Session 07 − **Endocrine and Gastrointestinal Pharmacology**

07.001

Role of the cholinergic/NO pathway in delayed gastric emptying of liquids induced by α-terpineol in awake rats. Bento-Silva MT[1], Marques RB[2], Oliveira FGV[1], Silva LL[2], Piauilino CA[2], Oliveira IS[2], Pinheiro, ADN[1], Santos AA[3], Oliveira FA[2], Almeida FRC[4] [1]UFC − Fisiologia e Farmacologia, [2]NPPM-CCS-UFPI, [3]UFC − Medicina, [4]UFPI − Bioquímica e Farmacologia

Introduction: Literature has shown that α-terpineol (α-TERP) provokes several vasorelaxant activities (Magalhães, Fundam Clin Pharmacol v.22, p. 161, 2008). A greater relaxing effect is noted in guinea-pig ileum due to α-TERP when combined with constituents such as methyl-eugenol and cineole (Magalhães, Phytother Res v.12, p.177, 1998). However, it remains unknown on the effects of α -TERP on gastrointestinal motility. AIM: To study the effect of α-TERP on gastric emptying (GE) of liquids in awake rats, as well as the possible involved mechanisms. **Methods:** This study was conducted on male Wistar rats (200-250g, n=8) kept under conditions of stable temperature (22 ± 2°C) and a 12 h light/12 h dark cycle with free access to tap water and food. All experimental procedures were assessed and had prior approval by the local animal ethics committee (Number #10/08). The study on GE was conducted as described by (Reynell, J Physiol v.131, 452, 1956) a widely used technique in our laboratory. After a fasting period of 24h, the rats were treated with α-TERP doses (12.5, 25 or 50 mg.kg-1 p.o) or vehicle (Tween 80 0.2%). After analyzing the three aforementioned doses, we chose to always work with 50 mg.kg-1 p.o. So as to investigate the involved neural mechanisms, a separate group of animals were pre-treated (30-min) intra-peritoneal with: Atropine 1mg.kg-1 (ATR), Guanethidine 10mg.kg-1 (GUA), Hexamethonium 20mg.kg-1 (HEX), L-NAME 5 mg.kg-1 (L -NAME), L-Arginine 300mg.kg-1 (L-Arg) and Methylene Blue 3 mg.kg-1 (MB). Next, the rats were gavage fed with α-TERP dose or vehicle and after 60-min, they were gavaged too with a liquid test meal of phenol red dextrose (5%), being sacrificed 10-min later so as to study postprandial GE. Data are expressed as mean ± SEM and compared by ANOVA followed by Tukey test. **Results and Discussion:** We observed a decrease in GE ($p < 0.05$) in rats treated with α-Terp-50mg.kg-1 compared to the vehicle (63.8 ± 3.6 vs 48.8 ± 2.0%). The pre-treatment with ATR abolished significantly ($p<0.05$) GE decrease, compared to α-TERP-50mg.kg-1 (46.5 ± 5.2 vs 63.8 ± 3.6%). Pre-treating with L-NAME reversed the effect of decreased GE induced α-TERP-50mg.kg-1 (49.1 ± 2.6 vs 63.8 ± 3.6%), an effect altered by administering L-Arg. All pre-treatments, with GUA, HEXA and MB were unable to change the decreased GE phenomenon induced by α-TERP-50mg.kg-1 (61.8 ± 2.6, 69.9 ± 3.5 and 62.0 ± 2.1 vs 63.8 ± 3.6%). Therefore, these results suggest that α-TERP-50mg.kg-1 decreases the GE via the cholinergic/NO pathway but neither through adrenergic nor ganglionic pathways. FINANCIAL SUPPORT: CNPq, CAPES.

07.002

Characterization of neuro-humoral pathways in increased gastric retention of liquids due to mechanical right atrium stretch in awake rats. Palheta Junior RC[1], Okoba W[1], Bento-Silva MT[1], Pinheiro ADN[1], Oliveira, FGV[1], Elias LLK[2], Rodrigues JA[2], Santos AA[1] [1]UFC − Fisiologia e Farmacologia, [2]FMRP-USP − Fisiologia

Introduction: Right atrium mechanical stretch (AS) increases gastric motility in anesthetized rats (Life Sciences 86, 2010). We aimed to study the effect of AS on gastric retention (GR) in awake rats and elucidate on involved neuro-humoral pathways. **Methods:** After approval by the local research ethics committee on animal use (Protocolo 02/2009- Comitê de Ética em Pesquisa com Animais da Faculdade de Medicina da UFC), male albino rats (N=156, 250-280g) had a silicone balloon inserted in the right atrium. After 24-h, the central venous pressure (CVP), heart rate (HR) and the mean arterial pressure (MAP) were monitored over the first 20-min, after which the animals were randomly pre-treated with: Saline (S, 0.1 ml/100g, i.v.), Atropine (A, 0.5 mg/kg, i.v.), Guanethidine (GT, 10 mg/kg, i.p.), Hexamethonium (H, 10 mg/kg, i.v.), NG-nitro-L-arginine methyl ester (L-NAME, 3 mg/kg, i.v.), L-Arginine (100 mg/kg, i.v.)+L-NAME (3 mg/kg, i.v.), Methylene Blue (MB, 3 mg/kg, i.v.), Glibenclamide (GB, 1 mg/kg, i.p.) or Glibenclamide+Diazoxide (GB+D, 3 mg/kg, i.v.), Anantin (ANT, 5mg, i.v.) or Atosiban (AT, 40 mg/kg/h, i.v.). Next, AS with saline zero (sham) or 50mL was performed in all animals during 5min. Consequently, each group (n=6), had rats gavage-fed with a liquid test meal (1.5mL containing 5% Glucose Solution with Red Phenol, 0.5mg/mL), 20-min after AS and sacrificed 10-min afterwards to study GR (J Physiol 131:452, 1956). Results Comparing to Sham, AS increases GR (44 ± 4.5 vs 69.8 ± 3.5% ($p<0.05$). Besides, in comparison to the basal period, AS increased CVP and HR (1.6 ± 0.6 vs. 4.7 ± 1.1 cmH2O and 365 ± 8 vs 426 ± 21 bpm, respectively). In relation to their respective controls, AS increased ($p <0.05$) GR in S (38.9 ± 4.0 vs 61.7 ± 2.7%), A (58.7 ± 2.0 vs. 72.3 ± 3.8%), GT (42.7 ± 2.7% vs 60.6 ± 2.8%), L-Arginine+L-NAME (49.2 ± 1.7 vs. 64.4 ± 4.6%), MB (41.4 ± 2.3 vs. 53.7 ± 4.5%) and in GB+D groups (45.2 ± 2.7 vs. 64.2 ± 1.6%). However pre-treatment with H (47.6 ± 2.1 vs. 42.6 ± 3.0%), L-NAME (51.7 ± 5.4 vs. 41.0 ± 4.0%), GB (40.2 ± 1.7 vs. 38.3 ± 3.2%), ANT (40.9 ± 3.2 vs. 35.8 ± 3.5%) or AT (33.8 ± 2.6 vs. 35.5 ± 3.0%) prevented the effect of AS on GR. **Discussion:** In reference to above results therefore, the increase in GR of fluids, which is mediated by cardiac low pressure receptors in awake rats, depends upon a cascade mediated by Oxytocin, Atrial Natriuretic Peptide and Nitric Oxide through K+-ATP dependent channels. Key Words: Gastric emptying, blood volume, atrial natriuretic peptide and oxytocin. Research funding: CAPES, CNPq, FAPESP and FUNCAP

07.003

Hypoglycemic activity of new derivates sulfonilhidrazonics in the animal model of type 1 diabetes. Oliveira LGT[1], Kartnaller MA[1], D´Andrea ED[2], Lima ML[2], Barreiro EJ[2], Sudo RT[1], Zapata-Sudo G[1] [1]UFRJ − Desenvolvimento de Fármacos, [2]FF-UFRJ

Introduction: Type 1 diabetes also known as juvenile diabetes or insulin-dependent diabetes is characterized as a metabolic disease with destruction of beta-pancreatic cells leading to an increase of blood glucose. When not properly treated can develop complications such as myocardial depression, renal failure, vision problems and neuropathy (KHAVANDI et al, 2009). The present work aims to identify new agents that reduce blood glucose on rats with diabetes induced by streptozotocin (STZ) which could delay the development of vascular and neural complications. **Methdology**: The synthesis of the derivates was performed using the reactions of interconversion of functional groups, starting from 4,3-methilenedyoxitoluene becoming the sulfonation followed by conversion into the potassium salt after chlorination. The hydrazinolysis resulted in the sulfonilidrazide which was the intermediary to obtain the final compounds: LASSBio-331, LASSBio-1470, LASSBio-1471 and LASSBio-1503. Type 1 diabetes was induced in Wistar rats (180 - 220 g) by intraperitoneal injection of STZ, 65 mg/kg (EREJUWA et al, 2010). Derivatives were administered intraperitoneally at a dose of 10 mg/kg in two experimental groups: 1. 3 days before inducing diabetes in animals and during the subsequent 7 days; 2. 15 days after induction of diabetes to determine the effect of derivatives on the already established hyperglycemia. Blood samples were obtained from the caudal vein and plasmatic glucose concentration was assessed using the system of Accu-Check® (Roche, Germany). This study was performed in compliance with the Animal Care and Use Comitte ate the Universidade Federal do Rio de Janeiro (UFRJ) under the protocol number DFBCICB013. **Results:** In the group of STZ-induced diabetes and treated with vehicle, blood glucose was increased from 111.3 ± 2.8 (control, day zero) to 484.0 ± 11.0 and 394.6 ± 38.9 ng/dL after 5 and 7 days after STZ administration. In the group in which derivatives were administered three days before STZ, only LASSBio-1471 and LASSBio-1503 reduced blood glucose levels of diabetic rats. Blood glucose was maintained at normal values of 123.7 ± 6.6 and 197.7 ± 59.9 ng/dL 5 and 7 after the beginning of treatment with STZ and LASSBio-1471. In addition, the group treated with LASSBio-1503 had the glucose concentration of 135.0 ± 4.2 and 147.7 ± 2.7 ng/dL. In contrast, LASSBio-331 and LASSBio-1470 did not interfere with the increase of glucose induced by STZ. After 7 days of administration of STZ, the blood glucose remained at 442.0 ± 28.4 ng/dL in animals treated with LASSBio-1470. Three hours after intraperitoneal injection of LASSBio-1471, hyperglycemia was reduced from 556.5 ± 4.5 to 346.0 ± 135.0 ng/dL ($P < 0.05$) 15 days after STZ-induced diabetes. **Conclusions:** Among the derivatives tested, LASSBio-1471 and LASSBio-1503 promoted hypoglycemic effect in type 1 diabetes induced in rats. **Financial support:** CAPES, FAPERJ, CNPq, FUJB, INCT.

07.004

Antiulcerogenic activity of ethanolic and aqueous extracts of the bark of *Terminalia catappa* in gastric ulcer induced by ethanol in *Rattus norvegicus*. Viana VSL, Brito-Júnior EC, Rabelo RS, Nunes-Filho DM, Maia EP, Martins MCC, Nunes PHM UFPI − Biofísica e Fisiologia

Introduction: Currently medicinal plants are the most attractive sources for new drugs available as therapeutic agents (Calixto J.B., *J Ethnopharmacol* 100, p.131, 2005). The plants Terminalia pallida Brandis. (GUPTA, M., J Ethnopharmacol 97, p.405, 2005), Terminalia fagifolia Mart. (AYRES, M., Quim. Nova, 32, p.1509, 2009) and Combretum leprosum (Nunes, P.H.M. et al, Pharmazie 64, p. 58, 2009) exhibit antiulcerogenic activity (AUA), indicating that this botanical family (Combretaceae) probably has others species with the same potential. The *Terminalia catappa* L., commonly known as "castanhola", has antioxidant and hepatoprotective effects (KINOSHITA, S., Phytomedicine, 14, p.755, 2007), but there is no reference to respect for his AUA. Therefore, the aim of this study is to evaluate the AUA of the aqueous (AETC) and ethanolic (EETC) extracts of T. catappa L. in *Rattus norvegicus*. **Methods:** The project was approved by the Ethics Committee for Animal Experimentation (CEEA) of UFPI (nº 042/09). *R. norvegicus* males and females (n = 6-10 animals per group) weighing 250-320 g, fasted for solids for 24 hours, received orally 5 mL/kg of water (control group), AETC (1 g/kg), EETC (250 and 500 mg/kg) or carbenoxolone (100 mg/kg) one hour before absolute ethanol (1 mL/animal, orally). Thirty minutes after administration of the ulcerogenic agent, the animals were euthanized with an overdose of sodium thiopental (100 mg/kg, i.p.). After laparotomy, the stomachs were removed and opened by its lesser curvature. The contours of the body of the stomach and areas of ulcerative lesion (AUL) were drawn on transparent sheets, and the AUL´s were calculated as percentage of body area of the stomach, using the software ImageJ. The values were analyzed by ANOVA followed by Tukey's post-test. The significance level was set at 5% ($p < 0.05$). **Results:** The AUL (Mean ± EPM) found in animals treated with carbenoxolone 100 mg/kg (4.0 ± 0.98, $p < 0.001$), AETC 1 g/kg (8.4 ± 2.18, $p < 0.05$) and EETC at doses of 250 mg/kg (2.5 ± 0.82, $p < 0.001$) and 500 mg/kg (0.07 ± 0.03, $p < 0.001$) were significantly lower when compared to the control (17.1 ± 2.06). The AUL were significantly lower for EETC 500 mg/kg group in relation with the carbenoxolone 100 mg/kg group. **Discussion:** The AETC and EETC showed antiulcerogenic activity on ulcers induced by ethanol. Moreover, the gastroprotection elicited by EETC at a dose of 500 mg/kg (99.9%) was higher than that of carbenoxolone (76.6%). Thus, one can infer the probable existence of active potential in EETC with AUA related to preservation of the mucus layer or on the control of gastric blood flow, known to be altered by ethanol (OATES, PJ, Gastroenterol., 94, p.10, 1988). **Support**: UFPI

07.005

Nitric oxide (NO) pathway influence the gastroprotection induced by carbon monoxide (CO) against ethanol- induced gastric damage in mice. Lucetti LT, Medeiros J-VR, Gomes AS, Santana APM, Soares PMG, Ribeiro RA, Souza MHLP UFC − Fisiologia e Farmacologia

Introduction: Carbon monoxide (CO) has been identified as the second gasotransmitter in the gastrointestinal tract, although the role and importance of CO is less well-studied than NO. Carbon monoxide and nitric oxide are gaseous messengers that possess anti-inflammatory action in the stomach and are involved in maintaining the integrity of the gastric mucosa (Fiorucci et al. 2005 and 2006; Gibbons & Farrugia, 2004). Several authors suggest that these molecules can interact in physiological and pathological conditions, having a regulatory role in various tissues. **Methods:** Swiss mice, (20-30g), previously fasted for 24h, were treated with: saline, ethanol 50% (0.5 ml/ 25 g, gavage), Hemin (3 mg/kg i.p)+ethanol or DMDC (CO donor, 12,5 µmol/kg, i.p)+ethanol. For other experiments, before 30 min, mice were pre-treated with L-Name (NOS inhibitor, 3 mg/kg, i.p.). After 60 min, the animals were sacrificed and stomachs removed for measuring the rate of gastric damage, The colorimetric assays were used to determine gastric tissue level of malondialdehyde (MDA) (Brzozowski, et al. 2002) and glutathione (GSH) concentration. To determine the index of gastric injury, stomachs were photographed with camera for subsequent digital analysis of the lesions using a computerized planimetry program (Ko JK-S & CH Cho, 1998). Data were analyzed using One-Way ANOVA and Newman- Keuls test. All animal treatments and surgical procedures were approved by the local ethics committee (protocol No 63/07). **Results:** Hemin (34.99 ± 5.74 mm2, N=6) and CO donor (DMDC =13.18 ± 2.68 mm2, N=6) significantly ($p<0.05$) decreased the gastric lesion induced by ethanol (103.9 ± 5.93 mm2, N= 6). The pre-treatment with NOS inhibitor before both Hemin (L-Name = 80.26 ± 6.83 mm2, N=6) and DMDC reversed the gastric lesion induced by ethanol. Malondialdehyde (MDA) levels was measured and the treatment with Hemin (55.29 ± 6.93 nmol/g, N=6) and DMDC (45.54 ± 5.66 nmol/g, N=6) decreased the levels of MDA as compared with ethanol (89.63 ± 10.47 nmol/g, N= 6). L-Name pre-treatment (75.30 ± 4.28 nmol/g, N=6) reversed the CO gastroprotective effect. The glutathione levels obtained for Hemin (523.3 ± 43.91 mg/ g of tissue) and DMDC (501.8 ± 25.24 mg/ g of tissue) treatments was increased when compared with ethanol (323.6 ± 23.89 mg/g of tissue). The pre-treatment with NOS inhibitor (L-Name = 352.6 ± 30.10 mg/ g of tissue) reversed the effect of both hemin and CO donor in glutathione levels, during ethanol induced gastric damage. CONCLUSION: The results suggest that the nitric oxide synthase interact with carbon monoxide in the protection of gastric mucosa against ethanol induced gastric damage. FINANCIAL SUPPORT: Capes, Funcap.

07.006

Role of nitric oxide synthase (NOS) in the gastroprotective effect of hydrogen sulphide (H_2S) in ethanol-induced gastric damage in mice. Lucetti LT[1], Medeiros J-VR[2], Gomes AS[1], Santana APM[1], Barbosa ALR[1], Soares PMG[3], Cunha FQ[4], Ribeiro RA[1], Souza MHLP[1] [1]UFC − Fisiologia e Farmacologia, [2]UFPI − Biologia, [3]UFC − Morfologia, [4]FMRP-USP

Introduction/Aim: The gastric mucosal defense is a combination of factors responsible for the ability of the mucosa to resist various insults such as bacterial products, NSAIDs and ethanol (AASE, Scand J Gastroenterol Suppl., v. 163, p. 17-23, 1989.; RICHARDSON, Am J Med., v. 79 p. 1-7, 1985).The gaseous mediators such as nitric oxide (NO) and hydrogen sulfide (H2S) are among the most important biological mediators in the human body, participating in numerous physiological and pathological processes. Recent studies suggest that NO and H2S should interact each other in defense of the gastric mucosa, acting in a synergistic pathway. H2S is formed in mammalian cells by the activity of two enzymes: cystathionine γ-lyase (CSE) and cystathionine β-synthetase (CBS) (Moore, P.K., Trends Pharmacol. Sci. 24, 609–611, 2003). The aim of this study was to evaluate the participation of NO in H2S gastroprotective effect in the ethanol-induced gastric injury in mice. **Methods:** Swiss mice, (20-30g), previously fasted for 24h, were treated with: saline (control), e NaHS (H2S donor, 150 µmol/kg, i.p). For other experiments, before 30 min, mice were pre-treated with L-NAME (NOS inhibitor, 3 mg/kg, i.p.). An hour after receiving ethanol 50% (0.5 ml/ 25 g, gavage). After 60 min, the animals were sacrificed and stomachs removed for measuring the rate of gastric damage, the colorimetric assays were used to determine gastric tissue level of malondialdehyde (MDA) (Brzozowski,T. Jour of physiol and pharmacol, 53, 4, 761.773, 2002) and glutathione (GSH) concentration. To determine the index of gastric injury, stomachs were photographed with camera for subsequent digital analysis of the lesions using a computerized planimetry program (Ko JK-S & CH Cho, Dig Dis Sci., v. 43, p. 1248-1257, 1998). Data were analyzed using One-Way ANOVA and Newman- Keuls test. All animal treatments and surgical procedures were approved by the local ethics committee (protocol No 63/07). **Results:** Treatment with H2S donor significantly ($p<0.05$) decreased the gastric lesion (NaHS + ETOH = 22.33 ± 7.05 mm2, N=6) as compared to vehicle-treatment (ETOH= 112.30 ± 7.44 mm2, N= 6). The pre-treatment with NOS inhibitor (L-NAME + NaHS + ETOH = 88.99 ± 16.96 mm2, N=6) reversed the NaHS effect in the gastric lesion by ethanol induces. The levels of malondialdehyde (MDA) was mensured and the treatment with H2S donor decreased the levels of MDA (NaHS + ETOH = 50.15 ± 2.36 nmol/g, N=6) as compared to inducer of injury (ETOH= 89.12 ± 5.63 nmol/g, N= 6).The pre-treatment with L-NAME (L-NAME + NaHS + ETOH = 78.44 ± 4.62 nmol/g, N=6) reversed the NaHS gastroprotector effect. Treatment with NaHS showed an increase in glutathione levels when compared to ethanol group (ETOH = 327.60 ± 21.84 mg/g de tecido; NaHS + ETOH =621.12 ± 58.12 mg/ g de tecido) and pretreatment with L-Name reversed this effect of NaHS (L-NAME + NaHS + ETOH= 388.54 ± 23.03 mg / g tissue).CONCLUSION: The presence of nitric oxide (NO) via NOS is important for the protective effect of H2S on gastric mucosal injury induced by ethanol, so there is interrelation between the gaseous mediator NO and H2S. **Financial Support**: Capes, Funcap.

07.007

A possible role of leptin and adiponectin during differentiation of monocyte in macrophage *in vitro*. Acedo SC[1], Gambero S[2], Cunha FGP[2], Lorand-Metze I[2], Gambero A[1] [1]UNIFAG-USF, [2]UNICAMP − Hemocentro

Introduction: Macrophage infiltration is increased in obesity and the ability to produce cytokines depends on its pro-inflammatory (M1) or anti-inflammatory (M2) phenotype. Cytokines produced by infiltrated macrophages (TNF-α, IL-6 and IL-1β) modify adipose tissue functions and it can results in obesity associated pathologies, such as insulin resistance. Substances produced in a microenvironment can interfere with monocyte/macrophage differentiation process and modify the cell phenotype. The aim of this work was to evaluate if adipocytokines such as leptin and adiponectin contribute to macrophage phenotype. **Methods:** Mononuclear cells were obtained from human blood by centrifugation in Ficoll-Paque®. CD14+ cells were isolated by MACS®. Classical differentiation stimulus IFN-γ (M1) and IL-4 (M2) were used as control. Leptin (25 and 50 ng/ml) and adiponectin (2.5 and 1 µg/ml) were also added to CD14+ cell cultures. Surface markers were analyzed by flow cytometry (FACsCalibur-BD). The basal and stimulated (LPS) cytokine production was measured by ELISA in culture supernatant. (Protocolo CAAE: 000.0.142.000-08) **Results:** M1 and leptin (25 and 50) cells express more CD14, CD163, CD80 and CD86 than M2 cells. However, CD36, CD40, CD200, CD209 and CD11b expression was intermediate between M1 and M2 cells. M2 cells produce less TNF-α (515 ± 132, 125 ± 39, 292 ± 92 and 219 ± 61 pg/ml in M1, M2, Lep25 and Adi1, respectively; $p<0.05$), IL-6 (299 ± 1,6, 132 ± 59, 222 ± 4 and 217 ± 54 pg/ml in M1, M2, Lep25 and Adi2.5, respectively; $p<0.05$), IL-1b (92 ± 24, 28 ± 7, 62 ± 1 and 96 ± 21 pg/ml in M1, M2, Lep25 and Adi1, respectively; $p<0.05$) and MIP-1α (12376 ± 4326, 1316 ± 440, 7866 ± 293 and 8356 ± 3231 pg/ml in M1, M2, Lep25 and Adi1, respectively; $p<0.05$) when stimulated by LPS. Basal MCP-1 production was only reduced in M2 (1285 ± 339 and 291 ± 136 pg/ml in M1 and M2, respectively; $p<0.05$). However, leptin and adiponectin cells had no ability to produce RANTES as M1 cells. IL-10 production was not changed, while IL-1ra was reduced in leptin cells (136 ± 46, 12 ± 4, 9 ± 2 pg/ml in M1, Lep 25 and 50, respectively; $p<0.05$). **Discussion:** The leptin presence during macrophage differentiation induces M1 surface markers expression. Leptin and adiponectin produce cells with high ability to release pro-inflammatory cytokines and chemokines similar to M1 phenotype. These two mediators secreted by adipocytes could contribute to generate infiltrated adipose tissue macrophages with a deleterious phenotype. **Financial support:** FAPESP

07.008

Gastroprotective effect of nitrosyl-ruthenium against the ethanol-induced gastric damage in mice. Santana APM[1], Torres JNL[1], Medeiros J-VR[2], Lucetti LT[1], Soares PMG[3], Wong DVT[1], Tavares BM[1], Saraiva MIR[1], Lopes LGF[4], Souza MHLP[1] [1]UFC − Fisiologia e Farmacologia, [2]UFPI − Biologia, [3]UFC − Morfologia, [4]UFC − Química Orgânica e Inorgânica

Introduction: Peptic ulcer is a chronic disease characterized by inflammatory and necrotizing lesions in the gastrointestinal mucosa due to an imbalance between protectors agents (mucus, bicarbonate, blood flow, prostaglandins, endogenous antioxidant system, cell renewal) and aggressive agents (HCL, pepsin , bile acids, NSAIDs, ethanol and stress). Nitric oxide (NO) mediates many protective effects on the gastrointestinal tract. Recently, it was develop new NO donors with ruthenium metal in its composition. These compounds are soluble in water and release NO, especially under the action of reducing agents, with higher chemical stability and lower toxicity. The aim of this study was to evaluate the gastroprotective effect of the nitrosyl-ruthenium against ethanol induced gastric damage in mice. **Methods:** Swiss male mice (25-30g) were handled in accordance with the ethical principles governing the use of experimental animals and the protocol was approved in the local ethics committee (Protocol 33/10). The animals were initially treated with nitrosyl-ruthenium (1 and 3 mg / kg) by gavage. After 30 min, it was administered 50% ethanol (0.5 ml/25g, vo). One hour later, mice were sacrificed and the stomachs removed and opened by the greater curvature to determine the injured area using a computerized planimetry program (Image J). Additionally, fragments of tissue were removed for microscopic analysis and determination of levels of glutathione (NP-SH) and malondialdehyde (MDA). **Results:** 50% ethanol caused macroscopic gastric damage (103.5 ± 14:36 mm2) and microscopic (loss of epithelial cells, edema, hemorrhage), and reduced glutathione (267.0 ± 26.2mg/g) and increased concentration of malondialdehyde (241.8 ± 19.0mg/g). The administration of nitrosyl-ruthenium at a dose of 1 mg / kg protected significantly the macroscopic gastric mucosal lesion induced by ethanol (28.5 ± 11.1 mm2). Therefore, also reversed the decrease of glutathione (535.2 ± 59.0 mg/g) and increase of MDA (184.8 ± 8.5 mg/g) induced by ethanol. DISCUSSION: These results demonstrate that the compound ruthenium-nitrosyl has gastroprotective effect and that this effect is due, in part, to decreased of the production of free radicals induced by ethanol. **Financial Support**: CNPq, CAPES, FUNCAP.

07.009

Perinodal adipose tissue and mesenteric lymph node cells interactions during inflammatory intestinal response: adipocytokine and polyunsaturated fatty acids profile.

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Introduction: Perinodal adipose tissue (PAT) is a depot of stored fat in which lymph nodes are embedded. There is increasing evidence that perinodal adipose tissue does not merely provide an energy resource but is also specialized to contribute to lymph node physiological mechanisms. PAT provides key fatty-acid and adipocytokines and by this way, can modify the lymph node cell activation status. The aim of this work was to evaluate the adipocytokine and polyunsaturated fatty acids (PUFAs) profile in mesenteric perinodal adipose tissue during experimental colitis. We also assessed the lymph node cell activation status. **Methods:** Colitis was induced by repeated intracolonic instillation (days 0, 14 and 28) of 3 mg of trinitrobenzene sulfonic acid (TNBS) to Male Wistar rats (CEA/USF Protocol s/n-2008). Control group was carried out by intracolonic saline instillation. After 35 days, rats were killed and PAT collected for adipocytokine quantification using ELISA kit (leptin, adiponectin, TNF-α and IL-10). PUFAs profile was analyzed in plasma and PAT after Folch's extraction method by gas chromatography (GC). Cellular suspension of lymph node cells were cultured and stimulated with LPS and anti-CD3ε. IFN-γ, TNF-α and IL-10 was quantified in the culture supernatant. Colitis was assessed by macroscopic damage score and MPO activity. **Results:** TNBS induces high level of MPO activity (1.22 ± 0.17 and 14.83 ± 3.29 U/mg in colon for control and colitis, respectively; $p<0.01$) and colonic macroscopic damage (0.8 ± 0.8 and 5.2 ± 1.3 for control and colitis, respectively; $p<0.01$). Lymph node cells from colitis animals produce more TNF-α after CD3ε stimulus (8.4 ± 1.0 and 16.7 ± 2.0 ng/ml; $p<0.05$) and IFN-γ after LPS (0.3 ± 0.2 and 1.2 ± 0.2 ng/ml; $p<0.01$) and, CD3ε stimulus (0.6 ± 0.1 and 0.9 ± 0.1 ng/ml; $p<0.05$). IL-10 production was not modified. PAT from colitis animals was also able to increase the production of leptin (2750 ± 563 and 6021 ± 548 ng/mg tissue; $p<0.05$), adiponectin (1863 ± 78 and 2321 ± 100 ng/mg tissue; $p<0.05$), TNF-α (56.9 ± 7.0 and 93.1 ± 8.0 pg/mg tissue, $p<0.05$) and IL-10 (773 ± 194 and 2055 ± 378 pg/mg tissue; $p<0.01$). ω6 PUFAs were increased after colitis in PAT (18:2(ω6): 28.05 ± 1.04 and 37.25 ± 1.10%($p<0.01$); 18:3(ω6): 0.61 ± 0.17 and 1.32 ± 0.06% ($p<0.05$); 20:4 (ω6): 0.21 ± 0.12 and 1.26 ± 0.14% ($p<0.05$) in controls and colitis). ω3 PUFAs were not modified. Plasma analysis shows that ω6 PUFAs were reduced. **Discussion:** Experimental colitis involves mesenteric lymph node activation and lymph node cells produce predominantly pro-inflammatory cytokines. Perinodal adipose tissue is also activated producing both pro- and anti-inflammatory adipocytokines in response to intestinal inflammation. Interestingly, the PUFAs incorporation was modified in PAT, resulting in ω6:ω3 ratio alterations. ω6 PUFAs seems to have a role in inflammatory events through generation of eicosanoid mediators and thus, PAT could act as an important source of arachidonic acid and others precursors during intestinal inflammation.

Financial support: FAPESP

07.010

Saccharomyces boulardii ameliorates gastric dysmotility and inflammation presents in intestinal mucositis induced by 5-fluorouracil in mice. Justino PFC[1], SILVA LM[1], Melo LFM[1], Costa JVG[1], Nogueira AF[1], Lucetti LT[1], Ribeiro RA[1], Souza MHLP[1], Soares PMG[2] [1]UFC − Fisiologia e Farmacologia, [2]UFC − Morfologia/Fisiologia e Farmacologia

Introduction: The treatment with of antineoplasic chemotherapy may lead to delayed gastric emptying, early satiety, anorexia, nausea and vomiting, described collectively as the cancer-associated dyspepsia syndrome (CADS). Data recently published (Soares et al. Cancer Chemother Pharmacol., 63(1):91-8, 2008) report a delay in gastric emptying during intestinal mucositis induced by 5-FU. Cytokines are demonstrated participated of the pathophysiological of the mucositis intestinal induced for radiotherapy and chemotherapy. *Saccharomyces boulardii* (SB) is a thermophilic non-pathogenic yeast that is used widely in the treatment of gastrointestinal disorders associated with diarrhea. Our hypothesis is that 5-FU increases the concentration of cytokines (IL-1b and CXCL1) and promotes gastrointestinal dysmotility and these findings can be reversed by treatment with Saccharomyces boulardii. **Methods:** Swiss male mice (30-35g) were treated with 5-FU (450 mg/Kg, i.p., only dose), 5-FU + SB [800 mg/Kg for 3 days (3D) or 6 days (6D)] or saline (control). On the third day after administration of 5-FU or 5-FU + SB, mice were sacrificed and samples of jejunum (J) and ileum (I) were collected for assessment cytokines (IL-1b, CXCL1; pg/ml) by ELISA. In order to study gastrointestinal motility, on the 3rd day after 5-FU or 5-FU + SB (3D and 6d) treatment, rats were gavage-fed (1.5 mL) with the test meal (5% glucose solution with 0.05 g mL(-1) phenol red) and killed 20 min later. Gastric emptying was measured by spectrophotometry. 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:** 5-FU increased the concentration of cytokines pro-inflammatory (jejunum: IL-1b: C= 589.33 ± 36.76, 5-FU= 1135.00 ± 90.70; CXCL1: C= 33.43 ± 23.42, 5-FU= 341.33 ± 146.43 and ileum: IL-1b: C= 420.00 ± 54.10, 5- FU= 673.66 ± 77.96; CXCL1: C= 170.76 ± 37.90, 5-FU= 663.66 ± 279.16). The treatment with SB reduced cytokines concentration (jejunum: IL-1b/5-FU+SB(3D)= 726.00 ± 98.43, 5-FU+SB(6D)= 624.33 ± 115.50; CXCL1/5-FU+SB(3D)= 66.30 ± 50.50, 5-FU+SB(6D)= 65.23 ± 30.19 and ileum IL-1b/5-FU+SB(3D)= 369.33 ± 54.90, 5-FU+SB(6D)= 396.00 ± 62.13; CXCL1/5-FU+SB(3D)= 125.53 ± 35.70, 5-FU+SB(6D)= 94.66 ± 24.67). Besides, the treatment with SB (3D and 6D) improved delay in gastric emptying (C= 25.21 ± 2.55%; 5-FU= 54.91 ± 3.43%; 5-FU+SB(3D)= 31.38 ± 2.80%; 5-FU+SB(6D)= 26.97 ± 2.72%) and gastrintestinal transit measured by center of mass (C= 2.32 ± 0.08; 5-FU= 1.83 ± 0.06; 5-FU+SB(3D)= 2.31 ± 0.06; 5-FU+SB(6D)= 2.40 ± 0.07). **Discussion:** Saccharomyces boulardii was able to reverse cytokines as well as improving gastrointestinal dysmotility. Thus, the treatment with Saccharomyces boulardii could be useful for gastrointestinal symptoms associated of antineoplasic chemotherapy drugs use. **Financial support:** CNPq

07.011

Intestinal permeability test as a useful tool to discriminate patterns of diarrhea due to cancer chemotherapy agents. Wong DVT[1], Bem AXC[1], Nunes LG[1], Leite LL[1], Noronha FJD[1], Barbosa CRN[1], Brito GAC[2], Souza MHLP[1], Lima AAM[1], Lima-Júnior RCP[1], Ribeiro RA[1] [1]UFC − Physiology and Pharmacology, [2]UFC − Morphology

Introduction: Diarrhea and the associated intestinal mucositis (IM) are common side effects (15-25%) of colorectal anticancer therapy with Irinotecan (IRI). Our group has demonstrated that IRI-induced IM is involved with inflammatory events, intestinal damage and dysfunction (diarrhea and intestinal overcontractility detected in vitro). We also have demonstrated that another antineoplastic agent, methotrexate, is able to induce intestinal barrier dysfunction which correlates with IM. Data from the literature suggest differential expression of inflammatory mediators indicating that the pathophysiology of IM vary with the anticancer agent employed. We then aimed to evaluate through a non-invasive method (intestinal permeability to sugars) the pattern of intestinal barrier dysfunction in mice with IRI-induced diarrhea. **Methods:** Male mice C57BL/6 (n=7) were treated for 4 days with sterile saline or IRI (75 mg/kg, i.p) and the patterns of weight loss, food intake, and diarrhea were obtained up to the 5th day post-first dose of IRI. On day 4, after an overnight fast, the animals received by gavage 0.25 ml of a solution containing lactulose (200mg/ml, presents a paracellular absorption) and mannitol (50mg/ml, presents a transcellular absorption). The urine was collected for the next 24 h in a flask with 25 µl of solution containing chlorhexidine (40mg/ml) to evaluate intestinal permeability to these sugars (Lactulose/mannitol, L/M ratio). The animals were then sacrificed and had intestinal sections collected to morphometric and myeloperoxidase acivity (MPO) measurements. Statistical analysis was performed with Student's t test or ANOVA/ Bonferroni's test as appropriate. $P<0.05$ was accepted. (CEPA: Protocol 02/04). **Results:** IRI induced significant ($P<0.05$) weight loss (7.3%) when compared on saline, a marked reduction in food (36.8%) and water intake (21,4%), and increase in diarrheal scores (1[0-3]) when compared with saline treated mice (0[0-0]). Irinotecan also showed a significant ($P<0.05$) increasing on MPO activity (17.94 ± 2.4) in comparison to saline treated group (4.62 ± 0.91). The L/M was significantly decreased in IRI treated group (0.026 ± 0.24) compared to the control (0.596 ± 0.21) when evaluated 5 days after the first dose of IRI. The villus/crypt ratio in the IRI-treated mice was significantly decreased on days 5 (1.38 ± 0.05) and 7 (1.21 ± 0.04) compared with mice given saline (3.59 ± 0.11), demonstrating the injurious effects of IRI on the intestinal epithelium. **Discussion:** In addition to causing morphological damage to intestinal mucosa, IRI also induces intestinal functional disruption. Our findings (a reduced L/M ratio) considerably differ from our previous study (Carneiro-Filho et al, Dig Dis Sci, 49(1): 65–72, 2004) that showed an increase in L/M ratio due to methotrexate administration, suggesting different mechanisms of intestinal damage regarding the drug employed. Such differences might have a true impact into the therapeutic aspects of anticancer toxicity. **Financial support:** CAPES/CNPq.

07.012

Mechanisms involved in delayed gastric emptying induced by thermogenic supplement in female ovariectomized mice. Sousa, LN[1], Santos, RGS[1], Oliveira, FGV[2], Silveira, GL[2], Bento-Silva MT[2], Monteiro FMF[1] Santos AA[2], Palheta Junior RC[1] [1]UNIVASF − Veterinary Medicine, [2]UFC − Physiology and Pharmacology

Introduction: Thermogenic substances capable of accelerating body metabolism such as Xenadrine, Ripped Fuel and/or Thermobuterol, most being amphetamines, have been used to increase body performance and weight loss (Green, GA. Clin J Sport Med 11: 51-6, 2001). It is a fact, that they are used in combination with strenuous exercise to synergize aforementioned results, a situation which predisposes the gastrointestinal tract to ischemia. Aim: To assess the effect of thermobuterol on gastric emptying of liquid in female ovariectomized mice and the possible involved mechanisms. **Methods:** After approval by the local research ethics committee on animal use (088180609), female, albino mice, (30-40g) were anesthetized, with ketamine (100 mg/kg, i.p) added to xylazine (5 mg/kg, i.p), subsequently subjected to ovariectomy. Seven days after the surgery, the animals received doses of thermobuterol (10 or 100 mg/kg p.o.) or saline. In order to investigate possible involved mechanisms, mice were pretreated 30-min, intraperitoneally with: Prazosin (PRAZ-0.25 mg/kg), Propranolol (PROP-2 mg/kg), Hexamethonium (HEX-10 mg/kg), and L-NAME (10 mg/kg), Ondansetron (ONDA-50µg/kg) or Para-chlorophenylalanine (PCPA-300 mg/Kg). After the treatment period, all animals were fed with a liquid test meal (0.3ml of 5%v GS with 0.5 mg/mL phenol red). Subsequently, they were sacrificed by cervical dislocation, 10-min post-prandial. This was followed by evisceration and the determination of the fractional dye retention of the stomach by spectrophotometry. The data (mean ± SEM) of each sub-group comprising of 6-7 animals were compared using the ANOVA one-away test and Student-Newman-Keuls test. **Results:** Comparing to their respective control groups, a dose of 100 mg/kg-Thermobuterol provoked a higher ($P<0.05$) gastric retention (49.8 ± 2.6% vs 66.7 ± 3.6%), however no significant difference was noted with 10 mg/kg-Thermobuterol treatment (49.9 ± 4.0%). Moreover, despite pre-treating the animals with PRAZ, PROP or L-NAME, 100 mg/kg-thermobuterol still maintained greater gastric retention in each group, just like 100 mg/kg-thermobuterol alone (66.0 ± 2.9%, 74.7 ± 5.0% and 68.9 ± 6.0% respectively). Contrarily, the pretreatment with HEXA, ONDA or PCPA prevented an increase in gastric retention due to 10 mg/kg-thermobuterol, (56.0 ± 2.8%, 42.6 ± 3.7% and 53.7 ± 5.8% vs 66.7 ± 3.6% $p<0.05$ respectively). Conclusion: Thermobuterol increases gastric retention in mice, a phenomenon mediated by the autonomic ganglia, through serotonergic pathways. Support: CNPq and UNIVASF

07.013

Metyrapone reverses effects of LPS on neuroendocrine response and maternal behavior of lactating female rats. Vilela FC, Melo CM, Andrade CAF, Giusti-Paiva A
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Introduction: Considering that it has been shown that the lipopolysaccharide (LPS) can modulate prolactin and oxytocin secretion, we investigated the effects of LPS on behavioral and hormonal responses of lactating female rats. Using metyrapone (a glucocorticoids synthesis inhibitor), we also investigated whether these responses are mediated by glucocorticoids release, since it also modulates prolactin and oxytocin secretion. Material and methods: Lactating female rats in the sixth or seventh lactation day were divided into three groups (n=7 per group): saline/saline, saline/LPS and metyrapone/LPS for evaluation of maternal behavior or hormone changes. In order to evaluate the maternal behavior lactating rats were treated with saline (1 mL/kg, i.p.) or metyrapone (50 mg/kg, i.p.) 10 and 2 hours before administration of the LPS (500 µg/kg, i.p.) or saline (1 mL/kg, i.p.). Then the litter of eight puppies was removed from the home cage and 2 hours after saline or LPS administration, pups were replaced in their home cage at the opposite side of previous nest and they were filmed during 30 minutes. In order to evaluate hormonal changes, blood samples were collected 15 minutes after the beginning of breastfeeding. All experiments were conducted with the approval of the Ethics Committee of Federal University of Alfenas (255/2009). **Results:** LPS treatment decreased period of licking pups (390.40 ± 33.19 to 168 ± 25.61 seconds; $p < 0.001$), decreased permanency in arched-nursing position (41.08 ± 2.74 to 20.35 ± 2.69 percent; $p < 0.001$), decreased total maternal behavior (28.02 ± 4.60 to 8.44 ± 2.57 percent; $p < 0.01$) and increased number of rearing (13.29 ± 1.01 to 26.13 ± 4.53; $p < 0.05$) when compared to control treatment. Pretreatment with metyrapone increased period of licking pups (168 ± 25.61 to 275.00 ± 51.25 seconds; $p < 0.05$), increased percentage of permanency in arched-nursing position (20.35 ± 2.69 to 59.44 ± 4.09 percent; $p < 0.001$), increased the percentage of total maternal behavior (8.44 ± 2.57 to 43.71 ± 6.73 percent; $p < 0.001$) and decreased the number of rearing (26.13 ± 4.53 to 9.33 ± 1.72; $p < 0.01$) when compared to LPS treatment. LPS treatment reduced oxytocin secretion (from 55.6 ± 16 pg/mL to 11.9 ± 3.8 pg/mL; $p < 0.05$) and prolactin secretion (545 ± 119 ng/mL to 167 ± 31 ng/mL; $p < 0.05$) during lactation when compared to control. Metyrapone treatment increased oxytocin secretion (from 26.1 ± 4.5 pg/mL to 49.7 ± 8.2 pg/mL; $p < 0.05$) and prolactin secretion (75.3 ± 29 ng/mL to 334 ± 37 ng/mL; $p < 0.05$) during lactation when compared to LPS treatment. Conclusion: LPS treatment attenuates maternal behavior in lactating female rats, followed by a reduction in prolactin and oxytocin secretion. These changes may be due to glucocorticoids release because metyrapone reversed the behavioral and neuroendocrine responses produced by LPS. Supported by: FAPEMIG, CAPES, CNPq, FINEP.