07. Endocrine and Gastrointestinal

07.001 Participation of nitric oxide on pathogenesis alendronate-induced gastric damage in rats. Silva RO[1], Nicolau LAD[1], Costa NRD[1], Lucetti LT[2], Santana APM[2], Aragão KS[2], Barbosa ALR[1], Ribeiro RA[2], Souza MHLP[2], Medeiros JVR[1] [1]UFPI – Experimental Physiopharmacology, [2]UFC – Pharmacology of Inflammation and Cancer

Introduction: Alendronate is a bisphosphonate that can cause serious adverse effects in patients, including ulcers, gastric inflammation, nausea and abdominal pain, although the mechanism underlying these reactions remains unknown (GRAHAM, D.Y., *Dig Dis Sci*, v.47, p.1665, 2002). The aim of this study was to investigate the effect of sodium nitroprusside (NO donor) in pathogenesis of alendronate-induced gastric damage in rats and the role of cytokines (TNF-α and IL-1β) in this effect. **Methods:** This study was approved by the local ethics committee (protocol N^{o} 0067/10). Wistar albino rats (170-200g) were administered alendronate (30 mg kg^{-1}) by gavage for 4 days, either alone or following treatment with sodium nitroprusside (10 mg kg^{-1}, p.o.). On the last day of treatment rats were killed and stomachs were removed. The gastric damage was measured using a computer planimetry programme. Samples of stomach were also taken for histopathological assessment, glutathione levels (GSH), malonyldialdehyde concentration (MDA), myeloperoxidase (MPO) activity and pro-inflammatory cytokines concentration. **Results and Discussion:** Chronic oral administration of alendronate induced macroscopic (38.7±7.2 mm^2) and microscopic (edema, loss of epithelial cells and inflammatory infiltrate) gastric damage. However the sodium nitroprusside prevented the macroscopic (10.8±2.7 mm^2) gastric damage and confirmed by histological evaluation of the samples from several rat stomachs. Similarly, the alendronate increased MDA levels (121.1±4.3 nmol g^{-1}), MPO activities (31.5±3.8 U mg^{-1}) and TNF-α and IL-1β concentration and decrease GSH levels (180.3±21.9 µg g^{-1}). Treatment with sodium nitroprusside changed all biochemical parameters, MDA (86.6±3.7 nmol g^{-1}), MPO (17.7±2.6 U mg^{-1}), tissue level of TNF-α and IL-1β (31% and 39% inhibition, respectively) and GSH (471.6±45.27 µg g^{-1}). The present study suggests that alendronate induces gastric damage by neutrophil infiltration, oxidative stress and increase of pro-inflammatory cytokines, and sodium nitroprusside protects this damage by antioxidant properties and anti-inflammatory actions. **Financial Support:** CNPq, FAPEPI.

07.002 Protective effect of H_2S donors against alendronate-induced gastric damage in rats. Santos MS[1], Silva RO[1], Nicolau LAD[1], Costa NRD[1], Lucetti LT[2], Santana APM[2], Aragão KS[2], Barbosa ALR[1], Ribeiro RA[2], Souza MHLP[2], Medeiros JVR[1] [1]UFPI – Experimental Physiopharmacology, [2]UFC – Pharmacology of Inflammation and Cancer

Introduction: Alendronate is a primary amino bisphosphonates and one of the most commonly used members of this group, however, oral administration has been associated with gastrointestinal adverse effects including gastritis, gastric ulcer, and erosive esophagitis (MARTENS, M.G., *J. Reprod. Med.,* v.48, p.425, 2003). The aim of the present study was to evaluate the gastroprotective effect of the Lawesson's reagent (H_2S donor) in the alendronate-induced gastric damage. **Methods:** This study was approved by the local ethics committee (protocol N^o 0067/10). Wistar albino rats (170-200g) were administered alendronate (30 mg kg^{-1}) by gavage for 4 days, either alone or following treatment with Lawesson's reagent (27 µmol kg^{-1}, p.o.). On the last day of treatment rats were killed and stomachs were removed. The gastric damage was measured using a computer planimetry programme. Samples of stomach were also taken for measurement of glutathione (GSH) and malonyldialdehyde (MDA), myeloperoxidase (MPO) activity and cytokines concentration. **Results and Discussion:** The alendronate administration induced macroscopic (38.7±7.2 mm^2) and microscopic (edema, loss of epithelial cells and inflammatory infiltrate) gastric damage. However the Lawesson's reagent prevented the macroscopic (18.1±5.3mm^2) and microscopic gastric damage. Similarly, the alendronate increased MDA levels (121.1±4.3 nmol g^{-1}), MPO activities (31.5±3.8 U mg^{-1}) and TNF-α and IL-1β concentration, whereas GSH levels (180.3±21.9 µg g^{-1}) were decreased. Treatment with Lawesson's reagent changed all biochemical parameters, MDA (78.5±7.6 nmol g^{-1}), MPO (29.9±2.0 U mg^{-1}), GSH (398.0±40.2 µg g^{-1}) and tissue level of TNF-α and IL-1β (36% and 30% inhibition, respectively). Based on the above shown results, we can suggest that H_2S donors play a protective role against alendronate-induced gastric damage through mechanisms antioxidant and/or inhibit of proinflammatory cytokines. **Financial Support:** CNPq, FAPEPI.

07.003 Protective effect of sulfated-polysaccharide fraction from red algae *Gracilaria birdiae* on naproxen-induced gastric damage in rats. Brito CFC[1], Silva RO[1], Carvalho NS[1], Bezerra TS[1], Oliveira CB[1], Damasceno SRB[1], Barbosa ALR[1], Souza MHLP[2], Medeiros JVR[1] [1]UFPI – Experimental Physiopharmacology, [2]UFC – Laboratory of Pharmacology of Inflammation and Cancer

Introduction: Naproxen, a representative of the NSAID family, causes gastric ulcers through various processes, including generation of reactive oxygen species (ROS), inhibition of PG synthesis and lipid peroxidation (YOSHIKAWA, T., *Gut*, v.34, p.732, 1993). The aim of this present study is to investigate the protective effect of the sulphate polysaccharide fraction (PLS) extracted from the *Gracilaria birdiae* against naproxen-induced gastric damage in rats. **Methods:** This study was approved by the local ethics committee (protocol N^o 0066/10). Male Wistar rats (120-150g) were pretreated with 0.5% carboxymethylcellulose (vehicle) or polysaccharide (PLS, 10, 30, and 90 mg kg^{-1}, p.o.) twice daily (at 9.00 h and 21.00 h) for 2 days. After 1 h, naproxen (80 mg kg^{-1}, *p.o*) was administered. The rats were killed 4h after the naproxen treatment and the stomachs was opened along the greater curvature. The gastric damage was measured using digital calipers (Mitutoyo®). Samples of stomach were also taken for histopathological assessment, assays of glutathione (GSH), malonyldialdehyde (MDA) and myeloperoxidase (MPO) activity. **Results and Discussion:** The naproxen administration induced macroscopic (12.9±4.0 mm) and microscopic (edema and loss of epithelial cell) gastric damage. Similarly, the naproxen reduced GSH levels (103.4.3±24.9 µg g^{-1}), increased MDA levels (401.7±60.5 nmol g^{-1}) and MPO activities (5.9.±1.7 U mg^{-1}). However, PLS pre-treatment prevented naproxen-induced macroscopic (0.8±0.1 mm) and microscopic gastric injury in a dose-dependent manner, reaching maximal effect at a dose of 90 mg kg^{-1}. Treatment with polysaccharide has changed all biochemical parameters, GSH (251.1±54.9 µg g^{-1}), MDA (151.0±29.8 nmol g^{-1}) and MPO (0.9±0.5 U mg^{-1}). Our results suggest that PLS prevented naproxen-induced gastric damage in rats. **Financial Support:** CNPq, FAPEPI.

07.004 Role of the NO/K_{ATP} pathway in the protective effects of sulfated polysaccharide fraction from algae *Hypnea musciformis* against ethanol-induced gastric damage in mice. Damasceno SRB[1], Rodrigues JC[1], Silva RO[1], Nicolau LAD[1], Chaves LS[2], Barros FCN[2], Freitas ALP[2], Souza MHLP[3], Medeiros JVR[1] [1]UFPI – Experimental Physiopharmacology, [2]UFC – Proteins and Carbohydrates of Marine Algae, [3]UFC – Pharmacology of Inflammation and Cancer

Introduction: Sulfated-polysaccharides are exploited as free-radical scavengers and antioxidants (Zhang, Q., *Carbohydr. Res.*, v. *339*, p. 105, 2004). However, studies of sulfated galactans role in models of gastric damage are rare in the literature. The aim of the present study was to investigate the gastroprotective activity of a sulfated-polysaccharide (PLS) fraction extracted from the marine red algae *Hypnea musciformis* and the mechanism underlying the gastroprotective activity. **Methods:** Protocol was approved in the local ethics committee (Protocol 0066/10). Male Swiss mice (25-30g) were treated with PLS (3, 10, 30 and 90 mg kg^{-1}, *p.o*). After 30 min, 50% ethanol was administrated by gavage. One hour later, gastric damage was measured using a planimeter. Samples of the stomach tissue were also obtained for histopathological assessment and for assays of glutathione (GSH) and malondialdehyde (MDA). Other groups were pretreated withL-NAME (inhibitor non selective of nitric oxide synthase, 10 mg kg^{-1}, *i.p*), aminoguanidine (inhibitor of NOS-induced, 100 mg kg^{-1}, *i.p*), or glibenclamide (blocks K_{ATP}-dependent channels, 10 mg kg^{-1}, *i.p.*). After 1 hour, PLS (30 mg kg^{-1}, *p.o*). was administrated. After 30 min, ethanol 50% was administered followed by sacrifice after 60 min. **Results and Discussion:** The ethanol administration induced macroscopic (72.2 ±20.4mm^2) and microscopic (hemorrhage, edema and loss of epithelial cells) gastric damage. PLS prevented ethanol-gastropathy in a dose-dependent manner, reaching maximal effect at a dose of 30 mg kg^{-1} (3.1±0.9mm^2). However, L-NAME (30.76±7.0mm^2) and glibenclamide (33.23±9.5mm^2) significantly reversed the protection afforded by PLS. When the animals were pretreated with aminoguanidine (3.5±1.1mm^2) it did not alter the protective effect of PLS. Ethanol administration also promoted reduction of the GSH gastric levels (178.6±19.8 µg g^{-1}) and increased the MDA concentration (230.1±31.6 µg g^{-1}). However, when the animals were pretreated with PLS 30 mg kg^{-1}, there was significant increase of the gastric levels of GSH (262.8±18.5µg g^{-1}) and concentration decrease of MDA (129.4±11.9µg g^{-1}). Our results suggest that PLS has a protective effect against ethanol-induced gastric damage in mice via activation of the NO/K_{ATP} pathway. **Financial Support:** CNPq.

07.005 Gastroprotective effect of heme-oxygenase 1/sGC/K_{ATP} pathway in alendronate-induced gastric damage in rats. Costa NRD[1], Silva RO[1], Nicolau LAD[1], Lucetti LT[2], Santana APM[2], Aragão KS[2], Barbosa ALR[1], Ribeiro RA[2], Souza MHLP[2], Medeiros JVR[1] [1]UFPI – Experimental Physiopharmacology, [2]UFC – Pharmacology of Inflammation and Cancer

Introduction: Recent studies show that CO, produced by heme-oxygenase 1 (HO-1), has anti-inflammatory properties and contributes to the gastroprotection (TAKASUKA, H., *J. Pharmacol. Experim. Therap.*, v.337, n.1, p.293, 2011). The aim of this study was to evaluate the protective effect of HO- 1/sGC/K_{ATP} pathway in alendronate-induced gastric lesions in rats. **Methods:** This study was approved by the local ethics committee (protocol N°0067/10). Wistar rats (170-200g) were treated with hemin (HO-1 inducer; 1, 3 and 10 mg kg^{-1}, *p.o*.) or DMDC (CO donor; 9, 27 and 81 µmol kg^{-1}, p.o). Another group, one hour before DMDC (81 µmol kg^{-1}, *p.o.*), received ODQ (soluble guanylate cyclase (sGC) inhibitor, 10 mg kg^{-1}, *p.o*) or glibenclamide (K_{ATP}-dependent channels inhibitor; 1mg kg^{-1}, *i.p.*). After 1h, the animals were treated with alendronate (30 mg kg^{-1}) by gavage for 4 days. The last day of treatment, four hours after the alendronate administration, the animals were sacrificed and their stomachs removed. Gastric lesions were measured using a computer planimetry program, and gastric corpus pieces were assayed for malonylaldehyde (MDA), glutathione (GSH), pro-inflammatory cytokines (TNF-α and IL-1β) or myeloperoxidase (MPO). Other group was used to measurement gastric mucus. **Results and Discussion:** Treatment with alendronate caused gastric damage macroscopic (49.5 ± 4.3 mm^2) and microscopic (loss of epithelial cells, edema, hemorrhage and inflammatory infiltrate), increased the expression of HO-1 in the gastric tissue. Similarly, the alendronate increased MDA levels (121.0±4.3 nmol g^{-1}), MPO activities (31.5±3.8 U mg^{-1}) and TNF-α and IL-1β concentration and decrease GSH levels (180.3±21.9 µg g^{-1}) promoted mucus depletion in the gastric mucosa. Pre-treatment with hemin reduced macroscopic (15.7.5 ± 4.8 mm^2) and microscopic gastric damage, MDA concentration (99.0.0±6.2 nmol g^{-1}) and MPO activity (11.9±2.2 U mg^{-1}), tissue level of TNF-α and IL-1β (51% and 57% inhibition, respectively), and increased GSH concentration (570.3±21.6 µg g^{-1}) in the gastric mucosa. DMDC also reduced gastric damage (11.1±1.1 mm^2), MDA concentration (58.5±10.4 nmol g^{-1}) and MPO activity (9.4±0.7 U mg^{-1}), and increased GSH concentration (482.1±73.8 mm^2) and prevent the depletion of gastric mucus in the gastric mucosa. ODQ (52.9±4.7 mm^2) and glibenclamide (40.7±10.7 mm^2) completely abolished the DMDC protective gastric effect. Our results suggest that heme-oxygenase 1/sGC/K_{ATP} pathway participates in the protection of gastric mucosa against gastric damage induced by alendronate. **Financial Support**: CNPq, FAPEPI.

07.006 Antioxidant activity of soy isoflavones in gastrocnemius muscle of thyrotoxic rats. Marinello PC, Bernardes SS, Guarnier FA, Cecchini R, Cecchini AL UEL

Introduction: Muscle weakness and atrophy are important complications of thyrotoxicosis. It is mainly caused by increased protein and muscle degradation and it seems that reactive oxigen species (ROS) are able to activate different proteolytic pathways. Since soy isoflafones (Iso) have been demonstrated antioxidant properties, the aim of the present study was to investigate its role on inhibition of oxidative response and muscle mass loss in a model of experimental thyrotoxicosis. **Methods:** Male Wistar rats weighing 250 g were used and divided in six experimental groups (n=6): control (C); thyrotoxicosis (T3); control Iso 1 (Iso1); thyrotoxicosis Iso 1 (T3iso1), control Iso 10 (Iso10) and thyrotoxicosis Iso 10 (T3iso10). Animals were treated intraperitoneally during three days (approval number of the Animal Ethic Committee: 14154.2011). Iso was administered at two different concentrations (1 and 10mg/kg body weight) and T3 at 1mg/kg. Animals were weighed daily to calculate the Weight Loss Index (WLI). The oxidative stress was evaluated by chemiluminescence to estimate lipid hydroperoxide and total antioxidant capacity (TRAP). TBARs was used to quantify malondialdehyde, and 2,4-dinitrophenylhydrazine (DNPH) derivatives to quantify carbonyl protein content, both spectrophotometrically. The proteasomal proteolytic activity was measured by chemiluminescence, using a commercial kit based on chymotrypin-like activity; Plasma glycerol levels and retroperitoneal fat were also measured to estimate lipolysis. Control and experimental groups were compared using the Student's unpaired *t*-test. Chemiluminescence curves were compared using *two-way* analysis of variance (ANOVA).The results were shown as mean ± SEM of 6 animals, and $p < 0.05$ was considered statistically significant. **Results and Discussion:** Thyrotoxicosis led to decreased body weight (WLI= 8.44%), increased oxidative stress, observed in chemiluminescence curves ($p<0.0001$), and TRAP analysis (1.8±0.3 to T3 and 1.1±0.06, $p<0.05$ to control). Lipoperoxidation also increased: MDA was 86.16±6.44 to T3 group and 60.7±7.7, $p<0.05$ to control. Carbonyl proteins, lipolysis and proteasomal proteolytic activity did not change. Iso treatment at 1mg/kg decreased the WLI ($p<0.01$), partially reversed lipoperoxidation, observed by MDA analysis and promoted an increase in lipolysis: glycerol levels increases in T3iso1 animals (2.7 ± 0.2) when compared with Iso1 (1.7 ± 0.2, $p<0.05$) and retroperitoneal fat decreased in T3iso1 group (1.0 ± 0.2) when compared with Iso1 group (1.7 ± 0.1, $p<0.05$). The proteasomal proteolytic activity decreased (T3iso1 group was 37070 ± 5162 and Iso1 was 54670 ± 5142; $p<0.05$). However, treatment with Iso at 10 mg/kg did not alter the thyrotoxic body weight loss and increased muscle oxidative stress, leading to increase malondialdehyde levels (88.8±4.7 to T3iso10 and 60.5±8.2 to Iso10 group, $p<0.05$) and carbonyl protein content (12.7 ± 0.02) when related with Iso10 group (8.3 ± 1.6, $p<0.05$). These findings show that Iso treatment is able to decrease muscle oxidative stress caused by thyrotoxycosis at low concentrations and, at higher concentrations, play a prooxidant role. Although the objective of statistics outline was not this, it emphasizes the idea that antioxidants should not be used indiscriminately. **Support:** CAPES

07.007 Activation of PPAR-gamma by rosiglitazone reduces the HPA axis hyperactivity in alloxan-diabetic rats. Torres RC, Prevatto JP, Telles TS, Martins MA, Silva PMR, Carvalho VF Fiocruz – Fisiologia e Farmacodinâmica

Introduction: Hyperactivity of hypothalamo-pituitary-adrenal (HPA) axis is associated with several diabetic complications, including neuropathy and wound healing deficiency. High levels of glucocorticoids are related with hyperglycemia and impaired stress responsiveness on diabetic patients (Chan, Endocrinology 143(5): 1761, 2002). PPAR-gamma agonists have been effective in reducing the hypercortisolism in other diseases associated with hyperactivity of HPA axis, as Cushing disease (Ambrosi, Eur J Endocrinol (151): 173, 2004). Therewith, our aim was to investigate the effect of the PPAR-gamma agonist rosiglitazone in the hyperactivation of HPA axis in diabetic animals. **Methods:** The animals were obtained from the Oswaldo Cruz Foundation breeding colony and used in accordance with the guidelines of the Committee on Use of Laboratory Animals of the Oswaldo Cruz Foundation (CEUA-FIOCRUZ, license LW 23/11). One single intravenous injection of alloxan (40 mg/kg) was used into fasted rats to induce the diabetic condition. PPAR-gamma agonist rosiglitazone (0.5 mg/kg) was administered i.p. after 3 days of diabetes induction, once daily, for 18 consecutive days. Some animals received the PPAR-gamma antagonist GW9662 30 min before the administration of rosiglitazone during all the study period. We determined the plasmatic levels of the PPAR-gamma ligand 15-deoxy-delta-12,14-PGJ2 (15-d-PGJ2) using an enzyme immunoassay (EIA) kit and the evaluation of plasma corticosterone and adrenocorticotropin hormone (ACTH) levels were made by radioimmunoassay (RIA). ACTH receptor (MC2-R) expression in adrenal and glucocorticoid receptor (GR) and corticotropin releasing factor receptor (CRFR1) expression in pituitary was assessed by immunohistochemistry. **Results and Discussion:** Diabetic rats presented hypertrophy of the adrenals and an increase in the circulating levels of ACTH and glucocorticoids in parallel with a high expression of MC2-R in the adrenals and a low expression of GR in the pituitary. Moreover, diabetic rats presented reduced plasmatic levels of the endogenous PPAR-gamma ligand 15-d-PGJ2 compared to non-diabetic rats (42 ± 42 and 383 ± 64 pg/ml, respectively; mean ± SEM). Treatment with a synthetic agonist of PPAR-gamma rosiglitazone was able to reduce both the adrenal expression of MC2-R and ACTH levels at diabetic rats. Additionally, we observed that diabetic animals treated with rosiglitazone presented reduction in adrenal hypertrophy reflecting in decreased glucocorticoids levels, although the reduced GR expression on pituitary of alloxan-diabetic animals was not reversed by the treatment with rosiglitazone. However, we showed that CRFR1 expression in the pituitary of rosiglitazone-treated diabetic rats was reduced when compared with untreated diabetic rats. Finally, we observed that rosiglitazone acts in HPA axis of diabetic animals by a mechanism dependent of PPAR-gamma, once the antagonist GW9662 suppressed the reduction on glucocorticoids levels mediated by rosiglitazone in diabetic rats. Together, these results indicate that PPAR-gamma activation decreases the HPA axis activation in diabetic rats by reducing expression of CRFR1 and MC2-R in pituitary and adrenals, respectively. **Keywords**: Diabetes, HPA axis, Glucocorticoids and PPAR-gamma. **Financial Support**: CNPq, FAPERJ and FIOCRUZ.

07.008 Stretch stress and sources of Ca^{2+} For ileum contraction in dystrophic mice. Alves GA[1], Silva LR[1], Ribeiro RF[1], Aboulafia J[1], Souccar C[2], Nouailhetas VLA[1] [1]Unifesp – Biofísica, [2]Unifesp – Farmacologia

Duchenne's muscular dystrophy (DMD) is an X-linked hereditary disease leading to the release of the protein dystrophin from the dystrophin-associated protein complex which links cytoskeleton to the plasma membrane. Although dystrophin has been well studied in the skeletal muscle, much less is known concerning its function in smooth muscle, particularly in the intestine. We thus investigated the role of dystrophin in $[Ca^{2+}]_i$ regulation and in contractile response under stretch stress condition. Male mdx and control mice (3-4 mo old, N=5 for each group) were used. Ileum isometric contractions were performed in the presence of Tyrode solution at 37°C, pH 7.4, bubbled with air. The loss of the contractile responses induced either by successive maximal KCl-depolarization or maximal concentration of CCh administrations were recorded immediately and at different time intervals after Ca^{2+} removal from the Tyrode solution. Half-life times ($t_{1/2}$) were determined from the adjusted exponential regressions to experimental data. Cumulative concentration-response curves to Ca^{2+} were constructed with tissue previously stimulated with either KCl (80 mM) or CCh (30 µm) in the presence of nominal Ca^{2+}-free medium in the absence or presence of nifedipine (1 µM), L-type Ca^{2+} VOC (LVOCC) blocker. Tissue recovery was also studied by measuring the contractile responses to repeated stimulations with either KCl or CCh initiated immediately after tissues being washed with normal Tyrode solution after a 15 min exposure to Ca^{2+}-free medium. Results are presented as percentages in comparison with control group. Comparisons were made using Two-way ANOVA or Student's t test ($P<0.05$ was considered significant). A slower rate of contractility loss was observed in the mdx ileum as compared with that observed in the control animals for KCl stimulation ($t_{1/2}$= 1.218 min in mdx and $t_{1/2}$= 0.429 min in control) but not for CCh-induced contraction ($t_{1/2}$=1.206 min in mdx and $t_{1/2}$=1.246 min in control). KCl-induced cumulative Ca^{2+}-contractile response curve was impaired in mdx group while curves in response to CCh were similar in both groups. In the presence of nifedipine we obtained similar concentration-contractile response curves in response to both KCl and CCh stimulations, indicating that LVOCC (and not stock or receptor-operated channels) might be altered in mdx mice intestine. In order to understand if dystrophin absence would prejudice ileum contractile response to stretch, we performed concentration-contractile response curves for KCl and CCh at distinct basal tension, the 0.5, 1 and 2 g. Mdx intestine was less susceptible to stretch-induced loss of contraction as KCl and CCh curves at 1 g-basal level (moderately stretched) were quite similar to those observed with both 0.5 g (standard control) and 2 g curves in the control group. Based on these results, we propose that calcium handling in the dystrophic mice is impaired just for eletromechanical coupling, probably to some modification of LVOCC. Interestingly, but unexpectedly, the intestine of dystrophic animal seems to be less sensitive to mechanical (stretch) stress than those from the control animals via a yet unknown mechanism. CEP/UNIFESP: 0097/08 FAPESP: 2007/59976-6, 2007/51343-4

07.009 Gastroprotective activity and mechanism of proteins from *Plumeria rubra* latex against ethanol-induced gastric ulcer in mice. Pinheiro RSP[1], Freitas LBN[1], Luz PB[1], Marques LM[1], Souza TFG[1], Carmo LD[1], Araújo ES[2], Couto TS[1], Rangel GFP[1], Ramos MV[2], Alencar NMN[1] [1]UFC – Fisiologia e Farmacologia, [2]UFC – Bioquímica e Biologia Molecular

Introduction: The *Plumeria* rubra, which belongs to Apocynaceae family, is a laticifer plant that is commonly known as frangipani or jasmine, is distributed mainly in tropical and subtropical regions, included Brazil. *P. rubra* has been widely used in traditional folk medicine to treat various diseases, such as fever and diarrhea. This study was performed to investigate the gastroprotective activity of a non-dialyzable protein fraction (PrLP) of *P. rubra* latex in gastric ulcer model induced by ethanol. **Methods:** Experimental protocols were registered on the Institutional Ethics Committee under number 57/2010. Swiss male mice, undergo fasting of 16h, were treated with PrLP at doses 0.5; 5 and 50 mg/kg (i.v.). After 30 min they received 0.2 ml of absolute ethanol per oral and after 60 min, the animals were sacrificed and stomachs removed and analyzed the lesion index and dosage of nitrate/nitrite. In order to investigate the involvement of NO, prostaglandins and potassium channels ATP-dependent (Katp), before treatment with PrLP animals received L-NAME(20mg/kg; i.p.) or L-arginine(600mg/kg; i.p.), Indomethacin(10mg/kg; v.o.) or Misoprostol(0.03µg/kg; v.o.), Glibenclamide(5mg/kg; i.p.) or Diazoxide(3mg/kg; i.p.). **Results and Discussion**: PrLP at the doses 0.5; 5 and 50 mg/kg was able to prevent injury in 84.5; 76.6 and 60% respectively ($p<0.05$). PrLP also restored the nitrite/nitrate levels in mucosa in 26% compared to the ethanol group. L-NAME, Glibenclamide and Indometacin were able to reverse the protective effect of PrLP, demonstrating the involvement of prostaglandins, NO and potassium channels in its mechanism of action. **Conclusion:** We can conclude that the PrLP has pharmacological activity with gastroprotetor effect in the gastric mucosa. This protection appears to be mediated in part by modulation of Prostaglandin/NO/Katp, which is of great importance in mucosal defense and in maintaining blood flow to the stomach. **Sources of research support:** CAPES and CNPq.

07.010 Gastroprotective activity of the hydroalcoholic extract of *Stryphnodendron rotundifolium* Mart. in rodents. Silva MR[1], Oliveira DR[2], Brito Júnior FE[2], Bento EB[2], Fernandes CN[2], De Souza HHF[2], Bezerra CF[3], Boligon AA[4], Athayde ML[4], Saraiva RA[4], Kerntopf MR[2], Costa JGM[2], Menezes IRA[2] [1]UFC – Farmacologia, [2]URCA – Química Biológica, [3]UFC – Farmacologia, [4]UFSM

Abstract: *Stryphnodendron rotundifolium* Mart. is native from the Cerrado of the Northeast of Brazil, and is used in folk medicine for the treatment of inflammation, infections, gastritis and other diseases. The research protocol was approved by the Ethics Committee for Animal Research of the Faculty of Medicine of Juazeiro do Norte (CEPA 2009_0433). **Methods:** The gastroprotective effect of the hydroalcoholic extract of *Stryphnodendron rotundifolium* Mart. (EHSR) in animal models of gastric lesions was evaluated. Swiss mice were divided into groups (n = 6), fasted for a period of 15 h and treated with EHSR (10, 25, 50, 100, 250 and 500 mg/kg, p.o.), omeprazole (30 mg/kg, p.o.), or vehicle (0.9% saline, 0.1 ml/10 g, p.o.) one hour before administration of absolute ethanol (0.2 ml/animal, p.o.). In the model gastric lesions induced by acidified ethanol, one hour after treatment the animals received 0.2 mL of 0.3 M hydrochloric acid (HCl) in 60% ethanol and were sacrificed 1 h later. After 30 minutes, the animals were sacrificed by cervical dislocation. Their stomachs were removed, opened along the greater curvature, rinsed with saline (0.9%) and digitized; the ulcerated area was expressed as a percentage relative to the total area of the gastric body using ImageJ software. **Results:** EHSR had antiulcerogenic activity, which was investigated by using different standard experimental models of induced acute gastric ulceration with absolute ethanol and acidified ethanol. Treatment with EHSR at all of the tested doses revealed a significant reduction of the area damaged in models. In the damage caused by ethanol model, the reductions of gastric ulceration were observed with 100 mg/kg (0.12 ± 0.62% and 99.41%), 250 mg/kg (3.32 ± 0.82% and 83.82%) and 500 mg/kg (0.23 ± 0.12% and 98.87%), demonstrating a significant reduction in the lesion area. In the acidified ethanol model, the results obtained with the dose of 100 mg/kg (1.22 ± 0.40% and 94.74%), 250 mg/kg (0.99 ± 0.30% and 95.73%) and 500 mg/kg (0.36 ± 0.16% 98.44%) also showed an important protection after administration of EHSR when compared with control group. **Discussion:** EHSR showed a significant gastroprotective effect. EHSR protect the gastrointestinal mucosa from lesions produced by experimentals ulcer models, against different necrotic agents. The results obtained from the administration of this natural product under *in vivo* models support the ethnopharmacological use of this species, represents a promising natural source of bioactive compounds and also corroborating its use in the folk medicine to treat of gastric lesions. **Keywords:** antiulcerogenic activity, intestinal motility, *Stryphnodendron rotundifolium* Mart. **Financial agency:** CNPq

07.011 Hypoglycemic activity of betulinic acid in mice with alloxane-induced diabetes. Freitas AMP[1], Dantas MB[1], Araújo VM[1], Morais TMF[1], Melo TS[1], Pereira NBS[1], Rodrigues HG[1], Maia AIV[2], Pessoa ODL[3], Queiroz MGR[1] [1]UFC – Análises Clínicas e Toxicológicas, [2]UFC – Química Orgânica e Inorgânica, [3]UFC – Química Orgânica e Inorgânica

Introduction: Alternative medicine is an increasingly popular option in the treatment of diabetes. Several natural products have been reported to possess hypoglycemic activity, especially terpenes—the largest group of secondary metabolites in plants. Among them, betulinic acid (BA), a lupane-type pentacyclic triterpene, displays a wide range of biological and pharmacological activities (Frighetto et al., *Rev. Bras. Farmacogn.,* v.15, p.338, 2005). Objective: To evaluate the hypoglycemic effect of BA on mice submitted to alloxane (ALX)-induced diabetes and the oral glucose tolerance test (OGTT). Methods: Male Swiss mice (25-35g) were used in both experimental protocols. The ALX protocol included five groups (n=8): negative control (NC), positive control (PC), 5mg/kg BA (BA5), 10mg/kg BA (BA10) and 20mg/kg BA (BA20). Following an 18-hour fasting period, the animals received an intraperitoneal injection with ALX (200mg/kg), except in NC. After 72 hours, the animals were fasted for 6 hours prior to blood draw for glucose measurement. Animals with glucose levels ≥250mg/dL were considered diabetic. BA was administered for 7 days, followed by fasting for 6-8 hours and a second blood draw to measure the levels of glucose, triglycerides and total cholesterol. The OGTT protocol included three groups (n=8): negative control (NC), positive control (PC) and 10mg/kg BA (BA10). The latter was the smallest dose capable of reducing glucose in the ALX protocol. Following pretreatment for 5 days with BA, the animals in Group BA10 and PC received glucose (2g/kg) p.o. Two hours after the last pretreatment (baseline) and at 30, 60, 90 and 120 minutes blood was collected to determine glucose levels (Melo et al., *Chem. Bio. Int*.,p.59 2010). The protocols were previously approved by the UFC Committee on Animal Research and Ethics under entry #03/11. The results were expressed as mean ± SEM. The groups were compared with the Newman-Keuls test followed by ANOVA, with the level of statistical significance set at 5% ($p<0.05$). Results: In the ALX protocol, glucose (mg/dL) was significantly reduced in BA10 (507.7±127.6) and BA20 (425.00±154.30), compared to PC (660.0±80.0). Triglycerides and total cholesterol were reduced at all BA concentrations tested. In BA10, the glycemic peak (mg/dL) was reduced after 30 min (190.0±29.3) and 60 min (171.0±16.9) of OGTT. Discussion: Alloxane has been shown in vitro to be selectively toxic to pancreatic beta cells, inducing cell necrosis and thereby raising glucose levels (Bellahcen et al., Phytother. Res., v.26, p. 180,2011). Our results match the findings of (Melo et al J. *Agric. Food Chem*..v.57, p.8776, 2009), who found BA to reduce glucose levels in obesity induced by a hypercaloric diet. BA displays a potential for the treatment of diabetes, but further studies are required to clarify the pharmacological mechanism involved. **Financial support**: CAPES

07.012 Protective effects of protein isolated from latex *Himatanthus drasticus* (MART.) *Plumel* (APOCYNACEAE) in mice gastric mucosa against injury induced by ethanol: involvement of NO/$_C$GMP/K_{ATP}. Souza TFG[1], Marques LM[1], Pinheiro RSP[1], Freitas LBN[1], Luz PB[1], Carmo LD[1], Alencar NMN[1], Matos MPV[2], Ramos MV[2] [1]UFC – Fisiologia e Farmacologia, [2]UFC – Bioquímica e Biologia Molecular

Introduction: The *Himatanthus drasticus* is a plant of Apocynaceae`s family and popularly known as janaguba. The latex produced by this plant is used in Brazilian local communities for medicinal purposes, as in inflammatory processes, treatment of cancer and ulcers. The aim of this study is to demonstrate the gastroprotective effect of proteins isolated from latex *Himatanthus drasticus* (HdPL) in gastric ulcer models and investigate the involvement of NO, cGMP and potassium channels with this activity. **Methods**: Animal handling and experimental protocols were registered on the Institutional Ethics Committee (CEPA) under number 43/2011. Swiss mice were used and divided into groups of 8 (n = 8), and undergo fasting for 16h, then treated with HdPL in different doses (0,5; 5; 50 mg/kg i.v.) or NAC (750 mg/kg p.o.). They received 0,2 ml of absolute ethanol per oral after 30 min. The animals were sacrificed and stomachs removed 30 min later, the lesion index were measured and GSH (reduced glutathione) were assayed. In order to investigate the involvement of NO, cGMP, and potassium channels, animals received L-NAME (20mg/kg i.p.), ODQ (10 mg/kg, i.p.) or Glibenclamide (5mg/kg, i.p) 30 minutes before treatment with vehicle, L-arginine (600mg/kg i.p.), HdPL (5 mg/kg i.v.) or Diazoxide (3mg/kg i.p). **Results**: HdPL's gastroprotective effect was observed only at a dose of 5mg/kg, which reduced by 83% and significantly ($p < 0.05$). In animals pretreated with L-NAME (20 mg / kg, sc), glibenclamide (5 mg / kg, ip), or with ODQ (10 mg / kg, ip), the gastroprotective effect of HdLP was inhibited and the injured areas in these groups increased significantly, 63%, 290% and 249%, respectively, compared to the group pretreated with only HdLP ($p < 0.05$). HdLP also restored the GSH levels in mucosa when compared to the ethanol group. **Discussion**: We can conclude that the HdLP has pharmacological activity with gastroprotetor effect in the gastric mucosa. This protection appears to be mediated in part by modulation of NO/cGMP/KATP, which is of great importance in mucosal defense and maintenance of stomach's blood flow. **Keywords:** protein isolated, *Himatanthus drasticus*, gastric ulcer.

07.013 Involvement of oxide nitric pathway, K_{ATP} CHANNELS and $TRPV_1$ receptors in NaHS-induced pyloric sphincter relaxation in mice. Lucetti LT[1], Medeiros J-VR[2], Santana APM[1], Carvalho ACS[1], Tavares BM[1], Soares PMG[3], Ribeiro RA[1], Souza MHLP[1], Cunha FQ[4] [1]UFC – Physiology and Pharmacology, [2]UFPI – Biology, [3]UFC – Morphology, [4]USP – Pharmacology

Introduction : Hydrogen sulphide (H_2S) has recently been shown to be involved in the regulation of smooth muscle tone, which suggests that endogenous H_2S may have modulating effects on intestinal motor function. This is likely through a neuromodulatory mechanism (Fiorucci et al., 2005; Kawabata et al., 2007; Teague et al., 2002), and at relatively high concentrations, H_2S relaxed vascular smooth muscle in a manner involving ATP-sensitive potassium channels (K_{ATP}) (Kubo et al., 2007; Zhao et al., 2001). We evaluated the effects of H_2S donors in pyloric sphincter muscle relaxation, and whether these effects were involved with nitric oxide and K_{ATP} channels or TRPV1 receptors. **Methods:** Mice (20-25g) (n=8) were euthanized, the abdomen was opened and sphincter pyloric was rapidly cut into segments of 1.0 – 2.0 cm in length. Circular muscle layers were mounted vertically in an organ bath containing Tyrode's solution bubbled with 95% O_2/ 5% CO_2 and maintained at 37°C, pH 7.4. The preparation was stabilized under an initial resting tension of 1g for 1 h before the experimental protocols. Active tension was developed isometrically using a force transducer connected to a computerized data acquisition system (LabChart 6.1; PowerLab, ADInstruments). Experimental protocols were initialized with a contraction control with KCl (80mM), following tissue washing with Tyrode's solution. After 1 h of equilibration, were added L-Name 300μM (NOS inhibitor), gliblenclamide 10μM (K_{ATP} inhibitor), capsazepine 3μM (competitive $TRPV_1$ receptor antagonist) or Tyrode´s solution. The drugs were allowed to incubate for 30 min before the administration of NaHS (H_2S donor) (10-1000μM) by a cumulative curve concentration-response. All animal treatments and surgical procedures were approved by the local ethics committee (protocol No 63/07). Data were expressed as percentages of the maximum relaxation obtain by papaverin (1 - 1000μM). All values are expressed as means ± S.E.M. The analysis Student's t-test was used to determine the statistical significance of differences between groups. Differences were considered as significant at $P \leq 0.05$. **Results:** We also observed that, in the pyloric sphincter, NaHS caused relaxation with a maximum value of 45.28 ± 2.97 % (1000 μM), when we compared with papaverin. However, the NaHS-induced pyloric sphincter relaxation (1000 μM NaHS) was abolished by glibenclamide 10 μM (11.12 ± 2.86 %) or capsazepine 3 μM (2.10 ± 1.90 %) and not by L-Name 300 μM (32.70 ± 7.25 %). **Discussion:** In summary, our results suggest that H_2S donors induced a relaxation of pyloric sphincter muscle and this effect occur by the activation of K_{ATP} channels and afferent neurons/$TRPV_1$ receptors and not include NO pathway. Financial Support: Capes/ CnPq/ Funcap.

07.014 Experimental outcomes of endogenous H2S in rats with acute pancreatitis evoked by secretory phospholipase A2 from *Crotallus durissus terrificus* (Cdt) venom. Zanoni CIS, Rodrigues L, Ekundi-Valentim E, Teixeira SA, Muscará MN, Costa SKP ICB-USP

Aims: During the past 20 years, there has been increasing evidences to suggest that the noxious gas H_2S is a gaso-transmitter, produced by various cells using the enzymes cystathionine beta-synthase (CBS) and cystathionine gamma-lyase (CSE), which plays an important role in inflammatory diseases. Whether H_2S plays a deleterious or a protective role in acute pancreatitis (AP), a painful and inflammatory disease of the pancreas, is not clear. This study aimed to investigate the effects of endogenous inhibition of H_2S in a model of AP in rats. **Methods and Results:** AP induced was evoked by injection of secretory PLA_2 from *Crotallus durrisus terrificus* (*Cdt*; 300mg/kg*)* snake venom into the common bile duct of rats, pretreated with an inhibitor of CSE, propargylglycine (Pgly; 50 mg/kg; i.p., -30 min) or vehicle (0.1 ml). Four hours later, rats were submitted to abdominal nociceptive behavioral test (von Frey) and euthanized. Blood and pancreas were collected and processed to measure plasma amylase, H_2S generation and inflammatory parameters (oedema, myeloperoxidase activity) in pancreas. All experimental protocols were approved by the CEEA/USP (n^{o}055 pg 44 book 2). Treatment with PGly markedly reduced inflammation in the pancreas of rats with pancreatitis, but neither increased serum amylase nor abdominal hyperalgesia was affected by this treatment. The kinetics of H_2S generation in pancreas revealed that CSE and CBS are both expressed in naive tissue, and a significant difference is seen between control and inflamed pancreas. Inhibition of CSE and CBS reduces H_2S generation in pancreas. **Conclusions**: endogenous H_2S plays a functional role in mediating inflammatory, but not nociceptive, mechanisms of PLA_2-induced AP. Thus, H_2S inhibitors may represent a potential (or complementary) therapeutic class for relief of pancreatitis symptoms. **Acknowledgments**: FAPESP, CNPq and CAPES for financial support. We thank MAB and IMG for technical assistance.

07.015 The Role of TRPV1 Receptors and GMPc in gastroprotective effect of β-ionone in models of acute gastric lesion. Olinda TM[1], Freitas LBN[1], Pinheiro RSP[1], Luz PB[1], Marques LM[1], Osório CBH[1], Couto TS[1], Carmo LD[1], Souza TFG[1], Sousa DP[2], Alencar NMN[1] [1]UFC – Fisiologia e Farmacologia, [2]UFS

Introduction: The β-ionone (4 - [2,6,6-cyclohexene-1-trimetill]-3-butene-2-one) is a sesquiterpene (degraded terpenoid - C13) present in the molecular structure of retinol, β-carotene and acid retinoic, formed from the mevalonate pathway in different types of plants. The research on the biological activities of β-ionone is still incipient and limited results with this compound are found in the literature. Objective: To demonstrate the gastroprotective of β-ionone in gastric ulcer models and investigate the role of TRPV1 and GMPc. **Methods**: Animal handling and experimental protocols were approved by the Ethical Committee for Animal Research/UFC under number 0286. Swiss mice were used, were divided into groups of 8 (n = 8), and undergo fasting of 16h, then were treated with BI in doses 12,5; 50; 100 and 200 mg/kg or NAC (750 mg/kg). After 30 min they received 0,2 ml of absolute ethanol per oral and after 30 min, the animals were sacrificed and stomachs removed and analyzed the lesion index. In order to investigate the involvement of TRPV1 receptors and GMPc, before treatment with BI animals received Capsazepine (5mg/kg) or Capsaicine (2mg/kg) and ODQ (10mg/kg). Results: In model of injury by ethanol, BI at the doses 12,5; 50; 100 and 200 mg/kg was able to prevent injury in 43,8; 81,2; 94,9; and 98,9% respectively. ODQ was able to reverse the protective effect of BI, demonstrating the involvement of GMPc in this mechanism of action. Capsazepine was unable to reverse the effect of BI, thus excluding a possible involvement of TRPV1 receptors. **Conclusion**: We can conclude that the BI has pharmacological activity with gastroprotetor effect in the gastric mucosa. This protection appears to be mediated in part by modulation of GMPc, which is of great importance in mucosal defense and in maintaining blood flow to the stomach. **Sources of research support**: FUNCAP, CAPES and CNPq.

07.016 Effect of nitrosyl-ruthenium on gastric inflammation model in mice – role of the cGMP-KATP pathway. Santana APM[1], Torres JNL[1], Tavares BM[1], Medeiros J-VR[2], Lucetti LT[1], Gomes AS[3], Soares PMG[3], Carvalho ACS[1], Silva FON[4], Lopes LGF[4], Ribeiro RA[1], Souza MHLP[1] [1]UFC – Physiology and Pharmacology, [2]UFPI – Biology, [3]UFC – Morphology, [4]UFC – Organic and Inorganic Chemistry

Introduction: Sodium nitroprusside (SNP), a NO donor, reduced the ethanol-induced gastric damage. Recently, new NO donors with ruthenium metal in its composition, which is soluble in water with higher chemical stability and lower toxicity, was develop. The aim of this study was compare the effects of nitrosyl- ruthenium (NR) and SNP against ethanol induced gastric damage in mice. Additionally, the participation of cGMP-K_{ATP} pathway was evaluated. **Methods**: Swiss mice (25-30g) were handled in accordance with the ethical principles (local ethics committee protocol 33/10). Mice were treated by gavage with saline, NR (3mg/kg, 4.5µmol/kg) or SNP (10mg/kg, 33.5µmol/kg). To evaluate the participation of K_{ATP} channels, glibenclamide (10mg/kg, i.p), a K_{ATP} channel blocker, was administred 1h before NR treatment. In another group of animals, ODQ (10mg/kg, gavage), a inhibitor of guanylate cyclase were injected 30min before NR (3mg/kg) administration. Thirty minutes after last treatment, ethanol (50%, 0.5 ml/25g) or saline was administered by gavage. One hour later, mice were sacrificed and the stomachs removed to determine the gastric damage. Additionally, tissue fragments were removed for histopathological analysis and measurement of glutathione (GSH) and malondialdehyde (MDA) levels. **Results**: Ethanol induced gastric damage (96.20±10.36mm^2), reduced glutathione (197.5±24.28µg/g) and increased MDA (78.8±7.4nmol/g) levels, when compared with saline (without gastric damage, MDA:36.06±3.4nmol/g and GSH:419.1±31.9µg/g). NR decreased in 92% (7.7±5mm^2) and SNP in 56.4% (41.98±14.02mm^2) the gastric damage induced by ethanol. Furthermore, both NR and SNP reversed the decrease in glutathione (NR=299.2±34.8; SNP=471.0±44.17mg/g) and increase in MDA (NR=34.32±4.4; SNP=50.15±2.36 nmol/g) levels induced by ethanol. Pre-treatment with glibenclamide (213±26.9mm^2) or ODQ (190.6±40mm^2), reversed the protective effect of NR (56.02±22mm^2) in ethanol-induced gastric lesion, furthermore, the effects of NR on glutathione (glibenclamide: 105.9±13.3, ODQ: 159.8±32.7, NR: 271.8±31.2mg/g) and MDA (glibenclamide: 68.6±3, ODQ: 66.5±8.8, NR: 32.1±4.24nmol/g) gastric levels was also reverted. **Conclusion:** These results demonstrated that NR compound prevent ethanol- induced gastrophaty, in part by decreased the ethanol- induced free radicals production. Furthermore, nitrosyl- ruthenium gastroprotective activity was observed at doses up to 22-fold lower than the gastroprotective dose of sodium nitroprussiate. This effect is likely due to activation of cGMP and the opening of K_{ATP}. **Financial Support**: CNPq, CAPES, FUNCAP.

07.017 Evaluation of gastroprotective activity of the ethanolic extract from *Pilosocereus gounellei*.
Sousa GA[1], Rocha FTA[1], Sousa-Neto BP[1], Freitas FFBP[1], Souza MFV[2], Oliveira FA[1] [1]NPPM-UFPI, [2]UFPB – Tecnologia Farmacêutica

Introduction: *Pilosocereus gounellei* (A. Weber ex K. Schum.) Bly. Ex Rowl, popularly known as "xiquexique", is a cactacea abundant in the semi-arid of Brazil. In traditional medicine, the aerial parts are used as ointment in the treatment of skin lesions. The aim of this study was evaluate, for the first time, the potential gastroprotective the extract ethanolic obtained from aerial parts of *P. gounellei* (EEPG) in a model of gastric lesions induced by absolute ethanol in mice. **Methods:** Male and female Swiss mice (25-35 g/n=6-8/group/The experimental protocols were approved by the Animal Care and Use Committee /UFPI/Process Nº. 077/11) were used in the assessments of acute toxicity (LD50) and gastroprotective activity of EEPG. In determining the LD50 animals were treated (p.o) with EEPG (500, 1000 and 2000 mg/kg) and observed for gross behavioural changes and mortality. In the model gastric lesions by ethanol, the animals were pretreated (p.o) with saline (0.9%), EEPG (100, 200 and 400 mg/kg) or carbenoxolone (100 mg/kg) and after 1 h received 0. 2 mL of absolute ethanol. After 30 min were killed, their stomachs removed, opened by the greater curvature and gastric lesion area determined by planimetry (mm²). A separate experiment was performed to examine the role of prostaglandins (PG) in the gastroprotective effect of EEPG (400 mg/kg) using the pre-treatment with ibuprofen (100 mg/kg, p.o) in the model of gastric lesions by absolute ethanol. Mice were pretreated (p.o) with ibuprofen (100 mg/kg) and after 1 h with EEPG (400 mg/kg), vehicle (0.9% saline) or carbenoxolone (100 mg/kg). After 1 hour the animals were treated absolute ethanol and they were euthanized after 30 min. The stomachs were excised and the mucosal lesion area was measured by planimetry (mm^2). Values are expressed as mean ± standard error of mean. Statistical analyzes were performed using ANOVA (one way) followed by Student Newman Keul test. **Results and Discussion:** The EEPG demonstrated no overt toxicity up to the dose of 2000 mg/kg and we were unable to establish its oral LD50 value. The model of ulcers induced by absolute ethanol promotes multifactorial necrotic lesions in the gastric mucosa. In this model EEPG showed significant gastroprotective effect ($p<0.05$) at doses of 100, 200 and 400 mg/kg (9.55 ± 2.11, 5.95 ± 0.54 and 3.10 ± 2.04 mm², respectively) compared to saline-control group (13.54 ± 1.41 mm²). Carbenoxolone, standard drug, was also able to reduce ($p<0.05$) gastric lesions (2.3 ± 1.08 mm²). Evaluating the possible involvement of PG in the gastroprotective effect of EEPG, the groups treated with EEPG (400 mg/kg) or carbenoxolone (100 mg/kg) exhibited significant reduction ($p<0.05$) of gastric damage compared the saline-control group (4.65 ± 2.5, 3.05 ± 1.61 and 12.60 ± 1.5). Likewise, pretreatment of mice with ibuprofen (100 mg/kg) reversed ($p<0.05$) the protective action of carbenoxolone (10.2 ± 4.40) but was unable to block effectively the gastroprotective effect of EEPG treated group (8.08 ± 2.62), suggesting no involvement of endogenous PG's in the gastroprotection EEPG. Studies are underway to investigate other possible mechanisms of action of the gastroprotective effect of EEPG. **Support**: UFPI-CAPES.

07.018 Irinotecan induces intestinal electrolyte secretion, bacterial translocation and toll-like receptor 4 activation during intestinal mucositis in mice. Wong DVT[1], Bem AXC[1], Costa ELF[1], Noronha FJD[1], Freire RS[1], Brito GAC[2], Souza MHLP[1], Carvalho CBM[3], Lima-Júnior RCP[1], Lima AAM[1], Ribeiro RA[1] [1]UFC – Physiology and Pharmacology, [2]UFC – Morphology, [3]UFC – Pathology

Introduction: Diarrhea and the associated intestinal mucositis are common side effects (15-25%) of colorectal anticancer therapy with Irinotecan (IRI). Gut injury induced by chemotherapeutic agents may result in bacterial/endotoxin translocation from the intestine to the systemic circulation. Then, we aimed to evaluate the intestinal electrolyte transport and the process of bacterial translocation and to characterize the profile of translocated microorganisms during IRI-induced intestinal mucositis. **Methods:** C57BL/6 mice (20-25g, n=7) were divided into groups: saline (5 mL/kg, i.p.); IRI (75 mg/kg/4 days, i.p). Diarrhea was assessed daily. Animals were killed on day 5 (D5) or 7 (D7). Intestinal tissue was collected to determine myeloperoxydase activity (MPO, U/mg tissue), morphometry, immunohistochemistry for Toll-like Receptor 4 (TLR4) and white blood cell count (cells/mm^3). In another experimental setting, in vivo intestinal perfusion was performed for evaluation of electrolyte transport (mEq/g/min). Bacterial translocation was also quantified by lymph node and liver cultures (CFU/g tissue) and bacteremia. Biochemical tests were used to bacterial identification. Statistical analysis was performed with Kruskal Wallis/Dunn's test or ANOVA/ Bonferroni's test as appropriate. $P<0.05$ was accepted. (CEPA 99/10). **Results:** IRI induced a significant ($p<0.05$) diarrhea (D5: 1,5[0-2]; D7: 2,5[1-3]), increased MPO activity (D5: 8,87±1,9), and leukopenia (D5: 1825±358.8; D7: 3467,9±506,8) compared with saline (0[0-0], 2,96±0,5, 9050±2789, respectively). The villus area and villus/crypt ratio in the IRI-treated mice was significantly decreased on days 5 (area: 11,10±0,5; ratio: 1,38±0,05) and 7 (area: 8,17±0,4; ratio: 1,21±0,04) compared with mice given saline (area: 19,9±0,5; ratio: 3.59±0.11). Additionally, IRI induced changes in gut injury index (D5: 3[1-4]; D7; 3,5[2-4]) versus saline group (0[0-0]). IRI significantly increased sodium (D5: 59,5%; D7:40,11%), potassium (D5: 160% D7: 156%) and chloride (D5: 26,2%) secretion versus control group. Bacterial translocation to mesenteric lymph nodes ($27,1x10^3$) and liver ($4,1x10^3$) was also significantly ($P <0.05$) increased only the IRI-D5 compared with the saline group ($5,8x10^3$, $1x10^3$, respectively). Bacteremia was also evidenced in IRI-D5 (25%) and D7 (42.85%) in comparison with the saline group. Biochemical identification of translocated bacteria revealed the presence of *Escherichia coli*, *Citrobacter sp*., Gram-Negative Bacteria non-fermentative and *Pseudomonas aeuriginosa* in the IRI injected mice. Immunostaining for TLR4 was significantly increased ($P <0.05$) in the intestine of IRI injected mice both at D5 (4[3-4]) and D7 (4[3-4]) versus saline group (1,5[1-4]). **Conclusion:** In addition to causing gut damage and electrolyte intestinal secretion, IRI causes gram-negative and non-fermentative bacteria translocation to the mesenteric lymph nodes and liver and activation of TLR4. **Support**: CNPq/FUNCAP/CAPES

07.019 ***Lactobacillus acidophilus*** **reverts gastric dysmotility and the inflammation present in intestinal mucositis induced by 5-fluorouracil in mice**. Justino PFC[1], Silva LMN[1], Melo LFM[1], Nogueira AF[1], Xavier AF[1], Souza EP[2], Souza MHLP[1], Ribeiro RA[1], Soares PMG[2] [1]UFC – Fisiologia e Farmacologia, [2]UFC – Morfologia

Introduction: Intestinal mucositis is a frequent side-effect associated to 5-fluorouracil (5-FU) clinical use and results in inflammatory events. It is characterized by epithelial ulcerations in the mucosa and clinical manifestations of abdominal pain, nauseas and diarrhea. *Lactobacillus acidophilus* (LAC) is a probiotic which has been shown to protect the gastrointestinal microflora from disequilibrium and from associated gastrointestinal disorders. Objective was to evaluate effect of *Lactobacillus acidophilus* in functional and inflammatory aspects of intestinal mucositis induced by 5-FU in mice. **Methods:** Swiss male mice (25-30g) were treated with 5-FU (450 mg/kg, i.p., only dose) or saline (C). One group, mice were treated with *Lactobacillus acidophilus* (LAC; 0,2 x 10^8 UFC) daily for 3 days. On the third day after administration of 5-FU or 5-FU + LAC, mice were sacrificed and samples of jejunum (J) and ileum (I) were collected for assessment of nitrite concentration for spectrophotometry and concentration of cytokines. To determination of gastric emptying (retention fraction %), phenol red (PR, 0.75 mg/mL, 300 µL) by gavage was administrated and mice were sacrificed 20 min later. The stomach and intestinal were processed measurement of PR concentrations by spectophotometry. All animal treatments and surgical procedures were approved by the local ethics committee (protocol 34/10). Significance statistics (tests ANOVA and Bonferroni), values considers with $p < 0.05$. **Results:** The treatment with LAC improved delay in gastric emptying (C=23.78±2.73%; 5-FU=49.06±5.42%; 5-FU+LAC =33.58±1.858%). The treatment with LAC reduced pro-inflammatory cytokines (pg/mL) induced by 5-FU (Jejunum: IL-1b: C=67.93±12.46, 5-FU=242.60±34.84, 5-FU+LAC=147.00±13.80; TNF-α: C=1230.00±125.90, 5-FU=1923.00±148.30, 5-FU+LAC=1288.00±156.90 and Ileum: IL-1b: C=304.40±34.88, 5-FU=540.60±45.35, 5-FU+LAC=375.60±48.79 ($p>0,05$); TNF-α: C=667.80±89.20, 5-FU=1242.00±197.50, 5-FU+LAC=345.20±90.26). LAC reduced the increase on the nitrite concentration (µM) induced by 5-FU (Jejunum: C=36.96±1.55, 5-FU=53.69±7.64, 5-FU+LAC= 27.68± 1.62; Ileum: C=33.84±1.36, 5-FU= 43.85±12.65, 5-FU+LAC=29.47±1.62). **Discussion:** Our results suggest that the treatment with *Lactobacillus acidophilus* reverted the gastric retention probably by reducing the inflammatory events on the intestinal mucositis induced by 5-FU in mice. Nitrite levels were lower in the ileum probably due to less reversal of cytokine concentrations compared to the jejunum. **Financial Support:** CNPq/FUNCAP.

07.020 Proliferative effect of alanyl-glutamine after *in vitro* rat intestinal cells injury promoted by enteroaggregative *Escherichia coli* (EAEC). Freitas REM[1], Silva VA[1], Cavalcante PA, Prata MMG, Lima IFN, Quetz JS, Lima AAM, Havt A UFC – Physiology and Pharmacology

Introduction: As diarrhea mortality rates decline worldwide. Persistent diarrhea becomes a concern since it is associated with various long-term problems such as weight loss, growth retardation and cognitive impairment (BHUTTA, A.Z. J Pediatr Gastroenterol Nutr, Vol. 39, P.711, 2004). Enteroaggregative *Escherichia coli* (EAEC) is a pathogenic bacterial strain not always associated with diarrhea cases in Brazil (ARAUJO, J.M., J. CLIN. MICROBIOL, V. 45, P.3396, 2007). It is known that the repair capacity of the intestinal epithelium when exposed to harmful stimuli and micronutrients deficiencies is an important factor against the persistent diarrhea development. In the event of an injury, new cells must be formed to replace the areas where the epithelium is discontinued. Moreover, glutamine, an important amino acid necessary to many enterocyte regulatory and metabolic functions, has low solubility and stability in aqueous medium, which limits its use as a therapeutic alternative. On the other hand, the dipeptide alanyl-glutamine (Ala-Gln) is soluble in aqueous medium, therefore serving as a glutamine stable store for cells (BRASSE-LAGNEL, C.G, *Biochimie*, V. 92, P.729, 2010).We aimed to evaluate the effect of alanyl-glutamine on the proliferation of rat intestinal epithelial cells when infected by enteroaggregative Escherichia coli (EAEC, strain 239-1). **Methodology**: IEC-6 cells (passages 30-33) derived from rat intestinal crypt were seeded in 96 well plate (2.5 x 10^4 cells /well) and grown in a humidified incubator at 37°C with 5% CO2 and 95% air. After 24h, the cells were infected or not with 100uL of an EAEC suspension (10^6 UFC/mL) for 3 hours and washed 3 times with PBS buffer. Subsequently, it were incubated with DMEM (containing 200µg/mL of gentamicin) supplemented or not with Ala-Gln in concentrations of 3 and 10 mM. After 24 h of incubation, all wells received 10µL of WST-1, remained in the incubator for 2 hours before reading the absorbance at 450 nm. Three independent experiments were performed in each group and done in quadruplicates. The results of absorbance are expressed as mean ± standard error of mean. The groups were compared using the Bonferroni test. Differences were considered significant with $P<0,05$. Results and discussion: The uninfected groups and the ones supplemented with Ala-Gln in concentrations of 3 mM (1.44 ± 0.09) and 10 mM (1.28 ± 0.10) showed significant proliferation when compared to the group without supplementation (0.73 ± 0, 06). The EAEC strain was able to negatively modulate the proliferation of intestinal epithelial cells (0.16 ± 0.03). However, the damage caused by the bacterium could be restored when infected cells were supplemented with Ala-Gln in concentrations of 3 (0.94 ± 0.22) and 10 mM (0.99 ± 0.25). The results demonstrate the dipeptide ability to stimulate intestinal epithelial cells proliferation as well as to repair lesions in the intestinal mucosa. Further experiments will investigate how Ala-Glu protects intestinal cells injury by maybe promoting cell proliferation interfering with one of the three MAP kinase pathways, ERK1/2, p58 or JNK/SAPK (ZHANG, W; LIU,H.M. Cell Research, V. 12:P.9, 2002).