an important health and economic component of biodiversity and also conservation and sustainable use. Medicine is used in all parts of the world and has a rapidly growing economic importance, mainly by the use of medicinal plants that have a respectable position today. Medicinal plants represent an important health and economic component of biodiversity and also conservation and sustainable use. The need to identify new anti-Leishmania compounds that are more effective and less toxic than conventional drugs has motivated research of substances derived from plant species. The *Platonia insignis* Mart has pharmacological properties with digestive, diuretic action and healing, as well as antioxidant activity. **Methods:** The extract tested was provided by the research group coordinated by Prof. Dr. Mariana Helena Chaves, Department of Chemistry of the Center for Natural Sciences, UFPI. All protocols were approved by the Animal Research Ethics Committee (CEEAPI no. 008/2012). Promastigotes in the logarithmic growth phase were seeded in 96-well cell culture plates at 1×10^6 Leishmania per well. Then, essential oil was added to the wells in serial dilutions of 800, 400, 200, 100, 50, 25, 12.5 and 6.25 $\mu g \cdot mL^{-1}$. The plate was kept at 26 °C in a biological oxygen demand (BOD) incubator. Leishmania was observed and counted by using a Neubauer hemocytometer after 24, 48, and 72 h to monitor growth and viability. Assays were performed in triplicate and were repeated 3 times on different days. Cytotoxicity of was assessed using the MTT test. In a 96-well plate, 100 µL of supplemented RPMI 1640 medium and about 1×10^5 macrophages were added per well. They were then incubated at 37 °C in 5% of CO_2 for 48, for each well at the tested concentrations (800, 400, 200, 100, 50, 25, 12.5, and 6.25 $\mu g \cdot mL^{-1}$). Cells were then incubated for 48 h. At the end of the incubation, 10 µL of MTT diluted in PBS was added at a final concentration of 5 $mg \cdot mL^{-1}$ (10% of volume, i.e., 10 µL for each 100 µL well) and was incubated for an additional 4 h at 37 °C in 5% CO_2. The supernatant was then discarded, and 100 µL of DMSO was added to all wells. The plate was then stirred for about 30 min at room temperature to complete formazan dissolution. **Results and discussion:** The (EtOH-CCB) in 24 hours inhibited the growth of the parasite in concentrations of 800, 400, 200 and 100 $mg.mL^{-1}$. Activity was also observed time-dependent, as in 48 and 72 hours, there was leishmanicidal activity in concentration of 800 $mg.mL^{-1}$, whereas the inhibition continued until the concentration of 50 $mg.mL^{-1}$. The IC_{50} value in 48 hours was 66.084 $mg.mL^{-1}$. However, they also show cytotoxicity on macrophages and the value of CC_{50} is at 321.401 $mg.mL^{-1}$. **Acknowledgement:** Capes, CNPq, UFPI.

09.129

Hydrogel of polyacrylamide-co-methycellulose containing extract *Calendula officinalis* L.

Castro LD, Bandeira ES, Gomes MF, Queiroz Santos GC, Ribeiro-Costa RM, Bastos GNT[1]
UFPA

Introduction The technology of hydrogels has high versatility in the pharmaceutical field, hydrogels are comprised of polymers that form dimensional networks, highly hydrophilic and has the characteristic of absorbing large quantities of water. It has biocompatibility, flexibility, wetting ability, biological fluids with swelling, low toxicity and biodegradable. Hydrogels are used as drug delivery system in which drugs can be inserted into the polymer network, resulting in a drug delivery system over time, improving the administration of drugs, and must be treatment skin ulcers. Among the medicinal plants used in herbal medicine the *Calendula officinalis* L. has highlighted to be used as anti-inflammatory, antiseptic and healing. Purpose of this study is develop phytotherapic with drug delivery system using hydrogel polyacrylamide-co-methylcellulose with dry extract of *Calendula officinalis* L. **Methods** The hydrogels of polyacrylamide-co-methylcellulose were obtained by free radical polymerization of acrylamide monomer with adsorption of the dry extract of *Calendula officinalis* L. The physicochemical characterizations were determined by the degree of swelling (Q), scanning electron microscopy (SEM) and infrared spectroscopy (FT-IR). To evaluate the acute dermal toxicity of hydrogel polyacrylamide-co-methylcellulose was used albino Wistar male rats and adults, following the protocol of OECD 402 and with the permission of the ethics committee at the N° CEPAE-UFPa: 131-13. The 17 rats were divided into three groups of five for testing hydrogels with acrylamide concentrations 3,6; 5,4 and 7.2% and two rats were randomly assigned to be the control used distilled with water, samples of hydrogels with 5 cm^2 were applied on the dorsum of the rat for 24 hours and posteriorly evaluated. **Results and discussion** From the study of the degree of swelling in which we compared hydrogels with and without methylcellulose, with methylcellulose presented the highest values of Q, which was attributed to increased water uptake of the hydrogel caused by the incorporation of hydroxyl groups from methylcellulose. The morphology observed in the SEM shows the porous structure of the material with the adhesion of the extract of *Calendula officinalis* L. on network. In IF-TR axial stretching of the region 3600-3200 cm^{-1} and 1607 cm^{-1}, indicating the presence of the group -OH,-NH-, and strong bands around 2900 cm^{-1} appear in almost all organic compounds resulting spectra the presence of C-H stretching. The adsorption of dry extract of *Calendula officinalis* L. in the polymer network has responded positively not hindering network formation. Regarding the acute dermal toxicity test did not show any visual perceptual change. Therefore, the biomaterial has a good chance of being a controlled release system. **Financial support:** CNPq, Capes, UFPA.

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Prospection of antitumor sulfated polysaccharides obtained of algae from the Brazilian coast. Assef ANB[1], de Oliveira CF[2], do Carmo LD[1], de Souza TG[1], Alencar NMN[1], Moreira TA[2], Faria CN[2], da Silva KT[2], da Costa BB[2], Cinelli LP[2], Costa-Lotufo LV[1], Wilke DV[1] [1]UFC – Fisiologia e Farmacologia, [2]UFRJ-Macaé – Glicofármacos

Introduction: Sulfated polysaccharides (SP) are a group of complex macromolecules with several biomedical activities reported including anticoagulant, antitrombotic, antitumor, antimetastatic and immunostimulating. **Objectives**: This study aimed to investigate the antitumor potential of four SP obtained from marine algae species, *Halimeda tuna*, *Gracilaria caudata*, *Dictyota mertensi* and *Penicilus capitatus*. **Methods**: The SP from *H. tuna*, (HTU), *G. caudata* (GCA), *D. mertensi* (DME) and *P. capitatus* (PCA) were obtained by protein digestion followed by precipitation using ethanol. The cytotoxicity was assayed through the MTT assay against a colon cancer cell line (HCT-116) at 50 and 100 µg/mL after 72h. The antitumor effect was evaluated on mice bearing Sarcoma 180 tumor. The mice (Swiss, n=8/group) were treated i.p. during 7 days with sterile saline or HTU, GCA, DME and PCA (25 and 50 mg/kg/day). A blood sample was used for biochemical and hematological analyses at day 8 post tumor implant. Mice were euthanized for collection and weighing of tumor, spleen, liver and kidneys. The animal handling procedures were performed in accordance with the Brazilian legislation for the use and care of laboratory animals ("Lei auroca" No 11.724/2008). This project was approved by the Animal Ethics Committee of the UFC (#NS50). **Results:** None of screened SP showed cytotoxicity against HCT-116 cells, nevertheless HTU caused an inhibition of 35% on the Sarcoma 180 tumor growth at both 25 and 50 mg/kg/day. HTU-treated mice also had monocytes and neutrophils counting diminished, while the lymphocytes counting were enhanced. Once HTU was not cytotoxic, but showed antitumor activity, this later could be related to an immunomodulatory effect. Besides that, the decreasing of circulating granulocytes and monocytes is feasible related to their migration to the tumor microenvironment. **Conclusion:** The sulfated polysaccharides – HTU, GCA, PCA and DME – were not cytotoxic, however HTU showed antitumor activity. Additional studies are ongoing to purify the most active fraction of SP in the HTU, as well the investigation of its possible effect on the immune system. **Support**- CNPq, Capes and Faperj.

09.131

Galetin 3,6-dimethyl ether activates K^+ channels and reduces Ca^{2+} cytosolic levels on guinea pig ileum. Vasconcelos LHC[1], Correia ACC[2], Souza ILL[1], Paredes-Gamero EJ[3], Buri MV[3], Rigoni VLS[3], Santos BVO[1,4], Cavalcante FA[1,5], Silva BA[1,4] [1]UFPB, [2]ICBS-UFAL, [3]DB-Unifesp, [4]DCF-UFPB, [5]DFP-UFPB

Introduction: *Piptadenia stipulacea* (Benth.) Ducke (Fabaceae) is a Caatinga tree from Brazilian northeast. The flavonoid galetin 3,6-dimethyl ether (FGAL) was obtained from its aerial parts and, in previous studies, inhibited both carbachol (CCh)- or histamine-induced phasic contractions on guinea pig ileum (MACEDO, J. Smooth Muscle Res., v. 47, p. 123, 2011). Thus, it was aimed to characterize the FGAL relaxant action mechanism. **Methods:** segments of guinea pig ileum were suspended in organ baths containing modified Krebs solution at 37 °C, bubbled with carbogen mixture under 1 g of resting tension, where isometric contractions were registered by force transducer. To cellular assays, myocytes culture was obtained from guinea pig ileum longitudinal smooth muscle layer. Viability protocol was performed using MTT assay, and cytosolic Ca^{2+} concentration ($[Ca^{2+}]_c$) with fluo-4, a Ca^{2+} indicator. All experimental protocols were approved by Ethical Committee on Animal Use of CBiotec/UFPB (Protocol 0705/13). **Results:** FGAL (10^{-10}-10^{-4} M, n = 5) relaxed pre-contracted ileum with histamine (10^{-6} M) either in absence (EC_{50} = 1.9 ± 0.4 x 10^{-7} M) or presence (EC_{50} = 1.1 ± 0.3 x 10^{-6} M) of CsCl, non-selective K+ channel blocker, and its relaxant curve was shifted to the right about 6-fold. In contrast, the relaxant curve of FGAL was not altered in the presence of apamin, SK_{Ca} blocker (EC_{50} = 1.6 ± 0.3 x 10^{-7} M), or TEA^+ 1 mM, BK_{Ca} blocker (EC_{50} = 2.6 ± 0.2 x 10^{-7} M). However, in the presence of 4-AP, K_V blocker (EC_{50} = 1.8 ± 0.2 x 10^{-6} M), or glibenclamide, K_{ATP} blocker (EC_{50} = 1.5 ± 0.5 x 10^{-6} M), relaxation curve of FGAL was shifted to the right about 10- and 8-fold, respectively. In cellular assays, viability of intestinal myocytes was not altered by FGAL (10^{-4} M, n = 3) either after 2 or 24 h. Furthermore, fluorescence intensity of myocytes stimulated with histamine (10^{-6} M) was attenuated in 88.3 ± 9.3% by FGAL (3 x 10^{-5} M, n = 3), similar to observed for verapamil (10^{-6} M, n = 3), which inhibited in 65.5 ± 5.9%. **Discussion:** the K+ efflux through its channels regulates the Ca^{2+} influx by Ca_V in smooth muscle and, thereafter, its contraction process (THORNELOE, Can. *J Physiol Pharm*, v. 83, p. 215, 2005). Since the relaxant curve of FGAL was shifted to the right in CsCl presence, it indicates the positive modulation of K+ channels by FGAL. As apamin or TEA^+ 1 mM did not change the relaxant potency of FGAL, it indicates that SK_{Ca} and BK_{Ca} are not involved on FGAL relaxant mechanism; however, as 4-AP or glibenclamide reduced the relaxant potency of FGAL, confirmed that FGAL positively modulates K_V and K_{ATP} to relax guinea pig ileum. Considering that FGAL did not alter the viability of intestinal myocytes, attention was turned to assess whether FGAL reduces its $[Ca^{2+}]_c$. The flavonoid attenuated the fluorescence intensity emitted by fluo-4 from stimulated myocytes with histamine as a result of $[Ca^{2+}]_c$ reduction. Thus, the relaxant mechanism of FGAL on guinea pig ileum involves the positive modulation of K_V and K_{ATP}, which, indirectly, attenuates the Ca^{2+} influx through Ca_V, leading to a reduction of its cytosolic levels. In view of these, FGAL could be an alternative for treatment of intestinal diseases. **Financial support:** CNPq, Capes, PgPNSB/CCS/UFPB.

09.132

Sulfated polysaccharides from *Dictyota caribaea* inhibit sarcoma 180 tumor growth.

Assef ANB[1], Celestino RCA[2], Cinelli LP[3], Carmo LD[1], Souza TFG[1], Alencar NMN[1], Santos GRC[4], Mourão PAS[4], Costa-Lotufo LV[1], Wilke DV[1] [1]UFC – Fisiologia e Farmacologia, [2]UFRJ-Macaé, [3]UFRJ-Macaé – Glicofármacos, [4]UFRJ – Bioquímica Médica

Introduction: Sulfated polysaccharides (SP) are a gifted group of bioactive molecules able to inhibit tumor growth, increasing the effectiveness of chemotherapy and reducing some of its common side effects. Phaeophyta algae produce SP with several biological activities such as anticoagulant, antitrombotic and antitumor. **Objectives**: This work aimed to investigate the antitumor potential *in vitro* and *in vivo* of SP from the *Dictyota caribaea* (DCA), a brown algae collected at Praia Vermelha – Paraty (RJ) (23° 11' 46" S e 44° 38' 38" O). **Methods**: DCA were extracted with proteolytic enzyme and supernatant was precipitated with increasing concentrations of ethanol. DCA cytotoxicity was tested *in vitro* by the MTT assay against a colon cancer cell line (HCT-116) at 50 and 100 µg/mL after 72h. Anticoagulant activity was tested by the aPTT assay. *In vivo* antitumor effect was evaluated on mice transplanted with Sarcoma 180 tumor (Swiss, n=8) treated i.p. during 7 days with sterile saline or DCA (25 and 50 mg/kg/animal). A sample of blood was collected for biochemical and hematological analysis at day 8 post tumor implant. Then mice were euthanized for collection and weighing of tumor, spleen, liver and kidneys. All animal handling procedures were performed in accordance with the Brazilian legislation for the use and care of laboratory animals ("Lei auroca" No 11.724/2008). This project was approved by the Animal Ethics Committee of the UFC (#NS50). **Results and discussion:** DCA did not exhibit cytotoxicity nor anticoagulant activity, however the tumor growth was inhibited by 50%. Besides that the spleen weight doubled on mice treated with DCA-treated mice, and they had monocytes counting diminished. Altogether, these results suggest that the DCA antitumor effect could be related with an immunomodulation effect. There are three major points to support this: 1- tumor growth was inhibited *in vivo*, although DCA was not cytotoxic itself; 2- spleen increased, a usual consequence of immunostimulation and; 3- decreased circulating monocytes could be related to the migration of these cells to the tumor microenvironment. **Conclusion:** DCA displayed host dependent antitumor effect, possibly due to an immunostimulation. Further studies are underway to better investigate the immunological effects of DCA and to elucidate its structure as well. **Support**: CNPq, Capes and Faperj.

09.133

Antihistaminic activity of *Schinus terebinthifolius Raddi* (Anacardiaceae) Bark extract.

Silva JL[1], Neto PAN[2], Sobrinho TJSP[2], Júnior ED[3], Costa-Silva JH[4], Araújo AV[5], Wanderley AG[2,1] [1]UFPE – Fisiologia e Farmacologia, [2]UFPE – Pharmaceutical Sciences, [3]Unifesp – Pharmacology, [4]UFPE – Physical Education and Sports Science, [5]UFPE – Nutrition

Introduction: *Schinus terebinthifolius* Raddi (Anacardiaceae), popularly known as "aroeira", is found throughout the Brazilian coast and has been used in traditional medicine, especially for the treatment of inflammatory, gastric and respiratory disorders[1,2]. The importance of this plant has promoted its inclusion in Brazilian Pharmacopeia[3]. The purpose of this work was evaluate the possible antihistaminic activity of the dried bark extract from *S. terebinthifolius* (St) using *in vivo* and *in vitro* experimental models. **Methods:** Phytochemical evaluation has been performed by HPLC analysis. *in vitro* assays were intended to investigate the effect of St on the simple or cumulative contractions induced in isolated guinea pig ileum by histamine, carbachol and potassium chloride. For evaluate the *in vivo* antihistaminic activity, the St was studied against hind paw edema induced by histamine in Wistar rats. All protocols were approved by the Animal Experimentation Ethics Committee of the UFPE (license no. 045543). **Results and discussion:** HPLC analysis revealed that gallic acid, ellagic acid, catechin and epicatechin are present in the sample. St (250, 500 and 1000 µg/mL) reduced the histamine-induced (1µM) contraction by 9.1 ± 1.8, 50.2 ± 2.0 and 68.9 ± 2.0%, respectively. However, there was no inhibition of the contractile responses induced by carbachol (1µM) or KCl (40mM). Hydroxyzine (0.125 and 0.250 µM/20 min), a H_1-selective antagonist, inhibited the responses of histamine (25.9 ± 3.1 and 51.2 ± 3.0%, respectively), but did not alter the contractions induced by carbachol or KCl. The association of St with hydroxyzine (250 + 0.125 or 500 µg/mL + 0.250 µM/20min) caused a significant potentiation of the inhibitory effect (67.0 ± 3.2 and 85.1 ± 2.1%, respectively). St also induced a shift to the right of the concentration-effect curves to histamine and, at concentrations 500 and 1000 µg/mL, reduced the maximal effect (65.7 ± 1.9 and 49.4 ± 2.5%, respectively). St (250, 500 and 1000 µg/mL/20min) produced a shift to the right of the concentration-response curves to 2-pyridylethylamine, a selective agonist of H_1 receptor and reduced the maximal effect (70.7 ± 3.3, 35.6 ± 4.3 and 17.1 ± 2.9%, respectively). In addition, St (100, 200 e 400 mg/kg) promoted a decrease of paw edema in the 1st hour after its induction (edema peak) of 33.9, 48.4 e 54.8%, respectively, whereas hydroxyzine (70 mg/kg) inhibited the edema by 56.5%. **Conclusion:** The data from this study suggests that the bark extract of *S. terebinthifolius* has antihistaminic effect (H_1), evidenced by selective antagonism of contractile responses induced by histamine and inhibition of paw edema. **References:** 1- Morton, Econ. Bot. 32: 353, 1978. 2- Medeiros *et al.*, Braz. J. Pharmacog. 17: 23, 2007. 3- Brandão *et al.*, Braz. J. Pharmacog. 16: 408, 2006. **Financial support:** FACEPE.

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Involvement of K^+ channels on spasmolytic effect of the new derivative of norlapachol on guinea pig ileum. Silva ACL[1], Vasconcelos LHC[1], Galvão JLFM[1], Ferreira PB[1], David CC[2], Camara CA[2], Cavalcante FA[3], Silva BA[4] [1]UFPB, [2]DCM-UFRPE, [3]DFP-UFPB, [4]DCF-UFPB

Introduction: Many pharmacological activities, such as spasmolytic, are related to lapachol and its natural and synthetic derivatives such as, norlapachol (CAVALCANTE *et al.*, Braz. J. Pharmacog., v.18b, p. 183, 2008; CAVALCANTE *et al.*, Z. Naturforsch., v. 65c, p. 627, 2010). From this perspective it was decided to investigate the possible effect spasmolytic of a new derivative of norlapachol, UFRPE 117 on isolated guinea pig ileum, as well as the mechanism of action of the compound. **Methods**: guinea pig ileum was suspended in organ bath containing modified Krebs solution (pH 7.4) at 37° C, gassed with 95 % O_2 and 5 % CO_2 mixture. Isometric and isotonic contractions were recorded. All experimental protocols were previously approved by Ethical Committee on Animal Use of CBiotec/UFPB (Protocol 0805/13). **Results:** UFRPE 117 antagonized phasic contractions induced by carbachol (CCh) 10^{-6} M (IC_{50} = 4.2 ± 0.6x10^{-5} M) or histamine 10^{-6} M (IC_{50} = 4.6 ± 0.6 x 10^{-5} M) and relaxed the ileum pre-contracted with KCl 40 mM, (EC_{50}= 3.3 ± 0,8 x 10^{-5} M and E_{max} = 91.6 ± 3.7%) or histamine (EC_{50} = 4.2 ± 0.9 x 10^{-6} M and E_{max} = 100%) in a concentration-dependent manner. UFRPE 117 also inhibited the cumulative curves to histamine, shifting them to right and in a nonparallel manner with E_{max} reduction. The spasmolytic effect (EC_{50} = 4.2 ± 0.9 x 10^{-6} M) was attenuated significantly in the presence of CsCl 5mM (CE_{50} = 5.1 ± 0.7 x 10^{-5} M), a non-selective blocker of K^+ channels, and its relaxant curve was shifted to the right. Interestingly, the relaxant curve of UFRPE 117 was not altered in presence of glibenclamide, K_{ATP} blocker (EC_{50} = 4.6 ± 0.8 x 10^{-6} M) but in presence of TEA^+ 1mM, BK_{Ca} blocker (EC_{50} = 2.0 ± 0.4 x 10^{-5} M) and apamin 100 nM, SK_{Ca} blocker (EC_{50} = 1.7 ± 0.06 x 10^{-5} M), relaxation curve of UFRPE 117 was shifted to the right about 4 and 5 fold, respectively. **Discussion:** Once UFRPE 117 inhibited the phasic contractions induced by carbachol or histamine with the same pharmacological potency and relaxed the organ when he was pre-contracted by KCl 40 mM or histamine, were investigating its mechanism of action. How UFRPE 117 inhibited histamine-induced phasic contractions and relaxed the ileum pre-contracted by the same agonist with a higher potency, we evaluated the involvement of histamine receptors in this effect. In according to curves profile it is suggesting a noncompetitive antagonism pseudo irreversible type. Since the spasmolytic potency of this compound was reduced when the ileum was pre-contracted with KCl 40 mM, we investigated the involvement of K+ channels, interestingly, the relaxant potency of UFRPE was attenueted around 12 fold in CsCl presence, it indicates the positive modulation of K^+ channels by UFRPE 117. In the glibenclamide presence, UFRPE 117 relaxant potency was not altered, discarding the K_{ATP} involvement but in the 1 mM TEA^+ or apamin presence the UFRPE 117 relaxant potency was attenuated around 4 to 5 folds, respectively, confirming the involvement of BK_{Ca} and SK_{Ca}. So, UFRPE 117 seems to involve a non-selective activation of K+ channels (BK_{Ca} and SK_{Ca}) that indirectly block Ca_v leading to relaxation of guinea pig ileum. **Support**: CNPq, Capes, PIVIC, PgPNSB/CCS/UFPB

09.135

Antitumor agents from *Actinomadura* sp. recovered from marine sediment collected at St. Peter and St. Paul Archipelago (SPSPA). Nunes LF[1,2], Ferreira EG[1], Pires K[1,3], Costa JFT[4], Torres MCM[4], Jimenez PC[1,5], Silveira ER[4], Pessoa ODL[4], Costa-Lotufo LV[1,6]
[1]LABOMAR-UFC – Instituto de Ciências do Mar, [2]UECE, [3]IFSC, [4]UFC – Química Orgânica e Inorgânica, [5]Unifesp – Ciências do Mar, [6]UFC – Fisiologia e Farmacologia

Introduction: Actinomycetes take up a prominent position in research to prospect for compounds with biomedical properties, such as antibiotic, antitumor and immunosuppressive activities, along with enzymes and enzyme inhibitors of great importance to the pharmaceutical industry. **Aim:** The aim of this study was to prospect antitumor compounds by bioassay-guided fractionation of an ethyl-acetate extract obtained from an actonomycete strain of *Actinomadura* sp. (BRA-177) recovered from sediment collected in the vicinities of SPSPA. **Methodology:** Bacterial growth was conducted in 20 x 500mL of A1 broth (soluble starch, yeast extract and peptone) supplemented with KBr,Fe_2SO_4 and $CaCO_3$ (0.4%) for 15 days under agitation of 200rpm at 28°C in 2000mL Erlenmeyer flasks (total of 10L). After the incubation period, the culture was extracted with ethyl acetate (EtOAc) for 3 hours under agitation. The concentrated extract yielded 270mg and was fractionated on silica gel using solvents of different polarities..The obtained fractions were analyzed by TLC and pooled to generate 11 fractions (A-L), and the active fraction (BRA-177E) was further purified by reverse phase column (C-18) using as mobile phase MeOH/H_2O (9:10) followed by MeOH 100%, from which 6 sub-fractions were obtained. The molecular identification of the strain was based on 16S rRNA gene sequencing and comparison using the EzTaxon-e database. The extract and all fractions were screened for their anti-proliferative activity on the human tumor cell line HCT-116 (human colon carcinoma) using the MTT assay. **Results:** The molecular identification of BRA-177 was consistent with *Actinomadura* genus. The crude extract showed a powerful cytotoxic activity against tumor cell line, with an average IC_{50} value of 0.13 µg/mL. The fractions BRA-177D, BRA-177E, BRA-177F, BRA-177H, BRA-177I and BRA-177J showed potent cytotoxicity against tumor cells, with IC_{50} ranging between 0.08 to 9.07 µg/mL, where BRA-177H was the most active. The cytotoxic compound cyclononilprodigiosin was isolated from sub-fractions (BRA-177E3) obtained from fraction BRA-177E and showed IC_{50} value of 1.10 µg/mL against HCT-116 cell line. Cyclononilprodigiosin was also identified in the fraction BRA-177J, while another prodigiosin derivative, nonilprodigiosin, was identified in the fraction BRA-177H. Prodigiosin and related compounds are well-know tripyrrole red pigments with immunosuppressive and anticancer activities, highlightining the biotechnological potential of the *Actinomadura* strain recovered from St. Peter and St. Paul Archipelago. **Support**: FUNCAP, CNPq and Capes

09.136

A role for the nitric oxide-cGMP pathway in the hemodynamic and vascular responses to *Lachesis muta* (South American bushmaster) snake venom. Dias L[1], Rodrigues MAP[1], Inoue BR[1], Rennó AL[1], Panunto PC[1], Rodrigues RL[1], Melgarejo AR[2], Hyslop S[1] [1]FCM-Unicamp – Farmacologia, [2]IVB – Zoologia Médica

Introduction: Peruvian *Lachesis muta* (bushmaster) venom causes hypotension in anesthetized rats. In this work, we examined the possible mediators involved in this response and in the effects of this venom in rat isolated aortic rings. **Methods and Results:** Male Wistar rats (300-400 g) were anesthetized with isoflurane (1.5% in air); the left carotid artery was cannulated for blood pressure measurement and a femoral vein was cannulated for venom injection (1.5 mg/kg; this dose caused significant hemodynamic alterations without killing the rats and was chosen based on initial experiments with 1, 1.5, 3 and 6 mg/kg). For experiments with isolated vessels, the thoracic aorta was removed from rats and placed in Krebs-Henseleit solution (composition, in mM: NaCl 125, KCl 4.8, $NaHCO_3$ 25, KH_2PO_4 1.2, $MgSO_4$ 1.2, glucose 11, EDTA 0.3, $CaCl_2$ 2.5, pH 7.4) continuously oxygenated with 5%CO_2-95%O_2 at 37 ºC. The vessels were cleaned of adipose and connective tissue and cut into 2-mm-long rings; in some rings, the endothelium was removed mechanically. The rings were mounted under a resting tension of 20 mN in 10 mL organ baths containing aerated Krebs-Henseleit solution at 37 ºC. The experiments were approved by an institutional Committee for Ethics in Animal Use (CEUA/Unicamp, protocol no. 2182-1). *Lachesis muta* venom was obtained from Centro de Extração de Toxinas Animais (Morungaba, SP, Brazil). The results (mean ± SEM) were analyzed using ANOVA followed by the Tukey-Kramer test, with $p<0.05$ indicating significance. Venom caused immediate hypotension that was maximal after 5 min [mean arterial blood pressure fell from 98.0 ± 5.9 to 41.2 ± 1.7 mmHg; n=7; $p<0.05$] but gradually returned to baseline over 60 min, decreasing again at 120 and 240 min. There were no significant changes in heart rate or respiratory rate. All rats injected with venom survived until the end of the experiment. Pretreatment with N^{ω}-nitro-L-arginine methyl ester (L-NAME, non-selective inhibitor of nitric oxide synthase; 20 mg/kg, i.v., 20 min prior to venom) and ODQ (soluble guanylate cyclase inhibitor; 5 mg/kg, i.v., 20 min prior to venom) potentiated the hypotension and lethality of the venom (mean time to death: 60 ± 1 min and 20 ± 1.5 min for L-NAME and ODQ, respectively; n=7/5). In contrast, SQ-22,536 (adenylate cyclase inhibitor; 5 mg/kg, i.v., 20 min prior to venom; n=6) had no effect on venom-induced hypotension or lethality. In aortic rings with endothelium (E +) *L. muta* venom (200 μg/mL) produced relaxation (18.0 ± 2.8%; n=6) that was significantly greater ($p<0.05$) than in rings without endothelium (E-) (6.0 ± 2.0%; n=6). Incubation with ODQ significantly attenuated the relaxation in E + rings (6.0 ± 0.8%; n=5; $p<0.05$). In contrast, preincubation of E + rings with H89 (a selective protein kinase A inhibitor) had no effect on venom-induced relaxation (19.0 ± 6.4%; n=6). **Conclusion**: In rats, *L. muta* venom causes hypotension predominantly by affecting the vasculature, with little cardiac involvement. The enhanced hypotension and greater lethality seen with L-NAME and ODQ *in vivo* and the inhibition of relaxation by ODQ *in vitro* indicate that the NO-cGMP signaling pathway has a modulatory role in venom-induced hypotension and vasorelaxation. The cAMP-PKA signaling pathway is apparently not involved. **Financial support:** Capes, CNPq, Fapesp.

09.137

Study of potential analgesic/anti-inflammatory ethanolic extract from different parts of the species *Plectranthus neochilus*. Trindade S, Azeredo JA, Cevada BA, Calheiros AS, Bozza PT, Castro-Faria-Neto HC, Frutuoso VS IOC-Fiocruz – Imunofarmacologia

Introduction: *Plectranthus neochilus* (Pn) is a plant species of medicinal use belonging to the family Lamiaceaes widely used in folk medicine for the treatment of liver failure and dyspepsia. Initial results showed that the ethanol extract of *Plectranthus neochilus* presents a significant analgesic/anti-inflammatory effect but there is no major scientific evidence supporting its use in the literature. For this purpose, our goal is to evaluate the analgesic/anti-inflammatory potential of the ethanol extract of *Plectranthus neochilus* from differents parts of the plant species. **Methodology:** To investigate the activities described above ethanol extracts from leaf (PnF) and branch (PnG) plant species *Plectranthus neochilus* were used. The cytotoxicity of differents concentrations of the extracts was performed by MTT assay (3 – (4,5-dimethylthiazol-2-yl) 2,5-diphenyltetrazolium bromide). The concentration of NO (nitric oxide) was determined using the technique of Griess in supernatant of peritoneum macrophages stimulated with lipopolysaccharide (LPS 500 ng/ml). In the same material we analyzed IL-6 and TNF-α (ELISA). The analgesic effect was evaluated by writhing model induced by acetic acid (10µL 0.8%/g) in Swiss mice (CEUA 033/09) pretreated (p.o.) 1h before of acid. **Results**: In the assessment of cytotoxicity was possible to verify that concentrations of 10 µg/mL, 1 µg/mL, 0.1 µg/mL, 0.01 µg/mL and 0.001 µg/mL of extracts, derived from the leaf (D.O. medium: 0.554; PnF10: 0.576) and branch (D.O. medium: 0.554; PnG10: 0.514) showed no cytotoxic effect on peritoneum macrophages due to maintenance of mitochondrial activity. Extracts by itself did not induce NO production for both leaf (control: 19.6 mM; PnF 10: 12.8 mM) and branch (control: 53.4; PnG 10: 21.1 mM) when compared to controls, also did not inhibited production when cells were pretreated with extracts and stimulated with LPS (LPS: 208.8 mM; PnF10 + LPS: 188.4 mM; PnG10 + LPS: 232.9 mM). The ELISA assay showed that the pretreatment with PnF and PnG extracts in macrophages stimulated with LPS was not show difference at concentration of IL-6 (leaf- control: 0.21; PnF10: 0.12; LPS: 4.95; PnF10 + LPS: 4.15 ng/mL; branch- control: 0.16; PnG10: 0.08; LPS: 1.43; PnG10 + LPS: 1.14 ng/mL) and TNF-α (leaf- control: 0.66; PnF10: 0.56; LPS: 2.37; PnF10 + LPS: 1.25 ng/mL; branch-control: 0.87; PnG10: 0.67; LPS: 2.72; PnG10 + LPS: 2.20 ng/mL) after 24 and 48 hours of stimulation. The evaluation of analgesic activity showed that treatment with the extracts at a concentration of 100 mg/kg led to a significant reduction in the number of writhings compared to the saline treated group (saline: 58 ± 7.8; PnF100: 28 ± 3.6; PnG100: 29.4 ± 6.71 – n = 6). **Conclusion**: Based on these data, we suggest that the ethanol extract of *Plectranthus neochilus* (leaf and branch) has a propensity to analgesic/anti-inflammatory activity, as evidenced by the writhing model. *in vitro*, we found that the extracts showed no cytotoxic effect on peritoneal macrophages, without altering the production of NO and pro-inflammatory cytokines IL-6 and TNF-α. **Financial support:** CNPq, PIBIC, Fiocruz/IOC.

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Assessment of cytotoxicity of polar and nonpolar fractions obtained from the latex of the plant *Synadenium umbellatum* in C6/36 cells. Ferreira PAB, Silva TFB, Penha JR, Pereira AS, Moreli ML, Gaban L, Ramos CDL UFG

Introduction: Dengue in a serious public health problem, because it affects millions of people throughout the world, and can lead to death. In Brazil, a dengue disease is one of a major public health problem. This disease can be caused by four serotypes of virus dengue (DENV1-4) infection. The virus is transmitted to humans by the bite from infected mosquitoes from species *Aedes aegypti* and *A. albopictus*. Actually, there is not approved vaccine or medicine for disease control. The vector control also depends on the conscientization of the population to suppress the spread of focus of mosquitoes, such as standing water. Beyond that, the dengue combat also depends of vector control by the use of insecticides and larvicides. However, these products can cause several harm to human health. Furthermore, the vectors may develop resistance against insecticides. The plant *S. umbellatum*, belong to Euphorbiaceae family, popularly known as "cola-nota", is popularly used for treatment of several diseases, such as infections or inflammatory conditions. The *Synadenium's* latex is used as a self mechanism of defense against insects, which presented cytotoxicity activity against them (Wahler, D. *Plant Physiol*, 151(1):334, 2009). This latex, in addition to protecting the plant, is also rich in bioactive compounds, which in recent studies demonstrated cytotoxic activity in mammalian cells (Cunha, L.C. *Braz J Pharmacog*, 19(2A):403, 2009). Considering that, this study sought to evaluate whether the polar and apolar latex fractions play a role against clone cells of *A. albopictus*, (C6/36). **Methods:** In order to observe if the polar (ethyl acetate and etanol) and nonpolar (chloroform and hexane) *S. umbellatum* latex fractions would be cytotoxic to C6/36 cells, they were exposed to different concentrations (50, 5, 0.5, 0.05 µg/mL) of the each fractions in the time of 2 h (non-adherent cells) and 48 h (adherent cells). After incubation in the specified time, the culture supernatant (2 h) or plate attached cells (48 h) were collected and stained with trypan blue. Negative control was performed by incubating these cells with culture medium only (L15). **Results and discussion:** The results showed that exposure to different concentrations of the polar and nonpolar fractions promoted cell death in acute situation (2 h), because they were potentially cytotoxic allowing a null cell viability when compared to the control that allowed an average of 91,33 ± 3,18 viability. In this time, the results shown that the four latex fractions tested prevented the attachment of the cells to the plate being highly cytotoxic to C6/36 cells. The chronic exposition (48 h) demonstrated that, when compared to control, ethyl acetate, etanol and chloroform fractions showed a statistically significant cytotoxicity. Nevertheless, the ethyl acetate fraction in the 0.05 µg/mL concentration and the all concentrations tested of the hexane fraction were not statistically significant when compared to the control group. Accordingly to this finding, the compounds present in the tested fractions provides a solid finding that support the subsequent studies aimed at dengue vector control. **Acknowledgement:** FAPEG.

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Viscoelastic properties of cells exposed to synthetic natriuretic peptide of *Crotalus durissus cascavella* venom. Neto JO[1], Ferreira MZJ[2], Silveira JAM[3], Monteiro HSA[3], Alencar AM[4], Evangelista JSAM[1] – [1]FV-UFC – Histology of Snake Venoms and Toxic Plants, [2]FEEC-Unicamp – Micro-Wave and Optics, [3]UFC – Farmacology of Venoms and Toxins, [4]IF-USP – Molecular Physiology and Microreologia

Introduction: The serpent *Crotalus durissus cascavella* is commonly found in the caatinga region in northeast Brazil. Several biologically active substances with different pharmacological activities are present in the venom produced by this and other serpents and among those is the Natriuretic Peptide (*NPCdc*). **Methods:** This rheological study was performed in Rat Aortic Smooth Muscle (RASM) cells which were exposed to the *NPCdc* and measurements were performed using the Optical Magnetic Twisting Cytometry (OMTC) method, which quantifies the elastic and viscous properties of the cytoskeleton. Cells were cultures in DEMEN with 10% fetal bovine serum and 100U/mL of penicillin. For the OMTC experiments, cells were conditioned in 96-well plates previously coated with porcine collagen at 8ºC. At 24h after plating, ferromagnetic microspheres of 4.5µm diameter were covered by a RGB sequenced peptide in order to the coupling with the integrin receptors at the cell surface could occur. A magnetizing system computer controlled initially magnetizes horizontally the microspheres and then the measurements are taken with a vertical homogenous magnetic field being applied which varies temporally in a sinusoidal form, generating torque. This equipment is attached to the platform of an inverted microscope with a video camera and a charge-coupled device (CCD). The magnetized microsphere movement is modulated by the viscoelastic properties of the cytoskeleton. An effective viscoelastic module $G^*(\omega)$ was calculated with the torque applied $T^*(\omega)$ and the resultant dislocation $d^*(\omega)$ of the microspheres, as demonstrated: $G^*(\omega) = G'(\omega) + j\, G''(\omega)$; where $G'(\omega)$ is the defined storage module related with the energy stored by the cell, $G''(\omega)$ is the loss module related with the dissipated energy within a cycle. **Results:** Three doses of *NPCdc* were used and for each the respective storage $G'(\omega)$ and loss $G''(\omega)$ were calculated. At the dose of 3.10^{-6} a $G'(\omega) = 0.1916$ and $G''(\omega) = 0.03727$ were found; at 1.10^{-5} resulted in $G'(\omega) = 0.29115$ and $G''(\omega) = 0.11277$; and at 3.10^{-5} the values of $G'(\omega) = 0.27254$ and $G''(\omega) = 0.03706$ were found. **Discussion:** There was an increase both in $G'(\omega)$ and in $G''(\omega)$ when the doses of 3.10^{-6} and 1.10^{-5} were compared, however the dose of 3.10^{-5} a considerable reduction in $G''(\omega)$ and a discrete reduction in $G'(\omega)$ was observed, which results in a reduction of $G^*(\omega)$ at this last dose. In conclusion, there are evidences that *NPCdc* acted in the lower dose as a constrictor and in higher dose with a relaxing effect, however the low amount of samples was insufficient for such a Conclusion: Therefore, further studies with this isolated substance using the OMTC method are necessary in order to better elucidate how it functions and its physiological characteristics. **Acknowledgments:** Capes, CNPq, Fapesp.

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Spasmolytic action of *Solanum paniculatum* L. on guinea pig ileum involves modulation positive of K^+ channels. Pereira JC[1], Vasconcelos LHC[1], Souza ILL[1], Ferreira PB[1], Sampaio RS[1], Araujo LCC[1], Silva TMS[2], Cavalcante FA[3,1], Silva BA[1] [1]UFPB, [2]DCM-UFRPE, [3]DFP-UFPB,

Introduction: *Solanum paniculatum* L. (Solanaceae) is used in folk medicine as antiinflammatory, diuretic and gastrointestinal disordens (RODRIGUES, Ciênc Agrotec, v.25, p.102, 2001). Clementino-Neto (Monografy, UFAL, 2012) showed spasmolytic and antidiarrheal activities for ethanolic extract from aerial parts of this species (SP-$EtOH_{AP}$). Thus, we aimed to investigate the spasmolytic activity of SP-$EtOH_{AP}$ on smooth muscle models (rat uterus, rat aorta and guinea pig trachea) and characterize its action mechanism on guinea pig ileum. **Methods:** the organs were suspended in organ baths on appropriate conditions. Isotonic and isometric contractions were registered. All experimental protocols were previously approved by Ethical Committee on Animal Use of CBiotec/UFPB (Protocol 0905/13). **Results:** SP-$EtOH_{AP}$ (243-729 µg/mL, n = 3) relaxed pre-contracted rat aorta with phenylephrine 3 x 10^{-7} M both in endothelium presence (E_{max} = 59.3 ± 7.1%) and absence (E_{max} = 58.3 ± 6.7%) as well as relaxed pre-contracted guinea pig trachea with carbachol (CCh) 10^{-6} M both in epithelium presence (E_{max} = 56.8 ± 2.7%) and absence (E_{max} = 30.0 ± = 1.5%). However, had no tocolytic effect on phasic contractions induced by oxytocin 10^{-2} IU/mL or CCh 10^{-5} M on rat. SP-$EtOH_{AP}$ (0.1-729 µg/mL, n = 5) relaxed pre-contracted guinea pig ileum with KCl 40 mM (EC_{50} = 47.5 ± 4.4 µg/mL), CCh 10^{-6} M (EC_{50} = 49.9 ± 7.3 µg/mL) or histamine 10^{-6} M (EC_{50} = 65.0 ± 5.3 µg/mL). Extract (27-729 µg/mL, n = 5) inhibited $CaCl_2$-induced cumulative curves in a depolarizing medium nominally without Ca^{2+}, shifting them to right in a non-parallel manner. SP-$EtOH_{AP}$ relaxant potency (EC_{50} = 65.0 ± 5.3 µg/mL) was reduced in CsCl presence, a non-specific K+ channel blocker (EC_{50} = 89.0 ± 3.0 µg/mL). **Discussion:** Clementino-Neto (2012) showed SP-$EtOH_{AP}$ antidiarrheal activity in mice and spasmolytic effect on guinea pig ileum, so we decided to expand these studies to other smooth muscle models. SP-$EtOH_{AP}$ did not presented tocolytic effect, but presented spasmolytic effect on other tested organs with low efficacy. When compared to previous results, the extract showed a selective spasmolytic action on guinea pig ileum. The next step carried out was elucidate the action mechanism underlying on this effect. Since on guinea pig ileum, the mechanisms involved on tonic and phasic phases of contraction are different (ABDELLATIF, Life Sci, v.45, p.757, 1989), we verified if SP-$EtOH_{AP}$ would relax pre-contracted ileum. The extract relaxed the pre-contracted ileum with KCl, CCh or histamine in an equipotent manner, indicating that SP-$EtOH_{AP}$ possibly acts on a common pathway, for example, voltage-gated Ca^{2+} channels (Ca_V). The $CaCl_2$ curves were shifted non-parallelly to right with E_{max} reduction, but without concentration dependence, suggesting a Ca_V indirect blockade. Since Ca_V could be modulated by K+ channels (THORNELOE, Can *J Physiol* Pharm, v.83, p.215, 2005), these channels were evaluated. Interestingly, SP-$EtOH_{AP}$ spasmolytic potency was attenuated in CsCl presence, confirming K+ channels participation. Finally, SP-$EtOH_{AP}$ showed to be a selective spasmolytic agent on guinea pig ileum what corroborate its medicinal indications to gastrointestinal disorders. **Financial support:** CNPq, Capes, PgPNSB/CCS/UFPB

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Evaluation of antibacterial and antifungal activity of chromatographic fractions obtained from the latex of *Synadenium umbellatum* Pax plant. Martins TMM[1], Gaban L[2], Braoios A[3], Ramos CDL[4] [1]LSC, [2]UFG – Patologia, [3]UFG – Microbiologia, [4]UFG – Farmacologia

Introduction: The use of medicinal plants for the treatment of several diseases is an ancient practice of humanity, and this practice currently known as phytotherapy (Zavariz, A. *Laes & Raes*. 205:162, 2013). An example is the antimicrobial activity own from some plants species, such as against deleteriously pathogenic microorganisms that may be harmful to human health. Among the plants used in the Brazilian herbal medicine belonging to Euphorbiaceae family The *Synadenium* plant genus that belongs to the this family is used by local population for several bacterial infections conditions (Costa, L.L.G. *Braz. J. Pharmacog*. 22(5):1070, 2012; Mota, M.F. *Braz. J. Pharmac. Sci.* 48(3): 497, 2012). *S. umbellatum* is widely used by the population situated in West-Central Brazil to control bacterial infections. However, there is no scientific evidence of any antibacterial effect (Cunha, L.C. *Braz. J. Pharmacog*. 19(2A):403, 2009). **Objective:** Investigate the antimicrobial activity of chromatographic latex fractions (hexane, chloroform, ethyl acetate and ethanol) belongs from *S. umbellatum*. **Methods**: The methodology applied was the Bauer-Kirby method to evaluate the antibacterial activity and Bauer-Kirby modified method for evaluating the antifungal activity. Briefly, experimental filter paper discs (6 mm diameter) impregnated with the chromatographic fractions from latex collected from *S. umbellatum* (hexane, chloroform, ethyl acetate and ethanol), in the concentrations of 500, 50 and 5 µg/disc. As positive control was used commercial discs impregnated with chloramphenicol (30 µg/disc). As negative controls was used filter paper without awareness and discs with brine and chloroform. The bacterial strains used were: *Serratia marcescens* (ATCC 8100), *Acinetobacter baumannii* (ATCC 19606), *Staphylococcus saprophyticus* (ATCC 15305), *Staphylococcus epidermidis* (ATCC 12228), *Proteus mirabilis* (ATCC 21100), *Salmonella typhimurium* (ATCC 14028). The fungus species used were: *Candida albicans* (ATCC 40175), *Candida tropicalis* (ATCC 13803) and *Candida parapsilosis* (ATCC 22019). The evaluation of the results was measured using the zone of inhibition of bacterial or yeast growth after 24 and 48 h of incubation in a microbiological greenhouse. **Results:** All positive control tested formed inhibition zone the growth of microorganisms, as expected. The inhibition zone diameter were measured and expressed as arithmetic means. In the other way, the latex fractions impregnated disks did not demonstrate any formation of halo of inhibition of bacterial or yeast growth in the tested concentrations. **Conclusion**: Even though the pure latex and the fractions of latex extracted from *S. umbellatum* not shown antimicrobial activity to herein tested strains, this study aimed to provide data for the research of herbal compounds widely used by population. Under the conditions tested, this work contributed to the demystification of the popular use of this plant for bacterial and fungal infections caused by the microorganisms strains here tested. We add that the study of the antimicrobial activity of the latex of this plant, using other microbiological techniques and others different bacterial or yeast strains are needed. **Financial support:** FAPEG

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Effect of centuroides Margaritatus scorpion venom on cardiac hemodynamics in anesthetized rats. Silva APG[1], Bindá AH[2], Valencia JMB, Vidal JTB[3], Cabral PHB[2], Nascimento NRF[1], Fonteles MC[1], Santos CF[1] [1]ISCB-UECE, [2]UFC – Farmacologia, [3]UniCauca – Farmacologia

The *Centuroides margaritatus* (Gervais, 1841) scorpion belongs to the Buthiadae family, the most numerous and widely distributed group of modern scorpions. *Centruroides margaritatus* is about 5 to 8cm long including the telson, and is native to Mexico, Central America and Northern South America (Venezuela, Colombia and Ecuador). The venom samples used in the present study were obtained from specimens of the region of Cauca (Colombia). A toxin isolated from *C. margaritatus* venom, Margotoxin, is a specific inhibitor of voltage-gated potassium channels (Kv1.1 Kv1.2 and Kv1.3). Kv1.2 is considered important for the repolarization phase of the cardiac action potential. This study was designed in order to evaluate the effects of the crude venom on the cardiac eletromechanical and hemodynamic parameters. All the protocols were approved by the Animal Ethics Committee of the Ceara State University under the protocol number 11221997-7/45. The crude venom was administered as intravenous bolus (1,10,100 µg) to pentobarbital anesthetized rats that were instrumentalized with microtip pressure-volume catheters. The crude venom increased the maximal left ventricle pressure (Pmax) of 8 ± 1%, 20.8 ± 4.2% and 41.8 ± 4.9%, respectively. Similarly, there was an increase in Dp/Dt max on the order of 16.2 ± 6.6%, 54.2 ± 9.9%, 83.4 ± 10.9% ($p<0.001$). On the order hand, these effects were accompanied by a slight drop in heart rate (around 8%) and an 18% increase in cardiac output. The venom induced, at 200µg, premature ventricular complexes. The crude venom of *Centruroides margaritatus* has a dose-related positive inotropic effect that is not related to increased heart rate. Higher doses induce arrhythmias. **Acknowledgment**: CNPq

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Evaluation of the leishmanicide activity and toxicity of bixin concentrate obtained of *Bixa Orellana L.* Vilar DA[1], Soares MSAV[1], Brito MT[2], Gonzaga JCO[3], Guimarães ARBV[3], Néris PLN[2], Oliveira MR[2], Barbosa filho JMB[2], Sobral MV[2] – [1]UFRN – Desenvolvimento e Inovação Tecnológica de Medicamento, [2]UFPB – Produtos Naturais e Sintéticos Bioativos, [3]UFPB – Farmácia

Introduction: The study of new therapies for the treatment of leishmaniasis has become important due to its high incidence, undesirable effects, high cost, parenteral, or teratogenicity of drugs that exist today (Kafetzis *et al.*, IJ AA, v.25, p .26-30, 2005). In this regard, bixin rich fraction obtained from *Bixa Orellana L.*, was tested against their potential for antileishmanial the development of a phytotherapy medicament.
Objectives: To evaluate the anti-leishmania activity and toxicity *in vitro* (in murine macrophages) and *in vivo* (oral acute toxicity) of bixin concentrate (BC) from *Bixa orellana L.* **Methods**: BC used was obtained by extraction with chloroform of the seeds of *Bixa orellana L.*, which had been extracted with hexane (Barbosa-Filho *et al.* RBF. v.7/8, p.41-47,1998). To assessment of the leishmanicide activity, the promastigotes of *Leishmania major* in log growth phase ($1x10^6$ cells/mL) were incubated in the presence of different concentrations BC, Glucantime ® (300 mg/ml) and Amphotericin B ® (1mg/mL). After 72 h, aliquots were removed and quantified to calculate the 50% inhibitory concentration (IC50). Cytotoxicity in murine macrophages (1x106 cells) was evaluated using the method of trypan blue, and the concentration which reduces cell viability of 50% (CC50) was calculated. For *in vivo* evaluation, female mice (*Mus musculus*) weighing 28-32 g were used, and a single dose of 2000 mg/kg (p.o., n=6) was given, in accordance with nº 423 of OECD/2001 protocol, with modifications. General behavior adverse effects and mortality were assessed over 14 days, as well as body weight, water consumption and feed, hematological, biochemical parameters and organ indexes. The indexes were obtained *in vitro* by Probit analysis (SPSS® program) (Amorim *et al.* Paras., v.140, n.1, p 29, 2013). In the case of *in vivo*, the results were expressed as mean ± standard error of the mean (SEM) and analyzed by Student's unpaired t test, followed by Mann-Whitney, and significant differences when $p<0.05$. All experiments were approved by the ethical committee for animal research of CBiotec / UFPB under CEPA No 0806/11. **Results**: BC inhibited the growth of *L. major* cultures at all concentrations tested, with IC50 for promastigotes of 2.16 ± 1.67 (µg/mL). The CC50 to macrophages was estimated to be 59.51 µg/ml. Regarding *in vivo* toxicity, there was evidence of depressive activity of the central nervous system, through the first 60 minutes of evaluation (ptosis, sedation and anesthesia), and effects on the autonomic nervous system (increased defecation) after administration of the sample. The LD50 was estimated to be greater than 2000 mg/kg. There were no clinically relevant changes in the evaluated parameters.So it is suggested low acute oral toxicity in the sample. **Conclusion**: BC showed significant activity against *Leishmania* promastigotes but high cytotoxicity, thereby decreasing its selectivity index. However, *in vivo* it was presented a low oral toxicity of the substance when administered to mice. **Financial support**: CNPq and Capes.

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Vasorelaxant effect of gallic acid in rat thoracic aorta. Oliveira LM, Oliveira TS, Bastos AM, Costa EA, Filgueira FP, Ghedini PC UFG – Ciências Fisiológicas

Introduction: Gallic acid (GA) is an organic acid found in foods such as blueberries, apples, flaxseeds, tea leaves, oak bark, walnuts and watercress. Studies showed that GA presents several pharmacological activities, such as anti-inflammatory, antimutagenic, anticancer, antioxidant and vasorelaxant effects (Gil-Longo *et al*., *J. Nutr. Biochem*. 21, 304, 2010). The present study was performed with the aim to characterize the action mechanisms involved in the vascular effect of GA. **Methods:** The vasorelaxant effect of the GA (0,4mM – 10mM) was evaluated in the preparations of the thoracic aorta rings from female Wistar rats (200-300g; n=5-7). 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). Data are presented as mean ± SEM of 5-7 experiments and compared by Student's t-test or one-way ANOVA when appropriated. P values less than 0.05 were considered significant. **Results and discussion:** The GA evoked concentration-dependent relaxation in aortic rings that were precontracted with phenylephrine. Aortic relaxation induced by GA was reduced by endothelium removal and by incubation of L-NAME (100μM) (a nitric oxide synthase inhibitor), ODQ (10μM) (a soluble guanylate cyclase inhibitor), calmidazolium (30μM) (a calmodulin inhibitor), TEA (1mM) (a non-selective K+ channel blocker), 4-aminopyridine (10μM) (a blocker of the voltage-activated K+ channel), and by chloride barium (10μM) (a rectifier K+ channel blocker). However, the incubation with wortmannin (30nM) (phosphatidylinositol 3-kinase – PI3K inhibitor), PP2 (10μ) (Src kinase inhibitor), glibenclamide (10μM) (ATP-sensitive K+ channels blocker), or indomethacin (10μM) (a cyclooxygenase inhibitor) had no effect on the GA-induced vasorelaxation. Taken together, the results reveal that GA induced endothelium-dependent and independent relaxation in the rat thoracic aorta and the vascular effect of GA involves stimulation of the nitric oxide/cyclic GMP pathway, activation of calmodulin and potassium channels. **Financial Support:** Capes, FAPEG, CNPq.

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Bothrops fonsecai snake venom toxicity and the use of commercial antivenom as a pharmacological tool. Collaço RCO[1], Tamascia ML[1], Silva IRF[1], Cogo JC[2], Rocha T[3], Hyslop S[1], Sanny CG[4], Randazzo-Moura P[5], Rodrigues-Simioni L[1] – [1]Unicamp – Farmacologia, [2]Univap – Estudos da Natureza, [3]USF, [4]OSU – Health Sciences, [5]PUC-SP – Farmacologia

Introduction: *Bothrops fonsecai* is a pitviper with a restricted distribution in montane regions in southeastern Brazil and its venom contains phospholipases A_2, metalloproteases, serine proteases and other toxin classes common to *Bothrops* venoms. Whereas the general biochemical composition of this venom is characterized, less is known of the biological activities and their neutralization by commercial equine antivenom (CAV). In this work, we examined the biological activities of *B. fonsecai* venom and their cross-reactivity with and neutralization by CAV. **Methods:** The neuromuscular effects and myotoxicity were assessed using isolated mouse extensor digitorum longus (EDL) preparations. Myotoxicity *in vivo* was examined in mouse gastrocnemius muscle. Hemorrhagic and edema-forming activities were assayed in rat dorsal skin and coagulant activity was assessed in poor-platelet plasma. PLA_2, proteolytic and esterase activities were assayed using phosphatidylcholine, casein and TAME, respectively. The neutralizing capacity of CAV (raised against a pool of venoms from *Bothrops jararaca*, *B. jararacussu*, *B alternatus*, *B neuweidi* and *B. moojeni*) was tested at venom:antivenom ratios of 5:1 (manufacturer's recommended ratio) and 5:2. Cross-reactivity with CAV was assessed by immunoblotting and screening of the SE-HPLC elution profile for immunoreactivity of the peaks with antivenom. The protocols involving animals were approved by an institutional Committee for Ethics in Animal Use (CEUA/Unicamp, protocol no. 2648-1). **Results**: *Bothrops fonsecai* venom had high PLA_2 activity that probably contributed to the neuromuscular blockade, myotoxicity and edema formation. PLA_2 activity, neuromuscular blockade and edema were partially neutralized by CAV at both ratios tested whereas myotoxicity was completely neutralized. The venom had low proteolytic activity that was not significantly inhibited by CAV. In contrast, hemorrhagic activity was totally neutralized by CAV. The esterase and coagulant activities of the venom were neutralized at both venom:antivenom ratios, particularly at the ratio of 5:2. The small reactivity between *B. fonsecai* and CAV were confirmed by SE-HPLC and immunoblotting when compared with *Bothrops jararaca* venom and specific antivenom; both assays showed than the CAV does not react with substances low molecular mass substances, as phospholipases and P-I metaloproteases. **Conclusion**: This results showed that CAV has low reactivity with *Bothrops fonsecai* venom showing that this venom differs from *Bothrops* venoms used to produce the CAV specially metalloproteases P-I and phospholipases A_2. **Financial support:** Fapesp, CNPq, Unicamp.

09.146

Spasmolytic effect of galetin 3,6-dimethyl ether involves histaminergic receptor antagonism and Ca^{2+} influx blockade on guinea pig ileum. Medeiros MM[1], Vasconcelos LHC[1], Souza ILL[1], Silva MCC[1], Araujo LCC[1], Ferreira PB[1], Santos BVO[2], Cavalcante FA[3], Silva BA[2] [1]UFPB, [2]DCF-UFPB, [3]DFP-UFPB

Introduction: *Piptadenia stipulacea* (Benth.) Ducke (Fabaceae) ia a tree from the Brazilian northeastern. In earlier studies, the flavonoid galetin 3,6-dimethyl ether (FGAL), obtained from aerial parts of this species, showed spasmolytic activity on carbachol- or histamine-induced phasic contractions on guinea pig ileum (MACEDO, J. Smooth Muscle Res., v. 47, p. 123, 2011). Thus, in this work was investigated the mechanisms underlying the spasmolytic effect of FGAL. **Methods:** ileum segments were suspended in organ baths, kept in modified Krebs solution at 37 °C, aerated with carbogen mixture under 1 g of resting tension. Isometric and isotonic contractions were registered. All experimental protocols were approved by Ethical Committee on Animal Use of CBiotec/UFPB (Protocol 0705/13). **Results:** FGAL (10^{-10} to 10^{-4} M, n = 5) relaxed the ileum pre-contracted with KCl 40 mM (EC_{50} = 2.6 ± 0.5 x 10^{-6} M), CCh 10^{-5} M (EC_{50} = 1.8 ± 0.4 x 10^{-6} M) or histamine 10^{-6} M (EC_{50} = 1.9 ± 0.4 x 10^{-7} M). The flavonoid (3 x 10^{-6} to 10^{-4} M, n = 5) rightward shifted the concentration-response curves for histamine, in a nonparallel manner, with reduction of the maximum effect (E_{max}). Moreover, FGAL (3 x 10^{-6} to 10^{-4} M, n = 5) inhibited the $CaCl_2$-induced cumulative-contractions, in depolarizing medium (KCl 70 mM) nominally without $Ca^{2+,}$ and rightward shifted these contraction curves, in a nonparallel manner, as well as the flavonoid (10^{-10} to 10^{-4} M, n = 5) relaxed the ileum pre-contracted with S-(-)-Bay K8644 3 x 10^{-7} M, a Ca_V1 agonist. **Discussion:** flavonoids are a wide class of plant secondary metabolites, for which has been described spasmolytic activity on intestinal smooth muscle (GHARZOULI, Pharmacology, v. 70, p. 5, 2004). Thus, it was appropriate to investigate the mechanism underlying the spasmolytic effect of FGAL on guinea pig ileum. Based on EC_{50} values, FGAL showed the same relaxant potency on ileum pre-contracted with both CCh or KCl, but presented higher potency when histamine was employed as contractile agent. Front to these results, it was decided to verify whether the flavonoid would be antagonizing histaminergic receptors. The profile of the FGAL-induced inhibition of histamine-induced cumulative contractions indicates a pseudo-irreversible noncompetitive antagonism, since histamine had its E_{max} abolished by the flavonoid (MAY, Annu. Rev. Pharmacol. *Toxicol*, v. 47, p. 1, 2007). Ileum contraction is totally dependent of the potential membrane variation, and the tonic component of its contraction is maintained almost exclusively by Ca^{2+} influx through Ca_V (REMBOLD, Biochemistry of Smooth Muscle Contraction, p. 227, 1996). So, it was hypothesized that FGAL could act blocking Ca^{2+} influx through Ca_V. Since FGAL inhibited the $CaCl_2$-induced cumulative contractions as well as relaxed the ileum pre-contracted with a Ca_V1 agonist, it indicates that FGAL attenuates Ca^{2+} influx through Ca_V1. Therefore, the spasmolytic effect FGAL on guinea pig ileum involves pseudo-irreversible noncompetitive antagonism of histaminergic receptors and attenuation of Ca^{2+} influx through Ca_V1, appearing as a promising drug for treatment of gastrointestinal disorders.

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Study of the gastroprotective activity of *Mauritia flexuosa* oil in acute model of ethanol-induced gastric ulcer in mice. Queiroz BCSH, Gomes AF, Sousa JA, Sousa GS, Neto BM FSA – Farmacia

Introduction: Buriti (*Mauritia flexuosa* L.), also known as coconut Buriti, miriti, Muriti, composes the vegetation of the flooded and humid regions of the Midwest, North and Northeast of Brazil (CUNHA *et al.*, 2012). Its pharmacological actions are attributed to the presence of carotenoids, fatty acids, flavonoids and tocopherol. Studies show the use of this product as an alternative therapy (ROSSO; MERCADANTE, 2007). It has, among other functions, the lubrication and regeneration of the skin hydrolipidic barrier frequently subjected to injuries, with healing effect on them, and antibacterial, antioxidant and anti-inflammatory effects (ZANATTA *et al.*, 2010). This study aimed to evaluate the gastroprotective activity of *Mauritia flexuosa* oil (MFO) in acute ethanol-induced gastric ulcer in mice of the species *Mus musculus*. **Methods**: This work was approved and recorded by the FSA Ethics Committee with Animal Research with the protocol number 01/2014. The first stage of the experiment consisted in the oil extraction from the zest of *Mauritia flexuosa*'s fruit, the Buriti, according to the methodology of Silveira *et al.* (2005), followed by completion of the acute gastric ulcers induced by ethanol protocol. As control groups were used saline (0.1 ml / kg) and cimetidine (1000 mg/kg) and Omeprazole (200 mg/kg), administered orally. At the end of each test, was performed euthanasia by cervical dislocation, preceded by the administration of anesthetic, thiopental sodium (30 mg/kg). The stomachs were removed, opened in the region of the greater curvature, fixed in petri dishes and drawn in transparency sheet, which were scanned for quantitation of the ulcerative lesions area (ULA), using the ImageJ software (ROBERT *et al.*, 1979). The obtained data were statistically analyzed by oneway ANOVA followed by Tukey's test, using the statistical program GraphPadPrism 6.0, and the results expressed as mean ± SEM (Standard Error of Mean). **Results:** It was observed that the MFO reduced the area of gastric mucosal injury. The oil's dose, 0.1 ml / kg, caused a reduction in ULA from 5.896 ± 0.598 mm^2 to 2.019 ± 0.584 mm^2 in control group that received saline. Animals receiving Cimetidine 1000 mg/kg had reduced lesion area to 2.534 ± 0.713 mm^2, and those that received Omeprazole 200 mg/kg reduced the area of injury to 1.216 ± 0.314 mm^2. **Discussion**: It is believed that the gastroprotective activity of MFO observed in this study is due to the large amount of phenolic compounds, especially flavonoids, in its composition, which have high antioxidant, anti-inflammatory and anti-ulcer activities proven. The inflamation caused by ethanol administration consists in an oxidative process that causes lesion on gastric mucosa, the MFO antioxidant compounds probably protected the mucosal tissue from injury. **Conclusion**: *From the results obtained in this study it can be concluded that the Mauritia flexuosa oil (MFO) at a dose of 0.1 mL/10g presented gastroprotective activity in acute model of gastric ulcers induced by absolute ethanol in mice, this having been activity, on average, higher than that of the H2 antagonist, cimetidine, at a dose of 1000 mg/kg, and smaller than the proton pump inhibitor, omeprazole, 200 mg/kg, compared to control (0.9% saline, 0, 1 mL / 10g).* **Agency Funding and Acknowledgements**: St. Augustine College, Teresina-PI. **References:** CUNHA, M. A. E.; NEVES, R .F.; SOUZA, J. N. S; FRANÇA, L.F.; ARAÚJO, M.E.; BRUNNER, G.; MACHADO, N. T. Supercritical adsorption of buriti oil (Mauritia flexuosa Mart.) in -alumina: A methodology for the enriching of anti-oxidants. Journal of Supercritical Fluids, v. 66, p.181-191, jun. 2012. ROBERT, A.; NEZAMIS, J. E.; LANCASTER, C.; HANCHAR, A. J. Cytoprotection by prostaglandins in rats. Prevention of gastric necrosis produced by alcohol, HCl, NaOH, hypertonic NaCl

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Functional chromatography as a strategy of target-directed prospection of bioactive natural compounds. Jimenez PC[1], Torres MCM[2], Pessoa ODL[2], Yeh K[3], Chapman E[3], La Clair JJ[4], Costa-Lotufo LV[5] [1]Unifesp – Ciências do Mar, [2]UFC – Química Orgânica e Inorgânica, [3]University of Arizona – Pharmacy, [4]Xenobe Research Institute, [5]UFC – Fisiologia e Farmacologia

Introduction: Natural products are an important spring of biologically active chemicals and remain as the leading source of novel pharmaceuticals. The oceans, a rather under-explored environment, are possibly the last frontier for the bioprospection of new chemical entities with biomedical potential. In this context, one search strategy involves that guided by a specific cellular protein or pathway as a biological target. Functional chromatography is a process of reverse affinity chromatography, which is applied, herein, as a new approach for target-directed screening of novel bioactive compounds. **Methods:** Briefly, recombinant target proteins, obtained by molecular cloning using an *E. coli* model, were incorporated into a resin, which, in turn, was incubated with a complex natural extract. Unbound compounds were washed away and the resin was extracted with ethanol to recover the retained material. The ethanolic extract was analyzed by LC-MS to identify signals of the molecules contained therein. These signals were translated in to molecular masses and compared in the AntiMarin database, in search of matches. In the present study, the cytotoxic acetate extract derived from the actinomycete strain BRA-213, recovered from the sediments surrounding the St. Peter and St. Paul Archipelago, was tested for its affinity to 6 proteins participating in different cellular pathways: the antioxidant repressors Keap1 and Kelch, the multifunction ATPase p97, and the histone deacetylases HSPA2, HSPA5 and HSPA8. **Results and discussion:** LC-MS analysis of the whole, pre-chromatography extract returned signals for about 83 compounds, while the material eluted from the resin bound with the Keap1 protein contained signals for 8 compounds, 7 of which were shared with that eluted from the Kelch resin. Four of these signals were matched in the AntiMarin database as romidepsin, malaysic acid, neomangicol B and PD 11.8576. The compound eluted solely from the Keap1 resin had an exact mass of 304.2998 and found no match in the database. The resins bound with the HSPs proteins retained, together, 17 compounds. The mass 453.1671, which corresponds to haterumalide X, was shared by all three. Masses 456.6017 and 498.2210 were shared between HSPA5 and HSPA8. The first mass showed no correspondence in the database, whereas the later matches sibiromycin. The resin bound to p97 retained 7 compounds, 4 of which were matched in the database. The unmatched mass of 414.3360 stood out as an interesting compound and was further investigated. A semi-purified fraction containing this mass was subjected to a 30 and 60 minutes p97 ATPase inhibition assay and showed IC_{50} of 31.78 and 34.78 ng/mL, respectively. **Conclusion:** It is clear that the next steps to be taken involve isolation of the retained compounds and validation of their modulatory effect upon the target using biological models. However, these preliminary results highlight the efficiency of this methodology in fishing out molecules from a complex crude extract that have affinity to a protein target and can be applied in bioprospection strategies of pharmacologically relevant natural products. **Support:** FUNCAP, Capes and CNPq.

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Cardiovascular actions of a cysteine-rich protein isolated from *Bothrops jararaca* snake venom. Tamascia ML, Silva IRF, Mocoso JAR, Antunes E, Hyslop S FCM-Unicamp – Pharmacology

Introduction: We have previously isolated and characterized an ~30 kDa non-hemorrhagic, fibrinogenolytic cysteine-rich enzyme from *Bothrops jararaca* venom that shows high homology with ablomin, a cysteine-rich venom protein from the Asian pitviper *Gloydius blomhoffii*. In this work, we investigated the myotoxicity of this protein and its action in isolated cardiac and vascular tissues. **Methods and Results:** All animals protocols were approved by the institutional Committee for Ethics in Animal Research (CEUA/Unicamp, protocol no. 2253-1). The protein was purified using a combination of gel filtration (Superdex 75) and ion-exchange chromatography (Q-Sepharose) and purity was confirmed by RP-HPLC and SDS-PAGE. Myotoxicity was assessed by injecting 40 μg of venom or protein (in 10 uL of 0.9% saline) into the right gastrocnemius muscle of Swiss white mice (25-30 g; CEMIB/Unicamp). Six hours later the mice were killed with an overdose of isoflurane and blood samples were obtained by cardiac puncture for the quantification of circulating creatine kinase (CK); the muscle was also removed for histological analysis. Control mice received saline alone. To assess the vascular actions, mesenteric arterial rings from isoflurane-anesthetized male Wistar rats (300-400 g) were mounted with intact endothelium (resting tension: 10 mN) in modified Krebs-Henseleit solution (composition, 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, ascorbic acid 0.14, pH 7.4 at 37 °C and continuous aeration with 5%CO_2-95%O_2). A cumulative concentration-reponse curve to phenylephrine was constructed before and after incubation with 1 μM (29 μg/mL) of purified protein. The influence of the protein (2 μM) on contractile responses to 60 mM KCl was also examined. Right and left atria were mounted in Krebs-Henseleit solution for the measurement of contractile force and right atrial rate. Left atria were paced electrically at a rate that matched the corresponding right atria. After equilibration (1 h), protein (1 μM) was added to the bathing solution and atrial responses were monitored for 60 min. Injection of purified protein in gastrocnemius muscle caused no hemorrhage or myonecrosis, but stimulated an inflammatory infiltrate, as assessed histologically. Similarly, there was no increase in CK release (basal: 109 ± 30 U/mL; venom – 40 μg: 960 ± 240 U/mL; protein – 40 μg: 141 ± 50 U/mL; n=5). The protein reduced the contractile force of left and right atria by 46 ± 6% (n=4) and 50 ± 17% (n=4), respectively, with a 17 ± 7% reduction in right atrial frequency. The protein also reduced the contractile response to KCl in mesenteric artery rings by 21 ± 7% (n=4) and caused a rightward shift in the concentration-response curve to phenylephrine (EC_{50} -0.93 ± 0.4 mM vs. 1.79 ± 0.4 mM in the absence and presence of the protein), without alterations in the $E_{max.}$ **Conclusion:** These results indicate that the cysteine-rich protein isolated from *B. jararaca* venom is not myotoxic but can adversely affect vascular and cardiac responses *in vitro.* These effects are similar to those of cysteine-rich proteins from other snake venoms. **Financial support:** Capes, CNPq, Fapesp.

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Pharmacological evaluation of standardized extract from *Cocos nucifera* L. (Palmae) *in vitro* and *in vivo.* Freitas RB[1], Freitas LBN[1], Lima EBC[2], Vasconcelos SMM[2], Leal LKAM[1]
[1]CEFAC-UFC –, [2]UFC – Fisiologia e Farmacologia

Introduction: *Cocos nucifera* L. (Palmae) is a plant widely distributed in tropical regions, including the Brazilian northeast and popularly known as "coco amarelo". Its ripe fruit has yellowish peel and a lot of fibers. Chemical studies revealed the presence of high content of pentosans, cellulose and lignin, as well as tannins in extracts obtained from fibers of *C. nucifera*. Pharmacological studies showed that these extracts present antibacterial, antifungal, antiviral and antioxidant activities. The aim of this study was to investigate the biological potential of the hydroalcoholic extract from *C. nucifera* (HAECN) evaluating the free radical scavenging activity, toxicity (human neutrophils) and its effect on Central Nervous System (CNS) behavior in mice. **Methods:** Animal handling and experimental protocols were registered on the Ethics Committee under number 32/2011. The alcoholic extract of Cocos nucifera (HAECN) was prepared with EtOH:water (2:1) in soxhlet extractor (6h). The antioxidant activity was performed by DPPH free radical scavenging assay (Saint-Cricq *et al.*,1999). The total phenols content was determined by Folin-Ciocalteu method (MAKKAR, 2000; SOUSA *et al.*, 2007). Polymorphonuclear cells, predominantly neutrophils (80-90%), with 95% of viability established by the Trypan blue assay were isolated from human blood (Boyum, 1968). The cellular viability of neutrophils (2.5x106 cells/mL), after their exposure to HAECN (50, 100 and 200 µg/mL), water (control) or 0.2% Triton X-100 (citotoxic standard) was evaluated by the MTT test. For analysis the effect on the CNS behavior was used the Open Field model which analyzed three parameters: horizontal locomotion (total, peripheral and central ambulation), rearing (vertical exploration) and grooming (self-cleaning) (MASUR, 1971).The animals were subjected to treatments with HAECN (50 and 100 mg/kg), vehicle (Tween 80, 4%) or diazepam (2 mg/kg). **Results and discussion:** The HAECN at concentrations ranging from 50-500 µg/mL reduced the DPPH radical since at lowest concentration, showing a free radical scavenging potential similar to α-Tocopherol (reference drug) reducing up to 86 % the free radical. The total polyphenol contents of the HAECN was 47.52 mg EAG/mL ± 0.155 and it was also detected the presence of tannins in the extract. The treatment of human neutrophils with HAECN at all the concentrations tested did not significantly reduce the viability of cells (viability 100.9 ± 1.3 %) as determined by the MTT test. In the evaluation of the locomotor activity,HAECN not showed depressant activity because did not change the locomotor movement when compared to vehicle (35.60 number of crossings/5minutes). In the rearing test, HAECN presented a decrease in the number of rearing in both doses and only at the highest dose increased the number of grooming. The results demonstrated that HAECN has the potential to scavenge free radicals and possibly part of its antioxidant activity may be related to the presence of phenols substances. In addition, the HAECN showed no toxicity in human neutrophils and not have effect on the CNS such as depressant although it showed a decrease of the rearing. However, further studies are necessary to complement this study. **Financial Agencies**: CNPq

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Anti-inflammatory activity of the extract of *Croton adamantinus* Müll. Arg. (Euphorbiaceae). Santos SM, Mendes RFV, Muniz RP, Silva METM, Domingos RF, Guerra ASHS, Oliveira TB, Silva TG, Albuquerque JFC, Ximenes RM UFPE – Antibióticos

Introduction: *Croton adamantinus* Müll. Arg is na endemic species of Caatinga found in the states of Pernambuco, Bahia, Rio Grande do Norte, Ceará e Piauí. It is used in local medicine as anti-inflammatory, analgesic, for wound healing and intestinal disorders. Antinociceptive and wound healing activities of the essential oil of leaves have been reported (Ximenes RM, J Nat Med, 67, 758, 2013). **Methods:** *C. adamantinus* were collected in Salgueiro/PE. A voucher specimen was deposited in the Herbarium of IPA (nº 85610). Stem bark was dried and extracted with ethanol under shaking during 3 hours. The procedure was repeated 3 times. Male Swiss mice (8 weeks old, n = 6) were used. All experiments were approved by Ethics Comission for Animal Use of UFPE (CEUA nº 23076.021665/2013-00). The anti-inflammatory activity was evaluated using 3 animal models: paw edema; vascular permeability; and air pouch. In all models, animals were treated with *C. adamantinus* ethanol extract (CaEE; 100, 200 and 300 mg/kg, i.p.), indomethacin (10 mg/kg, i.p.) or vehicle (10 mL/kg, i.p.) 30 min before the administration of the inflammatory agent. Paw edema was induced by the intraplantar injection of 50 µL of zymosan 2% in PBS in the right hind paw of the mice. Paw volume was measured immediately before and after 0.5, 1, 2 and 4 hours of zymosan injection using a plethysmometer (Ugo Basile, Italy). For vascular permeability assay, 30 min after treatment, animals were anesthetized (xylazine and ketamine; 8:2, i.m.) and received 0.2 mL of Evans blue 1% by the retro-orbital plexus, following the administration of acetic acid 1% (i.p.). Thirty min after the animals were killed with CO_2, and the peritoneal cavity was washed with 2 mL of saline. The exudate was collected and centrifuged at 2,000 rpm for 10 min. The absorbance of the supernatant was read at 610 nm in a multiwall plate reader. For the air pouch test, the animals received two subcutaneous dorsal injections of sterile air on day 1 and 3. On the seventh day, the animals were treated as previously described, and after 30 min, they were injected with 1 mL of carrageenan 1% into the pouches. After 6 h, animals were killed and the pouches washed with saline/EDTA for leucocyte count. Data are expressed as mean ± SEM and analyzed by ANOVA with Bonferroni post test. **Results and discussion:** In the paw edema assay, CaEE inhibited the edema induced by zymosan at the doses of 200 and 300 mg/kg. The lower dose (200 mg/kg) and indomethacin inhibited the edema in all evaluated times. Paw edema induced by zymosan has the participation of cellular and vascular events and is a useful model for screening of anti-inflammatory extracts or isolated compounds. On the vascular permeability assay, CaEE was not able to inhibit the increase in the vascular permeability induced by acetic acid as indomethacin did. In the air pouch model, all doses of CaEE were able to inhibit cell migration into the air pouch, with inhibition percentages ranging from 48.9 to 66.7%. Indomethacin was able to inhibit the cell migration at about 53.0%. These results point to a cellular action of CaEE instead of a vascular one.

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Effects of synthetic natriuretic peptide of *Crotalus durissus cascavella* venom in blood pressure and heart rate of rats. Lima DEV[1], Silveira JAM[2], Rodrigues FAP[2], Costa PPC[2], Morais GB[1], Evangelista JSAM[1], Monteiro HSA[2] [1]UFC – Histology of Snake Venoms and Toxic, [2]LAFAVET-UFC – Physiology and Pharmacology

Introduction: Natriuretic peptides have been described in the venoms of several venomous snakes. *Crotalus durissus cascavella* is a Viperidae snake found in the Northeast region of Brazil. Thus, the aim of this study was to evaluate the effects of synthetic natriuretic peptide of *C. d. Cascavella* venom (*NPCdc*) in Mean Arterial Pressure (MAP) and Heart Rate (HR) of rats. **Methods:** Were used, for the experiment, Wistar rats weighing between 280 and 300 g, submitted to cannulation of the right femoral artery and vein. The rats were divided in two groups and, in both groups, the control period of 15 minutes was followed. In the first group, the *NPCdc* was injected as a *bolus*, at dosis of 0.03, 0.1, 0.3 and 1 mg/kg, through the femoral vein with an interval between applications of 10 minutes. The second group received an isovolumetric injection of saline. Both groups contained six animals per group (n=6). Statistical analysis was performed using GraphPad Prism 5.0 software, using one-way ANOVA followed by Bonferroni post-test (α=0.05). This study was approved by the Ethics Committee on Animal Research of the Federal University of Ceará, with protocol number 79/08. **Results:** At dosis of 0.3 mg/kg and 1 mg/kg, the *NPCdc* decreased significantly the MAP (Control = 107 ± 8.3mmHg; $NPCdc_{(0.3\ mg/kg)}$ = 70 ± 5.6mmHg; $NPCdc_{(1\ mg/kg)}$ = 51 ± 9.3mmHg). After infusion of *NPCdc*, the HR at all dosis tested significantly reduced when compared to the control group (Control = 410 ± 21bpm; $NPCdc_{(1\ mg/kg)}$ = 290 ± 34bpm). **Discussion:** Thus, we conclude that the *NPCdc* presented a hypotensive effect, at the dosis of 0.3 mg/kg and 1 mg/kg, and reduction of heart rate at all dosis. More tests should be done to confirm the efficiency of *NPCdc*: Systemic hemodynamics, vascular contrality assays and histological analysis of organs submitted to tests. **Acknowledgments:** CNPq and FUNCAP for financial support.

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Study on the renal effects of the *Tityus stigmurus* venom. Maia GMP[1], Marinho AD[2], Morais ICO[3], Jorge RJB[2], Silva NA[4], Martins RD[4], Sousa PCP[2], Silveira JAM[2], Jorge ARC[2], Monteiro HSA[2] – [1]UFC – Histology of Snake Venoms and Toxic Plants, [2]LAFAVET-UFC – Pharmacology of Venoms and Toxins, [3]LCC-UFC – Clinical and Toxicological Analyses, [4]UFPE – Zoology

Introduction: Despite several reports on the toxic effects of poisons animals, these have also been widely recognized as a major source of biologically active molecules. The study of the therapeutic potential of toxins is increasingly gaining ground and arousing great interest from the scientific community as a source of molecular models for the design of new drugs. The objective of this study was to evaluate the renal effects of the venom of the scorpion Tityus stigmurus (TsV). **Methods:** Were used, for the experiment, male Wistar rats (n=6) weighing 250-300g, whose kidneys were isolated and perfused with Krebs-Henseleit solution modified, containing 6% w/v bovine serum albumin previously dialyzed. The experiments lasted on average 120 min. The evaluated parameters were Perfusion Pressure (PP), Renal Vascular Resistance (RVR), Urinary Flow (UF), Glomerular Filtration Rate (GFR) and Percentage of Total and Proximal Tubular Transport of Sodium (%TNa+ e %pTNa+). This study was approved by the Ethics Committee on Animal Research of the Federal University of Ceará, with protocol number 79/08. **Results:** The concentration of TsV 3 µg/mL caused significant reduction on the Transport of Sodium (%TNa+ 40' = 81.6 ± 3.46; TsV%TNa+ 40' = 72.7 ± 1.08) and Chloride (%TCl^-40' = 80.5 ± 1.40; TsV%TCl^-40' = 68.7 ± 1.74). The electrolyte potassium showed no significant difference. Urinary osmolality tended to increase back to normal levels in the remaining time (TsVUosm60' = 260.5 ± 5.62 vs. TsVUosm120' = 234.5 ± 2.25 $mL.g^{-1}.min^{-1}$). **Discussion:** This decrease in transportation may be related to the effects of toxic components of the venom on renal tubules and ion channels, reducing the amount of free channels for electrolyte binding. Scorpion toxins act directly on the sodium-potassium pump, resulting in the development of local and systemic symptoms. The massive presence of carriers in the basolateral membrane of the kidney contributes to generation of electrochemical gradient for sodium reabsorption, as well as the generation of free energy within the renal tubular cell. These factors are critical to the luminal transport of electrolytes, such as sodium and chloride. Blocking this active co-transport of sodium and potassium can promote changes in tubular transport of electrolytes, such as sodium and chloride, with a consequent increase in the excretion of the same. **Ackwnolegments:** Capes and CNPq for financial support.

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Cytotoxicity assessment of extracts obtained from actinomycetes recovered from the sediment of Paracuru Beach, Ceará, Brazil. Vasconcelos IMB[1], Guimarães LA[1], Ferreira EG[1], Jimenez PC[1], Freitas HPS[2], Sousa TS[2], Pessoa ODL[2], Lotufo LVC[5] [1]UFC – Ciências do Mar, [2]UFC – Química Orgânica e Inorgânica, [3]UFC – Fisiologia e Farmacologia

Introduction: The diversity of organisms in the marine environment has inspired researchers for many years to identify novel marine natural products that could eventually be developed into medicines. The coast of Ceará has been prospected for the pharmacological potential housed in sponges, tunicates and corals. This study focused on evaluating the cytotoxicity of organic extracts derived from actinomycetes isolated from sediments from Paracuru Beach, in Ceará, on the Northeast coast of Brazil. **Methods:** Sediment was hand collected at Pedra Rachada Beach (03°23'S; 39°54'O) and the processing of samples occurred by two different methods: dehydration (M1) and heating to 55°C (M2). Then, the sediments were plated in three different agar media: starch casein agar (SCA), trace minerals agar (TMA) and seawater agar (SWA). Individualized colonies were isolated based on actinomycete-like characteristics. Purification of the strains was performed by subcultures on plates with A1 solid medium (soluble starch, yeast extract and peptone). Pure strains were grown in 100 mL of A1 broth in Erlenmeyer flasks (28 °C/200 RPM/7 days), extracted with ethyl acetate (EtOAc), and vacuum-dried. The extracts were screened for cytotoxicity using the MTT assay on tumor cell lines (HCT-8, HCT-116 or PC-3M), either on a single concentration (50 µg/mL), for an initial qualitative analysis, or in a curve (0.016 – 50 µg/mL). The most active extracts were submitted to dereplication using HPLC and LC-MS followed by comparison of molecular masses in marine natural products databases. **Results and discussion:** A total of 26 strains were isolated. Method M1 was more effective for the isolation of actinomycete colonies, as over 69% of strains were obtained. SCA was the most efficient media, as almost 42% of strains were recovered. 16 extracts inhibited more than 65% of growth of HCT-116 cells and were selected for assessment of cytotoxicity in different concentrations. IC_{50} of extracts ranged from 0.07 to 42.13 µg/mL. The extract derived from the strain BRA-090, which exhibited the highest percentage of cell growth inhibition (98.38%), showed IC_{50} of 0.19 µg/mL. Dereplication applied to this extract led to identification of three known chromomycins (chromomycins A2, A3 and desmetilchromomycin A2) and also two masses regarding probable new molecules of the same class. Additionally, for the extract from BRA-148 (96% growth inhibition) were founded IC_{50} value of 0.07 µg/mL and the presence of staurosporines. Moreover, piericidin was identified within the extract of BRA-231 (IC_{50} = 2.94 µg/mL). These compounds are well known for cytotoxic activity and probably the responsible for the very potent cytotoxicity observed for these crude extracts. Nevertheless, these results highlight the potential of marine microorganisms in synthesizing various molecules with biomedical properties, and, most importantly, are great evidence that the coast of Ceará is a pertinent source of microorganisms with biomedical applicability. **Financial Support:** CNPq, Capes, FUNCAP and IFS.

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Cytotoxic effect and induction of apoptosis by venom of *Bothropoides pauloensis* in MDCK cell line. Macambira KDS[1], Marinho AD[2], Morais ICO[2], Jorge RJB[2], Menezes RRPPB[3], Sousa PCP[2], Silveira JAM[2], Lima DB[3], Martins AMC[3], Evangelista JSAM[1], Monteiro HSA[2] [1]HISTOVESP-UFC – Histology of Snake Venoms and Toxic Plants, [2]LAFAVET – Pharmacology of Venoms and Toxins, [3]LCC-UFC – Clinical and Toxicological Analyses

Introduction: The venom of snake *Bothropoides pauloensis* (BpV), popularly known as "jararaca-pintada" contains a variety of enzymes including proteases with haemorrhagic activity, fibrinolytic metalloproteinase, phospholipase A2 (PLA2), L-amino oxidase, and a variety of bradykinin potentiating peptides that may contribute to its biological action. The present study aimed to investigate the cellular changes induced by BpV on culture of renal tubular cells of MDCK type (Madin-Darby Canine Kidney). **Methods:** The determination of the cytotoxic potential of BpV at concentrations of 3.12, 6.25, 12.5, 25, 50 and 100 µg/mL was carried out after 6, 12 and 24h incubation using the MTT reduction Method: MDCK cells were grown in plastic bottles at 37°C in an atmosphere of 5% CO_2 with RPMI 1640 medium supplemented with fetal bovine serum 10%. Then, flow cytometry with Annexin V and Propidium Iodide was performed. For morphologic evaluation of MDCK cells after exposure to the test substance, the experiments were performed on the surface of glass slides for staining with May-Grunwald Giemsa. The experimental groups were analyzed by light microscopy and the most significant morphological characteristics were photographed. **Results:** Cytotoxic effect was observed at concentrations of time-dependent manner, with cell death. The BpV caused a decrease in cell viability showing IC50 of 7.5mg/mL in time of 12h. In flow cytometry with Annexin V and Propidium Iodide was demonstrated that cell death occurred by apoptosis predominantly. The treatment also led to depolarization of the mitochondrial membrane potential ($\Delta\Psi m$) and production of reactive oxygen species (ROS) results obtained by flow cytometry using TMRE for detecting $\Delta\Psi m$ and DCF-DA for ROS. Morphological changes such as the appearance of cellular debris and bare nuclei, vacuolated cells, reduced cell volume and increased cytoplasmic projections were observed. The analysis revealed the presence of apoptotic involvement in the cytotoxic effect of the substance. **Discussion:** These characteristics indicate that the BpV has a cytotoxic effect on MDCK cells, furthermore, there is an apparent involvement of apoptosis, concentration and time-dependent manner. **Acknowlegments:** Capes and CNPq for the Financial support.