11. Clinical Pharmacology, Pharmacokinetics, Pharmacogenomics and Preclinical Toxicology

11.001

Effect of Standardized Extract of Propolis (EPP-AF) on the *in vivo* activity of P-glycoprotein: clinical study in healthy volunteers. Vale GT[1], Lanchote VL[2], Coelho EB[3] [1]FMRP – Farmacologia, [2]FCFRP-USP – Toxicologia, [3]FMRP – Clínica Médica

The effect of a particular drug is closely related to its plasma concentration. The kinetics disposition of drugs may be affected by changes in its metabolism and excretion. However, changes in the activity of several membrane transporters may also influence on pharmacokinetic properties. Among these transporters, P-glycoprotein (P-gP) is a protein ATP-dependent extrusion widely expressed in various tissues and has been associated with cases of renal and biliary excretion, as well as intestinal absorption. Fexofenadine, an antihistamine that belongs to piperidines second generation class, is an important marker of the Pg-P. Fexofenadine is distributed in the clinic as a racemic mixture of the R-(+) and S-(-) and the last one is responsible for a better pharmacological activity and higher affinity for Pg-P. Propolis, the drug studied during this work, has several biological and pharmacological properties. From the pharmacological importance presented by standardized extract of propolis (EPP-AF®), the most objective of this study was evaluate the potential of this extract in altering the function "*in vivo*"of Pg-P, using the kinetic disposition of fexofenadine. With this purpose, a pharmacokinetic study, open and crossed, with 14 healthy volunteers in two phases, one with only fexofenadine and the other with fexofenadine + EPP-AF, after 7 days of using the extract of propolis. In both phases, a serial blood samples were taken immediately before administration of fexofenadine, and 0.5, 1, 1.5, 2, 3, 4, 6, 8, 12, 16 and 24 hours after administration of the drug. This clinical protocol was reviewed and approved by the Ethics Committee in Research of the Hospital of the Faculty of Medicine of Ribeirão Preto-USP (HCFMRP-USP), Hardcopy Cable Replacement Case 5912/2012, which is deposited in the registry of brazilian clinical trials platform rebec (Universal Trial Number U1111-UTN 1131-8461). For analysis of obtained plasma, an analytical method was developed in HPLC, based on ANVISA (National Health Surveillance Agency) rules, using a chiral column (Chirobiotic V) and a mobile phase of 97% methanol and 3% pure ammonium acetate 7mmol/L at pH 4.25 in the flow 0.7 mL/min. The detection system used was mass spectrometry (MS/MS). After comparing the pharmacokinetic disposition of the fexofenadine enantiomers it was observed that P-glycoprotein is an enantioselective enzyme, having higher affinity for S enantiomer, with an AUC (Area under the curve) ratio R/S of 1.96. Furthermore, it is noted that the present research shows a possible framework for induction of protein efflux after a week of treatment with the Standardized Extract of Propolis by presenting a significant decrease in AUC (700.4 vs 425.2 ng*h/mL of S-Fexofenadine; 1377 vs 787.6 ng*h/mL of R-Fexofenadine) and maximal plasmatic concentration (Cmax) (133 vs 84.5 ng/mL of S-Fexofenadine; 244.3 vs 149.4 ng/mL of R-Fexofenadina), as the same time, there is a significant increase in clearance (171.3 vs 282.3 of S-Fexofenadine; 87.15 L/h vs 151.4 L/h of R-Fexofenadine). These data suggest a possible enzymatic induction of P-glycoprotein by EPP-AF, showing that drugs from natural source, even when used popularly use may present clinically significant drug interactions.

11.002

Bacteria drug resistance in intensive care units: a systematic review. Luedy TA, Costa LS, Rodrigues CDM, Lima ISP, Júnior AGT, Lima MAP, Meireles CB, Linhares EHPCM, Simplício GN, Costa TR, Ferreira LFP UFCA

Introduction: Bacterial drug resistance has emerged as one of the most concerning problems for health care institutions in a global scale. Its clinical significance, especially over the last 10 years, has been propelled by its remarkable ability to hamper human treatment against bacterial infections and making it significantly more expensive. Furthermore, the bacterial drug resistance is transmitted in an indiscriminated way and can affect the entire global community. This manuscript focus on Intensive Care Units because is an epicenter of infections, due to its extremely vulnerable population (reduced host defenses deregulating the immune responses) and increased risk of becoming infected through multiple procedures and use of invasive devices distorting the anatomical integrity-protective barriers of patients (intubation, mechanical ventilation, vascular access). **Objective**: The aim of the present study was to conduct a systematic review of articles concerning bacterial drug resistance in Intensive Care Units (ICU). Also, this present study has a purpose to demonstrate the latest findings regarding the administration of antibiotics against multidrug-resistant bacteria. **Methods:**A systematic review of articles on bacterial drug resistance in ICU, published from January 1, 2009 to May 10, 2014, on SCOPUS and PUBMED databases was carried out. Search terms were "anti-bacterial agents"(Medical Subject Headings [MeSH]), "therapeutics"(MeSH), "Intensive Care Units"(MeSH) and "drug resistance"(MeSH). Of the 151 retrieved studies, only 40 met eligibility criteria. **Results**: Studies covered a wide range of aspects regarding bacteria drug resistance in ICU, such as broad spectrum *in vitro* activity of the carbapenem anti-bacterial agents. *Staphylococci* bacteria and their subgroups have an ability to develop resistance to methicillin and high sensitivity to drug Vancomycin. Recent scientific literature also found that patients infected by gram-negative pathogen *Acinobacter baumannii* required mechanical ventilation more often, mostly in the neonatal intensive care units. Moreover, the treatment with colistin has been tested and proved as outstanding, including when associated with the use of antibiotics. **Discussion:** This review showed that the problem related with bacteria drug resistance is affected for many factors, like the manner of use of the antibiotics or until the duration of the treatment, especially in intensive care units. Also, notes the urgency to efficient treatment of infections with a single one drug, mainly to combat gram negative bacteria responsible for the most cases of complications or even death of the patient.

11.003

Biochemical and hematological effects of acute administration to crude fraction from the leaves of *Celtis iguanaea* in female rats. Guex CG[1], Silva ARH[1], Noda JM[1], Pigatto GR[1], Rovani BT[1], Freitas RB[2], Froeder ALF[2], Athayde ML[2], Bauermann LF[1] [1]UFSM – Fisiologia e Farmacologia, [2]UFSM – Farmácia Industrial

Introduction: *Celtis iguanaea* (CI) is a plant belonging to the family Ulmaceae[1] and is popularly known as "esporão de galo"[2]. Based on its popular use, is therapeutically used for body aches, rheumatism, chest pain, asthma, colic, indigestion and as diuretic[3]. The use of plants to treat diseases is a very common popular practice and occurs with a few or no evidence of its pharmacological properties and toxics effects. Therefore, more studies are required to validate the efficacy and safety of these substances. The aim of this study was to evaluate the biochemical and hematological effects of acute administration of crude fraction from leaves of *C. iguanaea* in female rats. **Methods:** The leaves of CI were collected in the city of Jaboticaba, RS. It was performed an infusion and the aqueous extract was lyophilized to obtain powder. Adult female Wistar rats were obtained from Central Bioterio of UFSM (UFSM). Animals received a single dose of 2000 mg/kg of CI, dissolved in water, by gavage; control group was treated with water (10 mL/kg). The acute toxicity study was developed following the guidelines of OECD 423. After 14 days of administration, animals were subjected to a fasting period of 8h and then were sacrificed from the cardiac puncture. All animals were used according to Committee on Care and Use of Experimental Animal Resources from UFSM, Brazil (number 103/2013). The blood without the anticoagulant was allowed to clot before centrifugation (4000 x rpm for 10 min) to obtain serum, which was utilized for the assessment of glucose (GLU), total cholesterol (Chol), protein (PROT), blood urea nitrogen (BUN) levels, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities, by using Diagnostic Kits Laboratory Bioclin/Quibasa,Minas Gerais, Brazil and semi-automatic biochemical analyzer (Genz, Bioplus: Bio-2000). The anticoagulated blood was analyzed immediately for hematological parameters: leukocytes (WBC), erythrocytes (RBC), hematocrit (HCT), hemoglobin (HGB), were determined with the use of an automatic counter veterinary Mindray BC 2800. The data are expressed as mean ± standard deviation (SD). All the results were analyzed by one-way ANOVA followed by the Tukey *post hoc* test. The differences between the groups were considered significant when $p<0.05$. **Results and discussion:** The biochemical parameters, BUN and CRE levels (indicative of renal dysfunction) and AST and ALT activities (determination of hepatocellular damage) did not showed significant difference between control and treatment groups. Thus, acute treatment with CI did not showed kidney or liver damage. Also, there was no significant change in Chol and PROT levels when compared between groups. The GLU level increased in the group treated with CI as compared to the control group. This increase may be related to the plant constituents, which have yet to be elucidated for a better understanding. Hematological parameters, WBC, RBC, HGB and HCT did not showed significant change between groups. Thereby, CI did not exhibit significant toxicity when a single acute dose was administered. **References:** 1. CRONQUIST, A. An Integrated system of classification of flowering plants. New York: Col Univ Press, 1981. 2. SILVA, C. S. P. *et al.* Uso e disponibilidade de recursos medicinais no município de Ouro Verde de Goiás, GO, Brasil. *Acta Bot Bras*, São Paulo, v. 22, n. 2, 481, 2008. 3. PEREIRA, K. C. S. *et al.* Ausência de efeito genotóxico do extrato de esporão-de-galo em células somáticas de *Drosophila melanogaster*. Resumo do 54º Congresso Brasileiro de Genética. Salvador (BA), 111, 2008. **Financial Agencies and Acknowledgments:** UFSM, Capes.

11.004

Preclinical toxicological test for *Marrubium vulgare* on weaned calves. Schlemper V, Bernardo FD, Schlemper SRM, Franciscato C, Ambrosini F, Barichello D, Soares EL UFFS – Medicina Veterinária

Introduction: Male calves from dairy farming are discarded after birth because they have no economic value. Those non-ruminant animals can be used as experimental biological models for preclinical toxicological test with phytotherapy medication. To evaluate the model, we tested a medicinal plant *Marrubium vulgare*, previously studied in rodents. *M. vulgare* extracts and its phytochemical compound diterpene marrubiin had significant biological effects in rodent models (Schlemper *et al*., *Phytomedicine*, **7:** 103, 1996; Popoola *et al*., *Molecules*, **18**: 9049, 2013). This research has as objective to investigate clinically and with haematological/biochemical tests, signs of *M. vulgare* chronic toxicity in those animals, this way validating a preclinical toxicity method for phytotherapy medications. **Methods**: Jersey weaned calves (5-10 days, n= 6 – 8), weighting 15 to 20 kg, remained under adaptation for 7 days in individual stalls. They received colostrum during 5 days after birth, and from the 6th day they received 2.0 liters of milk with 50% of milk replacer in the morning and in the evening, which were incorporated 500 ml of *M. vulgare* infusion in increasing doses (1, 2, 4 and 8 g.kg^{-1}, 10 days each) to the studied group and equal amount of water to the control group. Calf feed was introduced from the fifth day *ad libitum* and animals were deprived of fodder for the maintenance of the nonfunctional rumen via esophageal groove, to the gastrointestinal absorption of the plant active principles. To carry out haematological/bio chemical tests, blood samples were obtained through jugular vein puncture. Through eritrograma, it was performed the red blood cells counting and the determination of hemoglobin concentration with an automated blood cell counter. Haematocrit measurement was made in a centrifuge (12.500 rpm/5 minutes). Mean corpuscular volume (MCV) and mean corpuscular hemoglobin concentration (MCHC) were calculated through standard formulas. For white blood cells count, leukocytes were taken in automated blood cell counter and the differential through stained smear by quick Panoptic method and microscopic analysis. Serum biochemical test was made through kinetic method in semiautomatic analyzer to evaluate aspartate aminotransferase (AST), gamma-glutamyl transferase (GGT), total protein and albumin for hepatic alterations, and the metabolites urea and creatinine to verify the renal function. Experimental protocols were approved by Ethics Committee for the Animal Use under No. 23205.004981/2013-40. **Results/Discussion**: Chronic administration of *M. vulgare* infusion inhibited significantly neutrophil ($p<0.01$ and maximal inhibition [MI] of 59.33 ± 4.91%) and monocytes ($p<0.05$ and MI of 56.01 ± 11.05%) counts up to the dose of 4 g.kg^{-1}. Enzymes tests (AST and GGT), proteins, albumin, as well urea and creatinine, had no alteration. The model proposed is suitable for preclinical tests in monogastric animals and may use parameters of toxicity and phytochemical compounds detection in blood. *M. vulgare* administered orally and chronically did not show adverse health effects in calves. Moreover, plant infusion inhibited neutrophils and monocytes circulating suggesting an immunomodulatory effect in high doses. **Acknowledgements:** Universidade Federal da Fronteira Sul-UFFS.

11.005

Juvenile Arthritis Rheumatoid therapeutics: A review of the last decade. Meireles CB, Simplício GN, Linhares EHPCM, Luedy TA, Costa TR, Ferreira LFP, Rodrigues CDM, Costa LS, Lima ISP, Júnior AGT UFCA

Introduction: The treatments of chronic inflammatory diseases such as juvenile rheumatoid arthritis (JRA) are advancing every year. It's important to review the literature for establishing a connection between standard therapy and those who are being researched in order to find the best therapy that promotes quality of life of the patient as quickly as possible. **Methods:** A review about JRA treatments on the last 10 years (01/01/2004 to 13/05/2014) was performed on SCOPUS and MEDLINE (review of medical literature and Retrieval System Online) databases using the MeSH (Medical Subject Headings) terms, "arthritis ", "juvenile ", "arthritis"and "therapy". Of the 167 studies reviewed, 27 met the eligibility criteria. **Results:** Studies show the effectiveness of immunosuppressants such as cyclosporine, and leflunomide wich dose values in pediatric patients with JRA to that in adult patients, should be adjusted modestly: 10 mg / d for 10-20 kg, 15 mg / d for 20: – 40 kg to 20 mg / day, > 40 kg. In patients with polyarticular JRA, methotrexate, which is an anti – metabolite, and leflunomide both resulted in high rates of clinical improvement, but the rate was slightly higher for methotrexate. Anti-inflammatory such as ertanecept, adalimumab and infliximab, also addressed in the literature, proved to be an effective alternative for treatment. Etanercept plus methotrexate have an acceptable safety and efficacy profile in children with selected categories of JRA, the improvement was maintained for 3 years who continued to receive medication. Infliximab 3 mg/kg and 6 mg/kg showed durable efficacy at 1 year, with a primary efficacy endpoint at 3 months did not differ significantly between patients treated with placebo and treated with infliximab. Moreover, there were complications caused by corticosteroid methylprednisolone pulse therapy including tachycardia, hypertension and flashing. In patients with rheumatoid arthritis in glucocorticoids with reduced muscle mass anti – tumor necrosis factor (TNF) with adalimumab generally has favorable effects. Furthermore, the levels of markers of bone physiology were significantly lower in children with JRA receiving calcium supplementation. **Discussion:** The studies show the effectiveness of medications for the treatment of JRA, and some make the comparison between drugs benefit and toxicity to obtain a better basis for pharmacological uses. It was noted that the therapeutic arsenal to combat JRA is quite broad, with representatives of the class of immunosuppressants, the group that stands out in the market as well as inhibitors and anti – TNF. The bibliography focuses analyzed immunosuppressive drugs in the treatment of JRA, so further studies should be guided in the growth prospects of this class of drugs and the discovery of new drugs that have different pharmacological properties.

11.006

Evaluation of acute toxicity of CeO_2 nanoparticles in mice. Beltrão DM[1], Xavier AL[1], Abrantes RA[1], Santos CCL[2], Keyson D[2], Sousa AG[2], Farias IAP[3], Albuquerque AJR[3], Sampaio FC[3], Sobral MV[1] [1]UFPB – Ciências Farmacêuticas, [2]UFPB – Química, [3]UFPB – Biotecnologia

Introduction: Concrete evidence that nanoparticles can have toxicological and environmental risks are reported (Oberdörster, G., Environmental Health Perspectives, 113:823, 2005). Whereas current knowledge in the toxicology of CeO_2 nanoparticles is emerging, it becomes imminent evaluate the acute toxicity of this nanomaterial. **Methods**: This assay was approved by the Animal Studies Committee of the UFPB (no. 0812110), and was performed according to the OECD protocol no. 423 (2001), with modifications. Swiss mice (*Mus musculus*), female, were submitted to single doses intraperitoneally (ip) (300 mg/kg and 2000 mg/kg, n = 6). In order to map possible central and autonomic behavioral changes, observation to detect toxic signs of general character was performed at the intervals: 0, 15, 30 and 60 minutes; after 4 hours and daily for 14 days (Almeida, R. N., *Rev. Sci Farmacogn*, 80: 72, 1999). After of the observation period, biochemical and hematological evaluations were performed. Furthermore, indexes of organs (organ mg/animal g), liver, heart, kidneys, spleen and thymus were evaluated to detect possible signs of toxicity. The results were expressed as mean ± standard error of the mean (SEM) and analyzed by Student 's Test and were considered significant when $p<0.05$. **Results**: The dose of 2000 mg/kg caused the death of one animal, while the dose of 300 mg/kg caused no deaths. Changes as writhing (up to 15 min), was present in all animals of the experimental groups. Using the guide (OECD, 423), the LD_{50} was estimated at > 2000 mg/kg for CeO_2 nanoparticles (5 nm). There was a decrease in food consumption in animals treated with both doses compared to controls. However, this effect not induced reduction of the animals' weight. The highest dose increased urea levels, the total number of red blood cells, as well as the index of liver. **Discussion:** The systemic toxicity of a substance is manifested by reduction in water consumption and diet, behavior modification, but also change the relative organ weights (Pires Júnior, H. B., Ci Anim Bras, 13:512, 2012). Treatment with CeO_2 nanoparticles caused reduced feed intake, however, was not accompanied by changes in body weight, parameter employed in toxicological to indicate, early, onset of toxic effects in the animal organism. However, this isolated finding has no clinical significance. Administration of 2000 mg/kg caused an increase in the urea levels, however, induced no change in creatinine. How to evaluate renal function, urea should be evaluated together with creatinine, it is concluded that this finding has no clinical importance. Furthermore, as the increase in erythrocyte count was not accompanied by elevation of hematocrit, erythrocyte changes may be associated with other factors unrelated to hematologic toxicity, including, stress, dehydration. Given the above, it can be inferred that the intraperitoneal administration of CeO_2 nanoparticles has low toxicity in mice. However, further studies are needed. **Financial support**: CNPq

11.007

Pharmacogenetic and pharmacogenomic effect of ala16val polymorphism – SOD2 cytotoxicity caused by methotrexate in human peripheral blood mononuclear cells (PBMCS). Machado AK[1], Barbisan F[1], Motta JR[2], Rogalski F[2], Teixeira C[2], Jung I[1], Dornelles E[3], Cruz IBM[2] [1]UFSM – Pharmacology, [2]UFSM – Biogenomics, [3]UFSM – Toxicological Biochemistry

Introduction: Several drugs directly affect oxidative metabolism. This is the case of methotrexate (MTX), a folic acid antagonist used in high doses as antimetabolite in anti-cancer treatment as well as in low doses for the treatment of rheumatoid arthritis and adults' psoriasis. Investigations described that MTX toxicity can be attenuated by antioxidant supplement. However the effect of chronic oxidative imbalance associated with gene polymorphisms is still not known. **Objectives:** Previous studies suggested that the Ala16Val superoxide dismutase manganese-dependent (SOD2) gene polymorphism is associated with some chronical diseases and presents toxicogenetic and pharmacogenetic effects (Bresciani *et al.*, 2013). Therefore, we postulated that Ala16Val-SOD2 polymorphism could present some impact on MTX toxic response on human peripheral mononuclear cells (PBMCs). **Methodology**: The research study described here was approved by the Ethics Committee of the UFSM (no 23081.015838/2011-10). Blood samples from donors carrying different Ala16Val-SOD2 genotypes (AA, VV and AV) were collected and cultured in controlled conditions (RPMI media, 10% fetal serum, 1% antibiotics, 37°C, 5% CO_2). Cells were exposed to different MTX concentrations (0, 0.1,1,10 and 100 µM) during 24h. Further, cytotoxicity, genotoxicity, modulation of oxidative and inflammatory molecules as well as modulatory effect on oxidative and apoptotic gene expression were analyzed and compared among genotypes. **Results:** MTX caused significant decrease in PBMCs viability in a dose-dependent way, as previously described in literature. However, this effect was significantly influenced by Ala16Val-SOD2 polymorphism ($p<0.001$). PBMCs carrier's V allele decreased the viability from 1 µM MTX whereas AA-PBMCs decreased the viability just from 10 µM MTX. PBMCs exposed to 10 µM MTX, presented the follow viability: AA-PBMCs = 97.1 ± 3.2%; VV-PBMCs = 65.6 ± 4.2% and AV-PBMCs = 71.37 ± 3.8%. The apoptotic caspases levels 1, 3 and 8 increased on all samples, but no genotoxic effect was detected in the surviving cells. A significant increase in protein carbonylation was observed just in PBMCs V allele carriers that present lower SOD2 efficiency. Differential antioxidant activity and gene expression triggered by MTX exposition was also Ala16Val-SOD2 dependent. **Discussion:** These results suggest that the SOD2 balance could play some pharmacogenetic or toxicogenetic role in the cellular MTX response. A robust number of studies have described that MTX causes oxidative stress in several types of cells. This observation was noted in the *in vitro* investigation performed by Chibbers *et al.* which showed that MTX alone or in combination with Cu (II) was able to inhibit scavengers of ROS and exhibit pro-oxidant action. PBMCs response to MTX exposition is directly influenced by SOD2 polymorphism. These results indicate that potential influence of antioxidant genetic variations in drug response needs to be more considered in pharmacogenetic and toxicogenetic studies.

11.008

Evaluation of rabbit vaginal permeability test applied to fenticonazole. Campos RM[1], Pissinati L[1], Rojas-Muscoso JA[1], Chen LS[2], Porto M[1], Gagliano TJD[3], De Nucci G[1] [1]Unicamp – Pharmacology, [2]Galeno Research Unit, [3]IBCCF-UFRJ

Introduction: Although fenticonazole has been indicated to treat vaginal infections during last decades, there are few studies evaluating the vaginal permeability of fenticonazole *in vivo* (Novelli *et al.*, 1991; Feng *et al* 2011). Permeability is a dynamic process, involving a great deal of blood perfusion of the tissues involved. Due to the presence of blood vessels networks in the submucosa space and few interference of the estrous cycle in the epithelium, we propose a rabbit model for evaluation of vaginal permeability and topical toxicity. A novel, specific and sensitive method for quantification of fenticonazole in rabbit plasma based on liquid chromatography was described. **Method:** Fenticonazole nitrate (Fentizol® or test formulation) was administered by vaginal route during eight days consecutive and plasma levels of fenticonazole were quantified by high performance liquid chromatography coupled to electrospray tanden mass spectometry. After sacrifice, macroscopic and histopatology analyze were performed. In order to evaluate the fenticonazole bioavailability a single dose of fenticonazole was administered by intravenous (2 mg/kg) or intravaginally (20mg/animal).The protocol in the present study was approved by the Ethics Committee for research of the State University of Campinas (protocol: 2997-1) **Results:** The mean data of the area under the curve were 326. ± 153.04 ng.h/mL for Fentizol®, 280.37 ± 86.14 ng.h/mL for the test formulation (F-test) for the first 2 hours after administration (unpaired t test Vs test formulation ;p= 0.80) and 409.6 ± 53.96 ng.h/mL for Fentizol® and 805.77 ± 252.48 ng.h/mL for F-test during 8th day of treatment (unpaired t test Vs F-test ;p= 0.20). The bioavailability of fenticonazole was 23.4%. In both groups, the vaginal histological architecture was conserved. Stratified squamous epithelium was present at the middle portion and a simple columnar epithelium was observed at upper vaginal section. One animal per group had discrete leukocyte infiltration in the upper vaginal portion. **Discussion:** The model is easy to perform, allows evaluation of fenticonazole vaginal permeability. The method suggested is usefull to determine drug safety for vaginal formulations, evaluating the presence of side effects and toxic sign. **Sources of Research Support**: This study was supported by CNPq/Brazil

11.009

Correlations among antiangiogenic factors and trace elements in hypertensive disorders of pregnancy. Rezende V, Palei AC, Barbosa Jr F, Tanus-Santos JE, Sandrim V FMRP-USP – Farmacologia

Although a number of studies have measured circulating levels of some trace elements in preeclampsia (PE) and compared to healthy pregnant (HP), there is no consensus yet about the deficiency of some metals and development of hypertensive disorders in pregnancy. The aim of this study was to compare plasmatic levels of Zn, Mn, Co, Cu, Se and Sr among non-pregnant (NP), healthy pregnant (HP), gestational hypertensive (GH) and preeclamptic (PE) women and to correlate these levels with plasma soluble endoglin (sENG) and soluble fms-like tyrosine kinase-1 (sFLT-1), two important antiangiogenic proteins related to PE. A total of 184 women were enrolled in this study (NP=35, GH=51, PE=37 and HP=61) and signed consent term (Ethics Committee number HCRP 4682/2006). Trace elements analyses were carried out with an inductively coupled plasma mass spectrometer (ICPMS). Soluble endoglin (sENG) and sFLT-1 plasma concentrations were measured by commercial ELISA kits. The most interesting result is that Sr is higher in PE (63%, $P<0.001$) compared to HP and their levels are positively correlated with sENG in all three groups of pregnant women. Moreover, we found a negative correlation between Zn and sENG in HP ($r=-0.43$, $P=0.003$). Regarding other elements, we found similar levels among pregnant groups. In conclusion, PE present higher Sr levels and that Zn may contribute to reduction of sEng in HP. **Financial Support:** This study was funded by the Conselho Nacional de Desenvolvimento Cientıfico e Tecnologico (CNPq) and the Fundação o de Amparo a Pesquisa do Estado de São Paulo (Fapesp-Brazil).

11.010

Toxic effects of OMC administration during development of rats in lactational period.

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Introduction: UV radiation might lead to several deleterious effects to human health, among them DNA damage, early aging, cataract, chemical unbalance e skin lesions, immunosupression, melanome. Because these effects there is the need to protect the population against UV radiation, by using cosmetics and other products that act as UV filters. The consuming market presents a series of these filters, which are classified as organic or inorganic ones. The octyl metoxi cinamate (OMC), an organic UV filter, is widely used in cosmetics, sunscreen, shampoos, among others, all of them registered in Brazil. Several studies have been shown that OMC is present in biological fluids, as blood, urine, milk, water as well as in chain food. The safety of use of these products is a great concern, once several studies have shown that OMC is a potential endocrine disruptive, especially on thyroid axis. Thyroid hormones are crucial during the development, the Central Nervous System one of the systems most affected by any disturbances in thyroid axis. Thyroid hormones have important role in proliferation, neuronal migration and maturation, and have been implicated in several cognitive deficits, as memory, learning and hyperactivity. **Methods**: All experiments receive approval from our ethical committee, number P-16/14.2. Wistar rats were exposed orally to OMC from P5-P22. The control groups receive oil corn (negative control), thyroxin (T4) (hyperthyroidism) or PTU (hypothyroidism). During treatment, weight was recorded daily and eye opening was evaluated from P10 to P16. On weaning one set of animals were euthanasiated for T4 dosage and organ collection and other set of animals were left to survive into adulthood for behavioral analyzes, as anxiety (elevated plus maze), exploration (hole board) and memory/learning (Morris water maze). **Results**: A decreased gain weight was observed in OMC/PTU groups during treatment, when compared to corn oil group (% of control), at both sexes: PTU 63.7% ± 1.1%; T4 99.73% ± 2.8%; OMC 85.2% ± 2.6% (ANOVAr; df = 3; F = 7,175; p=0,002). Regarding eye opening, chi-square analyses showed a mild delayed in OMC group compared to corn oil (X^2= 2.5; 0.1<p **Discussion:** Our results shown that OMC exposition statistically decreases gain weight during lactation, in a similar mechanism of PTU. This suggests a compromised thyroid axis development by OMC. A neurodevelopment milestone, eye opening, was mild delayed in PTU/OMC group. Regarding the immune system it was observed a decreased in thymus and spleen weight, as well as in splenocytes number in PTU/OMC groups, suggesting a toxic multisystemic effect. **Financial support**: Fiocruz, PAPES/CNPq

11.011

Does omeprazole association modulate digoxin pharmacokinetic in patients with heart failure? Souza FC, Barros RBM, Rocha RG, Baptista TM, Silva TA, Scaramello CBV UFF

Introduction: Knowledge of drugs' pharmacokinetic (PK) parameters in different patients is essential for safe therapeutics regimens establishment. Digoxin enhances myocardial contractility and is indicated for patients presenting heart failure (HF) with systolic dysfunction, associated with high ventricular rate observed in atrial fibrillation. It has a narrow therapeutic window and, according literature, digitalis intoxication may occur due to drug interactions or comorbidities. This study was divided into two steps (INC Ethics Committees id approval number: 0306/07-12-10). The aim of the first step was to determine patients prone to digitalis intoxication profile. According to previous data, male patients (79%), included into functional class (NYHA) III HF (65%), presenting renal failure (33%) are more susceptible to attain plasma concentration (Cp) out of therapeutic range.The second phase comprised the study of therapeutic association with omeprazole on digoxin pharmacokinetics because there is a case report of a 65-year-old woman on long-term digoxin therapy at a stable dose and stable blood levels that presented digoxin toxicity 3 months after initiation of omeprazole therapy (Kiley CA *et al*,South Med J. 100(4):400,2007). **Methods:** As the patients' profile prone to digoxin intoxication was determined, male subjects using digoxin, FC III, between 21-60 years-old were included into the study after informed consent signature. Individuals using digoxin 0,125-0,250mg were grouped according to therapeutic combination with omeprazole or not. Blood samples were collected at 6 different points along 24 h after digoxin oral administration. Measurements of digitalis Cp were performed using immuno-chemiluminescence Method: Data were presented as mean and standard error of the mean. Results: It was possible to allocate patients receiving digoxin in different doses in the same stratum because no significant differences were observed in terms of digitalis PK due to the dosage. There were no statistic differences between patients using omeprazol (AUC0-24 = 25.52 ± 2.87 ng.h/mL, Cmax = 1.38 ± 0.15 ng/mL, Tmax = 2.83 ± 0.92 h; CL/F = 0.11 ± 0.03 L/h/kg) or not (AUC0-24 = 21.47 ± 3.23 ng.h/mL, Cmax = 1.25 ± 0.17 ng/mL, Tmax = 1.67 ± 0.21 h; CL/F = 0.14 ± 0,03 L/h/kg) associated to digoxin. Discussion: This work suggests that omeprazole, a proton pump inhibitor widely used that is substrate of P-glicoprotein and citocrome P450 enzymes, does not influence on digoxin pharmacokinetics. **Financial support**: Faperj, CNPq, Capes, PROPPI/UFF.

11.012

Evaluation of streptokinase in biopharmaceutical formulations by *in vitro* bioassays. Cardoso Jr CDA[1], Schramm VG[1], Freitas GW[1], Walter ME[1], Xavier B[2], Dalmora SL[1] [1]UFSM – Ciências Farmacêuticas, [2]UFSM – Farmácia

Introduction: Streptokinase (STK) is obtained from culture filtrates of certain strains of haemolytic *Streptococcus* group C, that may also produce streptodornase and streptolysin as contaminants, interacts with and activates human plasminogen to form an activator. The recombinant STK has been expressed in *E. coli*. It consists of a 414 amino acids polypeptide chain, with a molecular mass of 47 kDa. It is clinically used as a thrombolytic agent for the treatment of patients with acute myocardial infarction, venous and arterial thrombosis.
The aim of this study was to perform the *in vitro* chromogenic assay to assess the biological potency of STK, and evaluate the content of streptodornase and streptolysin in biopharmaceutical formulations available for clinical use. **Methods:** Seven batches of biopharmaceutical products containing 1.5 million IU per vial of STK were evaluated against the 3rd International Standard for Streptokinase (WHO 00/464). Standard and samples were diluted to final concentrations of 2.5 to 40 IU/mL and the assay performed at 37°C, using chromogenic substrate (S-2251) and human plasminogen. The absorbances were measured in the plate reader at 405 nm. Streptodornase was assessed against a solution of sodium deoxyribonucleate in imidazole buffer solution pH 6.5 and the absorbance read at 260 nm. Streptolysin was evaluated using a 5 IU/ml reference standard solution of human antistreptolysin O, and erythrocytes suspension measuring the absorbances at 550 nm. Experimental protocol was approved by internal ethical commission of UFSM under the number 23.081.018819. **Results e Discussion:** The *in vitro* bioassays were applied for the potency assessment of streptokinase in biotechnology-derived products giving potencies between 92.50 and 104.30%, with fiducial intervals (P=0.05) between 80 and 125%, following the Pharmacopeial specifications. Besides, the activity of streptodornase was found variable according to the sample, but equivalent to a maximum of 10 IU of streptodornase activity per 100 000 IU of streptokinase activity. For the streptolysin the absorbances of the sample solutions were not more than 50 per cent greater than that of the reference standard solution, as recommended. **Conclusion**: The results obtained demonstrated the application of the *in vitro* assays to assess the biological potency of STK showing the quality of the biological medicines, which meet the specifications between 90-111%, of the stated potency. Besides, the levels of the contaminants of the process detected were according to the specifications. The combination of assays is necessary to assure the quality, and allows a great improvement which can be applied for the characterization of streptokinase, by ensuring batch-to-batch consistency of the bulk and finished biopharmaceutical products.
Acknowledgments: CNPq

11.013

Haloperidol-loaded polymeric nanocapsules prevents hepatotoxicity and DNA damage in rats.

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Introduction: Haloperidol (HP) is a typical neuroleptic drug widely used for treatment of psychiatric conditions due to its high potency and lower cost (Ponto; Methods Find. Exp. Clin. Pharmacol., 32:427, 2010). The long-term use of HP is related to serious side effects in vital organs as liver (Halici; Naunyn-Schmied *Arch Pharmacol*, 379:253, 2009). Polymeric nanoparticles have attracted attention as potential drug carriers by their property of enhancing the therapeutic efficacy of drugs, while are able to minimize the side effects of drugs associated to these systems (Callewaert; J Biomed Mater Res, 101:1319,2013). Considering HP toxicity in liver and in view of the lack of knowledge about safety of polymeric nanocapsules, our aim was to evaluate the effects of haloperidol-loaded lipid-core nanocapsules formulation on cell integrity and DNA damage in rats. **Methods:** For this study, 28 rats were assigned in four groups (n = 7) and treated with vehicle solution (C group- 5% polysorbate 80, v/v), free haloperidol suspension (FH group), blank nanocapsules suspension (B-Nc group) and haloperidol-loaded lipid-core nanocapsules suspension (H-Nc group). All suspensions were administered (0.5 mg/kg-ip) once a day, for 28 days. On the 29th day, all rats were anesthetized and euthanized. Livers were removed and sliced for cell integrity assay, which was quantified by measuring the reduction of 3-(4, 5-dimethyllthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) (Mosmann; Immunol Methods, 16:55, 1983). An aliquot of whole collected blood was separated for comet assay (Singh; Exp Cell Res, 175:184, 1988) and reminiscent was centrifuged for plasma achievement, and used for determination of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) by Bioclin® commercial kit. The experimental protocol of this study was approved by Animal Ethical Committee of UFSM (CIETEA- 22/2010). **Results:** Our results showed that free haloperidol (FH) was able to reduce the MTT levels in liver of rats and increase AST and ALT in plasma in relation to C and H-Nc groups. In addition, FH increased the damage index (DI) to DNA in comparison to others groups. **Discussion:** FH treatment caused an imbalance on the animals hepatic cell integrity, which was observed by reduced MTT levels and increased AST and ALT plasma levels. This experimental group also presented increased DNA damage index. On the other hand, the nanoencapsulation of the drug was able to prevent all changes observed with the FH group. We suggest that nanocapsules are drug delivery systems that may exert control and drug sustained release, preventing an excessive bioaccumulation in specific organs (Marchiori; Drug Dev Ind Pharm, 36:962, 2010), which is able to reduce the drug toxicity. Our study showed that sub-chronic administration of haloperidol-loaded lipid-core nanocapsules was no toxic to liver, indicating that further studies about this nanoparticles system on other vital organs are needed. **Acknowledgments:** The authors are grateful to CNPq and Capes for fellowships and financial support.

11.014

Inosine prevents MeHg induced neurotoxicity, hepatotoxicity and dyslipidemia in mice.

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Introduction: Methylmercury (MeHg), an environmental contaminant ubiquitously present in nature, induces toxic effects in both animals and humans. We have already been demonstrated that inosine, an endogenous purine, was able to prevent MeHg-induced neurotoxicity in a broad of behavioral and biochemical tests. Therefore, present study aimed to confirm neuroprotective effect of inosine and demonstrate its protective effect in face of MeHg-induced, hepatotoxicity and hypercholesterolemia in mice. **Methods:** Male Swiss mice (45-60 days old) were exposed to MeHg diluted in drinking water (40 mg/L, *ad libitum*) for 15 days. Concomitantly, animals received inosine intraperitoneally (10 mg/kg), once a day. Liquid ingestion and body weight were monitored daily. On the 15th day, animals were submitted to beam walking test in order to evaluate motor performance/coordination. After behavioral test, animals were anaesthetized with isoflurane and blood was collected using infraorbital puncture technique with heparinized capillaries. Subsequently, blood was centrifuged (3900 r.p.m. for 5 minutes) to obtain the serum, which was used for measurement of total cholesterol, HDL- and non-HDL cholesterol, urea and creatinine levels and ALT and AST activity, using specific commercial diagnostic sets and automated analyzer Mindray BS120. After blood collection, animals were euthanized by decapitation, cerebellum and liver were removed and weighed. Experimental procedures approved by CEUA/UFSC protocol PP00745. **Results:** Liquid ingestion and body weight did not differ between groups. MeHg daily dose ingested did not differ when compared MeHg-exposed groups treated with vehicle (10 ml/kg, i.p.) and inosine (10 mg/kg, i.p.) (mean = 6.70 ± 0.46 mg/kg *vs* 7.10 ± 0.45 mg/kg). Inosine prevented MeHg-induced motor performance/coordination deficit observed in beam walking test (87 ± 10%; mean=21.57 ± 3.06 s *vs* 46.88 ± 11.93 s) and prevented MeHg-induced reduction in cerebellum weight (71 ± 20 %, mean = 26.06 ± 1.26 % *vs* 22.33 ± 1.89 %). Moreover, inosine did not prevent MeHg-increased total cholesterol levels (mean = 128.0 ± 4.06 mg/dl *vs* 124.7 ± 3.93 mg/dl), however, was able to prevent MeHg-increased non-HDL cholesterol levels (83 ± 35 %; mean = 33.89 ± 7.68 mg/dl *vs* 52.29 ± 2.29 mg/dl) and promoted an increase in HDL cholesterol levels when compared with MeHg-exposed animals treated with vehicle (10 ml/kg, i.p.) (23 ± 11%; mean = 102.7 mg/dl ± 9.01 *vs* 83.53 ± 3.39 mg/dl). Inosine also prevented increase in ALT activity induced by MeHg (82 ± 16%; mean = 79.61 ± 7.32 U/L *vs* 116.6 ± 13.83 U/L), however was not able to prevent MeHg-induced AST increased activity (mean = 200.1 ± 15.45 U/L *vs* 178.3 ± 14.79 U/L). Finally, inosine prevented MeHg-induced increase in liver weight (42 ± 30%, mean = 4.46 ± 0.08 % *vs* 4.91 ± 0.16 %). **Discussion:** Inosine presented protective effect in face of MeHg-induced neurotoxicity, hepatotoxicity and hypercholesterolemia. our long-time goal is to provide initial subsidies to consider purinergic system, especially inosine, as pharmacological tool of interest in studies related to MeHg toxic effects. **Financial support:** Capes and CNPq.

11.015

Non-clinical toxicity and evaluation of the gastroprotective effect of bioproducts from *Psychotria carrascoana.* Almeida AC[1], Freitas LBN[1], Pierdoná TM[2], Filho JMSR[1], Nascimento RRG[3], Pimenta ATA[3], Lima MAS[1], Leal LKAM[1] [1]UFC – Farmácia, [2]UFC – Fisiologia e Farmacologia, [3]UFC – Química Orgânica e Inorgânica

Introduction: *Psychotria carrascoana* (Rubiaceae) can be easily found in the State of Ceará, being used in traditional medicine against dizziness, hallucination, dementia, rubella and pain disorders. The genus *Psychotria* has been regarded a promising source of alkaloids that have diverse pharmacological actions, such as antiplatelet, anti-nociceptive and anti-inflammatory activities. The aim of this study was to evaluate the toxicity and gastroprotective activity of bioproducts from leaves of *P. carrascoana* (ethanol extract – EE, alkaloidal fraction – AF and/or calycosidine, pyrrolidinoindole alkaloid – CAL) in mice or human neutrophils. **Methods:** Animal handling and experimental protocols were registered on the Ethics Committee under number NS29. To evaluate the gastroprotective effect, Swiss mice (n=8) were undergo fasting of 18h, then were treated with EE (50 and 100 mg/kg p.o.) or omeprazole (standard drug, 30 mg/kg p.o.) 60 minutes before receiving absolute ethanol (0.2mL, p.o.). The animals were sacrificed after 60 min and stomachs removed and analyzed the lesion index. The citotoxicity assays were carried out on the activity of the enzyme lactate dehydrogenase (LDH) as well as the MTT test in human neutrophils (2.5×10^6 cells/mL) with 95% cell viability (Tripan Blue exclusion technique). In this sense, before the toxic evaluation the cells were incubated with EE (50, 100 and 200µg/mL), AF (50, 100 and 200µg/mL), CAL (25, 50 and 100µg/mL), DMSO (vehicle/control), 0.2% Triton X-100 (citotoxic standard) or HBSS (negative control). **Results and discussion:** The treatment of the mice with EE (50 and 100 mg/kg) induced a gastroprotective activity by reducing the injury of ethanol in 77.0 and 88.8 %, respectively. Similar results were observed in the omeprazole group (3.7 ± 1.2%) The addition of EE (50, 100 and 200 µg/mL) or CAL (25, 50 and 100µg/mL) did not increase significantly (ANOVA, Tukey as post hoc test, $p<0.05$) the LDH activity (EE: 20.0 ± 7.0; 19.3 ± 5.7; 27.6 ± 6.9 U/L, respectively; AL: 32.6 ± 6.8; 29.9 ± 3.3; 22.1 ± 5.8 U/L, respectively) in cells suspension when compared to control group (38 ± 5.9 U/L). On the other hand, the AF since at concentration of 100 µg/mL (176.1 ± 39.6U/L) showed cytotoxicity when compared to control group (26. 2 ± 1.9 U/L). Similar results were found in the MTT test, where only AF showed toxicity in human neutrophils reducing the cell viability in until 53 %. These results suggest that among the bioproducts of *P. carrascoana* evaluated only AF seems to be toxic affecting the integrity of plasma membrane and the metabolism of human neutrophils. This toxic effect is possibly related to others alkaloids instead of calycosidine. The preliminary study provided evidence, for the first time, to support the gastroprotective effect of EE from *P. carrascoana*, which together with calycosidine were not toxic for human neutrophils. **Acknowledgements:** CNPq, FUNCAP and Capes.

11.016

Infection by *Paracoccidiodes brasiliensis* associated to use of adalimumab in arthritic patient: a case report. Caldas LM, Brito IRV, Vieira CJAB, Yamasawa RH, Borges VM, Bueno AN
HUAV-Unifenas

Introduction: Rheumatoid arthritis (RA) is a chronic inflammatory disease, which may commit small and large joints. Its treatment is complex and includes, in some cases, immunobiological drugs. One of them, adalimumab, humanized TNFα antagonist, can induce immunosuppression, and allow the development of opportunistic infections. This work presents a case report of a RA patient treated with adalimumab, who developed secondary infection by *Paracoccidioides brasiliensis*. Since it is the second report in the literature, it is important to be shared with the medical and scientific community. **Methods**: This study was conducted at Hospital Alzira Velano in Alfenas, Minas Gerais, Brazil, with agree of the Human Ethics Committee of the José do Rosário Velano University number 23/2014. Patient assessment was carried from his hospital medical history and conduct of clinical, laboratory and imaging tests, besides biopsy and hystopathological analysis of larynges tissue.
Case report: Patient, M.T.F., female, 55 years-old, smoker for 40 years and stopped in 2001, was diagnosed with rheumatoid arthritis in 2004. Firstly, patient received indication to nonsteroidal anti-inflammatory drugs (ibuprophen and naproxen). But, as the case had worsened, the second prescription included prednisone 15 mg/day. In 2005, presented Raynaud's syndrome, which result in extremities vasculitis, with subsequent amputation of the distal phalanges of second and third right chyro dactylo, and first and second left polydactylo. After this episode, patient received indication to use also chloroquine 250 mg/day. In 2008, were noted subcutaneous metacarpofalangeans nodules and joint deformities. Given this picture, was suggested the substitution of methotrexate by adalimumab 40 mg of 14 in 14 days. Followed this protocol, the patient, finally, went into remission of RA. However, in 2013, patient presented persistent cough, accompanied by hoarseness, presence of fine rales and expandability preserved, followed by weight loss. A rigid laryngoscopy was applied at 70°, which diagnosed a chronic laryngitis by pharyngolaryngeal reflux. Although the patient was treated with ciprofloxacin 500 mg 12/12 hours for 10 days, kept nocturnal episodes of fever, for which received a new indication for laryngoscopy, looking for collection samples from epiglottis and arytenoids for histopathological analysis. These procedure revealed findings consistent with infection by *P. brasiliensis*. The infection was monitored using itraconazole 200 mg daily, keeping the use of adalimumabe, resulting in maintenance of AR remission and allowed resolution of infection.
Conclusion: Knowing the importance of TNFα in the control of *P. brasiliensis* infection, and the fact that the patient not having any other co-morbidity causing immunosuppression, this paper reports that the antagonism of TNFα by adalimumab may predispose patients to secondary infections, reason by which, must be carefully evaluated case by case. **Acknowledgments**: José do Rosário Velano University (UNIFENAS) and Alzira Velano Hospital.

11.017

***In silico* drug discovery approach for new candidates on the treatment of Paracoccidioidomycosis disease.** Marschalk C[1], Seixas FAV[2], Cotica ESK[3] [1]LNBio-CNPEM-Unicamp, [2]UEM – Biochemistry, [3]UEM – Micology

Introduction: Paracoccidioidomycosis is an infection caused by the fungus *Paracoccidioides brasiliensis* and is considered one of the most important systemic mycoses in Latin America. It affects mainly farm workers causing severe skin injuries or even systemic involvement that can . The treatment is based on nonspecific antifungal medicines for an extended period, which is a major obstacle to cure the disease because of the cost and adverse effects of medication. The discovery of new molecular targets and therefore new treatment alternatives involving more effective drugs against paracoccidioidomycosis are therefore of great importance to the current socio-economic scenario of Latin America. In order to discover new drugs for the treatment of paracoccidioidomycosis, the enzyme Chorismate synthase (EC: 4.2.3.5) was used in molecular dynamics studies, virtual screening and docking of inhibitors. **Methods:** The tetrameric structure of *Pb*CS was built by molecular modeling (*Modeler* v9.11) using *S. pneumoniae* and *S. cerevisiae* CS bonded to FMN (cofactor) and EPSP (substrate) as templates. Over 200 000 ligands were screened on databases for Docking procedures and the the top-ranked candidates were selected by $\Delta G_{binding}$ score. Minimum inhibitory concentration (MIC) were evaluated by *in vitro* tests on Pb18 and Pb01 strains and Molecular dynamics were conducted afterwards to check the ligands stabilities on PbCS active site during the simulations. **Results and discussion:** Stereochemical evaluation of the *Pb*CS model showed all residues placed in allowed regions and sequence alignment with templates showed significant structural differences on the active site. Virtual screening selected 3 compounds (under patent process) named CP1, CP2 and CP3, with a $\Delta G_{binding}$ values of -13,3 Kcal.mol-1, -13,1 Kcal.mol-1 e -13,99 Kcal.mol-1 values respectively compared to the EPSP (12,7 Kcal.mol-1). *in vitro* tests showed 32 µg/mL MIC values for both CP1 and CP2 compounds and 512 µg/mL for CP3. Molecular dynamics suggests a more stable form on the tetrameric arrangement even in the presence of different ligands. The set of those results suggest promisor compounds for future *in vivo* tests as alternative candidates for the treatment of paracoccidioidomycosis. **Apoio Financeiro**: Fundação Araucaria, Capes e CNPq.

11.018

Active pharmacovigilance and study of a new oral anticoagulant dabigatran in Brazilian public hospital specializing in cardiology. Martins LB[1], Almeida FVS[2], Scaramello CBV[1] [1]UFF, [2]INC

Introduction: The National Institute of Cardiology (INC) belongs to sentinel hospitals Network of Brazillian National Health Surveillance Agency. Dabigatran, a new drug, was incorporated into the standardization of INC in 2012 as an alternative to warfarin for patients with nonvalvular atrial fibrillation (FA). **Methods:** This work constitutes a pharmacovigilance study that aimed to evaluate adverse events (EA) associated to this new oral anticoagulant in outpatients with FA into the six initial months of pharmacotherapy (CAAE: 03455512.5.0000.5272), being conducted at INC pharmacy from January to June of 2013. Patients on dabigatran 110mg and 150mg were followed by pharmaceutical interview during its dispensation and stratified according to the dose being analyzed about age, gender, comorbidities, other medications in use and reports of EA. **Results:** There were included 98 patients but 3 did not iniciate the treatment. Along the period evaluated, independent of the dose, patients majority were male (about 60%). Most patients on dabigatran 150mg (48.6%) had less than 65 years-old being younger than most users of 110mg dosage (42.9% aged between 65 and 75 years-old). Regardless of the dose of dabigatran, approximately 85% of patients received prior anticoagulation. However, the lowest dosage was applied specially to patients with highest bleeding score risk (52.4%). Only 12.3% of individuals who used 150mg dosage had a score risk that high. Patients, specially whose on dabigatran 110mg, showed several comorbidities such as hypertension (83.3% for 110mg vs. 63.9% for 150mg), stroke (55.6%vs.9.8%) and diabetes mellitus (55.6%vs.22.9%), justifying therapeutic associations with beta-blockers (71.4%vs65.7%), AT1 antagonists (66.7%vs.52.2%) and oral hypoglycemic agents (42.9%vs.10.5%). The main EA reported in the first trimester of pharmacotherapy by patients taking dabigatran 150mg was dyspepsia (male 57.1% and female 36.9%), mainly those beyond 75 years-old (55.6%). This EA frequency associated to the lowest dosage was not different between genders, being comparable to headache, fatigue and dizziness (28.6%) in females and more prominent in patients beneath 65 years-old (40%). In the second trimester, this EA was only related to 150mg dosage (5% males, 8.1% females and 15.8% individuals beneath 75 years-old). Therefore, after the first trimester, omeprazol or ranitidine combination was prescribed (14.9% individuals using 150mg and 4.8% patients on 110mg) and, irrespective of dosage, treatment interruption by patient's decision (14.3% for 110mg and 5.4% for 150mg) or medical suspension (19% for 110mg and 10.8% for 150mg) were noticed. It was also observed dabigatran dose decrease from 150mg to 110mg in both periods (5.4% and 7%, respectively). Deaths occurred just with the highest dose (1.3% in the first quarter and 1.7% in the second). **Discussion:** The main EA observed in this study had been already described in the literature (Connolly *et al.* The New Engl J Med, 361: 1139, 2009), however, our data show it in a greater frequency, justifying problems with adherence and treatment interruption. Considering this study it is possible to classify dabigatran's EA about its feature and occurrence enabling strategies development to optimize pharmacotherapy. **Financial support:** Capes.

11.019

Characterization of CYP2E1 polymorphisms in patients receiving treatment for TB. Santos EA[1], Gonçalves JCS[1], Fleury MK[2], Silva JRL[3], Estrela RCE[1] [1]FF-UFRJ – Fármacos e Medicamentos, [2]FF-UFRJ – Análises Clínicas e Toxicológicas, [3]UFRJ – Medicina

According the guidelines of the Ministry of Health in Brazil, currently, TB treatment consists in a single pill, which includes isoniazid – H, rifampicin – R, ethambutol – E and pirazidamide – Z, that can lead to hepatotoxicity. This risk could be related to genes expression encoding enzymes involved in the H biotransformation, considered the main anti-TB drug (Katsumi *et al*, 2008). Enzymes involved in the biotransformation of H are the NAT, CYP2E1 and GST. The H metabolism leads to the formation of Acetyl Hydrazine (Ac-H) by NAT acetylation. Ac-H is a substrate of CYP2E1 which produces active metabolites that are hepatotoxic. The SNPs present in the gene *CYP2E1* have been associated with high incidence and/or severity of adverse reactions of H. The objective of this study is to characterize the main effects of genetic polymorphisms in *CYP2E1* gene present in the hospitalized population during anti-TB treatment at the Hospital Estadual Santa Maria (RJ, Brazil) using PCR- RFLP. This study is an arm of the project Malnutrition and Immunogenetics in TB and HIV Infection approved by the CEP on 04/28/05 with number 004/05. Blood samples were collected from 40 patients and all signed an informed consent in the period from March to November 2012. The genomic DNA was extracted from total blood and amplification of the promoter and intronic region of the gene was made. Two fragments were generated 410pb and 373pb, respectively. The PCR product was subjected to enzymatic cleavage with restriction enzymes *RSA I* and *DRA I*. Thirty samples were analyzed, 3,34% of these samples are mutant allele of promoter region (CYP2E1*5A) and 8,33% of intronic region (CYP2E1*6). The frequency of genotypes were 10,0% for CYP2E1*1A/*6; 3,33% for CYP2E1*6/*6; 6,67% for CYP2E1*1A/*5A; 80,0% for wild-type CYP2E1*1A/*1A and none for CYP2E1*5A/*5A. In admission on the hospital, patients present profile of mild anemia, consistent with anemia of chronic disease (ACD) and according to the literature: a mean RBC 4,08 milion/mm³, hemoglobin concentration (CH) of 11,1g/dL, PCV of 34,8% and MCHC 31,2g/dL (Cançado *et al*, 2002). Ten to thirty days of treatment we observed a significantly reduction on RBC indices in CYP2E1*1A/1A genotype which reaffirm the characteristic of ADC; the wild-type shows slightly increased significantly on enzyme ALT, that represents hepatocellular lyses, since it is specific for the liver cells. This preliminary found is consistent with the literature, that associating increasing CYP2E1 activity with the wild type (Castelló *et al*, 2010). **Financial**: Faperj. **References:** Nota Técnica PNCT/DEVEP/SVS/MS, Katsumi. *F J Toxic Sci* Vol 33(2):187, 2008, Cançado. *Rev Bras Hemat Hemot* Vol 44(2):257, 2009, Castelló. *Toxicol Let* Vol 192:34, 2010

11.020

Evaluation of acute toxicity and determination of DL_{50} of a new derivative of ferulic acid.

Araruna Junior AA[1], Rêgo SC[2], Mata AMOF[2], Osório MS[2], Taimo MRD[3], Alencar MVOB[3], Gomes Junior AL[3], Freitas RM[3], Cavalcante AACM[4] [1]FSA, [2]Uninovafapi, [3]UFPI, [4]UFRGS

Introduction: Medicinal plants have great global importance. It is used for centuries as a therapeutic source and also as a raw material for a certain amount of conventional drugs used in clinical practice (HUBSCH, Z., *South African Journal of Botany*, v. 93, p 185-197, 2014). A study with plants has a certain amount of meaning. Among these meanings, it's presented the need of conducting research on their toxicity compounds in order to understanding their effects on organism (ZHAO, Z., *Food Chemistry*, vol. 109, p. 691-702, 2008). Ferulic Acid is a compound of natural sources which has an antioxidant action from the metabolism of phenylalanine and tyrosine. This acid also originates derivatives by esterification reactions, among them, the isopentanoyl ferulate (SÁNCHEZ, A., *Electrochimica Acta*, v. 133, p. 546-554, 2014). **Methods:** *Swiss* mice (25-30 g) of both sexes were divided into 2 groups of 5 animals. Ferulate was administered intraperitoneally (i.p.) on doses of 1000, 1500 and 2000 mg/kg and orally (v.o.) on doses of 1000, 2000 and 3000 mg/kg. The general behavior of mice was continuously monitored for 1 h after treatment and periodically during the first 24 h (with particular attention during the first 4 hours). Afterwards, it had a daily follow up for a total of 14 days. During this period, were evaluated the changes in general activity of mice; along with it were recorded the signs of toxicity or death. Ferulate's DL_{50} was calculated from mice treated acutely and intraperitoneally. This protocol was registered and approved by the Ethics Committee on Animal Experimentation – UFPI with the number: 030/13. **Results and discussion:** At doses of 1000, 2000 and 3000 mg/kg (v.o.) in acute treatment with ferulate there was no mortality in animals during 14 days of observation, but it has induced smaller effects of general activity, corneal reflex, piloerection and palpebral ptosis. There was no determination of the DL_{50} v.o. for testing the maximum allowed dose (BRAZIL, ver. 02, 2013), and no deaths were observed. In i.p. the first mouse died within 48 hours after injection of the dose of 1000 mg/kg and maximum frequency of death occurred in 2000 mg/kg. Of the 10 mice treated with a dose of 1500 mg/kg of ferulate, 4 animals died, 2 males and 2 females. With doses (i.p) the phenylpropanoid caused minor effects on spontaneous activity, ptosis, ataxia, analgesia, sedation, piloerection, hypnosis, panting. For ferulate, a dose of 1000 mg/kg i.p., was the minimum lethal dose tested (the lowest being first dose which induced death in mice) and 1500 mg/kg was determined as the DL_{50} (95% limit trust). Aftermath, by the results, were observed greater toxicity of ferulate when administered intraperitoneally.

11.021

Evaluation of amoxicillin generic brands by *in vitro* antibacterial activity method. Ignácio L[1], Silva MTG[2], Batista TGFM[2], Ferraris FK[1], Amendoeira FC[1] [1]INCQS-Fiocruz – Farmacologia, [2]INCQS-Fiocruz – Microbiologia de Produtos

Introduction: The emergence of generic drugs in 1999 provided greater access to the population to essential medicines. By definition, this product is similar to a reference product, which is intended to be interchangeable drug. It contains the same active ingredient, dose, pharmaceutical form, when administered by the same route and with the same therapeutic indication. The quality of generic drugs is guaranteed by Resolution RDC No. 17/2010 that regulates the practice for manufacture of drugs. Product verification, except for reasons of registration renewal is currently done only when there are many cases of complaints or adverse events about a drug. The successful use of antibiotics as treatment against infections depends on, among other factors, the plasma concentration achieved so that all microorganisms are affected. In cases of bacterial infections, the therapeutic activity has an outstanding importance to prevent bacteria develop resistance to the treatment and to promote a stronger infection, affecting more patient. Based on this, the purpose of post-marketing control is to ensure that the potency of antibiotics distributed to consumers is within acceptable limits for successful treatment. **Objective**: The objective of this study is to verify the potency of different samples of amoxicillin to create data to prove the efficacy of generic drugs. **Methods:** The determination of amoxicillin potency in oral suspensions was based in Brazilian Pharmacopoeia and the method used was the cylinder-plate. The cylinder plate assay of drug potency is a test based on measurement of the diameter of zone of inhibition of microbial growth surrounding cylinders (cups), containing various dilutions of test compounds. Solution of each concentration (0.1 µg/ml) of three generic amoxicillin brands and reference standards were added to the cups with a micropipette. The presence of definite zone of inhibition around the cup in an incubation time of 16 hours at 32°C indicated antibacterial activity. Strain of *Kocuria rhizophila* ATCC 9341 were used as a microorganism test. **Results and discussion:** The halo diameters measured from the standart amoxicillin was 145.22 ± 22.39 (n=18) and the commercial samples were A: 168.44 ± 24.99 (n=18), B: 168.88 ± 18.13 (n=18) and C: 165.33 ± 13.66 (n=18). It was concluded that samples of generic amoxicillin presented 90 to 120 % of the declared potency as preconized by the Brazilian Pharmacopoeia. The results suggest that the generic samples of amoxicillin were comparable with the reference standart. This assay can't guaranty the pharmacological efficiency just based on the potency, so it is necessary a pharmacokinetic study to compare the biological response of the generics formulations. **Financial support**: APQ1/Faperj and PAPES/CNPq

11.022

Evaluation of acute toxicity of HSE-07 in mice. Mangueira VM[1], Batista TM[1], Sousa TKG[1], Brito MT[1], Beltrão DM[1], Moura APG[1], Souza HDS[2], Souza RPF[2], LIRA BF[2], Sobral MV[1] [1]UFPB – Ciências Farmacêuticas, [2]UFPB – Química

Introduction: Selenorganics compounds have shown many biological activities such as antibacterial, antiviral, antifungal, antihistaminic, antiparasitic and anticancer (Athayde-Filho, P. F., Arkivoc, 22, 2004). The present investigation was carried out to evaluate the acute toxicity of HSE-07, a selenorganic compound, determining its potential toxicity after administration in mice. **Methods**: Acute toxicological test in mice was performed according to OECD "Guidelines for Testing of Chemicals"n. 423/2001, with modifications. It was approved by the Animal Studies Committee of the UFPB (no. 0801/14). Swiss mice (*Mus musculus*), female, were submitted to single dose intraperitoneally (i.p.) of 50 and 300 mg/kg (n = 6). In order to map possible central and autonomic behavioral changes, observation to detect toxic signs was performed at the intervals: 0, 15, 30 and 60 minutes; after 4 hours and daily for 14 days (Almeida, R. N., *Rev. Sci Farmacogn*, 80: 72, 1999). After of the observation period, biochemical and hematological evaluations were performed. Furthermore, indexes of organs (organ mg/animal g), liver, heart, kidneys, spleen and thymus were evaluated. The results were expressed as mean ± standard error of the mean (SEM) and analyzed by Student's T Test and were considered significant when $p<0.05$. **Results**: Animals treated with 300 mg/kg showed some central nervous system excitatory effects (tremors, convulsions), and depressants (ptosis, loss of corneal reflex and ear), preceding death of all the animals in the first minutes after administration. In contrast there was not observed effects after treatment with 50 mg/kg. Of all the parameters evaluated there was only a decrease in water consumption in treated animals at a dose 50 mg/kg (41.25 ± 2.31 mL) compared with control (34.58 ± 1.15 mL). None of the animals showed hematological or biochemical changes. Considering the OECD 423/2001 guide, it was possible to estimate the LD50 around 200 mg/kg. **Discussion:** The toxicological preclinical study of a product is an important initial stage, for its safe use, once it aims to characterize the toxic effects produced from its administration. Besides, studies like these aim to seek information about safe dose intervals to laboratory animals (Almeida, R. N., Guanabara Koogan, 357p, 2006). In order to evaluate one sample's toxicity in gastrointestinal system, metabolic parameters, such as drinking water and food, and weight assessment, should be analyzed in preclinical studies. However, an alteration in only one of these parameters is not enough to characterize toxicity in this system. Renal and liver function assessment can be performed by determining the levels of urea and creatinine; AST and ALT, respectively. HSE-07 50 mg/kg treatment did not significantly change theses parameters, compared to control group. Haematological alterations are another important group of parameters used to evaluate the toxicity of drugs in the body. No any alteration, such in size and count of blood cells, was observed in animals treated with the same dose. Thus, it's possible to conclude that HSE-07 shows moderate toxicity. **Financial support**: CNPq

11.023

Behavioral evaluation in mice treated with Artemether encapsulated in nanocapsules. Vidal AT, Souza ACM, Amancio GCS, Mosqueira VCF, Grabe-Guimarães A Cipharma-UFOP

Introduction: The neurotoxicity of the artemether (ATM), an antimalarial drug, is one of the aspects that diminish its therapeutic security. Therefore, the administration of ATM in nanocapsules (NC) could reduce its CNS side effects. The objective of this study was to compare the effect of the treatment with free ATM to the treatment with ATM encapsulated in NC over behavioral changes that indicates neurotoxicity. **Methods:** Female adult Swiss mice (20-25 g) received four doses of 20 mg/kg IV of ATM, either in its free or encapsulated form (ATM-NC), administered in 12-hours intervals. The animals of control group were treated with the corresponding volume empty NC or vehicle. Behavioral alterations related to the locomotor activity, anxiety, balance and muscular strength were evaluated and quantified using the open field, the elevated maze with four arms (two open and two closed), the rotarod and the traction instrument methods, respectively. The animals were subjected to evaluation both before treatment and two hours after the last dose administered. **Results:** In the open field, the number of fields invaded by the mice was similar between all treated groups (control=81.17 ± 6.5; NC=83.17 ± 5.85; free ATM=95.33 ± 6.21; ATM-NC=75.5 ± 8.25, P>0.05). There was not significant differences for grooming (control=1.66 ± 0.33; NC=2.5 ± 0.5; free ATM=2.5 ± 0.67; ATM-NC=3.6 ± 0.8, P>0.05), hearing (control=19.33 ± 3.54; NC=15.33 ± 1.94; free ATM=9.0 ± 2.04; ATM-NC=10.5 ± 1.23, P>0.05), and the number of fecal bolus (control=2.33 ± 0.66; NC=1.5 ± 0.71; free ATM=2.3 ± 0.66; ATM-NC=1.3 ± 0.49, P>0.05) counted during 5 minutes. No significant differences were observed in the exploration of open (control=12.67 ± 0.61; NC=9.66 ± 0.49; free ATM=11.17 ± 1.47; ATM-NC=12.33 ± 1.62, P>0.05) and closed (control=4.5 ± 1.05; NC=0.47 ± 0.71; free ATM=8 ± 0.63; ATM-NC=6.5 ± 1.3, P>0.05) arms by the animals in the maze. In the rotarod test, all the animals were able to remain in the rotating rod during the 2 minutes of experiment. For the muscular strength test, the animals of all groups were able to maintain themselves sustained in the metallic thread and touch it with the posterior paws, suggesting no alterations of the ATM over the muscular tone. **Conclusion:** These results suggest the absence of neurotoxic for both free and encapsulated ATM. However, as these are screening tests, it cannot completely be exclude the neurotocixity for this dose and via, with further histopathological analysis being required. **Ethics committee:** nº 13/2011 **Financial Agencies:** Capes; Capes/COFECUB (768/13); FAPEMIG; CNPq **Acknowledgments:** UFOP

11.024

Comparative bioavailability study between two sublingual formulations of ketorolac (Tablets 10 mg and 30 mg) and an intramuscular formulation of ketorolac (injectable solution of 30 mg/mL) in healthy volunteers of both sexes. Leite WS, Leite ALAS, Nascimento DF, Sales LC, Freire LM, Sena KS, Linhares AL, Gadelha EC, Pontes AV, Rocha MBS, Frota Bezerra FA, Moraes MO, Moraes MEA UFC – Fisiologia e Farmacologia

Introduction: Ketorolac is a nonsteroidal anti-inflammatory derivated of propionic cyclic acid that act inhibiting preferentially cyclooxygenase 1 that converts arachidonic acid into endoperoxides, precursors of prostaglandins, prostacyclin and thromboxane, consequently inhibiting the synthesis of prostaglandins responsible for pain. The aim of the study was to evaluate the bioavailability between two sublingual formulations of Ketorolac (tablets of 10mg and 30mg) versus an intramuscular formulation of Ketorolac (Toradol®) in healthy volunteers of both sexes. **Methods:** 36 healthy volunteers of both sexes aged between 18 and 50 years and BMI between 18.5-29.9 kg/m^2 were selected for the study after assessment of their health status by clinical evaluation and haematological, biochemical and routine urinalysis parameters. All subjects were negative for HIV, HCV and HBV (except for serological scar). All subjects gave written informed consent, and the Ethics Committee of the UFC approved the clinical protocol (Protocol n° 336.914). The study was conducted in accordance to the provisions of the Declaration of Helsinki (1964) and its revisions. The study was conducted in an open randomized, six sequences, three-period crossover balanced design with a 1 week washout period between the doses. During each period, the volunteers received 01 sublingual tablet of 10 mg (Test 1) or 01 sublingual tablet of 30 mg (Test 2) or 01 mL (30 mg) of injectable solution of the reference formulation (Toradol®-intramuscular) under fasting conditions. Plasma was obtained over a 36h interval. Ketorolac concentrations were determined by LC-MS/MS Method: **Results and discussions:** Thirty-six volunteers, 18 male and 18 female, ended the study. The mean age was 27.17 ± 6.64 years and the body mass index (BMI) was 24.64 ± 2.3 kg/m^2. The Ketorolac formulations were well tolerated. Regarding the pharmacokinetic parameters, in the statistical comparison, no difference was found in the AUC_{0-t}, AUC_{0-inf} and C_{max} between Test 1, and reference formulation, and Test 2 and reference formulation. The confidence interval for all parameters required to evaluate the bioequivalence were within the range of 80-125% as established by the National Health Surveillance Agency (ANVISA) and the Food and Drug Administration Agency (FDA). The geometric mean ratios (test:reference) for AUC_{0-t}, AUC_{0-inf} and C_{max} were 85.77% (90% CI, 81.98–89.74), 88.55% (90% CI, 84.72–92.55) and 99.52% (90% CI, 93.83--105.55), respectively for Test 1 versus Reference formulation, and 94.93% (90% CI, 90,74-99.32), 95.01% (90% CI, 90.9-99.31), and 100.91% (90% CI, 95.14-107.02), respectively for Test 2 versus Reference formulation. Thus, it is concluded that the two test formulations (sublingual tablets 10 mg and 30 mg) is bioequivalent in terms of both rate and extent of absorption to reference formulation (Toradol®). **Financial support:** Capes, CNPq, FUNCAP, InCB; MS-RNPC-UNIFAC-HM.

11.025

Pre-clinical monitoring of artemether-nanocapsules cardiotoxicity in rats. Vidal-Diniz AT[1], Grabe-Guimarães A[2], Richard S[3], Guimarães HN[4], Andrade RP[2], Borges RP[2], Mosqueira VCF[1]
[1]UFOP, [2]UFOP – Farmácia, [3]Université Montpellier – Physiologie & Médecine, [4]UFMG – Engenharia Elétrica

Introduction: Malaria, a parasitic disease, remains a major health problem in Brazil and worldwide. Modern strategies for its control include the development of new therapeutic agents of the optimization of the activity of already used drugs. Artemether (ATM) is effective against species and strains of *Plasmodium* and is used in malaria control. However, to improve the therapeutical use and safety of ATM some limiting issues have to be circumvented such as its short plasma half-life; low oral bioavailability; no intravenous (IV) formulation available and the risk of high toxicity particularly to the cardiovascular system. We developed a fast acting and safe formulation of ATM that could be administered IV to treat severe malaria. It has been shown that Nanocapsules (NC) present the potential to reduce cardiovascular toxicity of halofantrine, another antimalarial drug. Thus, the encapsulation of ATM in NC could also be useful to reduce its cardiovascular toxicity and/or to improve efficacy. **Methods:** All animals' procedures were approved by the UFOP Ethics Committee under number 03/2011. NCs of PCL containing ATM were prepared by the polymer deposition followed by solvent displacement method and characterized. The ATM loaded-NC or the free drug in solution were evaluated in anaesthetized Wistar rats (n=6), to determine the effects on ECG and arterial pressure (AP) upon IV injection. Two protocols were used to assess cardiotoxicity: the administration of a single high dose ATM (40 or 80 mg/kg) and the administration of multiple doses (4 doses – 20 mg/kg) simulating the therapeutic schedule used to treat malaria. The efficacy of the NC formulation to treat experimental malaria *in vivo* in *Plasmodium berghei* NK65-infected mice was investigated in two different treatment schedules (n=6). The first one soon after the mice infection and followed by the treatment with four doses once a day (4-day test) and the second one in animals with established infection and treated with one ATM dose (20 mg/kg IV). The Kolmogorov-Smirnov method was used to determine whether continuous variables were normally distributed. Results are expressed as mean of 6 animals. **Results and Discussion:** The obtained NC formulation presented high ATM concentration (4 mg/ml) and its characteristics were adequate to IV administration. The IV administration of free and encapsulated ATM at both doses induced a complete remission of parasitaemia, and no recrudescence was observed up to 60 days. ATM dose of 40 and 80 mg/kg induced the QT interval prolongation of 19.6 and 32.8%, respectively, and it was significantly reduced to 7.9 and 16.4% for ATM in NC. Similarly, after four doses of 20 mg/kg the QT prolongation were 22.7% for free ATM and 5.6% for ATM in NC. Severe hypotension and bradycardia were observed. The systolic arterial pressure (SAP) and diastolic arterial pressure (DAP) reduction were 17.8 and 41.4% for 40 mg/kg and 22.2 and 49.9% for 80 mg/kg after free ATM, respectively. For the ATM in NC these severe variations were not observed. In the four doses protocol, the free ATM induced significantly increases of the heart rate (HR) and AP, with important hypertension and tachycardia. For ATM in NC they were also reduced. These results indicate that ATM encapsulation was the main factor responsible for the significant prevention the cardiac toxicity observed. The reduction of toxic effects could be attributed to the smaller fraction of free drug in association with the cardiac tissue. It can be suggested that the incorporation of ATM in NC is an alternative to maintain the active drug

in the circulation, while provide a sustained release and minimize its toxicity. **Acknowledgments:** Capes-Cofecub (768-13), Fapemig, CNPq, CGPNCM/MS.

11.026

Evaluation of biochemical and hematological parameters of the acute administration of carvacryl acetate in mice. Oliveira RAM[1], Oliveira GLS[1], Saldanha GB[2], Sousa DP[3], Freitas RM[1]
[1]UFPI, [2]UFBA, [3]UFPB

Introduction: Carvacryl acetate (CA) is a semisynthetic monoterpenic ester (derived from the carvacrol) that has pharmacological properties as anxiolytic-like, antioxidant, anti-inflammatory and anti-nociceptive (PIRES *et al.*, 2013). Because of the need for toxicological studies, the objective of the present work was to evaluate the biochemical and hematological parameters in mice treated with CA. **Method:** The CA was prepared in our laboratory as previously described (PIRES *et al.*, 2013). The CA was emulsified in 0.05% Tween 80 dissolved in 0.9% saline (vehicle) and administered in single dose orally (p.o) and intraperitoneally (i.p) (1000, 2000 mg/kg). Were used *Swiss* mice of both sexes weighing between 25-30 g and all groups (5 males and 5 females per group) were observed for a period of 14 days. The controls groups received vehicle with the same volume (10 mL/kg, p.o and i.p). After 14 days of acute treatment, animals were anesthetized with sodium pentobarbital (40 mg/kg, i.p.) and then was made the collect of blood for determination of hematological and biochemical parameters as described previously (Almeida *et al.*, 2012). This study was approved by Ethics Committee on Animal Experimentation of the UFPI (UFPI, #013/2011). Results were expressed as mean ± SEM using variance (ANOVA) followed by t-Student-Newman-Keuls test as post hoc test ($p<0.05$). **Results:** In parameters of glucose, urea, uric acid, alanine aminotransaminase (ALT) and aspartate aminotransferase (AST) no significant differences were observed between the groups treated with CA and control group. However, was observed that the creatinine values (0,45 ± 0,06) were significantly ($p<0.05$) lower in males (2000 mg/kg, p.o) when compared to the control group (0,47 ± 0,02). Lower triglyceride levels ($p<0.05$) were identified in males (p.o=71,50 ± 10,78; i.p=75,00 ± 0,01) and females (p.o=72,3 ± 9,91; i.p=58,00 ± 0,05) treated in the dose of 2000 mg/kg, as well as to females mice (p.o=88,20 ± 30,01; i.p=68,26 ± 6,89) treated in the dose of 1000 mg/kg in relation to control [males (p.o=92,68 ± 6,22; i.p=96,75 ± 4,76) and females (p.o=106,00 ± 5,77; i.p=100,30 ± 8,99)]. For the values of total cholesterol, males mice treated with a dose of 1000 (p.o=75,01 ± 7,07; i.p=79,50 ± 4,17) and 2000 (p.o=65,28 ± 4,09; i.p=61,00 ± 0,04) mg/kg showed lower values ($p<0.05$) in comparison to the control group (p.o=103,60 ± 7,85; i.p=94,23 ± 5,64). The values of erythrocytes, hemoglobin, hematocrit, mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), platelets, leukocytes, neutrophils and lymphocytes showed no changes when compared to the control. **Discussion:** In general, the results of biochemical and hematological tests showed few changes between the groups treated with CA and vehicle for the majority of the parameters evaluated. These results are important as complement of the studies of Oliveira *et al* (2013), in which it was demonstrated a possible pre-clinical safety of CA in doses evaluated in this study. **References:** PIRES *et al.* Pharmacol Biochem Be, v. 112, p. 864, 2010. ALMEIDA *et al.* Brain Res, v. 1448, p. 56, 2012. OLIVEIRA *et al.* Bol informat Geum, v. 4, p. 28, 2013. **Acknowledgements**: Capes

11.027

The relative bioavailability / bioequivalence of two formulations of metformin 850 mg tablets coated in healthy volunteers of both sexes on fed condition. Sales LC, Leite ALAS, Nascimento DF, Freire LM, Sena KS, Leite WS, Linhares AL, Gadelha EC, Pontes AV, Rocha MBS, Frota Bezerra FA, Moraes MO, Moraes MEA UNIFAC-UFC – Fisiologia e Farmacologia – Unidade de Farmacologia Clínica ()

Introduction: Metformin is in a class of drugs called biguanides, that is used alone or with other medications, including insulin, to treat type 2 diabetes. The aim of this study was to evaluate the relative bioavailability/bioequivalence of two formulations of Metformin 850 mg film-coated tablets in healthy volunteers of both sexes under fed conditions. **Methods:** Twenty-eight healthy volunteers of both sexes aged between 18 and 39 years and BMI between 19-28.7 kg/m^2 were selected for the study after assessment of their health status by clinical evaluation (physical examination, ECG) and the following laboratory tests: blood glucose, urea, creatinine, AST, ALT, alkaline phosphatase, total bilirubin, albumin and total protein, triglyceride, total cholesterol, hemoglobin, hematocrit, total and differential white cell counts and routine urinalysis. All subjects were negative for HIV, HCV and HBV. The Ethics Committee of the UFC approved the clinical protocol (Protocol n° 46/12), and all subjects gave their written informed consent. The study was conducted in accordance with the provisions of the Declaration of Helsinki (1964) and its revisions. The study was open, randomized, two treatments, two-period crossover design, during which the volunteers were administered metformin 850mg (Test formulation) or metformin 850mg (Glifage® – MERCK S.A as Reference formulation) with a seven days washout period. Plasma was obtained over a 36h interval. The metformin concentrations were analyzed by combined reversed phase liquid chromatography and tandem mass spectrometry (LC-MS-MS) with positive ion electrospray ionization using selected daughter ion monitoring (MRM). The formulations were considered bioequivalent if the 90% CIs for the log-transformed values were within the predetermined equivalence range of 80%–125% for Maximum concentration (C_{max}) and Area under the curve (AUC), according to the guidelines of the Brazilian Healthy Surveillance Agency (ANVISA). The pharmacokinetics parameters are showed as geometric mean. **Results and discussions:** The method validation investigated the parameters recommended for the bioanalytical methods and got good results with limit of quantification of 5ng/mL. The retention time to Metformin was 2.10 minutes and to acyclovir (internal standard) was 4.01 min. The response was linear in the concentration range of 2-3000 ng/mL (r= 0.9959). The confidence interval for all parameters required to evaluate the bioequivalence were within the range of 80-125% as established by the National Health Surveillance Agency (ANVISA) and the Food and Drug Administration Agency (FDA). The geometric mean ratios (test:reference) for AUC_{0-t}, AUC_{0-inf} and C_{max} were 102.67% (90% CI, 98.45–107.07), 102.47% (90% CI, 98.43-106.68) and 102.29% (90% CI, 98.11-106.64), respectively . Thus, it is concluded that the Metformin test formulation is bioequivalent in terms of both rate and extent of absorption to reference formulation of Metformin (Glifage®), when administered in healthy volunteers. **Financial support:** CNPq, InCB, MS-RNPC-UNIFAC-HM, FINEP.

11.028

Preliminary studies of the LASSBio-1425, a potential anti-atherogenic compound, on the male gamete of rat. Mannarino LA[1], Fumian MM[1], Ribas JAS[1], Maia RC[2], Barreiro EJ[2], Brito FCF[1], Marostica E[1] [1]UFF – Physiology and Pharmacology, [2]UFRJ – LASSBio

Introduction: The pharmacological screening of a new series of phenylpyrazole derivatives pointed out the compound LASSBio-1425. This compound presents the phthalimidic group in its structure and previously we have demonstrated important analgesic and anti-inflammatory activities. Previous studies in rats fed with hypercholesterolemic di*et al*so showed that the treatment with LASSBio-1425 ameliorated the lipid profile and vascular reactivity, inhibited the production of serum inflammatory markers, such as TNF-α and IL-6, reduced the expression of NF-kB and increased eNOS expression in aorta, as well as decreased platelet aggregation induced by collagen (*Fumian, 2014 -Thesis UFF).* The data indicated the compound LASSBio-1425 as a promising prototype candidate for the treatment of atherosclerosis. Therefore, it is important to evaluate its toxicological profile. Thus, the aim of this study has been to evaluate the possible toxicological effects on male gamete of rat chronically treated with LASSBio-1425. **Methods:** (CEPA/UFF 287/12) Male Wistar rats (150-200g) were separated in five groups (n=8/group): CO- fed with commercial rat chow for 45 days; HC-fed with hypercholesterolemic diet for 45 days; CO + 1425- fed with commercial rat chow for 45 days and treated with LASSBio-1425 at the last 15 days; HC + 1425- fed with hypercholesterolemic diet for 45 days and treated with LASSBio-1425 (100 µmol/kg, i.p.) at the last 15 days; HC + SIMVA- fed with hypercholesterolemic diet for 45 days and treated with simvastatin (30 mg/kg i.p.) at the last 15 days. The animals were euthanized by cervical dislocation and decapitation under anesthesia effects. Testes, epididymis, kidneys and livers from different experimental groups were removed, weighed and processed. Gonadosomatic index (GSI) and organs relative weight were calculated and spermatic evaluation (total and progressive motility, vigor, hypo-osmotic swelling test and membrane integrity by using carboxyfluorescein diacetate/propidium iodide) was performed using sperm from epididymis cauda. The values are expressed as mean ± SEM; ANOVA, $P<0.05$. **Results:** The treatment with LASSBio-1425 did not alter the GSI when compared with control (CO: 0.5 ± 0.02; CO + 1425: 0.48 ± 0.01%). However, it was not able to modify the GSI increase caused by hypercholesterolemic diet (HC: 0.7 ± 0.04; HC + 1425: 0.69 ± 0.03%). Spermatic parameters were lower in HC and HC + 1425 groups than CO group. The treatment with LASSBio-1425 only partially recovered the progressive motility and sperm reactivity to host when compared to HC group (CO: 68.0 ± 1.9/58.6 ± 6.2; HC: 31.3 ± 0.3/25.5 ± 3.4; HC + 1425: 45.8 ± 2.7/41.2 ± 1.9 %). These parameters were similar after simvastatin treatment. On the other hand, LASSBio-1425 did not modify the percentage of injured cell increased by hypercholesterolemic diet (CO: 31.1 ± 2.3; HC: 69.0 ± 1.9; HC + 1425: 56.3 ± 2.1; HC + SIMVA: 38.0 ± 1.7 %). In addition, the treatment with LASSBio 1425 did not alter the relative liver weight when compared to its respective control group (CO: 3.18 ± 0.49; CO + 1425: 2.82 ± 0.83; HC: 5.34 ± 0.17; HC + 1425: 4.92 ± 0.39g) and there was no difference in the kidney relative weight among the groups. **Discussion:** Our preliminary results showed that administration of LASSBio-1425 did not cause more deleterious effect on the spermatic evaluation than simvastatin, an established anti-atherogenic drug. However, the new compound was not able to recover the harmful effect of hypercholesterolemia on male gamete. **Supported by:** Capes, CNPq, Faperj.

11.029

Urodynamic effects of the combination of tamsulosin and daily tadalafil in men with lower urinary tract symptoms secondary to benign prostatic hyperplasia: a randomized, placebo-controlled clinical trial. Sousa PRR[1], Sales LC[1], Santos LCO[1], Silva BGB[1], Marinho LB[1], Pamplona TL[1], Santos JMS[1], Segundo SAS[1], Silva Júnior JVM[1], Cerqueira JBG[1], Maia RR[1], Gonzaga-Silva LF[1], Regadas RP[1] [1]UFC – Cirurgia / Fisiologia e Farmacologia

Introduction: Recently, it has been observed an association between Benign Prostatic Hyperplasia (BPH) and Erectile Dysfunction (ED). It was reported that patients with ED treated with inhibitory phophodiesterase type 5 (IPDE5) improves erection and lower urinary tract symptoms (LUTS). The pathophysiology of LUTS is not completely known. However, despite the knowledge that there is improvement in LUTS, it is not known whether IPDE5 works during storage, emptying, or both. It is not yet known if the association IPDE5 with alpha blocker is better than its use alone or whether this association is safe. The aim of this study was to evaluate the safety of the combination of tamsulosin with daily tadalafil as well as its effect on lower urinary tract in human by urodynamic study (UDS). **Methods:** All patients underwent baseline UDS before randomization to tamsulosin 0.4 mg/tadalafil 5 mg (Group 1; n = 20) or tamsulosin 0.4 mg/placebo (Group 2; n = 20). End-of-study UDS were performed on completion of the treatment period. The primary end point was to demonstrate changes in urodynamic variables in the voiding phase, detrusor pressure at maximum flow (PdetQmax), and maximum flow rate (Qmax), from baseline to week four. The clinical study it was performed a randomized clinical trial, double-blind, placebo-controlled study during the period October 2010 to September 2011. All patients had LUTS associated with BPH and were evaluated with International Prostate Symptom Score (I-PSS) and urodynamic study at baseline and 30 days after treatment. The concentrations obtained by UDS were analyzed by combined reversed phase liquid chromatography and tandem mass spectrometry (LC-MS-MS) with positive ion electrospray ionization using selected daughter ion monitoring (MRM). The Ethics in Research Committee of the UFC – Walter Cantídio University Hospital approved the clinical protocol (Protocol n° 030.04.10), and all subjects gave their written informed consent. **Results and discussions:** In the clinical study, the age of patients (P=0.19) and the average volume of the prostate (P=0.28) were similar. The primary outcome measure of this clinical trial, PdetQmax, showed a significant reduction in tamsulosin/tadalafil group (13 ± 17.0%) compared to tamsulosin/placebo (-1.2 ± 14.35%) group (P = 0.03). Qmax increased in both groups, tamsulosin/tadalafil (1.0 ± 2.4%) and tamsulosin/placebo (1.4 ± 2.4%), but the difference was not significant between treatment groups (P = 0.65). The association of tamsulosin with tadalafil was more effective in improving the total score of the I-PSS and voiding sub-score, but comparing the groups there is no difference between the storage sub-score, quality of life, maximum flow and detrusor pressure. There were no major side effects. The combination of tamsulosin and tadalafil daily is safe and better than the isolated use of tamsulosin to treat patients with LUTS associated with BPH. **Financial support:** CNPq.