11 Clinical Pharmacology, Pharmacokinetics, Pharmacogenomics and Toxicology

11.001 Investigation of an association between a CYP1A2*1B polymorphism and response to clozapine. Rodrigues-Silva C[1], Neri HFS[1], Vianello RP[2], de Brito R[1], Ghedini PC[1] [1]UFG – Farmacologia, [2]Embrapa - Biotecnologia

Introduction: CYP1A2 is a relevant enzyme to the clozapine (CLZ) metabolism and polymorphisms in this isoform shown influence in the schizophrenia treatment response, as showed by CYP1A2*1F variant (de Brito et al., Schizophr Res. 169, 502, 2015). The mutation CYP1A2*1B is characterized by substitution of T5347C placed into exon 7 of the coding gene that influences the enzyme activity. Thereby this proposal intends identified if T5347C would be associated with CLZ treatment response in the super-refractory schizophrenia (SRS). **Methods:** Peripheral blood from 103 patients who received CLZ for at least 12 months was utilized to genotyping test. 61 individuals were diagnosed with TRS and 42 with SRS. CYP1A2*1B genotypes were determined by sequencing technique after that DNA genomic from blood having been obtained and submitted at polymerase chain reaction (PCR) with specific oligonucleotide flanking the polymorphism region. Comparative analysis of genotypic and allelic frequencies to TRS and SRS were performed by chi-square and Fisher´s exact test. Clinical data from patient records were compared by T test or ANOVA one-way, as well appropriate non-parametric test to compare TRS and SRS groups. All protocols were approved by Human Research Ethics Committee from Federal University of Goiás (process 1.483.734) and Health State Secretary of Goiás (process 1.537.538). Statistical tests were performed using SPSS software package (version 21.0; SPSS Inc., IL, US) and significance was considered when p-value <0.05. **Results:** No differences were observed to the genotype and allelic frequencies between TRS and SRS (P = 0.42 and 0.56, respectively). Frequencies of TT, TC and CC to TRS were 14.8%, 44.3% and 41.0%, and 11.9%, 59.5% and 28.6% to SRS, respectively. T and C frequencies were 36.9% and 63.1% in TRS; 41.7% and 58.3% in SRS, respectively. Gender, ethnicities, coffee consumption and smoking no showed statistic deviation between TRS and SRS (P = 0.21, 0.78, 0.22, and 0.48, respectively). BPRS scores and CLZ dosage were highest in SRS compared to TRS (P = 0.002 and P = 0.006, respectively). Age mean, time of CLZ usage and body mass index were not different between groups (P = 0.41, 0.10 and 0.66, respectively). **Conclusion:** CYP1A2*1B polymorphism is not associated with SRS. Further studies with larger sample of patients are necessary to consolidate these results. **Financial Support:** CNPq, Capes, Fapeg. **License number of ethics committee**: 1.483.734, 1.537.538 **Financial support**: CNPq, CAPES, Fapeg

11.002 Evaluation of the Pharmacokinetic Profile of the Analgesic Drug Prototype LQFM 020 in Rats by HPLC-PD. Oliveira LP[1], Oliveira Neto JR[1], Rodrigues CR[1], Fajemiroye JO[1], Marques SM[2], Naves LM[2], Pedrino G[2], Menegatti R[1], Cunha LC[1] [1]UFG – Ciências Farmacêuticas, [2]UFG – Ciências Biológicas

Introduction: The compound LQFM 020 contains a pyrazole ring that has anti-inflammatory and analgesic activity and is currently in the preclinical testing phase (Oliveira, 2017). **Objective:** To determine the kinetic parameters of LQFM 020 compound given orally at a dose of 35 mg/kg in rats using HPLC-PDA-validated bioanalytical methodology. **Methods:** Cannulas were introduced into the femoral artery of 3 Wistar rats (± 300 g). The solution of LQFM 020 in 5% DMSO at the dose of 35 mg/kg (v.o.) for each animal was administered via the cannulated artery. Venous blood samples (500 µL) were collected over 24h. The analyte was extracted from the plasma with ethyl acetate and quantified by a bioanalytical method validated by HPLC-PDA based on RDC 27/2012 ANVISA. Chromatographic conditions: mobile phase used was 0.2% formic acid: acetonitrile (70: 30, v/v), 1mL/min, ACE C18 column (150 x 4.6 mm, 5 µm), injection volume 50 µL, UV detection at 262 nm, internal standard piroxicam 20 µg/mL. The quality controls were 0.25 µg/mL (LLOQ), 0.75 µg/mL (LQC), 7.5 µg/mL (MQC), 15.0 µg/mL (HQC), and 30.0 µg/mL (DQC). Pharmacokinetic parameters were calculated from the concentration versus time, the curve was plotted using the PKSolver® extension. **Results:** The validated method showed to be selective, linear, accurate and exact in the tested concentration range (0.25 - 30 µg/mL). LQFM 020 after oral treatment presented a elimination half-life ($t_{1/2}$) of 6.48 h, total clearance (CL_T) of 1.66 (mg)/(µg /ml)/h and volume of distribution (Vd) of 1.47 (mg)/(µg/ml). C_{max} was 13.35 mg/mL and T_{max} was 5 h. **Discussion:** The evaluation of pharmacokinetic parameters showed that LQFM 020, besides having high tissue distribution, is largely eliminated. **Conclusion:** The LQFM 020 compound has high tissue distribution and rapid elimination. **Keywords:** LQFM 020; Pharmacokinetics; Bioanalytical validation. **References:** BRAZIL. Guide for validation of analytical and bioanalytical methods. Resolution RDC 27 of May 17, 2012. OLIVEIRA, LP et al. New pyrazole derivative 5- [1- (4-fluorophenyl) -1H-pyrazol-4-yl] -2H-tetrazole: synthesis and assessment of some biological activities. Chemical Biology & Drug Design, v.89, p.124, 2017. **License number of ethics committee**: 085/16 **Financial support**: Capes/Fapeg

11.003 Peanut leaves by-product extract: **Phytochemical characterization**, **cytotoxic evaluation**, **anti-inflammatory and antioxidant effect**. Cossetin JF[1], Dornelles RC[1], Maziero M[2], Casoti R[3], Brum ES[4], Antoniazzi CTD[1], Ramos AP[5], Pintos FG[5], Manfron MP[2], Marchesan SO[4], Sagrillo MR[5], Machado AK[5], Santos ARS[6], Trevisan G[1] [1]UFSM – Farmacologia, [2]UFSM – Ciências Farmacêuticas, [3] FCFRP-USP – Pharmaceutical Sciences, [4]UFSM – Bioquímica, [5]UFN – Nanoscience, [6]UFSC – Neuroscience

Introduction: *Arachis hypogaea* L. (Peanut) has remarkable economic importance, being one of the main crops cultivated and consumed in the world[1,2,3,4]. Peanut residues such as meals, grains, peels or vines could be exploited as a source of bioactive compounds for the production of nutritional or medicinal products2. Therefore, the objective of the present study was to describe the phytochemical composition of peanut extract (PLE) and evaluate its cytotoxic and antioxidant properties. **Methods:** The chemical analysis of the extract was performed by UHPLC analysis. The evaluation of the antioxidant capacity of PLE was by the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, or by measuring the PLE elimination of hydrogen peroxide (H2O2) and nitric oxide (NO). PLE cytotoxicity was evaluated in human peripheral blood mononuclear cells (PBMCs, CAAE: 31211214.4.0000.5306). For these assays, the MTT tests, free DNA quantification and determination of reactive species and nitric oxide production were performed. The same measurements were performed in a second reversal protocol pretreated with hydrogen peroxide. **Results:** The PLE dehydration by UHPLC allowed the identification of eight types of metabolites: organic acid, hydroxycinnamic acid derivative (HCAD), chromone, stilbenoid, flavonoid, isoflanoid, catechin and saponin. PLE exhibited antioxidant potential in the DPPH test [IC50 of 24.46 (16.14-37.07) µg / mL] or NO [IC 50 of 16.67 (11.17 - 25.170) µg / mL]. The PLE extract was not able to eliminate the oxidizing compound H2O2. In addition, PLE was non-cytotoxic at concentrations of 1, 10 and 100 µg / mL when incubated in PBMCs. In addition, this extract at concentrations of 10 and 100 µg / mL restored cell viability, free dsDNA levels in the extracellular medium, nitric oxide levels and the total rate of reactive substances. **Conclusion:** Our results revealed the promising potential of PLE as a source of antioxidant and anti-inflammatory products. **References:** [1]Lopes et al. 2011.J Agric Food Chem. 2011 May 11;59(9): 4321-30. [2] Zhao et al.2012 BMC Res Notes. 2012. 3 Foyer et al. 2016. Nat Plants. 2016 Aug 2;2: 1611. [4] Vinson et al. 2018. PLoS One. 2018 May 30;13(5): e0198191. **License number of ethics committee**: CAAE: 31211214.4.0000.5306 **Financial support**: capes

11.004 Exposure to Benzo(a)pyrene from juvenile to peri-pubertal periods cause negative impacts on reproduction in male rats. Reis ACC, Jorge BC, Sterde ET, Balin PS, Arena AC IBB-Unesp – Morfologia

Introduction: The Benzo(a)pyrene (BaP) is a potential endocrine disruptor formed by the incomplete combustion of complex organic compounds and is present cigarette smoking. Studies have revealed that the BaP can interfere in steroidogenesis process through its interaction with the StAR protein in leydig cells. This study evaluated the effects of exposure to Benzo(a)pyrene from juvenile until peri-pubertal periods on reproductive parameters of male rats in adult life. **Methods:** Juvenile male rats (23 post-natal days) were distributed in four experimental groups: a control group (corn oil + DMSO); and three groups treated with BaP: 0.1; 1 or 10 µg/kg/day, during 31 consecutive days (by gavage). During the treatment, clinical signs of toxicity and the age of the preputial separation were evaluated, while in adult life, male sexual behavior, fertility test with no-treated females, morphology, motility and sperm count in testis and epididymis were analyzed. **Results:** During the treatment period none animal presented alterations. There was an increase in the latency for the first ejaculation in the group treated with 0.1 µg/kg. This same group presented a biological decrease in the fertility potential and an increase in the pre-implantation loss. The sex ratio was altered in the all treated groups, as well as the weight distribution of the newborns, which showed an increase in the number of fetuses large for gestational age and decrease in the number of fetuses adequate for gestational age. Moreover, there were a decrease in the thyroid and seminal gland relative weights, in the morphology, motility and sperm count in the testis in all BaP-treated animals. **Conclusion:** Our results indicate that BaP acts as an endocrine disrupter, interfering with the reproductive system of the males exposed during the pre-pubertal period. Ethics committee: nº958/2017. **Financial support**: FAPESP/CAPES. **License number of ethics committee**: 958/2017 Instituto de Biociências de Botucatu **Financial support**: FAPESP/CAPES

11.005 Praziquantel tablet for pediatric: **A new model to testing palatability**. Garcia TA[1], de Oliveira KC[1], Teixeira RGS[1], Drummond D[2], Boniatti J[2], Guimarães TF[2], Dantas FML[3], Fonseca LB[4], Viçosa AL[2], Calil-Elias S[1] [1]UFF – Farmacologia, [2]Fiocruz – Farmanguinhos, [3]Fiocruz – Tecnologia, [4]Fiocruz – Equivalência e Farmacocinética

Introduction: Praziquantel (PZQ) has been the drug of choice for schistosomiasis control, but at the moment, it is widely used off-label to treat preschool-aged children. This treatment has been adapted from the commercial adult formulation and is not adequate for pediatric use. Taste plays an important role in the development of oral pediatric pharmaceutical formulations in relation to patient acceptability and adherence. Human panel is an important method for taste assessment and is an ethical issue challenge, especially when it involves children. Another method is the electronic tongue, but previous works mention that it was not applicable for PZQ analysis due to the non-ionic characteristic and the low solubility of the drug in water. Rat models designed for the evaluation of the palatability showed good correlation to human panels and quite promising for taste assessment of drugs like PZQ. The aim of the present work was the taste assessment using rat palatability model of commercial praziquantel tablet for pediatric off-label use. **Methods:** Sixteen female Wistar rats were used. The rats were maintained on a 12 h/12 h light/dark cycle, received a standard chow and water ad libitum except during the training and testing water restriction conditions as mentioned below. On the first day rats were deprived of water for a period of 22 h to motivate the licking behavior. On the second day water was offered for 30 minutes, and then the consumption was measured. Then water was offered to all animals on demand for 90 minutes. After this period rats were deprived again of water for 22 h. This procedure was repeated one more day, for animals training. On the fourth day, the animals were separated in 4 groups (n=4/group) and different solutions/dispersions kept in recirculation were offered for each group and the consumption measured after 30 minutes: Sweet and Bitter test solutions, PZQ (API) dispersion and water dispersion of the fourth part of the commercial PZQ 600 mg tablet equivalent to the dose of 150 mg of pure PZQ. **Results:** The mean total volume consumed at the end of the 30 min experiment was approximately 12, 14, 8, 9 and 5 mL for water, sweet and bitter solution, API dispersion and tablet dispersion, respectively. Neutral rats' taste perception was observed for water. Rats liked the taste of sweet test solution. Taste aversion behavior was observed such as animal retreating indicating that the rats did not like the taste of PZQ commercial tablets. An unexpected result was obtained for the API because of the poor water solubility of PZQ which did not provide a homogeneous dispersion. **Conclusions:** The rat palatability model used in this work may be a valuable tool for the taste assessment of pediatric pharmaceutical formulations based on bitter-tasting APIs. **License number of ethics committee**: 4119060418 **Financial support**: PAPES VII/CNPq/Fiocruz

11.006 The impact of active tubular transport and maturation processes on renal clearance in preterm neonates. Benzi JRL[1], Cristea S[2], Allegaert K[3], Krekels EHJ[2], Knibbe CAJ[21]FCFRP-USP – Análises Clínicas e Toxicológicas, [2]Leiden University - Biomedicine & Pharmacology, [3]University of Leuven; Sophia Children's Hospital - Development and Regeneration, Leuven, Belgium; Intensive Care and Pediatric Surgery, Erasmus University Medical Center, Rotterdam.

Introduction: In the pediatric population, processes underlying renal excretion of drugs undergo maturation, which results in high variability in renal clearance (CL_r) between individuals of different ages. The processes involved in renal elimination (glomerular filtration rate (GFR), active tubular secretion (ATS) and reabsorption) are anatomically and functionally immature at birth and these processes have different maturation rates. GFR (determined by inulin clearance) was reported to reach adult values (normalized to bodyweight) within the first six months of life (1). However, little is known about the maturation of active transporters and how this impacts drug excretion. Thus, the objective of the present study is to investigate the impact of ATS on CL_r in typical preterm individuals using a physiology-based pharmacokinetic (PBPK) kidney sub-model. **Methods:** CL_r simulations were performed in R using a published population model to describe GFR (2) integrated with ATS into a PBPK kidney sub-model (3). The ATS was calculated based on hypothetical intrinsic clearance (CL_{int}) values, reflecting a realistic range of transporters activity. Body surface area and cardiac output were used as input to calculate renal blood flow (4), which plays an important role in predicting CL_r. In the CL_r simulations, reabsorption was assumed to be negligible. Demographic characteristics for typical preterm neonates needed to calculate GFR, were taken from the WHO-UK growth charts and included: birth body weight (bBW) (595 - 2850 g), gestational age (23 - 36 weeks) and post-natal age (1 - 7 days) (5). For bBW, we used the average value between boys and girls. Maturation of ATS was included as hypothetical maturation percentages (MP). An MP of 100% represents a fully developed ATS, with CL_{int} being at 100% of adult values. MP values of 10, 50 and 100% of the ATS were tested for all investigated ages. Hypothetical drugs with different unbound fractions (Fu = 0.1, 0.5, 0.9) and CL_{int} values (CL_{int} = 2, 50, 100 µL/min) in adults were used for the CL_r simulations. **Results:** ATS has the lowest contribution (15 to 20% of CL_r) when CL_{int} is 2 µL/min and MP of 10%. The ATS contribution to CL_r increases with increasing MP and CL_{int}. When the MP is 100%, the ATS contribution reaches 75% of CL_r when CL_{int} is 2 µL/min and exceeds 90% when CL_{int} is 100 µL/min. The latter represents the highest contribution of ATS to CL_r. **Conclusion:** In pediatric patients, ATS becomes clinically relevant for renal excretion for drugs with medium to high CL_{int} or when a particular transporter is at least 50% mature since birth. These results are useful to identify scenarios for which ATS is clinically relevant or not, for the renal elimination of drugs. **References:** (1) ALCORN, Clin. Pharmacokinet, 41, 959, 2002; (2) De COCK, Pharm. Res., 31, 754, 2014; (3) ROWLAND-YEO, Expert Rev. Clin. Pharmacol, 4, 262, 2011; (4) JOHNSON, Clin. Pharmacokinet, 45, 931, 2006; (5) - WHO-UK - Growth chart - https: //www.rcpch.ac.uk/resources **Financial support**: FAPESP Nº 2017/12452-4

11.007 Reproductive impairment in the offspring mediated by paternal exposure to benzo(a)pyrene during pre-puberty in rats. Jorge BC, Reis ACC, Balin PS, Sterde ET, Barbosa MG, Inocencio LCL, Arena AC IBB-Unesp – Morfologia

Introduction: Developmental programming is defined how any injury that can alter normal trajectory of organism during a critical developmental window, such as the juvenile period and the puberty. Endocrine disrupting chemicals (EDCs) can change reproductive parameters and produce negative impacts not only in the exposed individual but also in the offspring and subsequent generations. The potential EDC evaluated in this study is the Benzo(a)pyrene (BaP), a substance present in cigarette smoke and burning of organic compounds, classified as persistent organic pollutants. Our objective was to evaluate the reproductive impacts mediated by the paternal exposure to BaP during pre-puberty in both female (experiment 1) and male (experiment 2) offspring. **Methods:** Male rats (23 days) were treated with BaP (0, 0.1, 1, or 10 µg/kg) for 31 consecutive-days, by gavage. In adulthood, these males were mated with no-treated females to obtain the offspring for this study. All fetuses were evaluated in the following parameters: classification according to their birth weight and relative anogenital distance (AGD) in post-natal day (PND) 1, 13 and 22. In the experiment 1, the puberty installation (vaginal opening and first estrous), estrous cycle, fertility test and ovary histology were analysed, while in the experiment 2, we evaluated the puberty installation (testicular descent and preputial separation), morphology and sperm count. **Results:** There was an increase in fetuses small for pregnancy age in all treated groups when compared to control. Males treated with the lowest and intermediate doses presented a decreased AGD in all measurements (PND 1, 13 and 22). In the experiment 1, the vaginal opening and the first estrous were anticipated in the group 0.1 µg/kg, and in this same group, the number of estrous and several fertility parameters were altered, such as placental efficiency, number of implantations and number of live fetuses. The ovary's histology was affected by the treatment in all groups, with increased atretic follicles and decreased corpora lutea. In the experiment 2, the age of the testicular descent was altered in all treated groups and the preputial separation in group treated with the lowest dose presented delay when compared with control group. The percentage of normal sperm and the number of spermatids in the testis were also reduced in male offspring. **Conclusion:** Paternal exposure to BaP causes several negative impacts on the reproductive parameters in both female and male offspring, suggesting epigenetic alterations provoked by this substance. Approved by ethics committee, nº 958/2017. **Financial support**: CAPES. **License number of ethics committee**: 958/2017 - Instituto de Biociências de Botucatu **Financial support**: CAPES

11.008 Development of ultrasensitive method to analysis of neurotransmitter by liquid Chromatography-Tandem Mass Spectrometry. Cardoso MS, da Costa JL Unicamp – Farmacologia

Introduction: Neurotransmitters (NT) are biochemical messengers responsible for information transference between neurons located in the central and peripheral nervous system and biological fluids of mammals. Disturbances in the production of NT are associated to neurological disorders and diseases caused by genetic of external factors. Therefore, there is an increased interest on the research of NT concentration in biological matrixes, and also its precursors and metabolites, as biomarkers to diagnostic, prognostic, therapy and investigation of the development and progression of pathological disorders. The aim of this work is to develop an ultrasensitive method based on liquid chromatography-tandem mass spectrometry (LC-MS/MS) and derivatization with benzoyl chloride (BzCl) for analyze of epinephrine (E), norepinephrine (NE), serotonin (5HT), dopamine (DA) and histamine (HIST). Method: For method optimization, 200mL of standard solution of NT (50ng/mL in methanol) were derivatized with 100mL of alkaline solution (100mmol/L, composition described below) and 100mL of BzCl (2% v/v, in acetonitrile). The mixture was vortexed for 10s, then 1mL injected onto LC-MS/MS system (Shimadzu LCMS-8060™) with electrospray ionization (ESI+) and multiple reaction monitoring (MRM) scan mode. The NT were separated using C18 column (Titan™1.9µm x 100mm x 2.1mm, Supelco). The mobile phase was composed of formic acid (0.1%, v/v) and ammonium formate (2mmol/L) in water (A) and methanol (B). The elution gradient was set as follow: 10% of B for 0.5min, followed by a linear increaseof %B until 95% in 4.0min, keep concentration for 2min, and returning to initial condition in 0.2 min (re-equilibration time of 2min). **Results:** To the derivatization reaction optimization, four adjuvant solutions were evaluated: sodium tetraborate (TS), sodium carbonate (CS), ammonium hydroxide (HA) and ammonium acetate (AM). Using HA and AM, a considerable decrease analytical signal (chromatographic peak areas) was observed (50% less than obtained with TS). There was a significant improvement of sensitivity to some NT: 281%, 260% and 227% for NE, E and DA, respectively, using CS instead of TS, which was selected to the continuity of the research. Different temperatures conditions and heating forms of derivatization (microwave at 100W/5min and heating plate at 40°C/10min) were evaluated, however it wasn't observed significant difference between the results of these conditions. Finally, ESI parameters were optimized (interface voltage, interface temperature, DL temperature, drying gas and nebulizing gas flow). The ESI optimization increased signal by 85%, when compared with the instrument standard condition. Our optimized method was able to detect the NT in concentrations as low as 0.1ng/mL (around 0.5nmol/L), using minimal sample volume. **Conclusion:** A high-sensitive method was developed for the detection of NT by LC-MS/MS. The method will be now applied to quantitative analyses in studies of CNS development e neurotoxicology, using zebrafish (Danio rerio) model exposed to drugs of abuse and other toxicants. To achieve the maximum sensibility, different analytical conditions will be evaluated. **Financial support** and acknowledgment: FAPESP, CAPES. **Financial support**: FAPESP; CAPES.

11.009 MEFAS: **A promissory antimalaric with low toxicity effects**. Silva GR[1], Lima DA[1], Ferreira FA[1], Gomes C[2], Dalsenter PR[2], Boechat N[3], Lourenço ELB[1], Lívero FAR[1] [1]UNIPAR – Preclinical Research of Natural Products, [2]UFPR – Reproductive Toxicology, [3]Farmanguinhos-Fiocruz-RJ – Organic Synthesis

Introduction: Malaria is a disease responsible for approximately 445 000 deaths worldwide annually and it is characterized as a serious public health problem, especially in developing countries. Due to the high prevalence and mortality of the disease, the search for new therapeutic agents against the protozoan *Plasmodium* is required. One of these agents is MEFAS, a hybrid of low cost developed from the association of artesunate and mefloquine, antimalarial agents. However, there is no information about its safety, a necessary step for a product to be used by the population, according to National Agency of Sanitary Surveillance (ANVISA) legislation. Thus, this research investigated the acute toxicity of MEFAS and the association of artesunate and mefloquine. **Methodology:** The institutional committee for the animal care approved all the procedures (number 27370/2015). The investigation of the acute toxicity of MEFAS was conducted according to the ANVISA guidelines. For this, 42 female Swiss mice of 3 months of age were maintained in control conditions (20 ± 2ºC, 12 hours light/dark cycle) with *ad libitum* access to water and food. The animals were divided into 7 experimental groups (n = 6) and treated orally by gavage, with vehicle (distilled water, negative control), MEFAS (50, 500 and 1000 mg/kg) and artesunate with mefloquine (50, 500 and 1000 mg/kg). After treatment, the animals were observed for the presence of ataxia, ptosis, tachypnea, piloerection and diminished touch response at times 15, 30, 60, 120, 180, 240, 480 minutes and once daily until the 14th day. On the 15th day the animals were weighed and submitted to euthanasia in a chamber saturated with isoflurane (1-3%) followed by cervical dislocation, with subsequent collection of liver, spleen and kidneys samples for determination of relative weight and histopathological analysis. Statistical analyses were performed using one-way analysis of variance (ANOVA) followed by Bonferroni's test and values of $p < 0.05$ were considered statistically significant. **Results:** The association of artesunate with mefloquine produced toxic effects in the animals when compared to the negative control group, reflected by clinical signs alterations and increased relative weight of the kidneys and liver. Hepatic histopathologic lesions (multifocal necrosis of hepatocytes, mixed inflammatory infiltrate with predominance of lymphocytes, perivascular inflammatory infiltrate and multifocal periportal and cholangiohepatitis) and renal (focal tubular degeneration) were also observed. Regarding MEFAS, no clinical changes were observed in organ weight or histopathology in the mice when compared with the negative control group. **Conclusion:** The administration of MEFAS was safer than the artesunate and mefloquine association in mice and may be a potential candidate for the treatment of malaria, due its low toxicity and cost. **License number of ethics committee**: 27370/2015 **Financial support**: CNPq and DEGPP-UNIPAR.

11.010 Occurrence of Ibuprofen in the waters of the Bengal River in Nova Friburgo. Fujimaki CM[1], Bernardo R[2] [1]UFRJ – Ciências Farmacêuticas, [2]UFRJ – Biofísica

The increasing use of pharmaceuticals throughout the world is generating a new environmental problem where they are found in low concentrations, but the long-term risks to various organisms, as well as human health, are not yet known. These drugs, as well as their metabolites, are introduced into aquatic environments through excretions or discharges and may cause the same exposure as POPs (Persistent Organic Pollutants) due to its continued entry into the environment. The aim of this work is to develop a sensitive analytical method for the detection of ibuprofen and its metabolites in the Bengalas river that crosses the city of Nova Friburgo in the state of Rio de Janeiro. Three collection points were analyzed monthly in the river from its source, as well as points of treated water throughout the city. The samples were collected monthly and lyophilized. Subsequently, they were subjected to a solid phase extraction (silica) with the solvent dichloromethane: hexane (1: 1; v/v). The samples were evaporated over a nitrogen atmosphere and subjected to a LC-MS (Liquid Chromatography with Mass Spectrometry) with 5% acetic acid gradient: acetonitrile as the mobile phase. In the search for drugs, there were found ions and fragments of ibuprofen (m/z 206, 205, 177) that were sought and compared to with their standard. These indicators can result in deleterious effects on aquatic life in these bodies of water, as well as on those who use this water from the Bengalas river.

11.011

Effect of chronic ethanol consumption on cyclophosphamide-induced bladder toxicity

Vale GT[1], Sousa AH[2], Gonzaga NA[1], Oliveira MG[3], Justo AFO[3], Antunes E[3], Tirapelli CR[1] - [1]Escola de Enfermagem de Ribeirão Preto - EERP-USP - Farmacologia, [2]Escola de Enfermagem de Ribeirão Preto - EERP-USP - Acadêmico, [3]Faculdade de Ciências Médicas-Unicamp - FCM-Unicamp - Farmacologia

Introduction: Chronic ethanol consumption increases reactive oxygen species (ROS) generation in distinctive organs. Cyclophosphamide (CYP) may also increase ROS generation in several organs, including the bladder. The present study aimed to evaluate whether ethanol consumption would aggravate the bladder damage caused by CYP. **Methods:** Male C57BL/6J mice were divided into four groups: 1) Control (C): mice received water ad libtum; 2) Ethanol (E): mice were treated with ethanol 20% (vol./vol.) for 10 weeks; 3) CYP (CC): water ad libtum and intraperitoneal injection of cyclophosphamide (300 mg/kg) 24h before euthanasia; 4) Ethanol + CYP (EC): ethanol 20% and intraperitoneal injection of CYP (300 mg/kg) 24h before euthanasia. The study was approved by the local ethics committee (#2017.5.93.22.5). Results were analyzed using Two-way ANOVA followed by Bonferroni's test ($p<0.05$). **Results:** CYP induced bladder edema, increased bladder basal pressure and micturition spots and reduced micturition volume. Chronic ethanol consumption increased bladder capacity while CYP treatment reduced it. CYP induced reduction of bladder compliance and increased bladder micturition pressure and frequency of non-voiding and voiding bladder contractions. No difference was found in threshold bladder pressure. Both chronic ethanol consumption and CYP treatment induced increase of bladder superoxide anion generation, but only CYP treatment reduced peroxide hydrogen, tiobarbituric acid reactive species and nitrite/nitrate bladder concentrations. Ethanol consumption and CYP treatment induced increase of Nox2 protein expression in bladder, but only CYP treatment increased Nox4 and iNOS protein expression in bladder. No difference was found in Nox1, NoxO1, p47phox and eNOS protein expression in bladder. Chronic ethanol consumption induced increase of SOD activity, but ethanol associated with CYP treatment reduced bladder SOD activity. No difference was found in SOD1, SOD2, and SOD3 protein expression in bladder. Chronic ethanol consumption and CYP treatment induced reduction of bladder GSH concentration, but only CYP treatment increased bladder catalase activity. Ethanol consumption and CYP treatment reduced carbachol-induced bladder contraction, but no difference was found in KCl-induced bladder contraction and isoprenaline-induced bladder relaxation. **Conclusions:** Chronic ethanol consumption induced bladder oxidative stress but did not aggravate the toxic effect of CYP in the bladder. Finnancial support: CNPq. **License number of ethics committee**: 2017.5.93.22.5 **Financial support**: CNPq