Section A
Morphology and Physiology
General Pathology
Biomaterials and Biocompatibility;
Biology of Development
Regeneration and Differentiation
Regenerative Medicine

A01 ALTERED PLACENTAL DEVELOPMENT TRIGGERED BY SUBCUTANEOUS INJECTION OF AN ORALLY TOLERATED PROTEIN IN MICE.
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Introduction: Oral tolerance is an immunological phenomena observed after ingestion of proteins, as in normal feeding. Parenteral (s.c., i.p.) injection of orally-tolerated antigens inhibits the triggering of immunological responses to the ingested protein and, unexpectedly, also responses to unrelated proteins, footpad inflammation by carrageenan injection (Immunoology 2008) and improve wound healing after incisional dorsal skin lesions (Wound Repair Regen, 2011). Eutherian embryonic development requires the proper development of placenta enabling maternal-fetal transference of nutrients and respiratory gases. The requirement of inflammatory cells, especially uterine NK cells, for proper placental development is currently under investigation. Objective: To investigate if the s.c. injection of orally-tolerated proteins inhibits inflammatory cell infiltration into decidua and alters placenta development in mice. Methods: BALB/c female mice in estrous were caged with male overnight and then examined for copulatory vaginal plug (1st day of pregnancy - dop). Experimental mice received a s.c. injection of 10 µg orally tolerated protein in Al(OH)3 adjuvant in the 3th dop; control mice received s.c. injection of saline. On the 11th dop, pregnant mice were sacrificed and ovaries and uteri were collected for macroscopic and microscopic analysis. Corpora lutea were counted. The embryo implantation sites (E.I.S) were photographed, counted, fixed by immersion in 4% paraformaldehyde and routinely processed for inclusion in paraffin. Results: Macroscopic evaluation showed that reabsorptions and also the percentage of females with altered E.I.S. were higher in experimental BALB/c mice. Histological analysis of these animals showed alterations in placental structure with reduction in the number of fetal vessels. Conclusion: Systemic effects triggered by parenteral injection of tolerated proteins disturb placental development in mice. Keywords: Oral tolerance, Placenta, uNK.

A02 EMPLOYMENT OF A COLLAGEN CONDUIT SOAKED IN AN ANGIogenic FRACTION DERIVED NATURAL LATEX IN THE REGENERATION OF SCIATIC NERVE OF RATS.
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Introduction: peripheral nerve injuries are very frequent in medical practice and although the use of autografts remains the standard procedure to repair the gap between the proximal and distal stumps, alternative techniques have been proposed to avoid complications to the donor site and speed up the nerve regeneration process. A membrane produced natural latex has been used successfully both experimentally (angioplasties, esophagus neoformation, reconstruction of the ocular conjunctiva) and clinically (myringoplasties, treatment of skin ulcers), showing angiogenic potential and leading to tissue neoformation. Objectives: study is to evaluate the capacity of a conduit made with collagen and soaked in an angiogenic protein extracted latex in accelerating and improving the regeneration after surgically sectioning the rat sciatic nerve. Methodology: adult Wistar male rats had the sciatic nerve sectioned under anesthesia with a subtraction of a 10mm nerve fragment. Then they received an autograft implant (inverted nerve fragment) or the interposition into the nerve gap of a tube made up of that collagen and soaked in an angiogenic fraction derived natural latex (P-1). At the endpoint of the experiments, the animals were submitted to neurological function evaluation, and killed by an overdose of anesthesia and exsanguination. The implants (collagen conduit or autograft) and the tibialis and gastrocnemius muscles were removed, fixed and processed with embedding in resin. Cross-section of implants and muscles were performed and prepared in histological slides to observation under light microscopy. Results: functio-
nal recovery was correlated with histopathological analysis. Both showed a significant better performance in rats that received implants with the collagen conduit soaked in angiogenic fraction derived natural latex. **Conclusion:** An absorbable conduit engineered and soaked with P-1 can be considered as a potential conduit for the regeneration of injured peripheral nerves in cases of traumatic damages.

**Keywords:** biomaterials, angiogenic fraction natural latex, nerve regeneration, Hevea brasiliensis. Supporting funding: CNPq

**A03 PARENTERAL INJECTION OF ALPHA-MELANOCYTE STIMULATING HORMONE IMPROVES CUTANEOUS WOUND HEALING.**

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**Introduction:** Skin wound healing is a complex process involving many types of cells and molecules and often Results in scar tissue formation in adults. However, scarless healing occurs in fetal skin and minimal scars may occur after cutaneous healing in the adult with reduced inflammation. Alpha-melanocyte-stimulating hormone (α-MSH) has strong anti-inflammatory activity. We asked whether parenteral (i.p) injection of α-MSH improves skin wound healing in adult mice. **Methods:** C57BL/6 young-adult mice (n=6) received an i.p. injection of 1 mg/kg of α-MSH and, 30 minutes later, two circular through-and-through holes (6.5 mm diameter) were made in their dorsal skin under anesthesia. Control mice were wounded and injected with saline. Mice were sacrificed 3 days after lesion for leukocyte and mast cell counts and 40 days after the lesion to measure the scar area and analyze the pattern of collagen deposition. Skin samples were fixed in formalin, embedded in paraffin, sectioned at 5 μm, stained with HE or toluidine blue for cell analysis, or Gomori’s trichome for extracellular matrix (ECM) analysis. Other samples were fixed in DMSO+methanol, embedded in paraplast and incubated with anti-collagen-I and anti-collagen-III for immunofluorescence analysis. **Results:** Alpha-MSH significantly reduced the number of leukocytes (α-MSH 2.05 ± 0.06 vs control 4.64 ± 0.34; means±SEM) and mast cells (α-MSH 5.5 ± 0.27 vs control 9.5 ± 0.95) in the lesion 3 days after injury. On day 40, α-MSH reduced scar area and improved the organization of the collagen fibers suggesting that it may direct the healing into a more-regenerative/less-scarring pathway. In addition, alpha-MSH increased collagen III deposition (α-MSH 0.1 ± 0.78 vs control 0.51 ± 0.06). **Conclusion:** Alpha-MSH decreased inflammatory cells and scar area, increased collagen III deposition and modified the pattern of collagen fibers to patterns structurally analogous to the ECM found in normal/un-injured dermis. **Keywords:** alpha-melanocyte-stimulating hormone, wound repair, ECM and fibrosis. Supporting funding: Fundação de Amparo à Pesquisa de Minas Gerais and Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil.

**A04 AGNOR COUNT IN ERLICH CARCINOMA CELLS AFTER TREATMENT WITH ORGANIC EXTRACT OF PSEUDOCIPHIHELARIA AURATRA.**

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**Introduction:** Pseudociphihelaria auratra is foliaceous lichen commonly found in hot and humid forests and occurs in the southern and southeastern part of Brazil. Several studies have focused in its phenolic composition, due to its anti-inflammatory, antimicrobial and antitumor effects. Nuclear organizer regions (NORs) are visible by silver staining (AgNORs) and it has been reported that the mean AgNOR number per nucleus (AgNOR count) is associated with tumor aggressiveness and the prognosis. **Objective:** To evaluate the effect in vivo of the treatment with Pseudociphihelaria auratra extract in the AgNOR count in murine Erlich carcinoma cells. **Methodology:** Erlich carcinoma cells (5.0 x 10^6 cells ml-1) were inoculated in the right dorsal region of 40 Swiss male mice. The treatment with Pseudociphihelaria auratra extract (200mg/kg) was initiated 24 hours after inoculation for 7 consecutive days (TG, n=20 animals). The control group were treated with 0.9% NaCl solution (CG, n=20 animals). Tumor biopsy specimens of euthanized mice were processed for histology. The histological specimens were stained with AgNOR technique, analyzed by light microscopy and photographed. AgNOR nuclear dots were quantified in 200 tumor cell per animal by using the software Imagej. For statistical analysis, the Student-s T test was performed. **Results:** AgNOR count was increased in tumor cells of treated animals (TG 4.99 x CG 4.03 ; p<0.001). **Conclusion:** The use of organic Pseudociphihelaria auratra extract modifies AgNOR count in murine Erlich carcinoma cells. **Keywords:** Mice, AgNOR, Lichen. Supporting funding: Federal University of Pernambuco, Academic Center of Vitoria, UFPE/CAV Brazil.

**A05 CRUDE EXTRACT AND FRACTION F1 OF Fusarium oxysporum ACT ON THE SKIN OF HEALTHY RATS CAUSING MORPHOLOGICAL DAMAGE.**

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Introduction: Fusarium oxysporum is a filamentous fungus that produces mycotoxins causing fusarioses in plants, animals and humans. The skin is one of the main gateways to fusarioses in humans.

Objective: The aim of this study was to evaluate in the skin of immunocompetent rats, the action of the crude extract and fraction F1 produced by F. oxysporum, comparing the morphology of tissues.

Methodology: The animals were injected intradermally with 50 μL of crude extract or fraction F1 at a concentration of 0.5 mg/mL, the control animals received the same volume of saline solution or polycrylamide gel, respectively. Three, six, 12 and 24 hours after application, the animals were killed with an overdose of anesthetic and skin samples were collected, fixed, embedded in paraffin and stained with hematoxylin and eosin for histopathologic study, stained with toluidine blue for quantification of mast cells with Masson’s trichrome for qualitative analysis of collagen and elastic fibers in the dermis and with sirius red for quantification of collagen fibers in the dermis and immunostained to detect expression of metalloproteinase MMP-9. The slides were examined under an optical microscope. Results: We observed the presence of an intense inflammatory response to the presence of polymorphonuclear and mononuclear cells at 6 and 12 hours after injection of the crude extract and fraction F1. In animals injected with the fraction we observed acidophilic and vacuolated keratinocytes and epidermal detachment, typical of necrosis. It was also observed a significant increase in the number of mast cells, structural changes in the dermal collagen and expression of MMP-9 in the skin, in both treatments. Conclusion: We conclude that the histopathological changes resulting F1 fraction injection caused drastic changes in tissue morphology compared with the injection of the crude extract.

Keywords: Fusarium oxysporum, Skin, Histopathology.

Supporting funding: CNPq

A06 EFFECT OF ALUMINUM CHLORIDE INLEYDIG CELLS

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Introduction: Hormesis is a quantitative process repairman that is adaptive in nature, reflected in body’s ability to allocate resources biologicals that are dependent on the disturbance experienced in a highly efficient way to repair damage caused by the toxic agent after a certain period of time. Objective: The aim of this study was to evaluate the recovery of damaged Leydig cells after exposure to different concentrations of aluminum for a prolonged period (112 days), considering histomorphometric parameters. Methodology: Sixty adult Wistar rats were divided into five groups (n = 12 animals/group). Animals of the control group received 1.0 mL of distilled water by gavage (Gv; 1 mL), while the other animals received 0.02 mg/L, 0.1 mg/L, 10 mg/Kg, and 40 mg/Kg of aluminum (AlCl3; Gv; 1 mL) daily during 112 days. Six animals per group were euthanized on the 113th day, while the other animals were euthanized one week later after the end of the aluminum treatment (121st day). The testis was removed, weighed, fixed in Karnovsky solution, and embedded in methacrylate plastic. The testicular histology was evaluated under light microscopy (Olympus BX-50). Images of the testicular tissue were analyzed using the software Image-Pro Plus, and were performed the following morphometric parameters of Leydig cells: nuclear diameter (μm), nuclear and cytoplasmic volume (μm3), the volume of nucleous and cytoplasm in the Leydig cell on the testis and per gram of testis (μm3), the total number of Leydig cells in the testis and per gram of testsis (106), and index leydigosomatic (%). The results were analyzed by ANOVA and Newman Keuls tests (P = 5%).

Results: The diameter and the volume of Leydig nucleous reduced in all animals exposed to aluminum and euthanized on the 113th day (P < 0.05). The volume of Leydig cells per testis was reduced in animals treated with 0.1 mg/L, 10 and 40 mg/kg aluminum, when compared to control animals (P < 0.05). The other parameters did not differ among treatments (P > 0.05). No difference among treatments were observed for those parameters of Leydig cells when animals were euthanized at 121st day (P > 0.05). Alterations observed in Leydig cells may have occurred because of they are close to blood vessels and they are the first cells to receive the aluminum transported by the blood. Conclusion: In conclusion, there were recovery in the nuclear diameter and volume of Leydig cells one week after the end of aluminum exposure.

Keywords: aluminum, histomorphometry, Leydig.

Supporting funding: FAPEMIG

A07 EFFECTS OF ALUMINUM ON TESTICULAR HISTOMORPHOMETRY OF ADULT WISTAR RATS.

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Introduction: Studies have shown that aluminum can cause a disruption in the architecture of the seminiferous tubules due to shedding of the epithelium with the presence of germ cells accumulated within the affected tubules and maturation arrest in some tubules and accumulate in the gonads.

Objective: The aim of this study was to evaluate the effect of aluminum on testicular histomorphometry in animals treated with aluminum chloride for a long-term (112 days). Methodology: Sixty adult Wistar rats were divided into five groups (n = 12 animals/group). Animals of the control group received 1.0 mL of distilled water by gavage (Gv; 1 mL), while the other animals received 0.02 mg/L, 0.1 mg/L, 10 mg/Kg, and 40 mg/Kg of aluminum (AlCl3; Gv; 1 mL) daily during 112 days. Six animals per group were euthanized on the 113th day, while the other animals were euthanized one week later after the end of the treatment (121st day). The testis were removed, weighed, fixed in Karnovsky solution, and embedded in methacrylate plastic.
The testicular histology was evaluated under light microscopy (Olympus BX-50). Testicular images were analyzed using the software Image-Pro Plus, and were performed the following parameters: gonadosomatic index (GSI; %), volume occupied by seminiferous tubules (mL), tubulesomatic index (TSI; %), total length of the tubules seminiferous (TL; m) and length of seminiferous tubules per gram of testis (TL/g; m). The results were analyzed by ANOVA and Newman Keuls tests (P = 5%). Results: No differences among treatments were observed for GSI, TSI, TL and volumetric proportion in animals euthanized on 113th and 121th days (P > 0.05). Animals treated with 40 mg/kg of aluminum and euthanized on the 113th day showed higher TL/g than animals treated with 0.1 mg/L. The absence of differences among animals for the most part of histomorphometric parameters indicated that there was no damage of testicular histology by aluminum. Conclusion: In conclusion, in our experimental conditions the aluminum did not cause testicular histomorphometric alterations, except length of seminiferous tubules per gram of testis.

Keywords: aluminum, histomorphometry, testis
Supporting funding: Fapemig

A08 GENE EXPRESSION EVALUATION OF IL1-β AFTER MUSCULAR IMPLANTATION OF A SYNTHETIC BIOMATERIAL: AN EXPERIMENTAL STUDY.
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Introduction: It is well known that implantation of biomaterials induces an immune response that comprises many steps, including inflammation. This process mediates cellular response of the implantation bed- cells, which in return influences the biomaterial integration, degradation and vascularization, by secreting modulatory cytokines and chemokines. This inflammatory reaction can jeopardize the clinical outcome. Objectives: We aimed to characterize the systemic differences (through whole blood study) in IL-1β pro-inflammatory mediator by gene expression analysis, assessed by the transcript levels after implantation of a synthetic bone graft material. Methodology: 5 Wistar rats with 12 weeks old were used. Prior to implantation procedure of the biomaterial, peripheral blood was collected through the tail vein. The biomaterial was implanted in muscle tissue, and after eight days, a second blood test was conducted. Total RNA was extracted from the samples and parameters of purity, integrity and quality analyzed, after which the cDNA synthesis was performed by reverse transcription. A 12 gene panel was used for normalization. The transcript levels were assessed by real time PCR using SYBR Green®. Results: The results of this study show that there is an increase of IL1-β gene expression after the biomaterial implantation procedure, compared to the levels present before the procedure, for the same animals (P = 0.0313). Conclusion: Our results suggest that bone regeneration procedures performed at a specific site of the body seem to release inflammatory mediators into the blood stream.
Keywords: IL1-β, biomaterials, gene expression

A09 HYDROXYAPATITE, β-TRICALCIUM PHOSPHATE (β-TCP) AND NIOBIUM PENTOXIDE COMPOSITE: A PROMISING BIOMATERIAL ON BONE REPAIR.
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Introduction: Biomaterials, of natural or synthetic origin, composed of one or an association of two or more substances, have been increasingly used for the purpose of aiding in the repair or replacement of bone. Objective: The aim of this study was to evaluate ex vivo biocompatibility, bioactivity and osteoconductive activity of a composite consisting of hydroxyapatite [Ca10(PO4)6(OH)2], β-tricalcium phosphate (β-TCP) and niobium pentoxide [Nb2O5] in the repair of critical size defects in the calvaria of rats. Methodology: Calvarial defects of 8 mm diameter were made in male Wistar rats. In the animals of the experimental group, the defects were filled with a tablet comprising the composite and in the control group, they were filled with a tablet of pure hydroxyapatite (HA). Prior to implantation, the physico-chemical properties of the tablets were determined by X-ray diffraction (XRD) and scanning electron microscopy (SEM), and have been tested for microhardness. The animals were killed 7, 15, 30, 45 and 60 days after surgery. Samples of the skull were collected and processed for paraffin inclusion and then stained with H&E and Azan for histopathological study or SEM. Results: Results indicated that both materials showed micropores. After sintering, the composite showed three distinct crystalline phases, with a predominance of β-tricalcium phosphate (β-TCP) (47.43%) and microhardness calculated at 66% higher when compared to HA tablets. The tablets of the composite remained more securely clicked into the defects after all the observation periods. They also presented biocompatibility, bioactivity and osteoconductivity activity similar to that of HA. There was no development of fibrosis nor inflammatory reaction. Conclusion: We conclude that the composite has biocompatibility, bioactivity and osteoconductive activity similar to that of hydroxyapatite, having a chemical composition with greater amount of β-TCP and higher microhardness.
Keywords: hydroxyapatite, β-tricalcium phosphate (β-TCP), critical size defect, bone repair.
Supporting funding: Fundação Araucária

A10 MORPHOMETRIC EVALUATION OF HEPATIC PROLIFERATION IN A MURINE IMPLANT
MODEL OF BIOMATERIAL.
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Introduction: Liver regeneration is a phenomenon known since the times of ancient Greece and is currently being studied associated with biomaterials due to the prospects that it can bring to the therapy of fulminant liver disease. Objective: To evaluate histological and morphometric parameters of liver tissue that proliferated in implants of polyester-polyurethane sponges inserted in the abdominal cavity of mice. Methodology: 36 mice were divided into experimental and control group. The control group did not undergo any surgical procedure, while the experimental group received the implant sponges above the liver capsule that were removed at predetermined intervals of times: 04, 08, 12 and 25 days post-implantation. Both control and animals in the experimental group were euthanized for removal of liver sample (control group) and the biomaterial (other groups). The implants collected were processed and stained by AgNOR techniques, hematoxylin and eosin, picrosirius and Schorr for histological analysis. Results: We observed an increase in the area of liver tissue in the matrix of the sponge implant and in the number of nucleolar organizer regions (NORs) present in the nuclei of these hepatocytes during the study period, however the amount of collagen and veins did not differ from the control liver, indicating that there were no histopathological changes in the new tissue that proliferated in the biomaterial. The analysis of fibrovascular tissue rich in vessels that infiltrates the polyester sponge implant showed maintenance of the number of vessels and an increase in the amount of collagen during the evaluation period, this is probably due the fact that this tissue functioned as a support for the migration and organization of liver cells. Conclusions: This work proposes an innovative and well-defined model for study cells and molecules involved in liver proliferation.

Keywords: Liver, proliferation, biomaterial.

A11 REPRODUCTIVE PARAMETERS OF FISH AS TOOLS FOR EVALUATION WATER QUALITY.
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Introduction: The aquatic environment represents a large part of our environment and resources, and therefore, the water quality is directly related to the safety of human health. Studies in controlled laboratory environments are important, however analyzes have the advantage in the field of measure biological pollutants available at realistic scale, integrating multiple effects and aid in the elucidation of their mechanisms of action. Changes in the aquatic environment may lead to behavioral modifications that are reflected in histology and reproductive physiology of fish. Objectives: The present study evaluated reproductive parameters in fish captured at two sites Itapecerica. Methodology: To achieve the proposed Hypostomus francisci were captured quarterly, between March/2010 to February/2012 in two sites of the Itapecerica River (P1 = urban perimeter with discharge of untreated wastewater and P2 = upstream of Divinópolis, MG). Ovary fragments were fixed in Bouins fluid, embedded in paraffin, sectioned and stained with hematoxylin and eosin. Results: The diameter of 30 oocytes in each stage of development was measured using a micrometric ruler coupled to the ocular of a light microscope. A subsample of the ovary middle region from 17 females mature were collected, and then kept in modified Gilson’s solution to promote the breakdown of the ovarian tissue. Total fecundity was calculated by the gravimetric method. In ovaries of fish were observed oogonia and ovarian follicles in different stages of development: initial and advanced perinucleolar oocytes, previtellogenic and vitellogenic oocytes. Besides, atretic follicles were found in ovaries from both sites. The follicle diameter was different comparing females from two sites in Itapecerica River. The smaller oocytes were recorded in P1. The batch fecundity ranged from 312 e 3466 oocytes. The batch fecundity of the females from (P1) was significantly higher when compared with the batch fecundity from (P2). In the urban environment, observed a higher fecundity and smaller diameters of oocytes in ovaries of H. francisci. Conclusions: These findings suggest that changes in reproductive patterns due to environmental conditions can influence the investment and energy metabolite of aquatic organisms and consequent human health.

Keywords: Teleost, Itapecerica River, Environmental impact, Reproduction
Supporting funding: FAPEMIG; CNPq; UFSJ

A12 PARENTERAL EXPOSURE TO A REGULAR DIETARY PROTEIN IMPROVES SKIN WOUND HEALING.
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Introduction: Specific inhibition of immune responsiveness is observed after ingestion of many different proteins (oral tolerance). Unexpectedly, parenteral injection of OVA (ovalbumin) into OVA-
-orally tolerant mice inhibits the initiation of immune responses to unrelated proteins (e.g., haemocyanin), footpad inflammation triggered by carragenin (Immunology 2008,126:354) and improves wound healing after incisional dorsal skin lesion (Wound Repair Regen, 2011,19:487). **Objective:** Herein, we investigated if the i.p. injection of a regular and daily protein component of the mouse chow (zein) inhibits inflammation and improves wound healing. C57BL/6 male mice (n=6) maintained according to the ethical rules, were grown in a commercial available animal chow that contains corn proteins, mainly zein. **Methods:** Two circular through-and-through (6.5 mm diameter) holes were made in the dorsal skin of young adult mice with a punch, under anesthesia. Experimental mice received an i.p. injection of 10 μg zein in Al(OH)3 immediately before the lesion; controls were i.p. njected with saline alone. Skin tissue samples were collected 7 and 40 days thereafter, fixed in formalin, embedded in paraffin, sectioned at 5μm and stained with HE or Gomori’s trichrome. **Results:** Significant reduction in inflammatory cell infiltration (1.029±0.056 versus 2.499±0.121, mean±SEM) and fibroblasts (2.328±0.193 versus 3.851±0.298, mean±SEM) were observed at day 7. Significantly, at day 40 the pattern of collagen deposition in experimental mice was more similar to that observed in intact skin. **Conclusion:** parenteral injection of a regular diet component reduced wound fibroblast and inflammatory cell number and improved wound healing in adult mouse skin. Keywords: Oral Tolerance, Wound Healing, Zein Supporting funding: Financial Support: Fundação de Amparo à Pesquisa de Minas Gerais Coordenação de Aperfeiçoamento de Pessoal de Nível Superior and Conselho Nacional de Desenvolvimento Científico e Tecnológico, Brazil.

**A13 PROLIFERATING CELL NUCLEAR ANTIGEN (PCNA) EXPRESSION IN INDUCED LESIONS IN MICE SKIN TREATED WITH PLANT EXTRACT.**

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**Introduction:** Since ancient medicinal properties are attributed plants, highlighting the healing activity. Studies have focused on plants with pharmacological active components in order to develop a more accessible, which reduces the healing time and achieve better Results in inflammation and remodeling of ulcers. In this study, two species of Achyrocline indicated as antiinflammatory were used, alone or in addition to platelets, wound healing induced. **Objective:** The purpose of this immunohistochemical study was to investigate PCNA in the process of healing in induced wounds. **Methodology:** It was performed a bioassay with 6 groups of 7 mice of Swiss line in which were caused cutaneous lesions. Daily treatment was applied topically and lyophilized consisted of extracts of the plant species, plant A (G2) and plant B (G4) in aqueous solution, the other groups received plant A and platelets (G3), plant B and platelets (G4) and control group received only water. The daily application of the treatments were performed on top of standardize circular wound about 2cm in diameter in the dorsal region of each animal. After 9 days of therapy the animals were anesthetized and killed. Tissues were subjected to histological techniques and included in paraffin and sectioned (4μm) in microtome for mounting slides. Immunohistochemistry was performed using a proliferation cell nuclear antigen (PCNA) at a concentration of 1:400. The immune expression of PCNA was analyzed by labeling index and the data was analyzed, once the statistical analysis was performed by test ANOVA one way test and then confirmed by Tukey-Kramer test. **Results:** After comparison studies between treatments by analysis of PCNA expression, significant differences were observed (P<0.01) for the 6 groups: 73.5 ± 2.2; 61.1 ± 3.1; 60.1 ± 2.1; 58.6 ± 3.1; 51.3 ± 2.9 and 48.2 ± 2.5, plant B and platelets, plant B, platelets, plant A and platelets, water based gel, plant A, respectively. **Conclusion:** The application of the plant B and platelets extract does accelerate the healing process of open wounds in mice and should be subject of further studies. Keywords: immunohistochemical, plant, healing Palavra-Chave: PCNA Supporting funding: FAPEMIG
Introduction: The species Byrsonima verbascifolia has not been extensively studied despite the popular use in various regions of Brazil. Objectives: To evaluate the anti-inflammatory activity and acute toxicity of crude methanolic extract of leaves of B. verbascifolia. Methodology: In the paw edema model, Swiss mice were divided in 5 groups and treated orally (p.o) or intraperitoneally (i.p.) with 20% DMSO or indomethacin 10 mg/Kg or extract in the doses at 50, 100 and 300 mg/Kg. Were also tested minor doses of the extract at 12.5, 25 and 50 mg/Kg (ip). After 60 min. (p.o) or 30 min. (i.p.) 30 µL of carrageenan was administered in the left hindpaw of the animals. In pleurisy model, Swiss mice were divided in 6 groups: (1) 20% DMSO (i.p.), or (2) dexamethasone 0.50 mg/Kg (i.p.) or fractions doses at (3) 50 (4); 100 and (5) 300 mg/Kg (i.p.) receiving injection of the 100 µL of carrageenan in the pleura, and the latter group receiving (6) 20% DMSO (i.p.) and injection of the 100 µL of PBS in the pleura. The total and differential counts of leukocytes in pleural lavage were performed. In hippocampic test (evaluation of acute toxicity) Swiss mice were treated with 5000, 500 and 50 mg/Kg (p.o) or 1000, 300, and 50 mg/Kg (i.p.) of the methanolic extract or with DMSO 20% (p.o. or i.p.). For 14 days were analyzed changes in clinical parameters and death. Results: In the model of paw edema the dose of 300 mg/Kg (p.o.) significantly reduced the edema but the effect was much delayed and fleeting. All doses of extract (i.p.) reduced the edema and the dose of 12.5 mg/Kg (i.p.) showed anti-inflammatory effect similar of indomethacin. In pleurisy model the extract at doses of 50, 100 and 300 mg/Kg (ip) reduced significantly and similarly neutrophil migration. In the test hippocampic the doses of 50 and 300 mg/Kg (i.p.) and 50, 500 and 5000 mg/Kg (p.o.) caused a slight reduction in motor activity and traction force. The dose 1000 mg/Kg (ip.) caused the death of all animals 1 hour after administration. No animals in the control group showed a change in the parameters analyzed. Conclusions: The crude methanolic extract of B. verbascifolia showed significant anti-inflammatory effect in both models and acute toxicity was significant only at the dose 1000 mg/Kg (ip.). Keywords: Byrsonima verbascifolia, toxicity, anti-inflammatory.

B02 CHARACTERIZATION OF POSSIBLE ANTI-INFLAMMATORY EFFECT OF FRACTION ENRICHED WITH FLAVONOIDS OF BYRSONIMA VERBASCIFOLIA (MALPIGHIACEAE)

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Introduction: Investigations phytochemical in leaves of Byrsonima verbascifolia revealed the presence of flavonoids that are described mainly by the anti-inflammatory action. Objective: To evaluate the anti-inflammatory activity of fraction enriched with flavonoids (butanolic fraction) of B. verbascifolia contributing to the scientific validation of popular use. Methodology: The crude methanolic extract of leaves of B. verbascifolia was fractionated using n-butanol and the butanolic fraction was obtained. The anti-inflammatory activity of butanolic fraction was assessed through models of paw edema and pleurisy. In the paw edema Swiss mice were divided in 5 groups and treated intraperitoneally (i.p.) with 20% DMSO or indomethacin 10 mg/Kg or fraction at doses of 12.5, 25 and 50 mg/Kg. After 30 min. 30 µL of carrageenan was administered in the hindpaw left of all the animals. Plethysmometric measurements were made in the 1st, 2nd, 4th and 6th hour after induction of edema. In pleurisy model, Swiss mice were divided in 6 groups: (1) 20% DMSO (i.p.), or (2) dexamethasone 0.50 mg/Kg (i.p.) or fraction doses at (3) 50 (4); 100 and (5) 300 mg/Kg (i.p.), every receiving injection of 100 µL of carrageenan in the pleura, and the latter group receiving (6) 20% DMSO (i.p.) and injection of the 100 µL of PBS in the pleura. After 4 hours, the animals were sacrificed and realized the total and differential counts of leukocytes in the pleural lage. Results: In the model of paw edema in the 2nd hour all doses of fraction tested caused a reduction of edema. In this phase the dose of 50 mg/Kg of fraction showed more reduction of the edema than indomethacin at dose of 10 mg/Kg. In the 4th hour all doses of fraction tested caused a reduction of edema. In this phase the dose of 12.50 mg/Kg reduced the edema similarly of the positive control. In the model of pleurisy, the butanolic fraction at doses of 50, 100 and 300 mg/Kg caused a reduction in migration of neutrophil in the pleural cavity. Conclusions: The anti-inflammatory activity of butanolic fraction of B. verbascifolia was evidenced by reduced paw edema and confirmed by a reduction in neutrophil migration for the pleural cavity of mice. This anti-inflammatory activity may be attributed to flavonoids.

Keywords: Byrsonima verbascifolia, anti-inflammatory, flavonoids.

Supporting funding: FAPEMIG

B03 DOWMODULATION OF NUCLEAR TRANSCLOCATION OF PPARGAMMA, INCREASED PRODUCTION OF IL-12 AND DECREASED PRODUCTION OF TNFALPHA AND IL-10 BY PERITONEAL MACROPHAGES FROM BALB/c MICE TREATED WITH ATAZANAVIR.

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Introduction: In vitro studies showed that HIV protease inhibitors may influence functions of the phagocytes. However, the influence of the in vivo treatment with these medicines on the phagocytes functions remains still unclear. Objectives: Evaluate the influence of the oral treatment with atazanavir on the macrophage functions. Methodology: BALB/c mice were treated once a day, orally, for ten days with 90 mg/Kg/day atazanavir (ATV) or saline (PBS) as control. Peritoneal macrophages were seeded on 13 mm-diameter glass coverslips in 24-well plastic plates, incubated for 30 h, at 37°C, with 5% CO2. The number of macrophages adhered to coverslips was quantified by light microscopy after staining with 10% Giemsa solution. The percentage of viable macrophages was assessed by fluorescence microscopy after staining with acridine orange (5 mg/mL). Nitric oxide (NO) and hydrogen peroxide (H2O2) production were determined by Griess reaction and Pick method, respectively. The production of IL-12, TNF-α and IL-10 was estimated by ELISA method. The lipid bodies expression was evaluated by optical and confocal microscopy after staining with Oil Red and Bodipy, respectively. The PPAR-gamma pathway was studied by confocal microscopy. Results: ATV treatment did not change the viability, adherence capacity, H2O2/NO production and lipid bodies expression by peritoneal macrophages from BALB/c mice. However, ATV enhanced the production of IL-12 (PBS=0.0 pg/mL; ATV=29.0 pg/mL; p=0.001, Mann-Whitney test), but downmodulated the production of TNF-α (PBS=49.0±6.26 pg/mL; ATV=28.0±4.24 pg/mL; p=0.0002, t test) and IL-10 (PBS=1.25±0.47 pg/mL; ATV=0.0 pg/mL; p=0.01, Mann-Whitney test) by macrophages. These changes were accompanied by the decrease of the mean fluorescence intensity of the total (PBS=6.45±2.14; ATV=2.34±1.34; p<0.001, t test), cytoplasmic (PBS=1.25±0.47; ATV=0.30±0.07; p<0.0001, t test) and nuclear (PBS=5.19±2.12; ATV=1.68±0.85; p<0.0001, t test) PPAR-gamma by macrophages from BALB/c mice treated with ATV. Conclusions: Our results suggest that ATV modulates cytokine production both of anti-inflammatory as inflammatory via. It is possible that this drug may influence the recovery of HIV+ patients through mechanisms unrelated to their direct effects on HIV replication, by modulating the immune system response. Keywords: atazanavir, PPAR-gamma, macrophage.

B04 EFFECTS OF MONASCUS PURPUREUS IN EXPERIMENTAL MAMMARY CARCINOGENESIS INDUCED BY DMBA.

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Introduction: Monascus purpureus has been used as traditional Chinese medicine in eastern Asia for several centuries. Recent studies have shown the beneficial effects of this specie in the cancer tre-

B05 STUDIES OF MUCOSAL PENETRATION OF CATIONTONIC NANOEMULSION LOADED WITH CHLOROALUMINUM PHTHALOCYANINE FOR USE IN EXPERIMENTAL LEUKOPLAKIA TREATMENT.

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Introduction: Chloroaluminum phthalocyanine (AIClPc) has interesting physical properties for the photodynamic therapy. Unfortunately, AIClPc is insoluble in biologically compatible solvents. To overcome this limitation, conventional and cationic nanoemulsions were used as drug delivery system. Objective: The main aim of this study was to compare the penetration of free AIClPc and incorporated in conventional and cationic nanoemulsions (NE) in the healthy mucosa of the tongue of hamsters. Therefore, this work aims to determine

Objective: Herein, we evaluate the effect of the Monascus purpureus extract on neoplastic mammary experimental specimens, as the macroscopic aspect, histological and immunohistochemical. Methods: Fourteen female Sprague-Dawley rats, virgin, with 45-55 days with food and water “ad libitum” in photoperiodic cycle of 12 h light and 12 h dark, at 22 ± 2°C. Mammary tumors were induced by administration of DMBA (7,12-dimethylbenz (a) anthracene), 100mg/kg diluted in 1 ml of soybean oil and administered intragastrically by gavage, a single dose. The animals were randomly divided into two groups: control group (n = 7) treated daily with filtered water; experimental group (n = 7) treated daily with aqueous Monascus purpureus (600 mg / animal), both administered by intragastric gavage during 30 days. After 31 days, animals were autopsied and the material processed for histopathological evaluation and immunohistochemistry. Differences between groups were evaluated by ANOVA, considering P value ≤ 0.05. Results: After 8 weeks of chemical induction with DMBA, the animals showed 1-3 macroscopic neoplasms, with total tumor volume of 11.89 cm3 in the experimental group, and 9.04 cm3 in the control. It was observed that 85.71% of the tumors were unilateral and 14.28% bilateral. After the fourth week of treatment, it was observed stabilization of tumor growth in control group. And the experimental group had continued increasing. Histologically, the tumors were classified as invasive ductal carcinoma in its pure form, or mixing composed tumors, and other types such as papillary carcinoma, lobular carcinoma, phyllodes tumor and metaplastic carcinoma, according to WHO criteria, 2012. There was a higher cell proliferation in experimental samples, characterized by at least 14% of positivity for Ki67. Conclusion: In this work, treatment with Monascus purpureus caused increase and proliferation of at least 14% of positivity for Ki67. Conclusion: In this work, treatment with Monascus purpureus caused increase and proliferation of neoplastic cells of the tumor mass. Keywords: Monascus purpureus, Mammary carcinogenesis, DMBA, Sprague-Dawley. Supporting funding: FAPEMIG
which formulation can be used in the treatment of experimental leukoplasia for photodynamic therapy. **Methodology:** The NE containing 0.2mg/ml of AlClPc were prepared by the spontaneous emulsification. The cationic lipid, stearylamine, was used to prepare cationic nanoemulsion. The size, polydispersity index and zeta potential were determined. For penetration studies, were used Syrian golden hamster (Mesocricetus auratus) in the experiments. The project was approved by the Animal Research Ethics Committee of University of Ouro Preto (2011/02). The free AlClPc and incorporated in NE were topically administered in hamster tongue. After application animals were killed, tongues were completely excised, histological sections stained with 4’-6-diamidino-2-phenylindole and analyzed in a Zeiss LSM 510 META Confocal Microscope.

**Results:** The mean diameter for AlClPc loaded in NE was 280nm and 268nm for conventional NE and cationic NE, respectively. The NE showed narrow size distribution with a mean polydispersive index of 0.15 and high zeta potential values. All NE formulations were able to cross the keratin layer of the mucosa reaching the epithelial region. The NE cationic, however, showed better adherence to mucosa, and should be considered as an effective strategy for delivering AlClPc. **Conclusion:** The formulations obtained were suitable for topical application in the oral mucosa and for use in the treatment of experimental leukoplasia.

**Keywords:** Chloroaluminium phthalocyanine, photodynamic therapy, nanoemulsion, leukoplasia.

Supporting funding: This work was also supported by FAPEMIG APO-4403-07, NANOBIOMG Network, CAPES, CNPq (481195/2011-4) and UFOP.

**B06 THE LIGNANA JUSTICIDIN B ISOLATED FROM PHYLANTHUS ACUMINATUS PRESENTS ANTI-LEISHMANIAL ACTIVITY.**

**Introduction:** Leishmaniasis are a complex diseases inserted in the group of neglected tropical diseases. The drugs used in its treatment are pentavalent antimonials and amphotericin B used for many years in this therapy. However, they are associated with serious collateral effects. Therefore, it is necessary to search for new antileishmanial drugs. **Objective:** Investigate the activity of lignin Justicidin B isolated from NE of Phyllanthus acuminatus. **Methodology:** The Justicidin B was isolated from the ethanol crude extract of the aerial parts of Phyllanthus acuminatus. Its structure was determined by the techniques of Nuclear Magnetic Resonance (+H and 13C) and mass spectrometry. Promastigotes forms of Leishmania amazonensis were cultured in complete Schneider (1x106 cells/mL) in the absence (control group) and presence of Justicidin B and ethanol extract of Phyllanthus acuminatus in different concentrations for 72h at 25°C. After 72h the promastigotes forms were quantified in a Neubauer chamber under an optical microscope. The parameter used to evaluate cell death was the comparison with the control. To evaluate the cytotoxicity of Justicidin B on murine peritoneal macrophages assays were performed using the vital dye Trypan Blue. **Results:** The analysis of the spectra led to the identification of the isolated substance, the lignan Justicidin B. Both the ethanol extract as Justicidin B inhibited the growth of promastigotes forms. However, Justicidin B showed an IC50 of 7.35 μg/ml while on the ethanol extract had an IC50 of 35.58 μg/ml. Cytotoxicity results demonstrated that the CC50 to Justicidin B was 63.69 μg/ml, while the ethanol extract presented a CC50 of 37.48 μg/ml. **Conclusion:** The lignan Justicidin B has activity against promastigotes forms of L. (L.) amazonensis presenting a greater cytotoxicity than murine macrophages

**Keywords:** Justicidin B, Leishmania amazonensis, cytotoxicity.

**B07 MORPHOLOGICAL ANALYSIS OF CYTOTOXIC ACTIVITY AND ANTIMICROBIAL LEAVES OF MIMOSA CAESALPINIFOLIA**

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**Introduction:** The Mimosa caesalpinifolia is a plant popularly known as 'Samson of the Field'. Its family and gender have pharmacological properties, such as anti-cancer, anti-inflammatory, and antimicrobial. **Objectives:** This work aimed at characterizing and assessing the antimicrobial and cytotoxic activities for human breast cancer cells (MCF-7) of the extract and fractions obtained from the leaves of M. caesalpinifolia. **Methodology:** Following their picking, the leaves were subjected to exhaustive extraction through percolating with 70% ethanol. In order to obtain more information on the chemical composition of the hydroalcoholic extract and identify further the constituents of M. caesalpinifolia, a LC-DAD-ESI-MS analysis was performed. In the microbial susceptibility profile, the assays were carried out through microdilution in a RPMI 1640 medium for yeasts as per protocol M7A6 and microdilution in an Mueller Hinton broth for bacteria, as per protocol M7A6. For the citotoxicity assay, the MCF-7 cells were grown (2x104 cam/mL) in a RPMI 1640 medium, with 20% calf foetus serum and antibiotics, on multiwell plates for the analysis of cellular proliferation and DNA extraction, or on Petri dish glass slides for morphological study. The cells were incubated for 24 and 48h with different concentrations (20-360 μg/mL) of the samples under study. In order to assess the anti-proliferation activity, the Sulforhodamine B assay was carried out. **Results:** All the samples displayed a growth-inhibiting activity against the microorganisms evaluated in 5 to 1,000 μg/mL concentrations, and a powerful anti-proliferation activity, and inducer of cell death, given that maximum activity was obtained at the smallest concentration of the ethyl acetate fraction. The DAD-UV spectra of the major compounds were characteristic of flavones and flavonoids. The combination of UV and
MS/MS fragmentation allows for an efficient dereplication of compounds. In the other hand, the UV and MS data of the compounds suggest a presence of usual compounds. **Conclusions:** These results suggest that the extract and the fractions, especially those obtained from medicinal plants, will continue to have an important place in the process of discovery of new drugs, particularly in the development of microbiological control and anti-cancer drugs.

**Keywords:** Morphological; Cytotoxic; Antimicrobial.

**Supporting funding:** FAPESP-BIOTA; FAPEMIG

### B08 ACTION OF EXTRACTS BARBATIMÃO IN ACTIVITY METALLOPROTEINASES.

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**Introduction:** The species popularly known as Barbatimão are used as healing due to its anti-inflammatory activity. There aren’t studies exploring its potential effects on cancer. It is known that matrix metalloproteinases (MMPs) are connected to the metastatic process. MMPs 2 and 9 degrade various substrates, among them collagen, an essential molecule in the extracellular matrix. **Objective:** Collect leaves, stem, seed, petiole, bark and fruit of barbatimão and produce crude extracts these parts to evaluate the potential of action it same on MMPs 2 and 9. **Methodology:** Upon obtained the hydro-alcoholic extract of leaf, stem, seed, petiole, bark and fruit (without seed) of ed species, lyophilization, was performed zymographic trials in duplicate to evaluate the action of extract of each plant on activity of the MMPs. The extracts were diluted in DMSO (0.001 g / mL). To each strip of the gel added 5μg/mL to each extract and 10 μg / mL sample. Electrophoresis was performed in 70V/4h at 4 °C. Washed the gels in 2.0% Triton X-100 (v / v) and put in Tris-HCl 0.05 M (pH 8.0) with 10 mM CaCl2 for activation of proteolytic activity at 37 °C (over night). The gels were stained and destained. Scanned images of gels in Xerox®, the white region the gel, corresponding to area that had degraded gelatin (MMP activity) were quantified by AxionVizion® Release 4.8.6 and thus, calculated the percentage inhibition of the activity of gelenolitic each extract. **Results:** We evaluated the inhibitory effects of extracts of barbatimão on activity of MMPs. According to our findings, the fractions of crude extract which had greater inhibitory effect on MMP-9 were: fruit (98.78%), petiole (98.3%), stem (98.1%). For MMP-2, were: leaf (63.51%) and fruit (55.92%). The other parts of the plant also showed inhibitory action, but in smaller amounts, with the exception of raw extract of seed showed the opposite effect, increasing the area degraded gelatin (at 32.84%), which corresponds to greater activation of MMP-2. **Conclusion:** The extracts of the fruit, petiole and stem were effective in inhibiting MMP-9 and leaf extract to inhibit MMP-2. Therefore, new research should be performed to determine the active components of the extracts ed in search of possible metabolites with potential therapeutic application. **Keywords:** Inhibition , Barbatimão, MMPs. **Supporting funding:** FAPEMIG

### B09 ACTION OF EXTRACTS OF BAUHINIA VARIEGATA IN VITRO.

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**Introduction:** Matrix metalloproteinases (MMPs) are key players in normal processes, as well as for pathologies of tumor cells (invasion and metastasis). The MMPs 2 and 9 have the function to cleave the type IV collagen, a structural component of the basement membrane. Studies have linked MMPs to cancer prognosis, associating higher expressions of these enzymes with increasing aggressiveness of tumors and the development of metastases. From antiquity many people opt for the base medicinal plants therapies as a complement to the treatment of various diseases. Bauhinia variegata (BV) is widely used in traditional medicine as an anti-diabetic and antioxidant agent. **Objectives:** Evaluate the effect of extracts of BVHN13 and recognizing groups of compounds responsible for this activity. **Methods:** Inhibition of activity of MMPs by the extracts of leaves, flowers, stems and fruit from BV was evaluated by gelatin zymography. The best results of the crude extracts underwent liquid-liquid partition, and the best results of these were fractionated by gravity column. The resulting fractions were analyzed by phytochemical study. The fractions that had great rates of inhibition of MMPs were selected for cytotoxic test on tumor cells from MTT and MTS. The best results of the cytotoxicity assay had their profile evaluated by High Performance Liquid Chromatography (HPLC). **Results:** Was observed that three fractions of the ethyl acetate partition of the stem of BV showed the highest rates of inhibition (100%, 100% and 85% of both enzymes), and the FR 16 fraction was selected for the cytotoxicity assay by having higher yield (g). After evaluation of cytotoxicity was observed IC50 of 0.012mg/mL in HN13 cells. The phytochemical study from this revealed the presence of triterpenes, phenolic compounds, tannins and coumarins. The HPLC profile showed six peaks, with substances medium polar, with retention between 20 and 40 min. Some peaks had similar spectra with phenolic compounds found in the literature. **Conclusion:** The FR16 of ethyl acetate partition of the stem of BV showed the highest rates of inhibition (100%, 100% and 85% of both enzymes), and is cytotoxic for cells HN13. It is suggested that further studies are performed to separate and elucidate the structures of the compounds present in these fractions. **Keywords:** Cytotoxicity, zymogram, phytochemical study.

**Supporting funding:** FAPEMIG and CAPES

### B10 ACTION OF FRACTIONS ETHYL ACETATE OF PLANT OF THE GENUS ANDIRIA IN THE GELATINOLYTIC ACTIVITY OF MMPS.

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**Introduction:** The MMPs 2 and 9 (gelatinase) are expressed in normal tissues and in disease processes such as carcinogenesis, having preferably the
collagen as a substrate, this being a constituent of basement membrane and MEC. So, the search for substances that inhibit MMPs may have therapeutic application. Objectives: To test the effect of 50 fractions ethyl acetate of the plant of genus Andira inhibition of gelatinases. Methods: The inhibitory action of ethyl acetate fraction of the plant Andira sp on the activity of gelatinases were assessed in tests zimográficos. Each well of the gel was loaded with 3.5 mg / ml of salivary samples and 5 mg / ml of the extract. The gels were washed in 2.5% Triton X-100 (v / v) and incubated at 37 °C over night in buffer containing 10 mM CaCl2, 0.15 mM NaCl and 50 mM Tris. The proteolytic activity was stimulated by incubation in activation buffer (TA), whereas the inhibitory capacity of the extracts was measured with the gel incubation in each extract (0.01g), together with TA and saliva. After the run, the gels were subsequently stained (Coomassie blue, for 1 h under gentle agitation) and bleached (for two hours). Digital images of gels were obtained by the program Lpix Image® (Locus Biotecnology®). From the results, was calculated the percent inhibition gelatinolytic activity caused by each extract. Results: The proteolytic activity of gelatinases was visualized by light bands on dark Introduction of the gels, and the sizes of the bands were inversely proportional to the inhibitory effect of each extract. From the analysis of the gel was observed that the fractions that promoted greater inhibition of these enzymes were the fraction 33 (45.87%) and 32 (38.04%). Conclusion: The constituents present in fractions 32 and 33 of the partition ethyl acetate are potentially effective in the inhibition of gelatinase activity, so other tests are necessary to identify the substances present in these fractions. Keywords: Metalloproteinases, Andira sp, protease inhibitor.

Supporting funding: CNPq, FAPEMIG, UFSJ

B11 ACTION OF PLANTS EXTRACTS AND HUMAN PLATELETS IN REMODELING PROCESS OF SKIN WOUND HEALING.

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Introduction: The wound healing follows a sequence of events that are didactically divided in four phases: hemostasis, inflammatory, proliferative and remodeling. When alterations occur in some via of repair process it can lead the development of chronic ulcers when the wound not heal or formation of keloids, when occurs some fibroproliferative disorder. Researchers have investigated new treatments to improve the repair process in these cases, based in natural products. Objective: investigate the effect of two plants of family Asteraceae in skin wound healing, associated or not to human platelets in remodeling process. Methodology: The mice were anesthetized, the dorsal thoracic skin was shaved and one excisional wound (1cm diameter) was made on the shaved region. The mice were split into six groups of ten animals each which received topical treatments: cutaneous base gel (G1); plant A extract in gel (G2); plant A extract + platelets in gel (G3); plant B extract in gel (G4); plant B extract + platelets in gel (G5); platelets in gel (G6). Half of animals of each group received the treatment during nine days, once a day, and the other half received the treatment during 21 days, once a day. After the two times of treatment the animals were sacrificed and wound tissue was taken to morphometric analyzes under light microscopy (LM) and the ultrastructural description by transmission electron microscopy (TEM). Results: The nine days treatment showed less deposition of reticular fibers in the groups treated with the plant A (G2) and plant B (G4) and at 21 days there was no difference. Regarding to the collagen fibers the deposition was higher and uniform among treated groups at nine days, highlighting a greater deposition in the group treated with plant A (G2) at 21 days. Although detected in all groups, myofibroblasts occurred less frequently in the treated groups. Conclusion: There was an effective extra-cellular matrix renewal and anti-fibrotic control during the tissue repair process after the treatments, and that the treatments with plant A and plant B showed better efficacy. Keywords: skin wound healing, plants extracts, platelets.

Supporting funding: FAPEMIG e CNPq

B12 ACTIVITY OF Tetradenia riparia ESSENTIAL OIL AGAINST Leishmania (L.) amazonensis AND L. (V.) braziliensis USING XTT METHOD.

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Introduction: Cutaneous leishmaniasis can present many cases of therapeutic failure and adverse effects. Recently, it was shown that the Tetradenia riparia essential oil has leishmanicidal activity against promastigotes of Leishmania (L.) amazonensis. Leishmanicidal assays on promastigote forms are based on colorimetric methods, including the XTT-based (sodium-2, 3 - bis [2-methoxy-4-nitro-5-sulphophenyl]-2H-tetrazolium-5-carboxanilide). This is a quick method, with high sensitivity and reproducibility. Objective: We evaluated the leishmanicidal activity of T. riparia essential oil on Leishmania promastigotes using the XTT method. Methodology: L. amazonensis and Leishmania (Viannia) braziliensis promastigotes in log phase of the growth were cultured in RPMI medium supplemented with 10% fetal bovine serum. The Tetradenia riparia e sential oil and amphotericin B (Amb, 4μg/mL) were added to the cultures. After 24, 48 and 72 hours at 25°C, a XTT solution (XTT tetrazolium and phenazine methosulfate) was added to the cultures, and they incubated by 3-5 hours at 37°C. The optical density was measured by spectrophotometer. The median lethal dose (LD, 50%; LD50/24, 48 e 72h) was calculated by linear regression of the death percentage. Results: The T. riparia essential oil showed leishmanicidal activity on L.amazonensis after 48h, which LD50 was 264ng/mL. LD50 Amb was 350, 428 and 351ng/mL after 24, 48 e 72 h. For L. (V.) braziliensis, LD50/24h T.riparia was 230ng/mL, and
Amb was 3.6mg/mL. After 48 and 72h, the parasite steps in the stationary phase and not maintained a stable viability. **Conclusion:** The Tetradenia riparia essential oil showed leishmanicidal activity on promastigotes of both species of Leishmania. The XTT method was effective to evaluate the leishmanicidal activity, and it can be used to study of compounds with potential biologic effect in leishmaniasis.

**Keywords:** Leishmania, Tetradenia riparia, colorimetric method, XTT.

**Supporting funding:** CNPq

**B13 ANTI-INFLAMMATORY ACTIVITY OF Tabebuia aurea ON THE PERITONITIS MODEL AND PAW OEDEMA INDUCED BY Bothrops neuwiedi VENOM IN MICE.**

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**Introduction:** Bothrops species are responsible for the majority of snakebites in Brazil. Several plants have been utilized in folk medicine as active agents against various effects induced by snake venoms. Tabebuia aurea is considered a plant with anti-inflammatory properties. Additionally, the inhabitants of the Pantanal and Cerrado region use the infusion of the bark of a plant popularly named -garrafada- to snakebites. **Objectives:** To evaluate the anti-inflammatory activity of Tabebuia aurea on the peritonitis model and paw oedema induced by Bothrops neuwiedi venom in mice. **Methodology:** Mice Swiss (n=7), male, 18-25g were pre-treated, 1h before, p.o.,10 mL/Kg, with hydroethanolic extract of Tabebuia aurea (HETa 100, 200 and 400 mg/Kg). Indomethacin, 15 mg/Kg (positive control). For the evaluation of leukocyte infiltration into peritoneal cavity, the leukocytes were harvested 4h after intraperitoneal injection of Bothrops neuwiedi venom (BnV,10mg) or sterile saline, by washing peritoneal cavities with saline solution. Another group received the injection intraperitoneal of incubated BnV:HETa (1:50, 30 minutes, 37°C). Aliquots were used to determine total and differential cell number (polymorphonuclear or mononuclear cells). The paw edema was assessed with the injection BnV or BnV:HETa in the subplantar region of the right hind paw. The left hind paw received an equal volume (40 mL) of saline. The volumes were measured by plethysmometry before and 0.5, 1, 2 and 4h after venom administration. Results were expressed as mean±EPM; ANOVA, Bonferroni test. **Results:** HETa inhibited the polymorphonuclear (PMN/mm3) migration in 32.06% (551±60, 100mg/Kg), 43.89% (455±94, 200mg/Kg), 43.77% (456±45, 400mg/Kg) and 52.03% (389±85, BnV:HETa) when compared with the BnV (811±66). BnV induced oedema peaking at 0.5h which decreased gradually over the following 4h. HETa inhibited the oedema at all measurements. At the first time post-BnV injection the inhibition was 24%, 34% and 25% for 100, 200 and 400 mg/Kg HETa, respectively. BnV:HETa inhibited in 22%, 20%, 33% and 40% at 0.5, 1, 2 and 4h, respectively. **Conclusions:** HETa inhibits the inflammatory response induced by BnV in mice. Investigation of snake venom inhibitors can provide useful tools to be used as models for development of agents in the treatment of snakebites.

**Keywords:** poisoning, folk medicine, phytotherapy. **Supporting funding:** CNPq, FUNDECT, UFMS.

**B14 IN VITRO CYTOTOXIC ACTIVITY OF AM11 AND SEMI-SYNTHETIC INGENOL DERIVED FROM Euphorbia tirucalli ON A LARGE PANEL OF HUMAN CANCER CELL LINES**

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**Introduction:** The attempt to improve the antitu-

**mor therapy with new antineoplastic agents from 

tural sources have revealed efficacy and offers a 

large field for scientific research. Euphorbia tira-

calli is used in traditional medicine in the northea 

region of Brazil as antimicrobial, antiparasitic, an 

ticancer and other disease. However, little is known 

about its anticancer properties. Aim: We aimed to 

study the antitumor effect of the tetracyclic interpe 

ne alcohol, AM11, and three semi-synthetic inge 

nol compounds derived from E tirucalli, ingenol A 

(ingenol-3-trans-cinnamate), ingenol B (3-caproyl-

-ingenol) and ingenol C (ingenol-3-dodecanolate), 

against a large panel of human cancer cell lines. 

**Methodology:** Anti-tumor effects of AM11 and in 

genol compounds in vitro were assessed using 

MTS assays on 80 human cancer lines from 15 

solid tumor models. Additionally, we evaluate the 

potential combinatorial value of this drug in the 

context of standard glioblastoma therapy (temozolomi 

de-TMZ). **Results**: AM11 and ingenol compoun 

ds exhibited dose- and time-dependent cytotoxic 

effects on human cancer cell lines. Amongst the 

derived tested, ingenol C displayed the best activity 

across the tumor cell lines. Esophageal squamous 

cell carcinoma and pancreatic carcinomas showed 

the most sensitive profile of response to compoun 

ds. In comparison with TMZ, ingenol C was more 

efficient than AM11. The ingenol C showed a me 

dian of 135 fold increase in efficacy, while AM11 

showed a median of 30 fold increase, in the glioma 

cell lines. However, we found that when combined, 

Ingenol C and TMZ treatments, the effect seems to 

be antagonistic (combination index >1) on the most 

glioma cells lines investigated while AM11 syner 

gistically sensitizes the most glioma cell lines to TMZ 

treatment (combination index <1). **Conclusions**: It 

could be concluded that AM11 and semi-synthetic 

ingenol compounds from E tirucalli, in particular the 

ingenol C showing a promising cytotoxicity effect 

against several cancer cell lines. Taken together, 

our findings may provide insight into the tailoring 

designing of AM11 and ingenol C-based therapies 

for cancer patients. 

**Keywords:** Euphorbia tirucalli; glioma cell lines; 

cytotoxic activity.

**B15 PARTIAL CHARACTERIZATION OF AN-

TI-OXIDANT AND ALPHA-AMYLASE INHIBITION 

PROPERTIES OF EXTRACTS FROM PULP, PE 

ELS AND SEED OF SOME FRUITS FROM THE
Introduction: Fruits of the Cerrado biome are promising sources of bioactive compounds with antioxidant and amylase inhibitors with potential to prevent chronic and degenerative diseases such as the Diabetes.

Objectives: The aim of this study was to evaluate the antioxidant capacity and alpha-amylase inhibition activity by aqueous and ethanolic extracts of the following fruits and its parts: pulps of Eugenia dysenterica DC (caigua), Brosimum guaduichaudii Trécul (mama cadela), Hancornia speciosa (mangaba), Butia capitata Bec (coquinho azedo), Genipa Americana L. (jenipapo), Byrsonima sp (murici) and Caryocar brasiliense (pequi); pulp and peel of Campomanesia sp (gabiroba); seed and peel of Anonna sp (araticum).

Methodology: Antioxidant activity was evaluated by the method of DPPH (2,2-diphenyl-1-pircrylhydrazyl) and inhibition of alpha-amylase with GalG2CNP [alpha-(2-chloro-4-nitrophenyl)-β-1,4-galactopiranosilmaltoside] as substrate for the enzyme alpha-amylase partially purified from human saliva samples. The IC50 values for antioxidant capacity was expressed in mg/mL with the following Results: a) aqueous extracts of araticum (99) and gabiroba (97), peel extracts and araticum seed (91). The percentage of alpha-amylase inhibition by aqueous and ethanolic extracts of caigua with IC50 of 3.60 and 3.63, respectively; b) aqueous extracts of gabiroba pulp (5.93), coquinho azedo (6.85) and murici (6.10); c) aqueous extract of araticum seed (7.15); d) mama cadela showed different values for the aqueous (32.80) and ethanolic (79.39) extracts; e) IC50 of 36.73 and 70.78, respectively for mangaba and pequi extracts. The salivary alpha-amylase inhibition activity, expressed in percentage of inhibition, by different fruit extracts at concentration of 100mg/mL, showed promising results such as found for the aqueous extract of fruit peel and seed as follow: araticum (99) and gabiroba (97), peel extracts and araticum seed (91). The percentage of alpha-amylase inhibition by aqueous extracts of pulps were the followings: coquinho azedo (94), murici (67), caigua (48), gabiroba (43), jenipapo (31), mangaba (23) and mama cadela (23). Conclusions: Therefore, these data corroborate to add values for the fruits of the Cerrado Biome as sources of bioactive compounds with antioxidant and inhibition of alpha-amylase properties. Studies in vivo and the identification of the active ingredients of these extracts will reveal their importance to promote health and the combat of diseases such as Diabetes and obesity.

Keywords: Fruits, Cerrado biome, antioxidant, alpha-amylase.

Supporting funding: FAPEMIG, PROEXT-MEC, CAPES and REDE FITOCERRADO

B16 THE POTENTIAL USE OF BIOCHEMICAL STUDIES OF XYLOPIA SP EXTRACTS UNDER METALLOPROTEINASES OF MATRIX.

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Introduction: The family of matrix metalloproteinases (MMPs) has classically been described in the context of extracellular matrix (ECM) remodelling, which occurs throughout life in diverse physiological and pathological processes. MMP-2 (gelatinase A) is related to tumour invasiveness and metastasis, as remodelling of the ECM is thought to be necessary for a tumour cell to advance. Thus, has increased the demand for substances that are effective in decreasing the degradation of ECM, in search of antineoplastic drugs. The Xylopias are a genus that has the antiproliferative activity against a panel of cancer cell lines. Objective: Identify fractions of extracts of Xylopias obtained from chromatographic columns of chloroform and hydroalcohol, which are effective in the inhibition of gelatinase A.

Methodology: Were prepared Chloroform and hydroalcohol fractions of Xylopias obtained from its crude extract (hydroalcoholic). The extract was eluted on a chromatographic column and had their points grouped according to the profile obtained by chromatography thin layer. After that, assays zymography were utilized to evaluate the activity of these under gelatinase A. Each well of the gel was loaded with 3.5μg/mL of salivary proteins and 5μg/mL of plant extract. After the electrophoresis (4h, 70V) the gels were washed with 2.5% Triton X-100 (v / v) and incubated at 37 ° C overnight in buffer activation (TA) [10 mM CaCl2, 0.15M NaCl, and 50 mMTris (pH 7.5)]. After this, the gels were stained and discolored. Digital images of gels were obtained by the program Lpix Images® (LoccusBiotechnology®), and the white area was quantitated by the program AxionVizion® Release 4.8.6 (6-2010). From the results, it was calculated the percent inhibition gelatinolytic activity caused by each extract.

Results: From the analysis of the gel was observed that the hydroalcoholic column fractions that promote inhibition of gelatinase A was 19, 35, 41-42, 43 and 44, representing 100%, 90%, 100% and 70% inhibition, respectively. Already column fractions of chloroform with improved inhibitory activity were 16-17 and 30, corresponding to 55% and 100%, respectively. Conclusion: The obtained fractions of the plant have inhibitory action of MMP-2, which may be important therapeutically. So, further test should be performed for the isolation and identification of the substances present in this fraction.

Keywords: Xylopias, neoplasms, gelatinases. Supporting funding: CNPq, FAPEMIG, UFSJ.
its popular use, there is a lack of information regarding the effects of AEV treatment in hepatic tissue. **Objectives:** The aim of this study was to evaluate the effects of AEV treatment in oxidative stress parameters of diabetic rat livers. **Methodology:** DM was induced by intraperitoneal injection of streptozotocin (40mg/kg). Animals were divided in six groups: non-diabetic control, diabetic control, diabetic + AEV (500 mg/kg), diabetic + glibenclamide (6 mg/kg), non-diabetic + AEV (500 mg/kg) and non-diabetic + glibenclamide (6 mg/kg). Each treatment lasted for 43 days. Measurements of glycemia and body weight were taken in the first and last days of treatment. Livers were removed, weighted and homogenized in sodium phosphate buffer and stored at -80°C. For oxidative analysis, CAT, GPx, GST and SOD activity of liver homogenates and hepatic mitochondrial fraction was evaluated, even as GSH levels and lipid peroxidation (MDA). **Results:** Diabetic group shows an increase in activity of antioxidant enzymes GPx, SOD and MDA for liver homogenate analysis, and decrease in GHS, GST rates. On the other hand, the AEV diabetic group had this oxidative parameters restored to normal levels. The oxidative stress analysis of the hepatic mitochondrial fraction revealed similar results of lower activities for CAT and SOD and higher GPx activity in diabetic control group. For treated diabetic group with AEV the activities of CAT, SOD and GPX were near to the activities found for non-diabetic control group. **Conclusions:** According to experimental conditions, the AEV was efficient to reduce hepatic oxidative stress induced by STZ. Animal care and experimentation were approved by CEUA-UFU (protocol 060/10).

Keywords: Vochysia rufa, diabetes, liver, oxidative stress.

Supporting funding: grant for FSE FAPEMIG; CA-PES fellowship for IBM, NMG and LKC; and PIBIC/CNPq fellowship for CMM.

**B18 CHANGES IN SPLEEN TISSUE OF MICE WITH SCHISTOSOMIASIS CALORIC AND PROTEIN MALNUTRITION INDUCED DURING LACTATION.**

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**Introduction:** Despite significant advances in the study of schistosomiasis, the relationship between schistosomiasis and malnutrition are still not completely understood. Being lactation period of development suggesting a “programming” (imprinting) on the metabolism of the individual adaptive response to environmental factors found in the initial periods of development. **Objectives:** This study aimed to evaluate the characteristics of the spleen in acute schistosomiasis infection in mice metabolically programmed by caloric restriction and protein restriction. **Methodology:** The spleens of animals euthanized in the 9th week of infection were subjected to histological sections (5μm) and stained with hematoxylin-eosin. We performed histopathological evaluation, morphometry and stereology. **Results:** Was observed structural disorganization of the white pulp and red pulp in groups programmed, regardless of the presence of infection. Infected animals showed hyperplasia and hypertrophy of the white pulp and a greater amount of dispersed pigments in the spleen tissue and the eosinophils on the inside of vascular structures. The white pulp of both infected groups of caloric restriction as restriction protein morphometric measurements showed higher when compared to uninfected groups. The stereological results showed that the calorie restriction group infected had lower volume density of red pulp, while no significant differences in the volume density of white pulp. Megakaryocytes were seen in greater quantity in the infected groups, with emphasis on group protein restriction. **Conclusions:** These data suggest that programming by maternal undernutrition during lactation and schistosomiasis infection causes disorganization of splenic tissue.

Keywords: Schistosomiasis infection, Metabolic programming, Splenic tissue, Histopathology

Supporting funding: CAPES.
Conclusions: In conclusion, the immune response of C57BL/6 mice induced by T. crassiceps cysticercus can be characterized by mixed Th1/Th2 profile. The mixed response is able to promote the destruction of the parasites IFNγ-dependent, which results in a minor injury and lower collagen production. In IFNγ-deficient, there is a profile that tends towards the Th2 immune response, with the presence of CD301+ cells and increased collagen production. Keywords: subcutaneous cysticercosis, granulomatous inflammation, immune response

B20 EVALUATION OF THE INFLAMMATORY RESPONSE IN EXPERIMENTAL SUBCUTANEOUS CYSTICERCOSIS BY TAENIA CRASSICEPS IN BALB/C MICE.

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Introduction: This study proposes a model of the subcutaneous cysticercosis that enables a comparison with human cysticercosis caused by Taenia solium, using the larval stage of Taenia crassiceps - Cysticercus longicollis - antigenically similar to the first. Objectives: To analyze the general pathological processes present in the subcutaneous tissue of BALB/c mice after the inoculation of T. crassiceps cysticerci. Methodology: BALB/c mice were inoculated with 0.2 mL of phosphate buffered saline (PBS; pH 7.2, 0.15M) containing 10 initial cysticerci of the ORF strain of T. crassiceps into the subcutaneous tissue of the dorsum of the mice. Control animals were inoculated with 0.2 mL of PBS. At 10, 90 and 300 days post infection (dpi), animals were then euthanized and the subcutaneous tissues were collected. The material was fixed in 10% neutral buffered formalin and routinely processed to evaluate the role of IFNγ in the inflammatory response, by making use of conventional C57BL/6 mice and C57BL/6 mice lacking the IFNγ gene (KO-IFNγ) inoculated intracranially with T. crassiceps cysticerci. Methodology: Mice were inoculated via intracranial injection with viable cysticerci and euthanized at 7, 30, 60 and 90 days post-infection (DPI). Their brains were removed and analyzed histopathologically. Results: In conventional animals, the presence of IFNγ induced ventriculomegaly, microgliosis, ependymitis and perivasculitis with greater intensity, due to the chronification of the inflammatory response with early destruction of the cysticerci. Conversely, in KO-IFNγ animals, the inflammatory response was less intense with prevalence of polimorphonuclear cells. The increase in the mononuclear cells occurred only at 90 DPI when the destruction of the parasites occurred. Conclusions: These results show that IFNγ plays a fundamental role in the stimulus of the inflammatory response against the intraventricular T. crassiceps cysticerci stimulating the microglia cells and the intensity of the inflammatory response in the host which induces the early destruction of the parasite. Keywords: Taenia crassiceps, neurocysticercosis, C57BL/6.

B21 IFN-GAMMA INDUCED EARLY DESTRUCTION OF THE CYSTICERCUS IN EXPERIMENTAL ENCEPHALITIS.

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Introduction: Responsible for over 50 thousand annual deaths, neurocysticercosis (NCC) is one of the main parasitosis of the central nervous system (CNS) and the main cause of active epilepsy in patients in neurologic clinics in developing countries such as Mexico and Brazil. Experimental studies demonstrate that C57BL/6 mice are more resistant to intraperitoneal infection with Taenia crassiceps cysticerci as they present a predominance of type 1 immune response. Objectives: This study aimed to evaluate the role of IFNγ in the inflammatory response, by making use of conventional C57BL/6 mice and C57BL/6 mice lacking the IFNγ gene (KO-IFNγ) inoculated intracranially with T. crassiceps cysticerci. Methodology: Mice were inoculated via intracranial injection with viable cysticerci and euthanized at 7, 30, 60 and 90 days post-infection (DPI). Their brains were removed and analyzed histopathologically. Results: In conventional animals, the presence of IFNγ induced ventriculomegaly, microgliosis, ependymitis and perivasculitis with greater intensity, due to the chronification of the inflammatory response with early destruction of the cysticerci. Conversely, in KO-IFNγ animals, the inflammatory response was less intense with prevalence of polimorphonuclear cells. The increase in the mononuclear cells occurred only at 90 DPI when the destruction of the parasites occurred. Conclusions: These results show that IFNγ plays a fundamental role in the stimulus of the inflammatory response against the intraventricular T. crassiceps cysticerci stimulating the microglia cells and the intensity of the inflammatory response in the host which induces the early destruction of the parasite. Keywords: Taenia crassiceps, neurocysticercosis, C57BL/6.

Supporting funding: CNPq.

B22 IMMUNE RESPONSE PROFILE IN EXPERIMENTAL NEUROCYSTICERCOSIS.

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Introduction: Neurocysticercosis (NCC) possess as an experimental model the infection of mice...
with Taenia crassiceps, which induces a similar disease to the human cysticercosis. In the initial stages of this disease, it is possible to observe a predominance of a Th1 immune response added to a greater destruction of the parasite and low parasitary burden. In advanced stages of cysticercosis the predominant immune response is of Th2 type with IL-4, IL-5 and IL-10 production (Robinson et al., 1997; Conti et al., 2003). **Objective:** To evaluate the immune response through the concentration of cytokines (IL) such as Interferon-gamma (IFN-gamma), IL-4 and IL-10 the culture of spleen cells dosed by ELISA. **Methodology:** A total of 24 female BALB/c mice of 8 to 12 weeks old were inoculated with 1 to 3 T. crassiceps initial stage cysticerci (translucid and infectant). For the inoculation a stereotaxic device was used with the aid of an anatomic atlas and the location of the hypocampus was determined the bregma according to the following coordinates (AP-3.0, lateral +2.0). By 7, 30, 60 and 90 days after the inoculation (DAI) the mice were euthanized and necropsied when the spleen was removed and its cells were cultured to dosage the cytokines IFN-gamma, IL-10 and IL-4 through ELISA. **Results:** At 7 and 30 DAI IL-4 presented concentrations below 0.1 ng/ml while at 60 and 90 DAI the mean concentration was of 0.3 ng/ml. The IL-10 concentration was lower than 0.5 ng/ml at 30 DAI and on the other experimental days the concentration values were above 1 ng/ml. The IFN-gamma concentration was higher than the IL-4 and IL-10 concentrations at all experimental days ranging 2.77 to 7.13 ng/ml. **Conclusion:** According to these results, the Th1 immune response represented by the IFN-gamma concentrations was the most predominant during all the analyzed period. **Keywords:** experimental neurocysticercosis, immune response, cytokines.

### B23 INTERACTION OF A NEWLY ATYPICAL BRAZILIAN STRAIN OF TOXOPLASMA GONDII WITH NEURONS AND GLIAL CELLS IN VITRO

**Introduction:** Worldwide approximately two billion people are infected chronically with Toxoplasma gondii, showing unknown consequences. The infection is extremely ubiquitous, surrounding the biological brain barrier and it Results in a chronic stage after the establishment of immune response. The chronic stage is characterized by the presence of cysts containing bradizoites in the nervous and muscular tissue. The transplacental infection causes damage in the central nervous system including hydrocephaly, brain calcification, retinochoroiditis and abortion. The reactivation of the disease in immunocompromised patients causes toxoplasmic encephalitis, that can lead to death. The toxoplasmosis treatment is limited and varies with different strains. In South America T. gondii shows a common genotype population different the ones circulating in USA and Europe, and it is known that the serious manifestations of the disease are more common in Brazil. We used a newly isolated and genotyped virulent strain (TgCTBr9) obtained the peripheral blood of human newborns diagnosed with congenital toxoplasmosis in 12 regions of Minas Gerais/Brazil. **Objective:** Verify if the strain TgCTBr9, that circulates among humans in Brazil, is able to infect neurons and glial cells in vitro. **Methodology:** The superior cervical ganglia will be removed newborns, C57BL/6 mice, and dissociated before being plated in a concentration of 2 x 104 cells/well in the presence of DMEM, NGF and matrigel. After a period of 24 hours the wells will be infected with the parasite at a concentration of 1:5, host cell/parasite. The cells will be left to interact with the parasite for two hours and after this period the wells will be washed and incubated with DMEM and NGF for 22 hours. Past this period the wells will be fixated with paraformaldehyde at 4% for 20 minutes. **Results:** The infected cultures presented neurons and glial cells containing parasitosorus vacuoles with taquizoites. **Conclusions:** Our results show that the virulent strain, TgCTBr9, is able to infect neurons and glial cells in vitro. This is an unprecedented result since there are no studies correlating this unusual strain and the pathogenesis of Toxoplasmosis in Brazil. **Keywords:** Toxoplasma gondii, Primary neuron culture, Atypical strain. **Supporting funding:** CAPES, FAPEMIG, CNPq

### B24 MACROSCOPIC AND MICROSCOPIC ASPECT OF LIVERS FROM SANTA INÊS SHEEP INFECTED WITH FASCIOLA HEPATICA.

**Introduction:** Fasciola hepatica is a trematode parasite of the bile ducts that infests ruminants, rodents, pigs, horses, goats, deer and primates including humans. This parasite causes considerable economic loss in domestic ruminants like sheep due to growth retardation, rejection of livers in the slaughterhouse, decrease in milk, meat and wool production, mortality and drug spending. **Objective:** In spite of the worldwide distribution, there is a few works about susceptibility of Santa Inês sheep to F. hepatica. **Methodology:** A total of six animals of both sex, eight months of age and approximately 35 kg of average weight were infected via oral with 250 metacercariae of F. hepatica. Euthanasia and necropsy were performed 90, 180 and 210 days post infection. After macroscopic examination, 4 fragments were randomly collected of liver parenchyma, fixed in 10% tamponed formol and processed according to standard technique of paraffin embedding. Paraffin containing tissue samples were cut at 6 µm and stained with hematoxylin and
eosin, Massons Trichrome and periodic acid-Schiff.

**Results:** Macroscopically, all livers showed volume increase, convex lobes and white spots distributed on the tissue surface. Biliary vesicles also showed an increased volume. Furthermore, livers had yellow spots and bile duct wall thickening after 210 days. Macroscopically, we observed multifocal and perivascular inflammatory infiltrates containing mononuclear cells. Necrosis areas, fibroses and hemosiderin deposition were also observed in tissue sections. We observed glycogen depletion inside hepatocytes in 210 days post infection. The infection was confirmed after liver and bile duct section, where we recovered a total of 364 parasites and eggs in biliary vesicles. **Conclusions:** The Santa Inês sheep showed susceptibility to F. hepatica infection with pathological changes typical of fasciolosis.

Keywords: Fasciola hepatica, sheep, pathology.

Supporting funding: CAPES; CNPq; FAPEMIG

**B25 PRESENCE OF AMASTIGOTES FORMS IN TISSUES SAMPLES OF MALE REPRODUCTIVE ORGANS OF DOGS WITH DIAGNOSIS OF VISCERAL LEISHMANIASIS WITH CLINICAL SIGNS OF LYMPHOPATHIA.**

**Introduction:** Canine visceral leishmaniasis (CVL) is a chronic parasitic disease caused by protozoa of the genus Leishmania. The disease has a worldwide distribution, and transmission occurs mainly by the sting of a hemophagous insect species Lutzomyia longipalpis belonging to the subfamily Phlebotominae. However, the vertical and venereal transmission of the CLVcan also occur. **Methodology:** We randomly selected 26 male dogs in the districts of Divinópolis-MG. For each captured animal, an epidemiological form was registered which had the following variables: symptoms, location of the coat, presence of alopecia, score ranking the dog’s body, the presence of onychogryphosis, signs of fasciolosis, presence of alopecia, score ranking the dog’s body, the presence of onychogryphosis, signs of fasciolosis, asymmetry in weight, score ranking the dog’s body, the presence of onychogryphosis, signs of fasciolosis, asymmetry in weight, score ranking the dog’s body, the presence of onychogryphosis, signs of fasciolosis.

**Results:** The indication of CVL in the dog was histologic confirmation, the absence of lymphopathia is an important indication as to the absence of parasites in the tissues of the reproductive organ of the dog and thereby decrease the likelihood of transmission of venereal disease in dogs without signs of lymph node involvement.

Keywords: Leishmaniasis, Lymphopathia, Dogs.

Supporting funding: CNPq

**B26 STUDY OF IMMUNE RESPONSE AND CAECAL LESION IN MICE C57BL/6 WILD TYPE AND CD1-/- INFECTED WITH ENTAMOEBA HISTOLYTICA.**

**Introduction:** The study demonstrated that Natural Killer T (NK T) lymphocytes are critical for preventing the development of amebic liver abscess. In this context, NK T lymphocytes are attractive due to the scarcity and relevance of studies in the area. **Objectives:** The aim of this study was to evaluate the infection, injury and immunity induced by E. histolytica in C57BL / 6 wild-type (WT) and C57BL / 6 CD1-/-.

**Methodology:** The procedures were in accordance with the standards of CETEA / UFMG. Female WT mice (n = 16) and CD1-/- (n = 16), 70 days old, were inoculated with E. histolytica and sacrificed after 48 hours of infection to collect the cecum. The cecum was used for morphometric analysis of lesion, trophozoites NK T lymphocytes and mucin MUC-2 by immunohistochemical reactions.

**Results:** The extension of necrosis of the mucosa and the number of trophozoites was larger in the group of mice Eh-CD1-/- (2,713±566.6 μm²), and (14.3±3.95) compared to group Eh-WT (575.4±69.73 μm²) (p <0.05). Qualitative analysis showed a significant increase in the number of NKT lymphocytes in group Eh-WT (121.1 ± 21.98) compared to the groups CTRL-CD1-/- (26.3 ± 2.95), Eh-CD1-/- (22.4± 4.23) and CTRL-WT (69.5 ± 7.17) (p <0.05). The area of mucosa group CTRL-WT (31,254 ± 1,273μm²) was higher than in the CTRL-CD1-/- (20,641 ± 1,885 μm²) (p <0.05). In animals of both groups CTRL-CD1-/- (391.0 ± 2.95), Eh-CD1-/- (750.0 ± 68.44 μm²) re-

**Conclusion:** Our results showed that the reduction of NK T lymphocytes in mice CD1-/- correlated with decreased mucus production, especially MUC-2, reducing the ability of the host to control infections. These lymphocytes appear to be involved in the inflammatory response triggered against infection by E. histolytica, as CD1-/- mice amoebic colitis developed more severe than WT animals. Correlation was also observed between normal mucosa with appropriate levels of mucus in WT mice, with larger protection against infection and the development of erosions and ulcers amoebic. Thus, our results suggest that NK T lymphocytes, the epithelial barrier and intestinal mucins produced by this epithe-
**B27 THE ABSENCE OF IL-4 INDUCES LOW TISSUE INFLAMMATION IN EXPERIMENTAL NEUROCYSTICERCOSIS.**

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**Introduction:** Neurocysticercosis (NCC) is a world-wide distributed disease caused by the larval stages of Taenia solium and is considered one of the most important infections of the central nervous system. Experimental models with Taenia crassiceps cysticerci have been used as a useful tool for studying the host-parasite relationships in cystercerosis. **Objectives:** This study aimed to analyze the influence of IL-4 in the inflammatory response and tissue injuries caused by the experimental model of NCC which uses T. crassiceps cysticerci. **Methodology:** We used BALB/c mice, both conventional or lacking the IL-4 gene (KO-IL-4), which were inoculated intracranially with viable cysticerci and euthanized at 7, 30, 60 and 90 days post-infection (DPI). **Results:** The absence of IL-4 induced greater parasitism. In the initial phase of the infection the KO-IL-4 animals presented lower inflammatory infiltration in the host-parasite interface with polymorphonuclear cells and intraparenchymatous edema. Also, in the late phase of infection in those animals the development of ventricularomegaly and inflammation in host tissues and meninges was lower, and cysticerci survival was favored. Both lineages were capable of destroying the cysticerci at 90 DPI, however, in the animals lacking IL-4 it was possible to find larval stage cysticerci during this period. **Conclusions:** According to the anatomopathological and inflammatory process analyses in KOIL-4 animal brains we believe that the absence of IL-4 reduces the intensity of the inflammatory infiltration, ventricularomegaly, gliosis and perivascularitis.

Keywords: Inflammation, neurocysticercosis, BALB/c.

Supporting funding: CNPq

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**B28 THE INFECTION OF SWISS MICE WITH LEISHMANIA (V.) BRAZILIENSIS RESULTS IN A CHRONIC INFECTION AND CONFER PARTIAL PROTECTION TO INFECTION BY L. (L.) AMAZONENSIS.**


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2. Biotério Thomas George, Centro de Biotecnologia/UFPB, Brasil

**Introduction:** The American cutaneous leishmaniasis (ATL) comprises a broad spectrum of clinical manifestations characterized by infections confined to the integument. It is documented that the species of Leishmania braziliensis and L. amazonensis, both associated with clinical forms of ATL, can be found together in some endemic regions of Brazil. **Objectives:** This study aimed to study the infection of Swiss mice with L. braziliensis and investigate whether prior infection with this species interferes with a subsequent infection with L. amazonensis. **Methodology:** Initially Swiss mice (groups of 10 animals) were infected in the left hind paw with promastigotes of two Leishmania species alone: L. braziliensis and L. amazonensis. Parallel groups of animals were coinfected: 14 promastigotes of two Leishmania species alone: L. braziliensis and L. amazonensis. Parallel groups of animals were infected: 14 weeks after infection with L. braziliensis the right hind paw were infected with L. amazonensis in the left hind paw. The development of lesions was monitored weekly with the aid of a caliper. **Results:** After two weeks of infection the mice infected only with L. braziliensis developed a small lesion at the site of infection (0,20 ± 0,05 mm), which remained without spontaneous cure throughout the observation period (18 weeks). Differently, mice infected only with L. amazonensis developed lesions with a continuous growth reaching a mean size of 4,32 ± 0,58 mm at 9 weeks of infection. However, in animals that were previously infected with L. braziliensis, the lesions due to infection L. amazonensis were significantly lower than in animals infected with only L. amazonensis. **Conclusion:** Swiss mice experimentally infected with L. braziliensis develop a chronic infection and partial protection from subsequent infection with L. amazonensis.

Keywords: Leishmania braziliensis, Leishmania amazonensis, coinfection.

Supporting funding: CAPES

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**B29 USE OF LED PHOTOTHERAPY λ846NM IN CUTANEOUS WOUND IN UNDERNOURISHED RATS: A IMMUNOHISTOCHEMICAL STUDY.**


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2. Oswaldo Cruz Foundation, Brazil.

**ABSTRACT**

**Introduction:** Undernourished patients often present larger and/or chronic wounds which is a main reason that lead to long term treatment at public or private health services. Phototherapies have been suggested as an effective method to improve wound healing. **Objective:** This study evaluated, by immunohistochemistry, the differences in the expression of ECM (extracellular matrix) proteins tenasin and fibronectin in healing of cutaneous wounds in nourished and undernourished rats treated or not with infrared LED (Light Emitting Diode) phototherapy (λ846nm). **Methodology:** 30 Wistar rats, aging 21 days, were randomly distributed into nourished (Standard diet) or undernourished (DBR-Diet Basic Regional) group. After 70 days diet, the rats had a standardized wound created on the dor...
ling n4 between 61 to 80 years. With respect to the
between 41 to 60 years, while cases in 57.15 total
20 to 40 years, as no 2, i.e. 28.57 ranging in age
males, n1 if 14.28 fall in the age group between
80 years of age. In the case of cases obtained from
n1 case, namely, 16.66, was around between 61 to
No 3, fall in the range between 41 to 60 years and
46.16. Of cases obtained from female n 6, 33.33, fit
53.84 belonged to male corpses, against female
rhythm aortic dissecting.

Introduction: The dissecting aneurysm of the aorta
is defined as the separation of the tunics that make
up the artery, evidenced by the formation of a vir-
ual light between the adventitia and the intima.
Objectives: to Correlate the clinical and epidemi-
ological findings of major cases involved dissecting
aneurysm by corpses of the thoracic segment. Me-
thodology: we conducted a study on the verifica-
tion service of deaths, at the Federal University of
Brasil

B30 CORRELATION CLINICAL-EPIDEMI-
LOGIC OF CASES DISSECATING ANEURYSM
OF THE AORTA HUMAN THORACIC SEGMENT.
Tenório, P.P.; Moura, M. R. C. G.; Souza, S. L.;
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Brasil

Introduction: the dissecting aneurysm of the aorta
is defined as the separation of the tunics that make
up the artery, evidenced by the formation of a vir-
ual light between the adventitia and the intima.
Objectives: to Correlate the clinical and epidemi-
ological findings of major cases involved dissecting
aneurysm by corpses of the thoracic segment. Me-
thodology: we conducted a study on the verifica-
tion service of deaths, at the Federal University of
Pernambuco, in which they were obtained autopsy
protocols of corpses affected by cases n13 aneu-
rysm aortic dissecting. Results: Of the dissecting
aneurysm involved aortas n13 of thoracic segment,
53.84 belonged to male corpses, against female
46.16. Of cases obtained from female n 6, 33.33, fit
in the age group between 20 to 40 years, while 50
No 3, fall in the range between 41 to 60 years and
n1 case, namely, 16.66, was around between 61 to
80 years of age. In the case of cases obtained from
males, n1 if 14.28 fall in the age group between
20 to 40 years, as no 2, i.e. 28.57 ranging in age
between 41 to 60 years, while cases in 57.15 total-
ing n4 between 61 to 90 years. With respect to the
location of the anterior chest pain seen in relation
to other organic sites in two genera considered. In
all, considering the two sexes, n=9 69.23 cases
had as introduction the systemic arterial hyperten-
sion (SAH), followed byn=6 smoking cases corre-
sponding to 46.15 and n5 38.46 alcoholism cases.
Conclusion: most of the cases occurred in males,
having as main introduction HAS, smoking and al-
coholism.
Keywords: Aneurysm, Dissecting Aneurysm, Epide-
miology.

B31 HISTOPATHOLOGICAL EVALUATION
AND COX-2 EXPRESSION IN SKELETAL MUS-
CLE LOCATED DISTANT BURN INJURY
IN YOUNG RATS.
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NT; Ribeiro, DA; De Oliveira, F
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Introduction: Burn injury (BI) in children is associa-
ted with a pronounced persistence of catabolism,
resulting in muscle wasting, tissue loss and inflam-
mati
DIETS. Taffarel, A.A.; Tomé, T.C.; Moretto, TL; Ribeiro, D.A.; De Oliveira C.A.; De Oliveira, F.; 1 Department of Biosciences, Federal University of São Paulo (UNIFESP), Santos-SP, Brazil.

Introduction: Diets with low protein content cause loss of muscle mass by catabolism state similar to fasting. Obesity is accompanied by generalized inflammation characterized by systemic inflammation. Cyclooxygenase-2 (COX-2) is an enzyme involved in different stages of inflammation. Herein, it would be interesting to know if, and to what extent, COX-2 expression is closely involved to different diets. Objectives The purpose of this study was to investigate the histopathology as well as COX-2 expression in skeletal muscle in mice submitted to hyperlipidemic and hypoprotein diets. Methodology: A total of 15 male C57BL/6 mice were distributed into three groups (n=5) according to diet administered for 12 weeks: Control (C) 17% of protein; Hypoprotein (HYPO) - low protein (6%); Hyperlipidic (HYPER) - high fat (34%). Procedures were approved by the Experimental Animal Use Committee of UNIFESP (CEUA 1750/11). After euthanasia, the medial part of gastrocnemius muscle were taken and the specimens were stained with H&E and Sirius Red to differentiate type I and III collagen. Results: COX-2 expression was assessed by immunohistochemistry. C group sections exhibited equidistantly polygonal muscle fibers with peripheral nuclei, characteristic of normal muscle. The HYPO group sections did consistently show these characteristics; many fibers in these sections exhibited a rounded contour, greater interfiber distances and smaller fibers. Sirius Red showed a substantial increase in the amount of type I collagen in the perimysia of HYPO muscle compared with C. HYPER group sections shows fibers with rounded contour, but with area similar to control group. Moderate quantity of inflammatory cells was observed in HYPER vessels and an increased type I collagen compared with C. In the same way of HYPO group, moderate COX-2 immunoreactivity was observed in vessels. Conclusion: HYPER and HYPO diets induced histopathological changes in skeletal muscle and COX-2 expression is abnormally present in vessel wall of these groups.

Keywords: low protein diet, high fat diet, skeletal muscle, COX-2.


B33 LACK OF CCR2 ALTERS THE PATTERN OF INFLAMMATORY ANGIOGENESIS IN MICE. Pollyana Ribeiro Castro; Suzane Mota Marques; Celso Tarso Rodrigues Viana; Luciola da Silva Barcelos; Silvia Passos Andrade. 1 Department of Physiology and Biophysics, Institute of Biological Sciences, Federal University of Minas Gerais

Introduction: The chemokine system is involved in the migration, activation, proliferation of leukocytes and endothelial cells and the chemokine ligand 2 (CCL2) is expressed in a number of inflammatory processes. However, the role of this chemokine in inflammatory angiogenesis has not been totally characterized. Objective: Study the role of CCR2 on blood vessel formation, leukocyte recruitment/activation and collagen deposition on the tissue induced by sponge implantation into mice with deletion gene for this receptor (CCR2-KO). Methodology: We have performed the kinetics of angiogenesis, inflammation and fibrogenesis in polyether-polyurethane sponge discs implanted subcutaneously in female C57/BL6 mice with and without CCR2 (CCR2-WT and CCR2-KO) (n=8). These parameters were evaluated in implants removed at days 1, 4, 7, and 14 post implantation. Results: Angiogenesis was delayed in implants of KO animals at day 14 as determined by hemoglobin (µg/mg) (KO=2.17+0.52 vs WT=3.95+0.38) and blood flow (perfused unit) (KO=123.1+6.44 vs WT=138.6+17.9). Neutrophils accumulation was increased in CCR2-KO implants at day 14, as determined by myeloperoxidase (OD/mg) (KO=24.6+0.24 vs WT= 5.21+1.12). On other hand macrophages accumulation was decreased in CCR2-KO implants at days 4 and 7, as determined by N-acetyl-B-D-glucosaminidase (OD/mg) (KO=2.70+0.30 vs WT=4.06+0.43; day 4). The levels of nitrite (µg/mg) were also reduced in CCR2-KO implants (KO=0.91+0.01 vs WT=3.27+0.50; day 7). CCL2/MCP-1 levels (pg/mg), however was higher in KO implants (0.06+0.003 x 0.05+0.002, day 14). Similarly, collagen deposition (µg/mg), fibrogenic parameter, was higher in KO implants compared with control animals (KO= 0.87+0.07 vs WT=0.64+0.04; day 14). Conclusion: We have shown that inflammation, angiogenesis and fibrogenesis are different between CCR2-WT and CCR2-KO mice, indicating that CCR2 is a critical endogenous regulator of the fibroproliferative tissue induced by the sponge implant. Keywords:Inflammation, angiogenesis, cytokines, CCR2.

Supporting funding: CAPES, CNPq and FAPEMIG-Brazil.

B34 THERMAL INJURY IN RATS PROMOTES LIVER HISTOPATHOLOGICAL CHANGES NOT RELATED TO CASPASE-3 EXPRESSION. Bortolin, JA; Quintana, HT; Silva, NT; Ribeiro; FAP; Ribeiro, DA; De Oliveira, F. 1 Federal University of São Paulo, Brazil.

Introduction: The trauma caused by severe burn injury induces a persistence of catabolism, resulting in systemic consequences that contribute to burn morbidity and mortality. The major frequency of this trauma occurs by scald hot liquids and lesions covering more than 40% of the body exhibit increased metabolism. Autopsies of burned individuals who died have shown significant alterations in liver tissue however. Several studies showed an increase of apoptosis in the liver of mice after severe burn injury. However, the apoptotic mediator Caspase-3 investigation in liver has been poorly documented. Objective: The purpose of this study was to investigate histopathological characteristics as well as Caspase-3 expression in a liver because of burn injury. Methods: First, all procedures were approved by the Experimental Animal Use Committee of UNIFESP (329/12) and after that, a total of fourteen 21 day-old male Wistar rats were distributed into two groups: Control (C) and submitted to scald burn injury (SBI). The burn area was 45% of the total body surface area. 14 days following the thermal injury, animals each group were
 euthanized, fragments of the liver were collected, and Caspase-3 expression was measured by RT-PCR. Histopathological analysis of the liver was investigated by means of H.E. stain. Results: In SBI, histopathological findings revealed morphological changes in hepatocytes and an increased number of Kupffer cells when compared with C. Data of Caspase-3 expression obtained C and SBI groups were submitted to Student t test. Although the histopathological findings, in Caspase-3 expression there was no difference (p>0.05) between C (33.98±0.96) and SBI (34.00±0.49) after injury. Conclusion: Therefore, was concluded that the liver SBI had hepatocytes altered but interestingly this changes were not related to apoptotic mediator Caspase-3. Further studies with are necessary to elucidate the issue. Keywords: thermal injury, liver, apoptosis. Supporting funding: Capes and FAPESP

B35 DEVELOPED OH LIPOSOOMES CONTAINING COMPOUND 2-PHENYL- 4-AMINO-6-P-FLUOROPHENYL-5-CARBONITRILE-PYRIMIDINE WITH ANTITUMORALPROPERTY.

Oliveira, J. V.1; Tavares, C. A.1; Melo, A. O.1; Silva, T. D. S.2; Santos, N. P. S.2 3; Falcão, E. P. S.1
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Introduction: The heterocyclic compounds such as 4-amino-pyrimidine-bissubstituítos (p-F-pyrimidine) have different biological and pharmacological properties. Within this group there are the pyrimidines and their derivatives that exhibit potent antitumor activity. Objective: Develop liposomes containing p-F-pyrimidine and investigate its antitumor action forward experimental solid tumor sarcoma-180. Methodology: Liposomes containing p-F-pyrimidine (Lipo p-F) were prepared by hydrating the lipid film and tested for accelerated stability and long term. Ascites tumor cells sarcoma-180 (5.0 x 106 cells ml-1) were inoculated subcutaneously in the right dorsal region of Swiss mice with a mean weight 30 to 35 grams. Treatment was initiated 24 h after inoculation for 7 consecutive days. Injection solutions of p-F-pyrimidine and Lipo p-F were administered intraperitoneally at a dose equivalent to 15 mg/ kg. The suspension of the p-F-pyrimidine solution was prepared in sterile 0.9% NaCl containing 2.5% Tween 80 (v/v). The control animals were treated with NaCl 0.9% sterile, under identical conditions. At the end of the experiment the animals were sacrificed. The tumor inhibition was determined the average weight of animals treated groups compared to the untreated control group. The histological sections of the tumor were stained with AgNOR technique. Results: After the preparation, the Lipo p-F shown to be homogeneous with Tyndall effect. The average diameter and the polydispersity of the vesicles were 298.75 ± 30.37 and 0.37 ± 0.01 nm, respectively. The encapsulation efficiency was 82.93 ± 0.04%. Treatment with Lipo p-F showed a significant tumor inhibition of 66.47 ± 26.8%, when compared to using the p-F-pyrimidine suspension which showed an inhibition of 50.46 ± 16.24% of the tumor mass. The histochemical analysis showed increased cell proliferation in animals treated with Lipo p-F. Conclusions: The encapsulation of heterocyclic compounds such as 4-amino-pyrimidine-bissubstituídos thus potentiate its antitumor effect. Keywords: pyrimidine, liposomes, cancer. Supporting funding: CAPES/REUNI

B36 FURTIVE LIPOSOOMES CONTAINING BARBATIC ACID Cladonia Salzmanii: DEVELOPMENT AND EVALUATION OF ANTITUMOR ACTIVITY.

Tavares, C. A.1 2; Silva, M. M. B. M 2; Campos, T. A.1; Silva, T. D. S.2 3; Oliveira, J. V.1 2; Ribeiro, J. S.2; Santana, M. C. S.2; Barbosa, J. A. P.2; Magalhães, N. S. S. 3; Santos, N. P. S.1 2 3
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Introduction: Barbatic acid (BA) is a lichenic metabolite that has many biological activity such as antimicrobial, cytotoxic and antitumor. The use of nanoparticulated system in order to directing and controlling the drug release contributes to a better bioavailability, reduced toxicity and increased biological activity. Objective: This work aimed to develop furtive liposomes containing barbatic acid and investigate its antitumor activity. Methodology: The Conventional and furtive liposomes (AB-LC and AB-LF, respectively) containing barbatic acid were produced by the method of hydrating lipid film. Ascites tumor cells sarcoma-180 (5.0 x 106 cells ml -1) were subcutaneously inoculated on right axillary part of female mouse with middleweight between 30.0 and 35 g. The treatment was begun 24 h after inoculation during 7 consecutive days. Barbatic acid injection in suspension, AB-LC and AB-LF, were intraperitoneal administrated at a dose of 20 mg/kg of body weight. The AB suspension was prepared in sterile solution of 0.9%NaCl with 0.05%Tween (v/v). The animals of control group were treated with sterile 0.9%NaCl, under identical conditions. At the end of experiment, the animals were sacrificed. Tumors were removed and weighed. The tumor inhibition was determinate through the middleweight of treated animals group and compared with control group. Results: After developmental process, the formulations AB-LC and AB-LF demonstrated to be stable and homogenous, with tyndall effect. The mean diameter and polydispersity index of vesicles were 125.2 ± 0.58 nm and 0.287±0.006 nm to AB-LC and; 107.8 ± 1.15 nm and 0.303 ± 0.03 to AB-LF. The obtained formulations were stable after 60 days, when stored at 4°C. The treatment of conventional and furtive liposomes containing barbatic acid against sarcoma-180 tumor showed 45.2 and 59.3% of tumor inhibition, respectively, as suspension of barbatic acid showed 42.3% of inhibition. Conclusion: These Results demonstrated that the barbatic acid encapsulation into liposomes provided an improvement in its bioavailability, increasing therefore its
antitumor activity.
Keywords: lichens, barbatic acid, liposomes, cancer.
Supporting funding: FACEPE

B37 ALTERATIONS IN THE RENIN-ANGIOTENSIN SYSTEM IN MICE SUBMITTED TO DIETS RICH IN FRUCTOSE AND SUCROSE.
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\textsuperscript{1} Laboratory of Morphometry, Metabolism & Cardiovascular disease - State University of Rio de Janeiro

Introduction: The chronic ingestion of diets high in simple sugars mimics components of the metabolic syndrome in C57BL/6 mice. Objectives: To evaluate the impact of intake of two diets rich in fructose and sucrose on blood pressure and renin-angiotensin system in the renalology. Thirty C57BL/6 mice were divided into 3 groups: SC (standard chow; 76% of corn starch); HFr (50% of fructose) and HS (50% of sucrose). The three diets were isocaloric (3802.8Kcal/Kg) and differed only in the type of carbohydrate offered. All diets followed recommendations of the AIN-93 M for rodents. Were assessed: body weight, food and water intake, oral glucose tolerance test (OGTT), total cholesterol (TC), triglycerides (TG), insulin, creatinine, urea and uric acid (plasma and urine), blood pressure (BP) and expression of renin, angiotensin II and AT1 receptor by western blot in the kidney. Results: There was no difference in body mass and energy intake of dietary groups studied. The water intake and urine volume were higher in HFr (107% and 136%, respectively; P<0.001) and HS (62% and 65%, respectively; P<0.05) compared to SC. The OGTT was higher in HFr (24%; P<0.05) and HS (25%; P<0.01). It has been observed hyperinsulinemia in groups HFr (95%; P<0.05) and HS (74%; P<0.05). The renin, TG and TG were higher for HFr (40% and 27%, respectively; P<0.01) and HS (35% and 28%, respectively; P<0.01). An increase in BP was observed in HFr and HS groups (18% for both; P<0.001) at levels greater than 140mmHg. An increase in creatinine clearance in the HFr (30%; P<0.001) and HS (10%; P<0.05); clearance of urea in HFr (190%; P<0.05) and HS (142%; P<0.05) and rate of glomerular filtration in HFr (50%; P<0.01) and HS (41%; P<0.05) compared to SC. The plasma and urinary uric acid were higher in HFr (57% and 222%, respectively; P<0.001) and HS (29% and 232%, respectively; P<0.05). The protein expression of renin, angiotensin II and AT1 were higher in HFr (449%, 604% and 832%, respectively; P<0.001) and HS (388%, 344% and 203%, respectively; P<0.05) when compared to SC. Conclusion: The intake of a diet rich in fructose and sucrose causes insulin resistance, dyslipidemia and changes in systolic blood pressure deficit in the regulation of the renin-angiotensin system in the kidney.
Keywords: Fructose, Sucrose, Kidney
Supporting funding: CAPES, CNPq and FAPERJ.

B38 CHANGES IN SALIVARY GLANDS HISTOLOGY AND SALIVARY A-AMYLASE EXPRESSION IN AN ANIMAL MODEL OF UNDERNUTRITION.
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\textsuperscript{5} Departamento de Biologia e Instituto de Ciências Agrárias e Ambientais Mediterrânicas (ICAAM), Universidade de Évora, Portugal;
\textsuperscript{6} ICAAM e Química Orgânica, Produtos Naturais e Agro-Alimentares (QOPNA), Universidade de Aveiro, Portugal.

Introduction: Undernutrition is one of the most common health problems affecting hundreds of millions of people worldwide, being estimated as the cause of about 53% of deaths in young children. Several studies report that undernutrition can affect salivary glands and saliva secretion, namely by reducing salivary flow. Objectives: To assess histomorphological changes and α-amylase expression in mice salivary glands induced by undernutrition.
Methodology: BALB/c female mice were divided into 2 groups: control (N = 5) and undernutrition (N = 5). Undernutrition was induced by administering 60-65% of mean food weight consumed by the animals from control group in the previous day. Care was taken for avoiding daily weight losses greater that 15% live body weight. After 2 weeks, animals were euthanized and major salivary glands removed and processed by routine histological techniques. Sections were stained with H&E, PAS, Alcian Blue pH 2.5 and Alcian Blue-PAS and immunohistochemistry was performed using anti-α-amylase primary antibody (Santa Cruz, sc-46657, 1:1000). Slides were observed under light microscope at 200X magnification, and images acquired by digital camera. Perimeter and area of serous and mucous acini were assessed using SigmaScan Pro 5.0 software. Data were statistically analysed using nonparametric tests and differences considered significant for P<0.05. Results: In mucous glands, higher intensities of Alcian Blue pH 2.5 and PAS staining were observed for undernutrition group, suggesting a greater production of both acid and neutral mucopolysaccharides comparatively to controls. The acinar area of parotid glands was 40% higher in the animals of undernutrition group. No apparent differences in α-amylase expression were observed. Conclusions: Salivary gland hypertrophy occurs in individuals subjected to undernutrition. Despite no differences in α-amylase intensity were observed, the higher acinar areas suggest that changes may be result from the need for greater production of saliva to optimize digestive processes at the oral cavity level.
Keywords: Undernutrition, salivary glands histology, salivary α-amylase
Supporting funding: Acknowledgments This work was funded by FEDER Funds through the Operational Programme for Competitiveness Factors - COMPETE and National Funds through FCT - Foundation for Science and Technology under the Strategic Projects PEst-C/AGR/UI0115/2011 and PEst-C/QUI/UI0062/2011.
We acknowledge also the financial support the Portuguese Science Foundation (FCT) in the forms of Post-Doctoral grants (SFRH/BPD/63240/2009) of E Lamy.

The funding sources played no role in the development of the present work or upon its submission for publication.

B39  EFFECTS OF METABOLIC PROGRAMMING BY MATERNAL HYPERGLICEMIA AND HIGH-FAT DIET ON PANCREATEIC ISLET OF FEMALE OFFSPRING FROM DIABETIC WISTAR RATS.

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Introduction: Maternal hyperglycemia during pregnancy and lactation increases the susceptibility of the offspring to develop morphological disorders that extend into adulthood. Aims: We sought to evaluate the effects of maternal hyperglycemia during pregnancy and lactation in the weight and diameter of the pancreatic islets of female offspring at 100 days of life. Methods: Twelve Wistar rats were divided into two groups: Hyperlipidic Group (GH), diabetic rats (glucose≥300 mg/dL) (n=6), experimentally induced by high fat diet (60% lipid) and streptozotocin (35 mg/kg) and Control Group (CG) (n=6), nondiabetic rats, that received during pregnancy and lactation fat diet (48% lipid) and a control diet based on casein, respectively. At weaning, the female pups (n=6 per group) were weighed and fed commercial diet up to 100 days of life, moment that they were euthanized to remove the pancreas, which was weighed and processed for histological analysis. The diameter of the pancreatic islets were measured with the help of the program ImageScope and were categorized as small (<125μm) and large (>150μm).

Results: During pregnancy and lactation the maternal glycaemia was 90.0±5.5 mg/dL for the GC and 438.5±3.5 mg/dL for GH (p<0.0001). The maternal hyperglycemia did not induce pancreatic hypertrophy in female rats at 100 days of life because the relative weights of the pancreas were similar in both groups (CG: 0.89 ± 0.1%, GH: 0.79 ± 0.1%, p = 0.2213). When evaluating the average diameter, the females from diabetic mothers showed hypertrophy of pancreatic islets (CG: 85.2 ± 1.9μm; GH: 125.3 ± 2.2μm, +46.2%, p<0.0001). When the islets were separated by small and large diameter, we observed that the percentage of small islets is lower in hyperlipidic group compared to the control group (CG: 82.1 ± 2.2%; GH: 60.1 ± 2.1%, -26.8%, p=0.0002). Regarding islets considered large, the hyperlipidic group presents a higher percentage than the control group (CG: 13.6 ± 2.5, GH: 30.5 ± 2.3%, +124.3%, p=0.0082).

Conclusion: The maternal hyperglycemia does not affect the pancreas weight of female offspring at 100 days of life, however the female offspring has hypertrophied pancreatic islets.

Keywords: Gestational diabetes, pancreatic islets, offspring

Supporting funding: CNPq e Faperj

B40  INFLUENCE OF THE COMPOSITION OF DIETARY LIPIDS IN DEVELOPING MURINE MAMMARY CARCINOMA 4T1.

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Introduction: Breast cancer is the second most common type of cancer in the world and among women is the most common. Dietary lipids have been shown to influence breast cancer development at several stages in the carcinogenic process. It is believed that dietary manipulation of fatty acid composition can produce greater benefits than changes in the amount of dietary fat. Objectives: We investigated the effect of dietary fatty acid manipulation on mammary carcinoma growth and metastasis, as well as mortality, in 4T1 murine metastatic breast cancer model.

Methodology: Twenty eight female Balb/c mice were distributed in four randomly groups: The control group (I) was fed with AI-N93G semi-synthetic diet, containing soybean oil (4g/100g); Groups II, III and IV were fed with AI-N 93G semi-synthetic diet containing canola oil, fish oil or flaxseed oil, respectively, replacing soybean oil. All groups were fed ad libitum for 50 days, but on the 30th day 4T1 cells (2.5 x 106 cells) were injected in the right posterior flank of the mice. Tumor growth, body weight and food intake were measured weekly. Complete blood count test was performed and total cholesterol and triglycerides contents were measured in serum. Results: Tumor growth, tumor weight, body weight and food intake did not differ between groups. Group IV showed increased serum triglycerides (192.90±15.27 mg/dL) and cholesterol levels (209.50±20.08 mg/dL) compared to control group (170.60±24.18 mg/dL; 133.00±28.39 mg/dL, respectively) and lower relative liver weight (0.0493±0.0040%) compared to Group II (0.0581±0.0080%). Group III showed elevated serum triglycerides levels compared to control group (186.50±18.58 mg/dL vs. 170.60±24.18 mg/dL, respectively). All groups had increased platelet counts (II=358.70±80.45; III=364.20±132.90; IV=357.40±180.60) compared to control group (186.50±18.58 mg/dL; 170.60±24.18 mg/dL, respectively).

Conclusions: Dietary manipulation of fatty acid composition produces metabolic changes in 4T1 metastatic breast cancer model. Tumor growth, body weight and feed intake were not different between groups. Group IV showed increased serum triglycerides and cholesterol levels compared to control group (170.60±24.18 mg/dL; 133.00±28.39 mg/dL, respectively) and lower relative liver weight (0.0493±0.0040%) compared to Group II (0.0581±0.0080%). Group III showed elevated serum triglycerides levels compared to control group (186.50±18.58 mg/dL vs. 170.60±24.18 mg/dL, respectively).

Supporting funding: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES)

B41  MORPHOMETRIC AND 8-OHdG IMMUNOHISTOCHEMICAL ANALYSIS IN SKELETAL MUSCLE OF MICE SUBMITTED TO HYPERLIPIDIC AND HYPOPROTEIC DIETS.

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**Introduction:** Fat diets induce obesity and this is associated with increased occurrence of numerous diseases including hypertension, atherosclerosis, insulin resistance and diabetes, comprising a metabolic syndrome and accompanied by generalized inflammation. Malnutrition causes skeletal muscle loss and promotes fiber atrophy. Hydroxy-2'-deoxyguanosine (8-OHdG) is one of the predominant forms of free radical that cause oxidative stress and therefore has been widely used as a biomarker for this kind of stress in the DNA. **Objectives:** The purpose of this study was to investigate morphometric changes and the 8-OHdg expression in a skeletal muscle in mice submitted to hyperlipidemic and hypoproteic diets. **Methodology:** A total of 15 male C57BL/6 mice were distributed into three groups (n=5), according to diet administered for 12 weeks: Control (C) - 17% of protein; Hypoproteic (HYPO) - low protein (6%); Hyperlipidic (HYPER) high fat (34%). Procedures were approved by the Experimental Animal Use Committee of UNIFESP (CEP 1750/11). After euthanasia, the medial part of gastrocnemius muscle was taken and the specimens were stained with H&E and 8-OHdG expression was assessed by immunohistochemistry. Morphometric investigation was performed through the analysis profile fiber area (PFA) of muscle fibers and the density of muscle fibers (number of fibers/mm²). Statistical analysis of data was performed using one-way ANOVA. **Results:** HYPO and HYPER diets induced increased 8-OHdG immunopexpression compared with C. In the morphometric analysis, the PFA obtained from the HYPO (1007.82 +/-345.94 μm²) was smaller (p<0.05) than HYPER (1143.88 +/-372.17 μm²) and C (1206.92 +/-430.01 μm²) groups. The density of muscle fibers of HYPO (920.20 +/-79.38 fibers/mm²) was higher (p<0.05) than the HYPER (720.20 +/-79.38 fibers/mm²) and C (830.40 +/-92.21 fibers/mm²) groups. **Conclusion:** HYPO diet induced morphometric changes in skeletal muscle and HYPE and HYPO diets are able to abnormally 8-OHdG expression. Keywords: diet, obesity, skeletal muscle, 8OHdG.

**Supporting funding:** CNPq and FAPERJ

B43 THE EFFECT OF TANNIC ACID ON THE HISTOMORPHOLOGY AND EXPRESSION OF Α-AMYLASE OF THE LINGUAL SALIVARY GLANDS IN MICE (MUS MUSCULUS).
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**Introduction:** Some studies showed that rats and mice fed diets containing tannins induce changes in serous cells of salivary glands and influence the secretion of several proteins, including α-amylase. Despite this protein being mainly produced by parotid, lingual serous glands (von Ebner’s) also produce it, but in these the effects are less studied. Moreover, most information concerns tannins in solid diet with fewer studies in liquids. **Objectives:** To assess histomorphological changes and the respective diets for 4 weeks; throughout the experiment, body mass, food intake and oral glucose tolerance were evaluated. After the mice were euthanized, the liver was removed and processed for histomorphometrical and molecular analysis. Blood samples were obtained for serum analysis. The data were tested by one-way ANOVA with a Holm-Sidak post-hoc test and were expressed as the mean ± standard error of the mean; the significance level was set at p < 0.05. **Results:** The HF (61.8 ± 2.5g) and HFHSu (62.4 ± 1.3g) groups were heavier than the SC (51.2 ± 0.8g) and HSu (50.8 ± 1.2g) groups. Animals from the HF, HSu and HFHSu groups presented glucose intolerance and the HFHSu group had poorer results in oral glucose tolerance test compared to the HF (+19%, p<0.0001) and HSu (+22%, p<0.0001) groups. Excessive fat and/or sucrose intake yielded hepatomegaly in the HF (+32%, p<0.0001), HSu (+23%, p<0.0001), and HFHSu (+51%, p<0.0001) groups when compared with the SC group. Furthermore, the HF, HSu and HFHSu groups (p<0.0001) had higher rates of hepatic steatosis and augmented hepatic triglycerides than the SC control group. The high-fat (+42%, p<0.0001) and high-fat-high-sucrose (+42%, p<0.0001) groups exhibited greater percentages of steatosis in comparison with the HSu group. There was an elevation in liver SREBP-1c, Glut-2, PEPCK, G6PASE, IRS-1, AKT protein expression and a reduction in PPAR-alpha expression in the experimental groups- livers compared to those of the SC group (p<0.0005). **Conclusions:** Administration of high-fat and/or high-sucrose diets promoted glucose intolerance and liver damage in adult male mice. Keywords: high-fat diet, high-sucrose diet, liver

**Supporting funding:** CNPq and FAPERJ
α-amylase expression in lingual salivary glands of mice induced by ingestion of tannic acid in solution.

**Methodology:** BALB/c-/- male mice divided into 2 groups, control (N = 5) and tannic acid (N = 5) in drinking water (1.0 mM), receiving the same standard diet. After 10 days of experiment, the animals were euthanized, tongues removed and processed by routine histological techniques. Sections were stained with H&E, PAS, Alcian Blue pH 2.5 and Alcian Blue-PAS and immunohistochemistry was performed using an anti-α-amylase antibody (Santa Cruz, sc-46657, 1:1000). Slides were observed under light microscope at 200X magnification, and images acquired by digital camera. Perimeter and area of serous and mucous acini were assessed using SigmaScan Pro 5.0 software. Data were statistically analysed through nonparametric tests, using SPSS 16 software. Differences were considered significant for P <0.05. **Results:** Groups did not differ in terms of histomorphometry of the serous and mucous acini of lingual salivary glands. Regarding α-amylase, positive staining was observed in serous acini of both groups but without apparent differences between them. **Conclusions:** Inversely to studies in major salivary glands, the results suggest that the administration of tannic acid in the drinking water did not induce morphological changes or different expression of α-amylase in lingual salivary glands.

Keywords: Tannic acid, lingual salivary glands, salivary α-amylase

Supporting funding: Acknowledgments This work was funded by FEDER Funds through the Operational Programme for Competitiveness Factors - COMPETE and National Funds through FCT - Foundation for Science and Technology under the Strategic Projects PEst-C/AGR/UI0115/2011 and PEst-C/QUI/UI0062/2011.

We acknowledge also the financial support the Portuguese Science Foundation (FCT) in the forms of Post-Doctoral grants (SFRH/BPD/63240/2009) of E Lamy.

The funding sources played no role in the development of the present work or upon its submission for publication.
C01  THE SEMI-SYNTHETIC INGENOL C DERIVED FROM EUPHORBIA TIRUCALLI INDUCES CELL DEATH VIA AUTOPHagy BUT NOT APOPTOSIS IN HUMAN GLIOMA CANCER CELLS.

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Background: Autophagy is originally named as a process of protein recycling and represents a major route for degradation of aggregated cellular proteins. Recently, the cytotoxicity of some anticancer agents is reportedly linked to autophagy induction.

Objectives: The present study aimed to investigate the cytotoxic activity of ingenol C (ingenol-3-do-decanoate), a semi-synthetic compound from Euphorbia tirucalli, in human glioma cancer cell lines.

Methodology: Glioma cell lines were treated with ingenol C, and their proliferative ability was analyzed by the MTS assay and the colony formation assay. Apoptosis was quantified by flow-cytometry after double-staining of the cells with Annexin V/PI. The cell cycle was evaluated by flow-cytometry after staining of cells with PI. Additionally, we evaluated the proteome profile analysis by apoptosis and stress cell arrays.

Results: Ingenol C exhibited dose-time-dependent cytotoxic effects on glioma cell lines and effectively reduced colonies formation on GAMG cells. Flow cytometry further revealed U373 and GAMG cells treated it that compound to be arrested in S phase and does not induces apoptosis. The expression of the autophagy-associated protein LC3-II, was analyzed by Western standard Charger la version blotting and it was demonstrated a markedly increase in Ingenol C-treated glioma cells. Furthermore, proteome profile analysis showed down-regulated expression of pro-apoptotic proteins (Cytochrome C, BAX and Hif1 alpha) whereas the anti-apoptotic protein p21CIP/WAF1 was upregulated in both GAMG and U373 cells. More importantly, cells treated with bafilomycin A1, a specific inhibitor of vacuolar type H(+)-ATPase that prevents autophagy at a late stage, showed an increase in the ingenol C cytotoxicity.

Conclusions: These results indicate that ingenol C induced cell death, autophagy but not apoptosis on glioma cells, supporting that activation of autophagy plays a crucial role on the mechanism of action of this drug. Autophagic cells death induction by ingenol C underlines the potential utility of its induction as a new cancer treatment modality.

Keywords: Euphorbia tirucalli; glioma cell lines; autophagy.

Financial Support: Amazônia Fitomedicamento and Barretos Cancer Hospital.

C02  MOLECULAR DETERMINANTS FOR PRIMARY RESISTANCE TO ANTI-EGFR DRUGS IN HEAD NECK CANCER CELL LINES

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Introduction: Head and Neck squamous cell carcinoma (HNSCC) is the sixth most common type of cancer worldwide. EGFR is a receptor tyrosine kinase that activates downstream signaling pathways, including the Ras-MEK-Erk and the PI3K-AKT pathways. EGFR is a target for targeted therapies in patients with HNSCC, being cetuximab, an anti-EGFR monoclonal antibody. However, there is a lack of predictive factors of cetuximab efficacy and new approaches for EGFR targeting in HNSCC tumors are needed. Objectives: To molecular characterize HNSCC cell lines and correlate with the response to anti-EGFR therapies.

Methods: Seven HNSCC cell lines were tested for the efficacy of cetuximab and AST1306 (an irreversibly anti-EGFR drug) by MTS assay. Their inhibitory effect on EGFR and its intracellular signaling pathways was assessed by western blot. Mutational status of EGFR, PTEN, PIK3CA, BRAF and KRAS was determined by direct sequencing. Results: We found that the cell lines depicted different sensibilities to the inhibitors, being overall more sensitive to AST1306 when compared to cetuximab, even that ones that were completely resistant to cetuximab. The HN13 cell line, resistant to both therapies, harbors an EGFR mutation (His773Tyr). The efficiency of the anti-EGFR drugs seems to be related to its capacity to inhibit the PI3K-AKT pathway, and the combination with an AKT inhibitor restores the sensitivity in HN13 cell line to anti-EGFR therapy.

Conclusion: Our work suggests that the pharmacological efficacy of anti-EGFR therapy is greater with an irreversible inhibitor and the combined inhibition of AKT phosphorylation protein appears to be fundamental for an effective anti-EGFR therapy response in HNSCC.

Key-Words: HNSCC, Anti-EGFR therapy, AKT

Acknowledgments: PAIP – Program of support for researchers – Barretos Cancer Hospital - SP

C03  EFFECTS OF VITAMIN D SUPPLEMENTATION ON CHEMICALLY-INDUCED PRENEOPLASTIC LESIONS DEVELOPMENT IN MALE Wistar Rats.

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Introduction: Chemically induced putative preneoplastic lesions (PNL) that express the placental enzyme glutathione S-transferase (GST-P) have been well characterized in the liver rat. Vitamin D has been categorized as a highly versatile molecule with emerging roles in fibrosis, fatty liver diseases, alcoholic liver diseases and cancer. Objectives: The modifying effect of active metabolite of vitamin D (1alpha,25-dihydroxyvitamin D3 - ViitD3) supplementation on GST-P-positive development was investigated in male Wistar rats.

Methodology: Male Wistar rats were randomly divided into six
groups (15 animals each), G1: untreated control group; G2-G6: groups received a single i.p. dose of diethylnitrosamine (DEN) and weekly intragastric administrations of carbon tetrachloride (CCl4); G3-G6: groups received VitD3 supplementation at doses of 250, 500, 1000 and 1500 mg/kg, respectively, on alternate days by gavage for 16 weeks. At the end of week 18, the animals were euthanized and blood (AST and VD3 serum levels analysis) and liver (GST-P-positive PNL and cell proliferation analysis) samples were collected. Results: Groups receiving 1000 and 1500 mg/kg vitamin D3 presented significantly lower mean body weight evolution and food intake in relation to groups G1 and G2. The relative liver weight was significantly higher in groups G5 and G6 when compared to the groups G1 and G2, but without a significant change in AST levels. The number of larger GST-P-positive PNL was significantly lower in the groups G3 to G6 when compared to the group G2, without a clear dose-response. In addition, the hepatic cell proliferation levels (Ki-67) were significantly lower in groups G5 and G6 when compared to the groups G1 and G2. Conclusion: High doses of VitD3 supplementation has beneficial effects against GST-P-positive PNL and cell proliferation in DEN/CCl4–treated animals, but resulted in systemic toxicity, as verified by body weight gain, reduced food intake and increased relative liver weight. Keywords: Hepatocarcinogenesis, Vitamin D supplementation, Preneoplastic lesions, Rat liver

Introduction: Ehrlich tumor is a species-specific, transplantable neoplasia malignant epithelium that corresponds to mice’s mammary adenocarcinoma. It is used to study biological and chemical components in cell proliferation, being excellent model for the search for natural compounds that have inhibitory power on tumor growth, which has motivated many researches in the field of oncology. Objective: To evaluate antitumor activity of X. aromatic in Ehrlich carcinoma. Methodology: This work was submitted and accepted by the ethics committee. Tumors were induced in mice by intraperitoneal injection of Ehrlich ascites carcinoma (EAC). For perform the tests, the cells were removed EAC. These cells were incubated with different concentrations of hydroalcoholic extracts leaves of X. aromatic [0.005mg/mL (B), 0.0007mg/ml (C) and 0.0003mg/mL (D)]. Cell viability was assessed by the tripan blue method. The cell count of the wells was performed every 2 hs after treatment application, and a count at 24 hours. To assess solid tumor growth, were inoculated 2.5 x 106 tumor cells in the right hind paw of each animal. The animals were divided into 3 groups of 10 animals each, as follows: one group received 30mg/kg another, 150mg/kg, both concentrations diluted in DMSO, the control group received DMSO at the same concentration diluted in water. The growth of tumors was monitored for 20 days. The tumor and popliteal lymph nodes was examined for histopathological study. The necrotic areas were quantified. Results: All concentrations of plant extract were effective in inhibiting tumor growth in vitro. In the evaluation of the proliferation curve of the Ehrlich tumor in vitro, it was observed that with the passage of time the number of tumor cells was declining. A remarkable fact was the reduction by over 50% in the number of viable cells in the first hour for the treatment of 0.005mg/ml concentration. The concentration higher decreased tumor growth in tumor-legged animals. The necrotic area in the control group compared to the treatment group was greater 150mg/kg also this concentration decreased tumor growth. Conclusion: The crude plant extract of leaves of X. aromatic inhibits cell proliferation and is cytotoxic. Treatment by gavagem showed lower area of necrosis in the concentration 150mg/kg.
Keywords: Tumor Ehrlich, Cell Culture, Plant extracts

Supporting funding: FAPEMIG

C06 ANALYSIS OF CDKN1A GENE EXPRESSION AND ITS RESPECTIVE PROTEIN IN FOLIC ACID FORTIFICATION IN COLORECTAL CARCINOGENESIS IN RATS

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Introduction: Experimental studies suggest that folic acid fortification may have a paradoxical role in the development and progression of colorectal cancer. Objective: Evaluate the effects of depletion, fortification and supplementation followed by folic acid fortification in cell cycle markers in colorectal carcinogenesis in rats. Methodology: Wistar male rats received one of four interventions: standard AIN-93M diet throughout the experiment (G1), folic acid free diet throughout the experiment (G2), diet with 3.25 mg of folic acid throughout the experiment (G3) or 5mg folic acid diet before induction of carcinogenesis followed with 3.25 mg of folic acid to the end of the experiment (G4). Colorectal carcinogenesis was induced by 4 intra-rectal doses of MNNG (5mg/mL) twice per week for 2 weeks. Analysis: P21 protein expression by western blotting; proliferating cell nuclear antigen (PCNA) expression by immunohistochemistry; CDKN1A gene expression analysis by RT-qPCR. Statistical analysis: ANOVA, p <0.05. Results: G4 showed increased expression of p21 than G2 (p <0.05). G3 showed higher CDKN1A gene expression than G1 and G4 (p <0.05). G3 showed lower expression of PCNA among the groups studied (p <0.05). Conclusion: Depletion with folic acid (G2) appears to stimulate the cell cycle without affecting cell proliferation. The fortification, despite being able to modulate the gene expression of CDKN1A does not increase the expression of their respective protein and decreases the expression of PCNA, suggesting the occurrence of a post-transcriptional or post translational modification that could be protective.

KEYWORDS: Folic Acid; p21; Colorectal carcinogenesis

Supporting funding: FAPESP

C07 ANALYSIS OF CYTOKINE SYNTHESIS AFTER IMMUNOTHERAPY WITH DENDRITIC CELLS IN MICE WITH BREAST CANCER INDUCED BY 4T1 CELLS.

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Introduction: Although uncommon, breast cancer in men and women is similar and is most frequent associated to hormone receptor positivity. Risk factors include family history, testicular disease, benign breast conditions, age and the Klinefelter syndrome. Objectives: Analyze the modulation of the immune system from the treatment with vaccines of DCs by cytokines: IFN-γ, TNF, IL-10 and IL-12, in a male model of breast cancer. Methodology: The experimental model of present study is male Balb/c mice (10 wks old), divided into three groups: Control Group, Tumor Group (induced tumor and treated with saline solution 0.9%), Treated Tumor Group (induced tumor and treated with vaccine). After 7 days of induction of the breast tumor, applying 4T1 cells, held three doses of vaccine DCs, DCs with 5.0 x106 cells for mice in the group treated tumor, weekly. After 1 week from the last vaccination the animals were sacrificed. Cytokine secreted by spleen cells were analyzed by flow cytometry. Results: Was observed a trend to decreased IFN-γ and TNF in treated group compared to untreated tumor group (p=0.367 e p=0.732 respectively). With relation to IL-12, a trend towards to increase in the treated group compared to the control group, without statistical difference between the groups. Regarding the group treated tumor, IL-10 demonstrated a tendency to increase, when compared to untreated tumor (p=0.367). Conclusion: This study indicates that the dendritic cell vaccine is able to enhance the production of IL-12, however further studies are needed.

Keywords: Dendritic Cells, Immunotherapy, Cancer Supporting funding: Fapemig, Capes, Cnpq, Finep e Funepu.

C08 ANALYSIS OF THE EFFECTS OF IMMUNOTHERAPY WITH DENDRITIC CELLS BY THE EXPRESSION OF CYTOKINES IN THE CULTURE SUPERNATANT OF SPLEEN CELLS IN EXPERIMENTAL BREAST TUMORS.

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Introduction: The immunotherapy has been studied as an alternative treatment of lots of types of cancer. The studies with vaccine of dendritic cells in experimental models are used to understand the mechanisms of the immune system against tumors. Objective: The objective were to analyze the influence of vaccine with dendritic cells, through the cytokines synthesis in animals induced by the treatment with 7,12 - DIMETIL-BENZANRACENO to develop tumor. Methodology: We used 42 females mice BALB/c with 8 weeks. DMBA was administered weekly during six weeks. Then the animals passed by a period of incubation (16 weeks). And then followed a treatment: control group (no tumor), tumor group (tumor induced and treated with saline solution) and treated tumor group (tumor induced and treated with vaccine). Four doses of vaccine with DC or physiologic solution, were applied in each animal with an interval of 15 days. After the 4 doses of vaccine the animals were euthanized, the spleen removed and the cells obtained was maintained in culture. Was evaluated by ELISA, the synthesis of the major cytokine profile Th1, Th2 and Treg cells: IL-2, IL-4, IL-10, IL-12, IFN-γ, TNF-α and TGF-β. Results: There was increase in the concentration of TGF-β in relation IFN-γ / TGF-β in the tumor group compared to the group Tumor Tre-
aty, with statistical significance, and consequently the synthesis of IFN-γ was lower than in group Tumor Treaty. The relationship between IL-12 / IL-4, showed an increase, without significant differences in the group Tumor Treaty. Conclusion: The immunotherapy with DC was able to decrease the Treg profile with an increase of Th1 profile in the treated tumor favoring the effectiveness of the response antitumoral. Keywords: Immunotherapy, Dendritic cells, Cancer Supporting funding: Fapemig, Capes, CNpq, Finep e Funepu.

C09 ANTI-TUMOR POTENTIAL OF PSORALEN-RELATED COUMARIN DERIVATIVES IN AN IN VITRO MODEL OF GIOBLASTOMA. 
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Introduction: Brain tumors have been studied for a long time, nevertheless the impact of technological advances on clinical outcome has not been satisfactory. Then, new therapeutic approaches, treatments and drugs are necessary. Objectives: The purpose of this study was to evaluate the antiproliferative activity of coumarin derivatives in an in vitro model of glioblastoma, since it was previously demonstrated that 8-methoxypsoralen (a furanocoumarin derivative) acts as antiproliferative drug on this model. Methodology: Rat glioma C6 cells and human glioblastoma GL-15 cells were used in tests with compounds at increasing concentrations, as well as rat astrocytes for comparison. Cell viability was measured in sub-confluent cultures by the MTT assay and cell morphology was accessed by phase contrast microscopy. Results: All coumarin derivatives showed more significant activity in tumor cells than in normal ones. The coumarin core alone did not show considerable activity, while the non-methoxylated psoralen was toxic for both tumor cells and astrocytes. If, however, the furan ring is removed the structure, but the oxygen atom preserved, the activity remains restricted to tumor cells. Moreover, the presence of polar groups such as methyl or prenyl seems potentiate the effect. Besides decreased cell viability, some compounds also promoted visible changes in the morphology of tumor cells. Conclusions: Our data pointed coumarin derivatives as a possible new class of antiproliferative agents, and gave relevant information about structure-activity relationship of these compounds. Keywords: coumarin, glioma Supporting funding: CNPq

C10 ANTITUMOR ACTIVITY OF ALBENDAZOLE AGAINST ASCITIC ERHLICH TUMOR. 
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Introduction: The dependence of tumor growth on the development of neoangiogenesis is characteristic of cancer biology. The resulting tumor vasculature governs the pathophysiology of solid tumors and thus tumor growth, invasion, metastasis and malignant ascites. The formation of ascites in patients with advanced-stage cancer is a difficult problem to manage in clinical oncology. Study in OVCAR-3 tumor-bearing nude mice treated with intraperitoneal albendazole (ABZ), a widely used benzimidazole carbamate anthelmintic, demonstrated the supression of malignant ascites. Objectives: In the present study we tested the efficacy of ABZ against tumor growth, ascitic development and local angiogenesis on mice bearing the ascitic Ehrlich tumor (AET). Methodology: Twenty four BALB/c female mice 8 weeks old were inoculated with 5 x 106 tumor cells, and six animals were preserved without manipulation. The inoculated mice were randomly distributed in two groups (12 mice each): SHAM, receiving 1,0 mL hidroperoxi-metil-ceulose(HPMC)/ip.; and ABZ, receiving 1,0 mL albendazole (150mg/kg) suspended in HPMC/ip. The animals were sacrificed 3 and 5 days after treatment. The ascitic fluid were collected and quantified, and the tumoral cells counted. The peritoneal walls were fixed in 10% formalin, histologically processed and factor VIII antibody was applied to sections by streptavidin/biotin/HRP method. Results: The tumor cellularity of ABZ animals was reduced and the peritoneal vesselarity increased when compared with SHAM group (p<0,001, ANOVA), without effect upon malignant ascites. Conclusions: ABZ in a single intraperitoneal dosis inhibits in vivo tumor growth in AET bearing mice. Further studies will be necessary to elucidate the exact mechanisms involved. Keywords: albendazole, ascitic Ehrlich tumor, mice Supporting funding: PIBIC/Santander
pretorium (25mg/kg, 50 mg/kg and 100 mg/kg) or the vehicle were administered orally for 7 days. On the 8th day, the mice were sacrificed and the organs were excised, weighed and examined macroscopically. The blood was collected by cardiac puncture. Tumor inhibition ratio (%), changes in body weight, food and water consumption during the experiment were calculated. Statistical evaluation was performed using the statistical program GraphPad Prism® 5 with Student’s t test for unpai red data or ANOVA followed by the Tukey’s test. The values were expressed as mean ± SEM with the level of significance set at p < 0.05. Results: Tumor inhibition rates were 19%, 44% and 77% at 25 mg/kg, 50 mg/kg and 100 mg/kg doses, respectively. A significant decrease in water consumption at a dose of 100mg/kg was observed. There was a significant reduction in stomach weight, a significant increase in the number of monocytes and lymphocytes and decrease of neutrophils in the treated group at a dose of 50mg/kg. In macroscopic evaluation of organs, it was observed the existence of a possible spontaneous metastasis in the lungs of an animal in the control group. Conclusion: The results of this study showed that A. vepretorium has a potentially useful inhibitory effect on tumor growth. Keywords: Annona vepretorium, Antitumor activity, Sarcoma-180

C12 ANTITUMOR ACTIVITY OF THE CRUDE EXTRACT PLANT X. AROMATIC IN EHLICH SOLID TUMOR. Silva, A. G. 1; Moreira, G.A. 1; Gomes, I.N.F. 1; Cipriano Junior, N. M. 1; Santos, H.B. 2; Thomé, R. G. 2; Ribeiro, RIMA 1
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Introduction: The search for new substances with antitumor activity has been growing, with a view that cancer is the second leading cause of death by disease in the world. It is reported in literature that the plant X. aromatic has substances with antitumor activity in some strains of human cancer. Ehrlich tumor has been referenced in research on plant extracts due to its rapid growth. Objective: To evaluate the antitumor activity of the crude aqueous ethanol extract of X. aromatic in Ehrlich solid tumor. Methodology: A total of 60 male mice of Swiss species were utilized. First, 5x106 Ehrlich tumor cells were inoculated into the footpad of all animals. After that, the mice were divided into 3 groups of 20 animals each group, subdivided into a control group treated with the diluent (DMSO and water) and a group treated with the plant extract 150mg/kg. The application was made daily by gavage. Each group of 20 animals was divided according to evaluation time of the extract activity on the tumor. In group I, the animals were sacrificed four days after the application of the inoculum; in group II eight days and Group III 12 days. This division was necessary to determine, through blood biochemical and organ histologic analysis, stages when the plant extract had effect. During the treatment period, the control and treatment animals paws were measured using a caliper every 24 hours to assess the evolution of the tumor. Results: In sacrificed animals after four and eight days, there was no effective activity of the plant extract on the tumor. However, from the tenth day, the tumors on treated animals showed size significantly smaller than those in the control group, effect only observed in group III. Conclusion: From these results, it is believed that the plant needs a minimum time to perform minimum biological activity, which slows down tumor growth. Thus, this work represents the first step towards the possible isolation of a compound of X. Aromatic with antitumor activity. Keywords: antitumor, X. aromatic, Ehrlich tumor

C13 CAFFEINE POTENTIATES CITOTOXICITY OF DACARBazine ON MURINE MELANOMA CELL, B16F10. Fagundes, T.; Luiz, R. C. 2; Sanches, L. J. 3

Introduction: The cutaneous malignant melanoma is considered a serious type of cancer due to the high risk of metastasis and low efficiency of the therapies used. To increase the efficiency of anti-neoplastic chemotherapy, adjuvants are used to potentiate the toxic effects on the tumor. Caffeine (CAF) has been proposed as a potentiating agent in the treatment of solid tumors in combination with various anticancer agents, but so far there is no report of the potentiating effect of CAF on melanoma treatment. Objective: The present study aimed to investigate the potentiating effect of CAF on murine metastatic melanoma cells (B16F10) treated with dacarbazine (DAC) - a genotoxic antitumoral agent used in melanoma chemotherapy. Methodology: To assess the antiproliferative capacity of CAF, the cells were treated for 24 hours. After that, trypan blue exclusion assay, cell counting and MTT reduction assay were performed. The Results were compared to control (p<0.05). In the present work CAF was tested in the concentrations of 1, 5, 10, 20 and 40 µM (based on human serum concentrations). Concentrations equal to or higher than 10 µM of CAF revealed a cytostatic effect, cells counting reduced but cell viability was not affected. This effect was previously reported for JB6 CI 41 and myeloblastic leukemia ML-1 cells, and is associated with TP53 expression upregulation and G1/S cell cycle arrest. For potentiating effect evaluation, we used 24h pre-treatment or post-treatment protocols with DAC (250 or 500 µM, 4 h). Cell viability was evaluated by MTT reduction assay. Results: We observed an increased cytotoxicity of DAC, mainly for the pre-treatment protocol with CAF, even at the lowest tested concentration. This result can be explained by the ability of CAF to inhibit DNA repair mechanisms. Our Results indicate that adjuvant treatment with CAF can be an alternative to improve the efficiency of chemotherapy of melanoma, being necessary to conduct further experiments in vitro and in vivo to elucidate the mechanisms involved. Keywords: caffeine, melanoma, dacarbazine

C14 CD133 AND CD34 WITH POTENTIAL MARKERS FOR IMMUNODETECTION OF BREAST CANCER STEM CELLS IN A COMPARATIVE IN VITRO AND IN VIVO STUDY OF EXPERI-
Mental Mammary Carcinogenesis.

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Objective: To determine whether oral administration of different concentrations of grape juice exerts a protective effect in rats treated with azoxymethane (AOM). Material and Methods: Forty male Wistar rats were divided into seven groups: G1, SHAM; G2, treated with AOM; G3, AOM + 1% grape juice administered before AOM; G4, AOM + 1% grape juice administered after AOM; G5, AOM + 2% grape juice administered before AOM; G6, AOM + 2% grape juice administered after AOM; G7: 2% grape juice. The effects of grape juice were evaluated by analyzing the multiplicity of aberrant crypt foci (ACF), COX-2 mRNA and protein expression, and DNA damage by the comet assay. Results: Grape juice at concentrations of 1% and 2% reduced the multiplicity of ACF (foci > 10) (p<0.05) in G4 and G6. COX-2 mRNA was expressed differently (p<0.05) in G3 and G6. Immunohistochemical analysis showed higher COX-2 expression in the group receiving 2% grape juice (p<0.05). No significant differences in genotoxicity were observed between groups (p>0.05). Conclusions: The results suggest a chemoprotective effect of grape juice during the initiation phase of carcinogenesis. Keywords: aberrant crypts, azoxymethane, grape, COX-2.

C16 Citotoxicity Assays of Salazinic Acid from Ramalina Complanata (Lichen).

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Introduction: The neoplastic diseases affect millions of people and the treatment is usually laborious, in many cases with poor prognosis. The search of news antineoplastic compounds are extremely important. Lichen secondary metabolites are known by its biological properties as antimicrobial, anti-inflammatory, antiviral and also antineoplastic. Objective: The aim of this work was isolating and purifying the salazinic acid Ramalina complanata and cytotoxicity against Hela cells were evaluated. Material and Methods: The identification and purification of salazinic acid were performed in thin layer chromatography and a high performance liquid chromatography. The cytotoxicity evaluation of salazinic acid was carried out in Hela cells (human breast adenocarcinoma) in exponential phase of growth. Cells lines were maintained in Dulbecco’s MEM with 10% fetal bovine serum and 1% penicillin/streptomycin at 37°C in 5% CO2. Aliquots of Hela cells (1x105 cells/ml) were distributed into 96
C17 CYTOKINES EXPRESSION IN SPLENIC CELLS OBTAINED FROM MICE WITH BREAST TUMORS EXPERIMENTAL SUBJECT TO IMMUNOTHERAPY WITH INTERFERON ALPHA.
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Introduction: Currently several cancer treatments, including active immunotherapy has been applied with the aim of stimulating the immune system to produce an effective response against tumors. Therefore, immunotherapy with interferon-alpha (IFN-α) have demonstrated efficacy in anti-tumor response in animals and humans. This cytokine has an antitumor effect by inducing apoptosis and suppression of a wide variety of tumor cells and also by inhibiting the process of angiogenesis. Objective: Evaluate by ELISA (enzymelinked immunosorbent assay), the synthesis of cytokine profile Th1/ Th2 and Treg (IL-10, IL-12, IFN-γ, TGF-β) in the culture of splenocytes of mice groups. Methodology: After gavage weekly with DMBA, respect to a range 16 weeks, relative to the period of tumor development. The treatment was given then by the application of 35 vaccine doses in both groups, being three applications for week containing SF 0.9% in the tumor group, and 35 doses of vaccine, with a concentration of 2.1 x10⁵ IU for mice in the group treated tumor, in both groups the immunization was realized by subcutaneous. Results: Analyzing the relation of cytokine synthesis profiles Th1, Th2 e Treg (IL-10, IL-12, IFN-γ, e TGF-β), was demonstrated that after the period of treatment with IFN-α, occur an increase of IFN-γ in the group treated tumor compared to control group. There was also increased expression of IL-12, relative to the other groups, associated with decreased IL-10 and TGF-β, when compared control and tumor groups. Conclusion: In Conclusion, immunotherapy with interferon alpha was able to assist the antitumor immune response with the increase of IFN-γ and IL-12 mainly, favoring the Th1 response. Keywords: Interferon alpha, Immunotherapy, Cancer

Supporting funding: Fapemig, Capes, CNpq, Finep e Funepu.
tem providing a likely response against the tumor. Keywords: Dendritic cell, Tumor, Physical activity
Supporting funding: Fapemig, CNpq, Capes, Finep, Funepu.

C19 DISTINCT TYPES OF TUMORS EXHIBIT DIFFERENTIAL GRADE OF INFLAMMATION AND ANGIogenesis IN MICE.
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Introduction: Inflammation, angiogenesis and cytokine production are common features of almost, if not all tumors. However, the extent of these processes induced by different types of tumors has not been evaluated. Aim: Our aims were to identify the pattern of these processes in tumors of differing origin simultaneously and to characterize the influence of the tumors locally (cutaneous tissue) and systemically (cytokine levels). Methods and Results: We investigated the growth pattern of the experimental metastatic tumors, B16F10 melanoma, CT26.WT colon and 4T1 mammary cells inoculated (106 cells) in the flank of syngeneic mice (n=10) and determined the degree of inflammation, angiogenesis, and production level of pro-inflammatory and pro-angiogenic cytokines within the tumors. The weight of tumors 15 days post-inoculation of 106 cells was markedly different. Melanomas were 2 and 10-fold heavier than colon and mammary tumors, respectively. Locally, CT26.WT tumor cells induced more vessels in cutaneous tissue adjacent to the tumors but systemically, the plasma levels of VEGF were higher (approximately 2-fold) in 4T1 tumor-bearing mice compared with the other two tumors. Mammary tumors presented the most prominent inflammatory content as assessed by a range of markers (inflammatory enzymes and cytokines). The vascular index, as determined by the intra-tumor amount of hemoglobin and number of vessels in hot spot areas, was also higher (approximately 2-fold) in melanomas compared with the other two tumors. Conclusion: These findings showing that distinct tumor types determine differential grade of inflammation, angiogenesis and host interaction in mice may provide new insights to tailor differential therapeutic approach based on the status of tumor biomarkers.
Keywords: melanoma, colon cancer, mammary tumor, cytokines
Supporting funding: CNPq, CAPES, FAPEMIG-Brazil

C20 EFFECT OF ELECTROCHEMOTHERAPY AND CHEMOTHERAPY WITH BLEOMYCIN ON EHRLICH TUMOR GROWTH AND IMMUNITY.
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Introduction: Electrochemotherapy (ECT) is a combined modality treatment using chemotherapy and electroporation. Electroporation is a physical method that involves the application of high-voltage direct-current electric pulses to cells or tissues that cause the permeabilization of the plasma membrane to increase cell uptake of molecules such as enzymes, dyes and drugs. Although various anticancer drugs have been employed in ECT, the cytotoxic effect of bleomycin has been more significantly enhanced by electroporation than that of any other anticancer drug. In parallel, a possible involvement of antitumor immunity in the effects of ECT remain unclear. Objectives: In the present study we evaluate the effects of ECT and chemotherapy with bleomycin on Ehrlich tumor growth, and lymphoid tissues. Methodology: The study involved 40 male BALB/c mice, divided in groups control-SHAM, experimental non-treated, chemotherapy and ECT with bleomycin. The treatment was applied after 30 days of tumor development in the solid form, and the mice were sacrificed 7 days after treatment. At necropsy we collected the tumor and lymphoid tissues, that were weighted and histologically processed. The proliferative activity were immunohistochemically evaluated by anti-PCNA antibodies.
Results: In contrast with the chemotherapy group, the animals submitted to ECT presented a regression in tumor size. All the tumor bearing mice had an increase in splenic size. No differences were observed on proliferative activity in tumor, splenic periarteriolar lymphoid sheath and thymic medulla between the groups. Conclusions: Electrochemotherapy, but no chemotherapy, effectively antagonized tumor development without evidence of disrupting the proliferative activity, on tumoral and lymphoid cells.
Keywords: Ehrlich tumor, electrochemotherapy, immunity
Supporting funding: PIBIC/CNPq

C21 EFFECT OF INTRAPERITONEAL ADMINISTRATION OF ETHYL ACETATE (AC) FRACTION FRUITS OF SOLANUM SP. ON THE SOLID EHRLICH TUMOR IN MICE.
Morais, M.G. 1; Costa, G.A.F. 1; Silva, I.C.A. 1; Pereira, A.F. 1; Ferreira, J.M.F. 1; Pinto, F.C.H. 1; Lima, L.A.R.S. 1
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Introduction: Cancer is one of the leading causes of mortality worldwide. Many chemotherapeutic agents used in cancer treatment have significant side effects on normal cells with high cytotoxicity. New drugs generated secondary metabolites of plants represent an alternative source of anticancer agents, which frequently seem to be less effective and less toxic. Objectives: The aim of this study was to investigate the effect of intraperitoneal administration of ethyl (Ac) fraction fruits of Solanum sp. on the growth of solid Ehrlich tumor. Methodology: A total of 12 Swiss female mice, were divided into two groups of 6 animals each: A and B. The animals in group A received purified water (control group). The first day, the animals of group B (preventive fraction AC) received daily, for 42 days of the experiment, 100 mg/kg of ethyl acetate fraction by administration intraperitoneal. Furthermore, at the twenty-one day, all mice were inoculated sub-
cutaneously with 2 x 106 Ehrlich tumor cells (0.05 mL) on the footpad. The tumor growth was evaluated by measuring the paw thickness, using a digital pachymeter, were performed seven readings with an interval of 48 hours. At the forty-two day, the animals were euthanized. Results: The tumor growth was similar (P > 0.05) between A and B groups, in the first, second, third, fourth and fifth readings, but in the sixth and seventh readings differences were statistically significant (P < 0.05). Group B shows the suppression of tumor growth in the fifth reading. In the seventh reading, group A showed the to paw thickness of 6.2 ± 1.27 mm, while group B of 3.15 ± 1.47. Conclusion: The results demonstrated anti-inflammatory activity of 2 mg digoxin plus liposome. While digoxin 2 mg showed antiproliferative effects on G2.

Keywords: Tumor Ehrlich, cardiac glycosides, liposomes

Supporting funding: CAPES, FAPEMIG

Introduction: Rosmarinus officinalis, belongs to the family of Lamiaceae, popularly known as rosemary, is a common household plant grown in many parts of the world. Constituents in rosemary have shown a variety of pharmacological activities for cancer chemoprevention and therapy in vitro and in vivo models. Objectives: The aim of this study was to investigate the effect of oral administration an ethanolic extract of Rosmarinus officinalis on growth of solid Ehrlich tumor. Methodology: A total of 18 Swiss female mice, two months old, were divided into three groups of 6 animals each: A, B and C groups. The animals of the A group received purified water (control group). At the first day, the animals of the B group received the daily dose of 100 mg/kg of Rosmarinus officinalis ethanolic extract by gavage. At the twenty-one day, the animals of the C group received, by gavage, the daily dose of 100 mg/kg of Rosmarinus officinalis ethanolic extract. Additionally, at the twenty-one day, all mice were inoculated subcutaneously with 2x106 Ehrlich tumor cells (0.05mL) on the footpad. The tumoral growth was evaluated by measuring the paw thickness, using a digital pachymeter. At the forty-two day, the animals were sacrificed; the paw and the popliteal linfonode were evaluated by histology. Results: The tumoral growth was similar (P>0.05) between all groups. At the end of experiment, the measures of footpad were: A- 4.65±0.26; B- 4.64±0.64; C- 4.99±0.69. The histopathological analysis of the paw of mice of the groups A, B and C showed tumoral cells with delicate stroma, pleomorphism, numerous and evident nucleoli and scarce cytoplasm. The metastasis in popliteal nodes of the tumor occurred in all animals. Conclusion: The oral administration an ethanolic extract of Rosmarinus officinalis did not affect the proliferative potential of tumor and histological pattern of tumor cells. Keywords: Ehrlich tumor, Rosmarinus officinalis, histopathology, chemoprevention

Conclusion (s): The study demonstrated anti-inflammatory activity of 2 mg digoxin plus liposome. While digoxin 2 mg showed antiproliferative effects on G2.

Keywords: Tumor Ehrlich, cardiac glycosides, liposomes

Supporting funding: CAPES, FAPEMIG

C23 EFFECT OF ORAL ADMINISTRATION OF ROSMARINUS OFFICINALIS EXTRACT OF THE SOLID EHRLICH TUMOR IN MICE.

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Introduction: The cardiac glycosides are a class of molecules derived from plants known for their cardioactive activity. It has been reported that cardiac glycosides may reduce the growth of various cancers, including breast, lung, prostate cancer, and leukemia. However there are no studies concerning the action of anticancer therapy cardiac glycosides on the Ehrlich tumor. Objectives: To investigate the antineoplastic action of two cardiac glycosides (digoxin and a derivative of digoxin, the DGB1) on the growth of Ehrlich solid tumor with or without the use of liposomes. Methodology: We adopted these strategies in rosemary have shown a variety of pharmacological activities for cancer chemoprevention and therapy in vitro and in vivo models. Objectives: The aim of this study was to investigate the effect of oral administration an ethanolic extract of Rosmarinus officinalis on growth of solid Ehrlich tumor. Methodology: A total of 18 Swiss female mice, two months old, were divided into three groups of 6 animals each: A, B and C groups. The animals of the A group received purified water (control group). At the first day, the animals of the B group received the daily dose of 100 mg/kg of Rosmarinus officinalis ethanolic extract by gavage. At the twenty-one day, the animals of the C group received, by gavage, the daily dose of 100 mg/kg of Rosmarinus officinalis ethanolic extract. Additionally, at the twenty-one day, all mice were inoculated subcutaneously with 2x106 Ehrlich tumor cells (0.05mL) on the footpad. The tumoral growth was evaluated by measuring the paw thickness, using a digital pachymeter. At the forty-two day, the animals were sacrificed; the paw and the popliteal linfonode were evaluated by histology. Results: The tumoral growth was similar (P>0.05) between all groups. At the end of experiment, the measures of footpad were: A- 4.65±0.26; B- 4.64±0.64; C- 4.99±0.69. The histopathological analysis of the paw of mice of the groups A, B and C showed tumoral cells with delicate stroma, pleomorphism, numerous and evident nucleoli and scarce cytoplasm. The metastasis in popliteal nodes of the tumor occurred in all animals. Conclusion: The oral administration an ethanolic extract of Rosmarinus officinalis did not affect the proliferative potential of tumor and histological pattern of tumor cells. Keywords: Ehrlich tumor, Rosmarinus officinalis, histopathology, chemoprevention

Conclusion (s): The study demonstrated anti-inflammatory activity of 2 mg digoxin plus liposome. While digoxin 2 mg showed antiproliferative effects on G2.

Keywords: Tumor Ehrlich, cardiac glycosides, liposomes

Supporting funding: CAPES, FAPEMIG

C24 EFFECT OF THE ENVIRONMENTAL ENRICHMENT IN THE DEPRESSIVE-LIKE BEHAVIOR AND DEVELOPMENT OF EHRLICH
TUMOR IN MICE.
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Introduction: Depression and stress are psychic disturbances whereon the oncologic patients are more susceptible, and which have relation with the immune response and social environment. Rats living in an enriched housing environment were less anxious, more curious, and quicker to resolve mazes than were animals left in the lab. The growth of cancers is dependent in part on their microenvironment, but the effect of the macroenvironment on tumor, specifically an individual’s interaction with its physical living is very important. Mice living in enriched environment (EE) showed reduced tumor growth. Objectives: This study proposes to establish a relation among the environmental enrichment, the development of Ehrlich-s tumor and the depression in mice bearing Ehrlich tumor. Methodology: Male mice were divided in four groups each, since three weeks of age and submitted to the environmental enrichment (EE), with tunnels, nesting material and toys and housed in larger groups, or not enriched (NEE) housed in control cage, for a period of four weeks, and inoculated with Ehrlich tumor suspension in dorsal subcutaneous or with buffered salin suspension. Before tumor inoculation they were submitted to the Open Field Test (OFT) and Forced Swimming Tests (FST). In the 18º day after inoculation of the Ehrlich tumor, all the groups were evaluated again in the OPT and SFT. Later, they were euthanized and the tumor was excised from the back, evaluated as its volume and conducted for histological evaluation. Results: The locomotion in OPT was lower in the EE groups than NEE, before (EE: 74,6±5,38/NEE: 134,15±10,54 quadrants invaded) and after tumor inoculation (EE: 65,6±5,28/NEE: 137,11±11,71 quadrants invaded). The immobility in FST was greater in the EE group inoculated with tumor (165,11±14,47 seconds) compared to NEE group without tumor (124,78±9,76seconds). The immobility in FST was lower in the EE group (EE: 110,29±12,76/NEE: 165,11±14,47 seconds) only 18 days after tumor inoculation. There wasn’t any significant difference in the evaluation of Ehrlich tumor volume, in EE and NEE groups. Conclusions: The environmental enrichment has a positive effect in the mice’s well-being and revert depression behavior, but in the period of four weeks, it wasn’t effective to decrease the neoplastic growth in a significant way.

Keywords: Depression, Environmental Enrichment, Ehrlich tumor
Supporting funding: CNPq FUNDAC-UNIVERSIDADE METODISTA

C26 EFFECTS OF WATERCRESS CONSUMPTION ON GROWTH OF SOLID EHRLICH TUMOR IN MICE.
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Introduction: Colorectal cancer is the third most common cancer in the world. The consumption of foods containing molecules with chemopreventive properties may decrease its incidence and risk of recurrence. Objectives: The aim of this study was to evaluate the effects of grape juice on colon cancer induced by azoxymethane (AOM) in Wistar rats by determining the number of aberrant crypts and RNA expression of NF-kB, iNOS and TNF-α.

Methodology: Forty male Wistar rats (5-6 weeks) were divided into 7 groups: G1 (n = 6): control; G2 (n = 5): 15 mg/kg AOM; G3 (n = 6): 1% grape juice 2 weeks before AOM; G4 (n = 6): 2% grape juice 2 weeks before AOM; G5 (n = 6): 1% grape juice 4 weeks after AOM; G6 (n = 6): 2% grape juice 4 weeks after AOM; G7 (n = 5): 2% grape juice without AOM. The colon was removed for analysis of aberrant crypt foci (ACF). RNA expression of NF-kB, TNF-α and iNOS was evaluated by real-time PCR. Results: No ACF were observed in G1 and G7. The number of crypts per focus was higher in G2 compared to G4 (p=0.004). G4 presented a smaller number of crypts per focus than G5 (p=0.009) and G6 (p=0.026). Small ACF (1-3) were more frequent in G4 compared to G2, G5 and G6 (p=0.009, p=0.009 and p=0.041, respectively). RNA expression of iNOS or TNF-α did not differ between groups. RNA expression of NF-kB was lower in G3 and G4 compared to G2 (p=0.004 and p=0.002, respectively). A positive correlation was observed between TNF-α and NF-kB gene expression (p=0.002).

Conclusions: The administration of 2% grape juice before the induction of carcinogenesis with AOM did not interfere with the formation of ACF, but reduced their multiplicity, attenuating carcinogenesis. Lower expression of NF-kB was observed in animals exposed to grape juice for a longer period of time, regardless of its concentration (1% or 2%). Inhibition of the NF-kB signaling pathway may be one mechanism by chemopreventive and chemotherapeutic agents such as concentrated grape juice interfere with colon carcinogenesis.

Keywords: azoxymethane, grape juice, RT-PCR, colorectal cancer
Supporting funding: FAPESP
Cruciferous vegetables such as watercress (Nasturtium officinale) contain relatively large amounts of isothiocyanates (ITCs). ITCs are highly effective in affording protection against chemically induced cancers in animal models. Objectives: The aim of this study was to investigate the effect of oral administration of watercress on growth of solid Ehrlich tumor. Methodology: A total of 18 Swiss female mice, two months old, were divided into three groups of 6 animals each: A, B and C groups. The animals of the A group received purified water (control group). At the first day, the animals of the B group received daily dose of 0.05 ml aqueous of the Nasturtium officinale (25mg/ml) by gavage. At the twenty-one day, the animals of the C group received, by gavage, the daily dose of 0.05 ml aqueous of the Nasturtium officinale (25mg/ml). Additionally, at the twenty-one day, all mice were inoculated subcutaneously with 2x106 Ehrlich tumor cells (0.05ml) on the footpad. The tumor growth was evaluated by measuring the paw thickness, using a digital pachymeter. At the forty-two days, the animals were sacrificed and the paws were collected to the histology analysis. Results: From the 10th day after tumor inoculation, animals of the B and C groups showed a suppression of tumor growth than that obtained for the control group (ANOVA, p<0.05). The histopathological analysis of the paw of mice with the Groups A, B and C showed tumoral cells with delicate stroma, pleomorphism, numerous and evident nucleioli and scarce cytoplasm. Quantification studies the necrosis showed that mice showed the groups B and C had a significant reduction in areas of necrosis when compared to control group (p<0.05). Conclusion: The watercress is a source of bioactive substances such as isothiocyanates with anti-tumor properties, which can be induced suppression of tumor growth, possibly via induction of apoptosis. The results provide further as to how cruciferous vegetable consumption contributes to chemoprevention of cancer.

Keywords: Ehrlich tumor, Nasturtium officinale, watercress

C28 EVALUATION OF BLOOD BIOCHEMISTRY OF ANIMALS WITH EHRICH TUMOR TREATED WITH SOLID CARDIAC GLYCOSIDES. Freitas, A. J. 1; Carvalho, N. M. 1; Nunes, N. A. M. 1; Alves, S. L. G. 2; Villar, A. F. P. 2; Barbosa, L. A. de O. 2; Maia, G. A. S. 3; Santos, H. de L. 3; Ribeiro, R. I. M. A. 3
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Introduction: The cardiac glycosides (GCS) are a class of molecules known due to its cardiotonic activities. It was recently reported that in addition to treating cardiovascular disorders, they also possess antitumor activity. Objective: To evaluate the toxicity of digoxin and DGB1 in Ehrlich tumor bearing mice. Methodology: The ethics committee on animal use of the Federal University of São João Del Rei (UFSJ) approved the methods used in this study (CEUA-07/2013). For the experiment, 25 mice (Swiss) were randomly divided into five groups: G1 (control - no treatment), G2 (Digoxin 2mg), G3 (Digoxin 5mg), G4 (DGB1 2mg), G5 (DGB1 20 mg). The solid Ehrlich tumor was obtained by inoculating 50 l of a solution containing 2.5 x 106 tumor cells in the footpad animal rights. For the evaluation of toxicity of digoxin and DGB1, blood samples were collected by cardiac punctu-
After animals had been anesthetized. We used the kit’s “Gamma Glutamyl transferase” and “Urea Enz Color” (line Bioliquid®). Results: The results obtained in this experiment were that the treated animals, the amount of urea and Gamma GT, although the values were different from those of the control group, there was no statistical difference between them. Therefore, digoxin and DGB1 in the doses used, did not seem to cause liver and kidney dysfunction. Conclusions: Thus, we can conclude that the results were not satisfactory in relation to this standard medication running this experiment. Keywords: Cancer, Ehrlich tumor, Digoxin. Supporting funding: Universidade Federal de São João Del-Rei. Campus Centro-Oeste Dona Lindu.

C29 EVALUATION OF SURVIVAL RATE AND TUMOR PROGRESSION OF 4T1 MAMMARY CANCER.

Moreira, G.V.¹; Reis, D.C.¹; Pinto, S.G.B.¹; Campos, L.C.¹; Silva, A.C.A.²; Lopes, M.T.P.²; Ferreira, E.; Cassali, G.D.¹.
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Introduction: Breast cancer is the second leading cause of cancer-related deaths among women worldwide. 4T1 cells are transplantable mouse mammary carcinoma cells and are poorly immunogenic with growth characteristics and resembling exactly to that of stage IV in woman breast cancer. These cells are highly invasive and primary tumor metastasizes as early as 2 weeks (after inoculation) to lungs, liver, bone, and brain. Objectives: The aim of the study was evaluate the survival rate and metastasis in animals injected with 4T1 tumor cells.

Methodology: Twenty-one female BALB/c mice (6-8 week-old) were obtained from the Central Animal Facility-Institute of Biological Science-Federal University of Minas Gerais; Belo Horizonte-Brazil. 4T1 cells (American Type Culture Collection, Manassas, VA) were maintained in RPMI 1640 medium (Hyclone, Logan, UT) containing 10% FBS. The cultures were maintained at 37ºC in humidified atmosphere of 5% CO2. 4T1 cells in log phase of growth were harvested and suspended in PBS at a density of 5x10⁶ cells/100mL. Cells suspension was injected in the posterior left -ank of all animals to obtain the solid tumor. To determine the survival rate of animals, mice were followed until the day of death. Tumor and organs (lung, liver, spleen and kidney) were removed and fixed by formalin for histopathological analysis. Likewise, tumor was weighted and measured. All animal experiments were approved by the Institutional Animal Care and Use Committee (CETEA 261/212). Results: The animals presented a media survival rate of 32 ± 2.24 days. The tumor weight and volume showed media of 2.00g ± 0.63 and 4.00 ± 2.58 mm³ respectively. Lung metastasis was observed in all animals. However, liver, kidney, spleen, adrenal and heart metastasis were also found in animals with different survival rate. Only renal metastasis was correlated with animals of high survival (r = 0.55; p<0.05). Lung and liver metastasis was correlated with low survival and related to death of the animal. Nevertheless, no statistical significance concerning time of survival was observed. Conclusion: 4T1 mammary carcinoma apparently present spreading characteristics that make it a suitable experimental animal model of metastasis for breast cancer and can be used in targeted therapy studies of advanced stages of cancer. Keywords: breast, cancer, model

Supporting funding: FAPEMIG, CNPq e CAPES.

C30 EVALUATION OF THE EFFECT OF DIGOXIN And DGB1 IN EHRICH-S TUMOR SOLID.

Ferreira, A. S.¹; Carvalho, N. M.²; Nunes, D. A. F.²; Faria, I.²; Lopes, G. F.²; Vilas, A. F. P.²; Barbosa, L. A. de O.²; Santos, H. de L²; Ribeiro, R.I.M.A.².
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Introduction: The digoxin and its derivatives belong to the class of cardiac glycosides. These molecules are used in the treatment of cardiovascular disorders and, in addition, has shown antitumor action. However, no studies on the action anticancer therapy using Ehrlich-s tumor, a type of neoplasm experimental transplantable species-specific mice. Objective: Evaluate the growth of solid tumors in mice-s paw treated with digoxin and a derivative of digoxin, the DGB1. Methodology: The 25 Swiss mice were randomly divided into five groups: G1 (control-no treatment), G2 (Digoxin 2mg), G3 (Digoxin 5mg), G4 (DGB1 2mg), G5 (DGB1 20 mg).

On the 1st day of the experiment were inoculated 50 μL of a solution containing 2.5 x 10⁶ tumor cells in the footpad animal rights. The next day started daily treatment. As Paw was measured with the aid of a digital caliper. Furthermore, we evaluated the weights of the animals and also clinical signs: walking, agitation or lethargy, breathing, salivation, cyanosis, mortality, tearing and eye color and hair. Results: In relation to body weight, G4 lost less weight, however, this was also the group with the highest tumor growth, which was similar to the untreated group. Regarding the thickness of the tumor, the G2 and G3 groups obtained the lowest values. Finally, with respect to clinical parameters observed, G2 and G3 showed the best evaluation. Conclusion: From the results the DGB group animals were not harmed by the treatment, since the days of treatment, they have not lost weight, compared to groups Digoxin. All groups showed similar tumor growth, and DIX groups. 2mg and DIXG. 5 mg had the lowest values. Finally, with respect to clinical parameters, we conclude that the treatments groups DIXG. 2mg and DIXG. 5mg, led to no improvement in clinical status of these animals. Keywords: cardiac glycosides, Experimental Pathology, Ehrlich tumor

Supporting funding: Fapemig

C31 EVALUATION OF THE EFFECTS OF METHOTREXATE-LOADED POLY (E-CAPROLACTONE) IMPLANTS (MTX PCL IMPLANTS) IN SOLID EHRICH TUMOR.

Pereira, A.F.¹; Silva, A.G.¹; Vidigal, P.V.T.²; Ribeiro,
Introduction: Implants are defined as controlled sustained release delivery systems of therapeutic agents incorporated or dispersed into a polymeric carrier. These systems can be implanted in specific organs and delivered by the antineoplastic agents at the target site to treat cancer. Objectives: The aim of this study was to evaluate the effects of methotrexate-loaded poly(e-caprolactone) implants (MTX PCL implants) in inhibiting Ehrlich solid tumor bearing mice. Methodology: Implants were prepared by fully blending MTX particles with melted PCL and then molding the blends into spherical implants. PCL implants without MTX were also produced. In vivo release of MTX from PCL implants was evaluated in the subcutaneous tissue of mice. A total of 20 Swiss female mice, two months old were divided into two groups of 10 animals each: A and B groups. At the first day, all mice were inoculated subcutaneously with 2*10⁶ cells Ehrlich tumor cells (0.05 mL) on the footpad. At 5 days post-inoculation, the animals of the A and B groups received PCL implants loaded with MTX PCL implants, respectively, in the subcutaneous tissue. The tumoral growth was evaluated by measuring the paw thickness, using a digital pachymeter. At 12 days post-implantation, the animals were sacrificed and the paw was collected to the immunohistochemical analysis of the expression of PCNA and quantitative analysis of necrotic area. Results: PCL implants provided the controlled MTX release within subcutaneous tissue of mice. Animals of the B group showed lower values of tumoral growth than that obtained for the A group (p<0.05). At the end of experiment, the measures of footpad were: A: 6.28±0.26; B: 3.50±0.64. The tumor proliferation rate, assessed by the PCNA marker, was similar in both groups. Quantification studies demonstrated the necrosis showed that received the implants MTX mice had a significant reduction in areas of necrosis when compared to untreated group (p<0.05). Conclusion: The MTX-loaded PCL implants demonstrated to be as effective in suppression the tumor growth, resulted in a significant reduction in tumor size associated with a significant reduction in necrotic area observed in treated group mice and data suggest that the diminished tumor growth was not associated with a proliferation rate. Keywords: Methotrexate, implants, Ehrlich tumor Palavra-Chave 4: PCNA

C33 GASTROINTESTINAL METASTASIS OF SPONTANEOUS MAMMARY TUMOR IN SWISS MICE.

Veloso, E. S.¹; Reis, D.C.¹; Cassali, G.D¹; Ferreira, E.¹

Introduction: Mammmary tumors of the mice has been induced by chemical carcinogens, estrogenic hormones, implantation of pituitary glands by and polynoma viruses. These tumors, usually occurring after the second parturition and metastasis are rarely and occasionally occurring in lung. Objectives: The aim of the study was describe the presence of gastrointestinal metastasis of spontaneous mammmary adenocarcinoma in Swiss mice. Methodology: Female Swiss mice, 13,5 months old, was ob-
tained the Institute of Biological Science - UFMG; Belo Horizonte - Brazil and maintained as a matrix in reproductive in the General Pathology Depart-
ment, presented after second mating nodular les-
ions of fast growing in the left flank. The animal
was monitored during the period of approximately
45 days after the appearance of the tumor and
euthanized for autopsy and research achievement of
metastases. Tumor and organ were removed and
fixed by formalin for histopathological analy-
sis. Results: During the monitoring, the primary
lesion progressed with increasing tumor mass and
the appearance of a second nodular lesion in the
right axillary region. Necropsy analysis showed
masses presented whitish and solid consistency,
blackish and greenish colours, and, measuring
about 2.5x3.0x4.0cm and 1.5x1.5x2.0cm, respecti-
vely. It was also observed macroscopic changes in
the spleen, liver, kidney, stomach, duodenum and
lung. In the spleen, liver and kidney were observed
whitish spots, delimited, millimeter in the external
surface and cutting the organ. In the stomach was
visualized in the body region, the presence of infil-
trating solid mass, firm, whitish measuring 1.0cm
in diameter. In the proximal duodenum was observed
a sessile nodule in the mucosa, blocking the
intestinal lumen, whitish, measuring approximately
1cm in diameter. Microropscopic analysis of the prima-
ry tumor showed cell proliferation compatible with
spontaneous mammary tumor. The organs analys-
es showed epithelial cell neoplasia consistent with
first tumor. Lymphocytic inflammatory infiltrate was
observed associated with tumor formation. Con-
clusion: The present results show that sponta-
neous mammary tumor presente metastatic spread to
different organ including to gastrointestinal sys-
tem. Thus, demonstrating to be a promising model
in the study of neoplastic progression and systemic
metastasis. Keywords: Mammary, mice, tumor
Supporting funding: FAPEMIG, CNPq e CAPES.

C34 Glioblastoma: A Fatal and Heter-
ogeneous Tumor Interacting with the
Parenchyma.
Lima, F. R. S. 1; Kahn, S. A. 1; Garcia, C. 1; Fonseca,
A. C. 1; Alonso, R. H. 1; Spoehr, T. 1; Santos Jr, F. A.
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Introduction: Glioblastoma cells (GBM), one of the
deadliest human cancers, are highly proliferative,
with aggressive invasiveness and interactions with
the parenchyma. Microglia, for instance, is attrac-
ted to GBM tissue and can infiltrate into the GBM
ccontributing to tumor proliferation, via secreted
factors. However, little is known with regard to the
immune performance and interactions of the micro-
glia with GBM. Objective and Methodology: In this
regard, using cell culture, immunocytochemistry and
electrophoresis methods, we first demonstrated
the result 1: effects of the microglia via stress inducible protein 1 (ST11) and MMP-9 on the proli-
feration and migration of human GBM. In addition,
the cellular heterogeneity of GBM may be due to
the presence of GBM stem cells (GBMSCs) in the
tumor mass. Objective and Methodology: Using the
same methods, our second aim in the present stu-
dy was to demonstrate a high expression of stem
cells markers in GBM after xenotransplantation in
mice. GBMSCs are highly resistant to current can-
cer treatments. Indeed, drugs ultimately used to kill
the majority of tumor cells, fail in GBM treatment
because they do not eliminate GBMSCs, which sur-
vive. In this respect, we used equinatoxin II (EqTx-
II), a pore-forming toxin from the sea anemone
Actinia equina, to potentiate the cytotoxicity indu-
ced by temozolomide (TMZ) and etoposide (VP-16)
GBM treatments. Objective and Methodology: Our
third aim, using the in vitro methods indicated abo-
veas well as by magnetic resonance imaging (MRI)
in vivo, Result 2: we demonstrated that, a non-cyto-
toxic concentration of EqTx-II potentiated the VP-16
and TMZ effects inhibiting the GBM growth. This
effect is selective regarding GBM cells and occurs
via the PI3K/Akt pathway. Conclusion: Our results
suggest that cytolyisins can be a potential new tool
for GBM treatment. Key words: Glioblastoma, heterogeneous tumor
MRI.
Supporting funding: INNT-INCT-CNpq-MCT; CA-
PES; FAPERJ; UFRJ.

C35 Grape Juice Concentrate Modu-
lates Inflammatory Mediators Follo-
wing Rat Tongue Carcinogenesis Indu-
ced by 4-Nitroquiline-1-Oxide.
De Jesus, GPP 1; Moura, CFG 1; Ribeiro, FAP 1;
Gollucke, APB 2; Ribeiro, DA 1.
1 Federal University of São Paulo, Brazil; 2 Catholic
University of Santos, Brazil
Objective: This study aimed to evaluate the che-
mospreventive potential of concentrated grape jui-
ce (G8000TM) following rat tongue carcinogenesis
induced by 4NQO by means of histopathological
analysis and gene expression of inflammatory me-
diators such as eNOS, Inos, TNF-alpha and COX-
2.
Methods: Wistar rats were distributed into 5
groups: Group 1 - received G8000TM for 8 weeks;
Group 2 - received 4NQO during 8 weeks and tre-
ated with G8000TM from 1st to 4th week; Group
3 - received 4NQO for 8 weeks and treated with
G8000TM from 5th to 8th week; Group 4 - received
4NQO for 8 weeks; and Group 5 - did not recei-
ve any treatment. G8000TM was given 1.18 mg/
45 days after the appearance of the tumor
and TMZ effects inhibiting the GBM growth. This
third aim, using the in vitro methods indicated abo-
ve, was well as by magnetic resonance imaging (MRI)
in vivo, Result 2: we demonstrated that, a non-cyto-
toxic concentration of EqTx-II potentiated the VP-16
and TMZ effects inhibiting the GBM growth. This
effect is selective regarding GBM cells and occurs
via the PI3K/Akt pathway. Conclusion: Our results
suggest that cytolyisins can be a potential new tool
for GBM treatment. Key words: Glioblastoma, heterogeneous tumor
MRI.
Supporting funding: INNT-INCT-CNpq-MCT; CA-
PES; FAPERJ; UFRJ.

C34 Glioblastoma: A Fatal and Hete-
rogenous Tumor Interacting with the
Parenchyma.
Lima, F. R. S. 1; Kahn, S. A. 1; Garcia, C. 1; Fonseca,
A. C. 1; Alonso, R. H. 1; Spoehr, T. 1; Santos Jr, F. A.
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Introduction: Glioblastoma cells (GBM), one of the
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ted to GBM tissue and can infiltrate into the GBM
ccontributing to tumor proliferation, via secreted
factors. However, little is known with regard to the
immune performance and interactions of the micro-
glia with GBM. Objective and Methodology: In this
regard, using cell culture, immunocytochemistry and
electrophoresis methods, we first demonstrated
the result 1: effects of the microglia via stress inducible protein 1 (ST11) and MMP-9 on the proli-
feration and migration of human GBM. In addition,
the cellular heterogeneity of GBM may be due to
the presence of GBM stem cells (GBMSCs) in the
tumor mass. Objective and Methodology: Using the
same methods, our second aim in the present stu-
dy was to demonstrate a high expression of stem
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Introduction: The growth of most malignant tumors is accompanied by elevated expression of cyclooxygenase enzymes (COX’s) responsible for the modulation of inflammatory chemical mediators. Objectives: The aim of this study was to evaluate the effect of treatment with non-ine inhibitory of cyclooxygenase on the development of Ehrlich solid tumor, correlating the tumoral development with fibrosis, participation of macrophages and vascular endothelial growth factor (VEGF). Methodology: The study was conducted with 107 inoculating tumor cells into Swiss mice subcutaneously. After 24 hours of the tumors cells implantation, the animals were treated with indomethacin (2mg/kg of weight, ip., 1x/day) or diluent (0.1 ml intraperitoneally, 1x/day) were euthanized with lethal dose of xylazine and ketamine for removal of the tumoral mass after seven, fourteen or twenty-one days. To evaluate the parameters total area, area of necrosis was employed morphomorfic study of sections stained by HE. To quantify macrophage density distribution of VEGF and collagen type I was employed immunohistochemical study. Results: The non-ine inhibition of cyclooxygenase resulted in a significant reduction of the total area at 14 and 21 days of treatment (2.81 ± 0.85 and 5.58 ± 1.32 respectively), a significant increase in necrotic areas at 7 and 14 days of treatment (0.66 ± 0.24 mm² and 2.25 ± 2.32 mm² respectively), reduction of the parenchyma after 7 (1.67 ± 1.71 mm²) and 14 days (1.70 ± 1.30 mm²). We observed an increase in collagen production in the initial period (18.8 ± 9.4 mm²) and the final period (13.0 ± 7.9 mm²). The density distribution of VEGF significantly increased in the initial period of treatment (11.7 ± 7.0 mm²). The influx of macrophages was not affected by treatment. Conclusions: The non-ine inhibition of cyclooxygenase was effective in controlling tumor growth at 14 days and in this period, the low production of collagen type I is involved in this contention, pointing to an important role of fibroblasts. But the suggestion that macrophages are not involved at least numerically, on this contention of Ehrlich solid tumor. Additional studies are needed to clarify the fact that the treatment in the initial period stimulated the production of VEGF, important for the formation of a well vascularized tumor for deployment.

Keywords: Cyclooxygenases, Type I collagen, VEGF, Ehrlich solid tumor

C37 IMMUNOHISTOCHEMISTRY EXPRESSION OF GSK3β AND p-GSK3β SERINE 9 IN ORAL SQUAMOUS CELL CARCINOMA.
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Introduction: Squamous cell carcinoma represents more than 90% of oral cancer. In most cases the diagnosis is detected in a advanced stage and hence the patient has a poor prognosis. The canonical Wnt signaling pathway has been prominent in the development of many types of tumors, including oral squamous cell carcinoma. However the results are inconclusive and there are few studies that relate all components of Wnt signaling in tumor progression of oral squamous cell carcinoma. One of the target proteins of this pathway is Glycogen Synthase Kinase 3 beta (GSK3β), a serine/threonine protein kinase that may play an important role in development of this cancer. Objective and Methods: This study aimed to investigate by immunohistochemistry the expression of GSK3β and p-GSK3β Serine 9 in 89 patients with oral squamous cell carcinoma and relate the expression of these proteins with tumor progression and clinical pathological factors of prognostic importance. The tissues sections were performed by the TMA (tissue microarray) technique, in duplicate. The identification of protein expression was performed using immunohistochemistry for the biotin streptavidin peroxidase. Results: Presence of GSK3β in all cases, regarding p-GSK3β Serine 9 protein was identified low expression, with most of the cases (83.9%) with low or negative expression. The difference between the expression of both proteins was significant with p<0.0001. Conclusion: There was no association between clinical pathologic factors with the expression of both proteins, as well as for tumor progression and survival of patients. Therefore, the present study demonstrated that GSK3β and p-GSK3β Serine 9 proteins were not associated with tumor progression and prognosis of patients, possibly due to the predominance of the active form of GSK3β, p-GSK3β Tyrosine 216. However more studies are needed to elucidate the role of Wnt signaling pathway in oral squamous cell carcinoma.

Keywords: Oral Cancer, Squamous Cell Carcinoma, GSK3β

Supporting funding: FAPEMIG CAPES

C38 IN VITRO CYTOTOXIC EFFECTS OF CITRAL AGAINST MURINE MELANOMA B16F10
Sanches, L.J.¹; Fagundes, T.R¹; Martinello, P.C¹; Bernardes, S.S¹; Luiz, R.C.²
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Introduction: Citral, a flavoring and scenting agent with lemon odor, has been described as potent anticarcinogenic agent. It is also citotoxic against human leukemia (NB4) and breast cancer (MCF7)² cells. Metastatic melanoma is one of the most lethal types of cancer, and it is resistant to conventional chemotherapy. Objectives: The present work was to investigate a possible cytotoxic effect of citral against murine metastatic melanoma (B16F10) in vitro. Methodology: B16F10 cells were treated 24 h with citral (0.05, 0.1, 0.5, 1.0, 2.5µM). Cellular viability and proliferation were evaluated by cell counting. Trypan blue exclusion assay, MTT reduction assay, Lactate dehydrogenase (LDH) release assay, Cell death pattern with Ethidium bromide/ Acridine orange staining. Citral results were compared to control (p<0.05). Results: concentrations equal or higher than 0.5 µM reduced cell counting
and cell viability in a dose dependent manner. LDH release increased with citral 1.0 and 2.5 µM. All concentrations increased apoptotic cells, only the highest concentrations increased necrotic cell death pattern. Autophagic pattern was not observed. **Conclusions:** these results suggests that citral is cytotoxic against B16F10 cells, this effect can be associated with the ability of citral to induce caspase 3 activity, downregulate bax expression, upregulate bcl-2 and NFKB expression, as described in scientific literature. To elucidate cytotoxic effect of citral, more experiments are being performed. **Keywords:** Citral, Cytotoxic, B16F10

C39 **INFLUENCE OF IMUNOTHERAPY WITH DENDRITIC CELLS IN THE EXPRESSION OF CITOCYNE IN THE SUPERNATANT CULTURE OF PERITONEAL MACROPHAGE IN BREAST EXPERIMENTAL TUMOR.**

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**Introduction:** The immune system is essential for the cancer control. In this context the dendritic cells are recognized as the principal cells which present the antigen, considering its capacity of crossed presentation and activation of auxiliary lymphocyte and cytotoxic. The immunotherapy with dendritic cells is considered promising specially when cancer is in the beginning. **Objective:** We are supposed to analyze the synthesis of principal cytokines of the profile Th1, Th2 and Treg as: IL-4, IL-10, IL-12, IFN-γ, e TGF-β in the culture of peritoneal macrophages, after immunotherapy with dendritic cells. **Methodology:** After gavage with DMBA weekly, the interval of 16 weeks was respected, corresponds to the period of tumor development. The treatment was conducted with 4 doses of dendritic cells vaccine 5,0x10⁶ cells/animal. **Results:** There was an increase in IL-12 in group treated with dendritic cells and a reduction in the synthesis of IL-12, IL-10 and TGF-β. Checking highest ratio in the group treated tumor compared to tumor group, the trends being presented statistical difference for IL-10 and TGF. Similarly in relation IFN-γ/TGF-β, we observed an increase in the synthesis of IFN-γ with statistical significance. **Conclusion:** Our findings suggest that the vaccine with dendritic cells is able to increase the synthesis of IL-12 and IFN-γ in peritoneal macrophage, which is essential for an effective antitumoral response. **Keywords:** Dendritic cells, Immunotherapy, Cancer

C40 **INFLUENCE OF ORAL ADMINISTRATION OF ETHANOL EXTRACT FLOWERS OF Pyrostegia venusta ON THE GROWTH OF SOLID EHRLICH TUMOR.**

Sant'Ana, P.G.S.¹; Pereira, A.F.¹; Costa, P.M.¹; Lima, L.A.R.S.²; Castro, A.H.¹; Da Silva, G.R.¹; Pinto, F.C.H.¹

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**Introduction:** The Pyrostegia venusta species, belonging to the family Bignoniiaceae, is native to the Brazilian Cerrado and popularly known as “cipó-de-são-jão”. Although ornamental, this species has some medicinal properties, and evidence that has potential anticarcinogenic. **Objectives:** The aim of this study was to evaluate the effect of ethanol extract obtained flowers of Pyrostegia venusta on the growth of solid Ehrlich tumor. **Methodology:** A total of 18 Swiss female mice, two months old, were divided into three groups of 6 animals each: A, B and C. The animals in group A received purified water (control group), the first day of experiment, the animals in group B received a daily dose of 100 mg/kg of ethanol extract of Pyrostegia venusta by gavage. The 30th day, the animals in group C received, by gavage, a daily dose of 100 mg/kg of ethanol extract of Pyrostegia venusta. At the thirty day, all mice were inoculated subcutaneously with 2x10⁶ cells of Ehrlich tumor cells (0.05 mL) on the footpad. The tumoral growth was evaluated by measuring the paw thickness, using a digital pachymeter. At the sixty day, the animals were sacrificed; the paw and the popliteal lymph node were collected to be evaluated by histology. **Results:** The 16th day after tumor inoculation, the animals of group B (preventive), which started the intake of the extract before the tumor inoculation, showed a suppression of tumor growth than that obtained for the other groups A and C (ANOVA, p≤0.05). Histopathological analysis of the paw of mice in groups A, B and C showed tumor cells with delicate stroma, pleomorphism, numerous and evident nucleioli and scant cytoplasm. The metastasis in popliteal nodes of the tumor occurred in all animals. **Conclusion:** The ingestion of Pyrostegia venusta extract before tumor inoculation, showed a capacity chemoprotective, tumor growth was probably controlled by the major compounds present in the extract (allantoin, β-sitosterol and hesperidin), which may be related to antitumor activity, highlighting antigenotoxic and antioxidant potential. **Keywords:** Ehrlich tumor, Pyrostegia venusta, histopathology

C41 **INFLUENCE OF PHYSICAL ACTIVITY IN SYNTHESIS OF CYTOKINES BY DENDRITIC CELLS IN EXPERIMENTAL MODEL OF BREAST CANCER.**

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²Discipline of Gynecology and Obstetric;
³Discipline of Immunology.

**Introduction:** Dendritic cells are key in the activation of the immune response against tumors, but neoplastic cells inhibit the dendritic cells using several escape mechanisms. Physical activity in turn can alter the functioning of the immune system, including the role of dendritic cells. **Objectives:** The aim of this study was to investigate the influence of physical activity in expression of cytokines in supernatant of dendritic cells differentiated in culture in the presence of mammary tumors. **Methodology:** For this study, we used female Balb/c mice (8 wks old), the mice were divided into four groups: a control group; a non-tumor and trained; another with tumor induced and non-trained group; and the last
tumor induced and trained group; the tumor were induced by 7,12-dimethylbenzanthracene (DMBA; 1 mg/ml weekly for 6 wks) and the animals were trained subjected to swimming (30 ± 4 °C) for 45 min, 5 times per week for 8 wks. After the experimental period, the animals were euthanized and their bone marrows were collected from the femur and tibia bones. The bone marrows were homogenized and washed by centrifugation in saline. The cells were counted and resuspended in complete IMDM and distributed in plates and received stimulation with IL-4 and GM-CSF after 2 days and 5 days after the procedure, the cells were stimulated with TNF-α and LPS. The supernatant were collected and the cytokines (IFN-γ, IL-4, IL-12, and TGF-β) measured by enzyme immune-linked assay. The results shown that the groups submitted to exercise have a higher IFN-γ, and the presence of the tumor when not associated with physical activity reduce significantly the synthesis this cytokine (p < 0.05). As regards the IL-12, the animals submitted to exercise have higher values being in the presence or absence of tumor. Synthesis of IL-4 by DCs in the presence of tumor and absence of physical activity increases significantly (p <0.05) and compared to the group with tumor and practiced physical activity reduce the levels significantly (p <0.05). Seen that similar behavior in the synthesis of TGF-β, but not statistically significant. Conclusion: The physical activity in the presence of tumors, is able to enhance the production of cytokines anti-tumoral and reduce cytokines pro-tumoral, so this can be considered a possible adjuvant treatment in fighting cancer.

Keywords: Dendritic cell, Breast cancer, Physical activity

Supporting funding: Fapemig, Capes, Cnpq, Finep e Funepu.

C42 MODULATION OF Glioblastoma CELLS MOTILITY VIA INTEGRIN PATHWAY
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Introduction: Glioblastoma (GBM) is considered the most aggressive brain tumor. The main features of GBM are the high proliferation, apoptosis resistance and surrounding brain infiltration. One of the cell signalling pathways that is under research is an integrin’s pathway. Objectives: The main objectives of this work were to evaluate: 1) the integrins expression in the human glioma cell line U-118; 2) the contribution of integrins in glioma motility, proliferation and survival using the αvβ3 integrin blocker, Shikonin; 3) the existence of a synergistic effect between Shikonin and temozolomide (TMZ), the gold standard in GBM treatment. Methodology: To attain these objectives, glioma cells were incubated with shikonin alone and in combination with TMZ. Integrin expression was evaluated by western blot using specific antibodies. Cell migration was analysed by the scratch assay. Cell proliferation and apoptosis was determined using BrdU/propidium iodide and annexin V assay, respectively. Results: Our results showed that U-118 cells express mainly the alpha V, beta 3 and alpha 5 integrin subunits. However, in cells treated with shikonin we observed a decrease in integrin expression which was accompanied by a significant reduction in the glioma cells migration and proliferation and by an increase in apoptosis. Moreover, the glioma cells were treated simultaneous with shikonin and TMZ in lower doses, comparing with monotherapy doses, suggesting a synergistic effect between these two drugs. In glioma cells, the decrease of integrin expression is due to shikonin treatment, which leads to the reduction of cell migration, proliferation and induces apoptosis. Conclusion: These results suggest that shikonin can be a useful therapeutic strategy for GBM treatment.

Key words: Brain Tumor, Temozolomide, shikonin.

Financial support: Universidade de Coimbra, FCT, INN-INCT-MCT

Supporting funding: Universidade de Coimbra, FCT-Portugal, Brazil: INNT-INCT-MCT

C43 PHOTODYNAMIC THERAPY WITH CHLOROALUMINUM PHTHALOCYANINE LOADED SURFACE-MODIFIED NANO CAPSULES INDUCES NECROSIS IN TUMORBEARING MICE.

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Introduction: Chloroaluminium phthalocyanine (AlClPc) present favorable photophysical properties for application in photodynamic therapy. However, its poor solubility in water impairs their parenteral administration. Nanocapsules of polyethylene glycol coated poly(D,L-lactide) (PLA-PEG NC) were used to disperse AlClPc in water permitting the i.v. administration. Surface modification polyethylene glycol increases the half-life time of their circulation in the blood stream. Objective: The present work, concerns the evaluation of the antitumoral efficacy of AlClPc loaded in the PLA-PEG NC against Ehrlich solid tumor-bearing mice. Methodology: Ehrlich tumor cells were transplanted subcutaneously (1x106 cells/animal) in the flank of female Swiss mice. The mice were divided in two groups: Control group (without treatment) and treatment group. The animals of treatment group received 6.0 mg/kg i.v. of AlClPc- PLA-PEG NC. The neoplastic lesion was irradiated at 670nm (800mW, 50 J/cm2). After 7, 14 and 21 days of treatment, five mice for group were sacrificed, tumors were excised and histological sections stained with hematoxilin and eosin. All histological sections were scanned in ScanScope FL and tumor/necrotic areas were quantified in Aperio Image Scope v11.2.780 software. Results: The mice treated showed a continuous degenerated necrotic area extended ulcerated surface to tumor center. Necrosis was characterized by an amorphous eosinophilic tissue without cytoplasmatic membrane integrity and absence of cell nucleus. Necrotic areas quantified after 7, 14 and 21 days of treatment were 68%, 67% and 41% of tumor, respectively. The area of necrosis determine for the
C44 REDUCTION OF CD3+ LYMPHOCYTES AFTER TREATMENT WITH THALIDOMIDE IN MICE 4T1 MURINE MAMMARY CARCINOMA.

Pinto, S. G. B.¹; Reis, D. C.¹; Moreira, G. V.²; Araújo, J. A.³; Silva, A. C. A.; Lopes, M. T. P. L.; Ferreria, E.¹; Cassali, G. D.¹

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Introduction: Thalidomide, a synthetic derivative of glutamic acid, has immunomodulatory properties associated with their antiangiogenic and anti-inflammatory ability, and is capable of inducing apoptosis in an already established neovascularization. It is therefore a promising therapeutic treatment against tumors and cancers. It has been reported that the co-stimulator function of CD3+ lymphocytes in patients with myeloma treated with thalidomide, however this relationship in solid tumors is not well established.

Objectives: Quantify T CD3 lymphocytes presence in inflammatory infiltrates of 4T1 murine mammary carcinoma in animals treated with thalidomide.

Methodology: Balb/c mice were divided into two groups of eight mice. After 5 days the treated group received, orally, 150mg/kg of thalidomide for 11 days. Tumors were measured every 48 hours until the end of treatment. The immunohistochemistry technique was used to determine the expression of T lymphocytes in the inflammatory infiltrate using CD3 antibody (CD3-12, University of California, Davis / CA, USA).

Results: Mononuclear lymph-plasmocytic inflammatory infiltrates were identified in all pulmonary metastases in intratumoral and peritumoral distribution. We observed a minor presence of CD3+ lymphocytes in metastases of animals that have undergone surgical resection (6.08 ± 5.33) compared to the animals that did not undergo surgical resection (4.93 ± 5.25). However, no significant difference was observed between groups (p=0.40).

Conclusion: Our results suggest that there is no evidence of a relationship between the infiltration of CD3+ with the development of lung metastasis after surgical resection of the primary tumor.

Keywords: mice, experimental model, cancer
Supporting funding: FAPESP, CNPq and CAPES

C46 RELATIONSHIP OF Ki-67 INDEX AND P63 EXPRESSION IN PLEOMORPHIC ADENOMA OF THE SALIVARY GLAND.

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Introduction: The pleomorphic adenoma (PA) is the most frequently identified mixed tumor in the salivary glands and has a tendency to recur and also progress to malignancy. Neoplastic cells have great morphological variability, presenting epithelial or myoepithelial components. One of the main myoepithelial markers, P63, that can be used as a prognostic tool and to define biologic profile of these tumors. Objectives: Observe and analyze the correlation of the expression of p63 and Ki-67 in...
epithelioid cells in pleomorphic adenoma of the salivary gland. **Methodology:** Twenty cases of pleomorphic adenoma were retrieved from Otolaryngology and Pathological Anatomy of Divinópolis, Brazil (CLAIP). All cases were submitted to immunohistochemical staining for P63 (4 A4, Neomarkers, 1:100) and Ki-67 (Dako, 1:100) that was performed at Comparative Pathology Laboratory, ICB - UFMG. The number of p63 reactive epithelioid cells were assessed semiquantiatively using a scoring system: - = no staining, + = focal stained or 5% cells stained, ++ = between 5 and 50% cells stained, and +++ = tumors with 50% cells stained. To evaluate immunostaining for Ki-67 the index (in percentage) was calculated by counting 500 epithelioid cells per case. Ki-67 median values were used to obtain two groups of patients: + when the proliferative index was lower than median; ++ when it was higher than the median. The results were analyzed by Spearman Rank correlation. **Results:** Among the twenty cases (12 female and 8 male, age range of 35.4 ±10.82 years) of PA, the positive expression for Ki-67 was on average a 1.6 ±1.144% and for P63 two cases were evaluated as++, five as +++, five as + and eight as -. The correlation between the expressions of p63 and Ki-67 was positive, but there was no statistically significant difference (r= 0.12 ; p= 0.6). When Ki-67 was categorized and correlated to p63 positivity the same correlation was observed (r=0.01190; p= 0.96). **Conclusion:** Our data support the notion that the decrease of p63 expression is correlated with decrease of proliferation index of the neoplastic cells in pleomorphic adenoma, which seems to be an important factor in the tumoral progression. Keywords: Pleomorphic adenoma, salivary gland, P63, Ki-67

Supporting funding: FAPEMIG e CNPq

**C47 SYNTHESIS OF CYTOKINES BY PERITONEAL MACROPHAGES IN MICE WITH BREAST TUMOR AND SUBMITTED TO PHYSICAL ACTIVITY.** Alves EAR¹; Abdalla DR³; Alexoo AAR¹; Cunha A¹; Silva SFM¹; Murta EFC²; Michelin MA¹ ²
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**Introduction:** The interest in studies correlating exercise and immune response to promote beneficial effects in preventing and fighting cancer is increasing. The role of macrophages in antitumoral immune response is important because these cells present in differentes patterns of response: M1 (IFN-γ and IL-12) and M2 (IL-1β, IL-4, IL-6, IL-10 and TGF-β). **Objectives:** The aim of this study was to investigate cytokine synthesis by peritoneal macrophages in the presence of mammary tumors in mice submitted to the physical activity. **Methodology:** For this study, we used female BALB/c mice (8 wks old), divided into four groups: a control group; a non-tumor and trained; another with tumor induced and non-trained group; and the last tumor induced and trained group; the tumor were induced by 7,12-dimethyl-benzanthracene (DMBA; 1 mg/ml weekly for 6 wks) and the animals were trained subjected to swimming (30 ± 4 °C) for 45 min, 5 times per week for 8 wks. After the experimental period, the macrophages were collected from peritoneal macrophage, maintained in culture (37°C and 5% CO2), and stimulated with lipopolysaccharide (LPS - 10 μg/ml). The cytokines were measured by enzyme immune-linked assay (IFN-γ, IL-4, IL-10, IL-12, and TGF-β). **Results:** In morphological analysis the DMBA was able to induce carcinogenesis in breast tissue, developing carcinomas in situ in and the group submitted to physical activity presenting minor changes in the breast ducts. The results show that tumor induction by DMBA provided a reduction in the expression of IFN-γ, the physical activity was able to minimize this reduction, but without statistical significance (p> 0.05). The synthesis of IL-12 remained unchanged across the groups. The expressions of IL-4 and TGF-β in the tumor/non-trained group showed a significant increase when compared to the control, non-tumor/ trained and tumor/trained groups (p<0.05). The expression of IL-10 by peritoneal macrophages was similar to the synthesis of IL-4 and TGF-β, however per tumor/trained group were elevated values compared to groups without tumor (p<0.05). **Conclusions:** The tumor induction, in the absence of swim training, reduced M1 cytokine levels while increasing the presence of M2 cytokines. Physical activity in mice with tumors promoted immune system polarization toward an M1 response pattern profile. Keywords: Immune response, Cancer, Physical activity

Supporting funding: FAPEMIG, FUNEPU, CNPQ, CAPES, FINEP

**C48 THALIDOMIDE TREATMENT IN 4T1 MURINE MAMMARY CANCER: ANALYSIS OF TUMOR GROWTH AND CLINICAL PARAMETERS AT DIFFERENT DOSES.** Reis, D.C.¹; Silva, I. L. D.¹²; Luna, A.¹; Pinto, S.G.B.¹; Campos, L.C¹; Souza, C.M.¹; Silva, A.C.A.¹; Lopes, M.T.P.¹²; Ferreira, E.¹; Cassali, G.D¹; ¹Laboratory of Comparative Pathology, Institute of Biological Sciences, Federal University of Minas Gerais, Brazil; ²Fundação Comunitária de Ensino Superior de Itabira, Itabira, Minas Gerais; ³Laboratory of Tumoral Substance, Institute of Biological Sciences - Federal University of Minas Gerais, Brazil. Email: cassalig@icb.ufmg.br

**Introduction:** The thalidomide is a synthetic derivative of glutamic acid, has low solubility in water, found in the equivalent mixture of two enantiomers of course that interconvert under physiological pH and has antiangiogenic and immunomodulatory properties. Studies in experimental oncology thalidomide are suggested in order to develop new therapeutic strategies most suitable and efficient. **Objectives:** Evaluate the effect of different doses of thalidomide on tumor growth in experimental 4T1 cells and verify their toxicity. **Methodology:** A total of 32 BALB/c mice previously inoculated with neoplastic cells of experimental mammary tumor 4T1 in the region of the left flank. The animals were divided into a control group and three treatment groups. From the 5th day of inoculation, the ani-
mals of the treatment groups received daily doses of 50mg/kg, 100mg/kg and 150 mg / kg VO until the 28th day. The control group received saline in the same period. Measurements were made every 48 hours for evaluation of tumor growth. On the 29th day all animals were euthanized by collecting and weighing the primary tumor and organs (lung, liver, heart, kidneys and spleen). For hematological evaluation were collected 500µL of whole blood in EDTA 10%, blood smears were also prepared and stained with Giemsa for differential leukocyte count. For biochemical analyses, were collected 500µL of whole blood in a dry tube for blood gases, nitrogen, creatinine, AST, ALT and GGT. **Results:** We observed a lower tumor growth in animals receiving 150mg/kg of Thalidomide compared to animals treated with 100mg/kg, both with significant difference compared to the control group (p <0.05). The hematological, biochemical and weight of the animals showed no significant differences between the treated and control groups. **Conclusion:** The use of thalidomide for the treatment of experimental tumor 4T1 cells demonstrated effectiveness and low toxicity when used in doses of 150mg/kg. Histopathological and molecular studies should be conducted to determine its direct action on the reduction of tumor growth. Keywords: mice, thalidomide, experimental cancer Supporting funding: FAPEMIG, CNPq and CAPES

**Introduction:** The interest in understanding the role in the prevention of several chronic diseases, such as cancer, diabetes and cardiovascular disease, has increased in recent decades, particularly the bioactive compounds. These substances play an important role in the prevention of several chronic diseases, such as cancer, diabetes and cardiovascular disease. **Objective:** The aim of the study was to evaluate the potential anti-inflammatory apple extract against chemically induced oral carcinogenesis by 4-nitroquinoline 1-oxide in rats. Ribeiro, FAP¹; Moura, CFG³; de Jesus, GPP¹; Gollucke, APB³; Ribeiro, DA¹

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² Catholic University of Santos, SP, Brazil.

**Methodology:** Animals were exposed to different CdCl2 (cadmium chloride) concentrations focusing their dose and time-dependent responses. The erythrocytes were analyzed in periods of 48, 96 hours of exposure and also after 48 hours of depuration. **Results:** The results showed selectivity in NAs frequencies related to time and concentration effects, being associated positively or negatively with different NAs in exposed animals. The decrease of NAs after 48 hours of depuration in exposed animals, displayed different patterns showing distinct survival for each specific NAs. Correlations were observed among the NAs lobbed and bud and the membrane permeability in depuration period for the group exposed to 2.5 mg.L-1 of CdCl2. No changes on NAs nor in permeability were found amongst the control samples. **Conclusions:** The data obtained suggested that high cadmium doses arrests the cells cycle affecting directly the NAs frequencies and are associated to clearance of cells containing NAs during depuration period. Moreover some NAs could replace micronuclei frequencies on genotoxic studies. Keywords: Genotoxicity, cell membrane integrity, nuclear alterations.

**Organ Financiador:** Capes, Fapemig

**C50 SPECIFIC NUCLEAR ALTERATIONS DYNAMICS SURROGATES GENOTOXIC EFFECTS IN ERYTHROCYTES OF ORECHROMIS NILONICUS EXPOSED TO CADMIUM**

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**Introduction:** The quantification of nuclear alterations (NAs) has been considered an excellent marker of exposure to heavy metal and genotoxic evaluation besides membrane permeability determination. They could report the Results of oxidative stress generated by ROS on cellular membranes and the inhibition of DNA damage repair system whose have been proposed as major factors underlying cadmium genotoxicity in vertebrates. **Objectives:** In this study we characterized the morphological changes of nucleus and membrane integrity of erythrocytes in O. niloticus by using morphological and flow cytometer procedures. **Methodology:** Animals were exposed to different CdCl2 (cadmium chloride) concentrations focusing their dose and time-dependent responses. The erythrocytes were analyzed in periods of 48, 96 hours of exposure and also after 48 hours of depuration. **Results:** The results showed selectivity in NAs frequencies related to time and concentration effects, being associated positively or negatively with different NAs in exposed animals. The decrease of NAs after 48 hours of depuration in exposed animals, displayed different patterns showing distinct survival for each specific NAs. Correlations were observed among the NAs lobbed and bud and the membrane permeability in depuration period for the group exposed to 2.5 mg.L-1 of CdCl2. No changes on NAs nor in permeability were found amongst the control samples. **Conclusions:** The data obtained suggested that high cadmium doses arrests the cells cycle affecting directly the NAs frequencies and are associated to clearance of cells containing NAs during depuration period. Moreover some NAs could replace micronuclei frequencies on genotoxic studies. Keywords: Genotoxicity, cell membrane integrity, nuclear alterations.

**Organ Financiador:** Capes, Fapemig

**C51 GENE AND PROTEIN EXPRESSION IMPLICATED IN LIVER CELL DEATH IN ACETAMINOPHEN (APAP) INTOXICATION**

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**Introduction:** The JNK (c-Jun N-terminal kinase) pathway is related with cell death by modulation of AP-1 transcription factor in acute response, induc
cided by TNFR1 expression in APAP acute intoxication. **Objective:** Evaluate the effect of APAP acute intoxication in mice hepatocytes death. **Methodology:** C57Bl6/J male mice was euthanized 24 and 48 hours after APAP (ip. 250mg/kg) application. Analysis: Evaluation of liver necrosis area, TNFR1 expression by immunohistochemistry and c-Jun gene expression by RT-qPCR. Statistical analysis: Student t test (p<0.05). **Results:** After 48 hours of APAP application, it was observed an increase in c-Jun gene expression, TNFR1 protein expression and liver necrosis (p<0.05). **Conclusion:** The APAP administration induces the activation of transcription factors that modulate the expression of cell death proteins, leading to the increase in liver necrosis. 

**KEYWORD:** acetaminophen, liver necrosis, molecular modulation

**Supporting funding:** FAPESP

**C52 ANTI-OXIDANT ENZYMES IN ORGANS AFTER EXPOSITION TO ALUMINUM CHLORIDE.**

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**Introduction:** Hormesis is a process repairman that is adaptive in nature, reflected in body’s ability to allocate resources biologicals that are dependent on the disturbance experienced in a highly efficient way to repair damage caused by the toxic agent, like aluminum, after a certain period of time. **Objective:** The aim of this study was to evaluate the recovery of organs after exposure to different concentrations of aluminum for a prolonged period (112days) considering antioxidant enzymes. **Methodology:** Sixty adult Wistar rats were divided into five groups (n=12 animals/group). Animals of the control group received 1.0mL of distilled water into gavage (Gv;1mL), while animals of the other groups received respectively 0.02mg/L, 0.1mg/L, 0.5mg/L, and 10mg/L aluminum for a prolonged period (112days) considering antioxidant enzymes. 

**C53 ARSENIC INCREASE THE BLOOD VESSELS PROPORTION ON THE INITIAL SEGMENT REGION OF EPIDIDYMIS.**

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**Introduction:** Arsenic is a widespread environmental contaminant with mutagenic, carcinogenic and angiogenic effects. Arsenic exposure in the drinking water may promote vascular changes in different tissues through mechanisms unresolved. **Objectives:** The aim of this study was to compare the effect of exposure to different concentrations of arsenic in trivalent (arsenite, As+3) and pentavalent forms (arsenate, As+5) on the initial segment of epididymis. **Methodology:** Were used 30 Wistar rats randomly divided into five experimental groups (n = 6 animals/group). Animals of the control group received saline, while treated animals were exposed orally to sodium arsenate (As5+) and arsenite (As3+), testing for each chemical form with concentrations of 0.01mg/L and 10mg/L, daily for 56 days. The rats were euthanized and their epididymis were analyzed using the Image Pro-plus software, and the results were estimated using methacrylate plastic (Historesin). The following measurements were performed: tubular (TD; μm) and luminal diameters (LD; μm), epithelium height (EH; μm), and volumetric proportion (%), considering tubular (epithelium, lamina propria, lumen with spermatzoa and lumen without spermatozoa) and intertubular compartments (blood vessels and connective tissue). The data were obtained 10 random microscopic fields per segment at 100x magnification, using a 266 point-grid test system. All the measurements were estimated using the Image Pro-plus software, and the results were analyzed by ANOVA and Newman Keuls tests (P = 5%). **Results:** There were no differences among experimental groups for TD, LD, EH, and the percentage of lumen with spermatzoa, lumen without spermatzoa, epithelium, lamina propria and connective tissue (p>0.05). The percentual of blood vessels at 0.01mg/L As5+ (1.76±0.76), 0.01mg/L As3+ (1.90±0.85), and 10mg/L As5+ (2.16±0.96) was higher than the control group (1.05±0.46). **Conclusions:** We concluded that the administration of arsenic resulted in significant alteration on the percentage of blood vessels. **Keywords:** arsenic, epididymis, initial segment

**Supporting funding:** Fundação de Amparo à Pes-
Introduction: The hematolgy on animal research is very used and it is considered a routine procedure on diagnosis methods of environmental impacts. DNA damages are one of the main effects caused by genotoxic agents, leading to cell death induction or mutational events. The armored catfish, Hypostomus francisci (Siluriformes: Loricariidae), presents a multibatch spawning and sedentary, and ilioophage feeding habit. It is widely found in the Itapecerica river and arises as a study model for biomonitoring of the aquatic environment. Objectives: This study aimed to evaluate the micronuclei frequency and nuclear abnormalities, as cellular biomarkers of environmental impact in erythrocytes peripheral blood of H. francisci in the Itapecerica river, MG, Brazil (Ethics Committee, protocol 49/2010 CEPEA / UFSJ). Methodology: To achieve the proposed objectives, two collecting were performed during the rainy season (November/2011 and February/2012) in two sites of the Itapecerica river (P1 = urban perimeter and P2 = upstream of Divinópolis, MG) that shows different environmental conditions. The smears of peripheral blood were obtained by the puncture of the tail vein H. francisci and two samples were prepared on glass slides for each animal. The material was fixed in absolute methanol and stained with Giemsa. The micronuclei frequency and nuclear abnormalities were calculated analyzing 2000 cells by blood distention and submitted to a statistical analysis with mean and standard deviation. The nuclear abnormalities were grouped only into one class, independent of the form. Results: The micronuclei frequency and nuclear abnormalities found did not present significant differences between the two sites of the river: 2.9 ± 2.1 the micronucleus frequency and 8.8 ± 5.9, the nuclear abnormalities frequency at P1, and 2.6 ± 2.1, the micronucleus frequency, and 8.1 ± 5.5, the nuclear abnormalities frequency at P2. Conclusions: The two sites of Itapecerica river had a high micronuclei frequency and nuclear abnormalities when compared with peripheral blood in other teleosts. Moreover, it was observed that micronuclei frequency and nuclear abnormalities was higher in urban perimeter of Divinopolis.

Keywords: Micronuclei, Hematology, Biomarkers

Supporting funding: Funding support: FAPEMIG (APQ 00837/09; PIBIC/UFSJ) CNPq (Processo 482826/2010);
ability to inhibit enzymes and/or to their antioxidant properties, and are able to regulate the immune response. The grape is considered a major source of phenolic compounds when compared to other fruits and vegetables. Objectives: The aim of this study was to evaluate whether phenolic compounds present in grape juice could reduce the noxious effects induced by experimental colitis. Methodology: A total of 41 male Wistar rats were randomized into seven groups, as follows: G1 - Sham group: sham induced-colitis rats; G2 - TNBS group: non-treated induced-colitis; G3 - 2% grape juice control group; G4 - 1% grape juice 24h after TNBS colitis induction; G5 - 1% grape juice on day 7 after colitis induction; G6 - 2% grape juice 24 h after colitis induction; G7 - 2% grape juice on day 7 after colitis induction. Results: The grape juice at 1% dose in the last 7 days of treatment as well as grape juice at 2% dose decreased the peripheral blood genotoxicity. The immunoexpression of TNF-a in the TNBS nontreated induced-colitis group increased significantly when compared to 1% grape juice on day 7 after colitis induction (p<0.05); the immunoexpression of iNOS was reduced in the 1% grape juice treated groups (1.66±0.95 ; 1.02±0.57 respectively). Conclusions: The grape juice at 1% dose, exerts anti-inflammatory effects in chronic colitis caused by TNBS as a result of down regulation in the expression of pro-inflammatory cytokines and reduction of genotoxicity in peripheral blood cells. Keywords: inflammatory bowel disease; TNBS -colitis; grape juice Supporting funding: This work was supported by the FAPESP and CAPES. DAR is a recipient of a CNPq fellowship.

C57 EFFECTS OF CADMIUM, LEAD AND ZINC IN RATS EPIDIDYMIS WISTAR.

Ribeiro, S.P.; Pacheco, C.M.; Lima, G.D.A.; Menezes, T.P.; Mouro, V.G.S.; Cupertino, M.C.; Neves, M.M.; Matta, S.L.P.1, 2
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Introduction: There is interest in understanding how exposure to heavy metal acts in the male reproductive system, as it can cause damage to germ cells. Objectives: Therefore, we evaluated the effects of cadmium (Cd), lead (Pb) and zinc (Zn) in the epididymis of rats. Methodology: The study was divided into three experiments using rats of reproductive age. To evaluate the effects of Cd, 30 animals were divided into four groups, one control and three treated, receiving, respectively, 1.1, 1.4 and 1.8 mg / kg of CdCl2 intraperitoneally in a single dose. For the evaluation of the effects of Pb 25 animals were divided into 5 groups, one control and four receiving Pb by gavage at concentrations of 16, 32, 64, 128 mg / kg / day, respectively. In the evaluation of the effects of Zn 28 animals were divided into 4 groups and the three groups Zn administered in the drinking water at concentrations of 5, 10 and 20 mg / day. Animals receiving Cd and Zn were euthanized after 66 days of treatment, while animals subjected to Pb were euthanized at 30 days. The following analyzes were carried out in the epididymis: tubular diameter (DT), lumen diameter (LD), height of epithelium (AE) and volumetric proportions (%) of the epithelium (Ep), connective tissue (CT), with luminal sperm (LCE) and lumen without sperm (LSE), the initial segment (IS), head (Cb) and tail (Ca) of the epididymis. Results: The Cd caused an increase in AE Head of epididymis and increased DT and DL in the tail of the epididymis. Furthermore parameters were reduced in proportion Ep Head and Tail. Pb have an effect on the cauda in all groups, significantly reducing the DL and DT, while the head caused an increase in the EA. The effects of Zn, significant reductions were observed AE in both the segment initial, head and tail of the epididymis and the DL and DT increased in the Tail. Conclusions: Thus, we believe that the three evaluated metals caused alterations in the three analyzed regions of the epididymis. Keywords: Heavy metal, Epididymis, Toxicity Supporting funding: CAPES

C58 ESPERMATOGONIUM MORPHOLOGIC EVALUATION IN A MODEL OF 1-NAPHTHYL METHYL CARBAMATE INTOXICATION.

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Introduction: Carbamates are a group of pesticides that include the 1-naphthyl methylcarbamate (1NC) this compound can be obtained by hydrolysis of the ester group of the carbamate resulting in degradation of hydrolyzed fragments. Regarding its solubility, 1-naphthyl methylcarbamate is moderately soluble in water, but it has a good solubility in several organic solvents. Objectives: The aim of this study is to evaluate the seminiferous tubules morphologic changes, measuring the area occupied by the spermatogonia, in the experimental administration of 1-naphthyl methylcarbamate. Methodology: The control group consist of ten male Wistar rats 8 weeks old, with no manipulation during 8 weeks. The test group, also consisting of ten male Wistar rats 8 weeks old, was subjected to administration of 60 mg / l of 1-naphthyl methylcarbamate in water during 8 weeks. All the animals were sacrificed on the 30th day, by an overdose of anesthetics and a complete necropsy was performed. The fragments collected were studied under light microscopy, stained by Haematoxylin & Eosin. The testes slides were submitted to morphometric analysis to evaluate the area occupied by spermatogonium compartment, using imageJ software. Results: The medium compartment of spermatogonium is 16653.29 ± 5702.52 μm2 and 18055.9 - 20726.2 μm2 , for a confidence interval of 95% , respectively for the
control group and test group. The percentage of the area of compartment of spermatogonium in the seminaliferous tubes is 19,58 - 20.78 μm² and 24.53 - 25.99 μm², for a confidence interval of 95%, respectively for the control group and test group. The graphic of the test group presents a peak of cases with greater areas than the control ones. Conclusions: These Results suggest that the spermatogonium compartment is changed by administration of 1-naphthyl methylcarbamate (1NC). Keywords: 1-naphthyl methylcarbamate, spermatogonia, seminaliferous tubes

C59 EVALUATION OF MUTAGENIC AND ANTIMUTAGENIC EFFECTS OF BANANA PEEL, IN VITRO.
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Introduction: The food intake is an important route of human exposure to different compounds able to interact with cellular DNA, induce mutations and cancer initiation, what happens in the spontaneous cases of colorectal cancer (CRC). For this reason, the use of foods with antigenotoxic compounds, such as alimentary fibers, has been proposed for the prevention of CRC. Banana peel is an important byproduct of the consumption of bananas and is generally discarded by the population. It is rich in fiber, and has been indicated as a alimentary supplement. Objectives: The aim of this study was to evaluate the mutagenic and / or antimutagenic effects of ripped Cavendish banana peel in vitro, using benzo [a] pyrene (b[a]P 50μM) and diethylnitrosamine (DEN 50μM) well established food contaminants - as DNA damage agents. Methodology: Ethanolic extract was obtained from dried ripped banana peels, and the solvent was subsequently evaporated. The Comet assay and the micronucleus assay were used for evaluations. Human colorectal cancer cells, HT-29, were used in the present work. Results: The highest concentrations of ECB 7.5 mg / ml revealed genotoxic and mutagenic effects, with no chemopreventive effects. Moreover, the concentrations of ECB 1.25 and 2.5 mg / ml (equivalent to 1/2 and 1 banana peel / L) did not show activity genotoxic and had a protective effect (almost 50%) against b[a]P and DEN, only in simultaneous treatment protocol. These biological effects are probably related to the presence of dopamine, catechins and condensed tannins in banana peel which have antioxidant activity, reducing the damage caused by free radicals during metabolism of both tested genotoxic agents. Tannins are also able to adsorb chemical compounds reducing their availability, but in higher concentrations tannins can generate oxygen reactive species in vitro. Conclusions: Our results demonstrate that banana peel is a promising chemopreventive agent against spontaneous CRC, but its consumption should be done cautiously by human population. Keywords: banana peel, mutagenesis, antimutagenesis

C60 HEART AND VITAMIN D TOXICITY.
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Introduction: Vitamin D3 or colecalciferol is a calcitrophic hormone crucial on mesenchymal stem cell differentiation into osteoblastic tissue. The role of vitamin D3 is generally studies on cell cultures to identify specific molecule interactions with genes and receptors, but few in vivo studies have been performed disabling a whole better understanding. In vivo studies complications are related to the risk of hypercalcemia dystrophic calcification with direct heart impairment. In addition to vitamins D3 direct hypercalcemia, some of its metabolites are also biologically active improving toxic effects mainly on kidneys and heart. Objective: Test the effect of dexamethasone on colecalciferol cardiototoxicity. Materials and Methods: 15 Sprague dawley female rats with 25 weeks of age were randomly distributed into three groups of 5 animals. The group I had no manipulation. The group II received colecalciferol 2.5mg/rat by intramuscular injection (IM) weekly. The group III received colecalciferol 2.5mg/rat IM plus dexamethasone 0.1mg/rat IM weekly. All animals were maintained in biotery controlled conditions for 4 weeks. The euthanasia was ethically performed followed by a full necropsy. The hearts were fixed in 10% formalin and the H&E histopathological preparations were evaluated on light microscopy. The alterations were registered on an enhanced pathology grading system (from 0 to 4). Results: The hearts of group I had no pathological changes. The hearts of the group II had perivascular edema and inflammatory infiltrate with a median grade of 2.6 whereas the same parameter of group III had a median grade of 1.6. The presence of chronic inflammatory cells on myocardium had a median grade of 3.1 for group II and a median grade of 2 for group III. The dystrophic and metastatic calcification were present on both groups, but on group II there was a slight increase on the intensity of myocardial necrosis and fibrosis. In vivo studies complications are related to the risk of hypervitaminosis D. This finding is useful to facilitate in vivo studies of bone differentiation mediated by vitamin D. Keywords: Vitamin D, Dexamethasone, Heart

C61 HISTOLGICAL CHANGES IN THE GILLS OF DANIO RERIO UNDERGOING EXPOSURE TEMEPHOS.
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Introduction: Temephos 500 EC® is an organophosphorus pesticide used to kill or regulate the growth of biological organisms. The release of these chemicals into the environment creates a potential adverse impact to both humans and non-target wildlife. The absorption of pollutants in fish occurs through two main ways: gills surface and gut tract. The gills are responsible for gas Exchange and contribute to osmoregulation being important targets of toxicological studies. The cyprinid, Danio rerio is tropical Asia, and due to its easy management in captive has become one of the most widely used models in toxicological assays. Objectives: In this context, this work aims to analyze histological changes in the gills of D. rerio subjected to Temephos. Methodology: To achieve the proposed objectives, after obtaining the LC50 100ppm, 24 adult of D. rerio purchased commercially and acclimated for a week with controlled temperature and photoperiod were divided (six in each tank) into a control group and treatment with a concentration of 100ppm of the organophosphate during 160h. The animals were fed twice daily with commercial feed for fish. Fragments were collected gills, fixed in Bouin’s fluid, embedded in paraffin and stained with hematoxylin-eosin. Results: The gills exposed to Temephos showed a histological architecture changed in relation to the control. Areas of necrosis were observed in several regions of gills, inflammatory processes, fusion the secondary lamellae, dilated venous sinus (hyperemia) and apoptosis. Conclusions: The results indicate that the concentration of 100ppm caused histological changes in the gills of D. rerio. Similar results were observed in other studies with gills of Oreocharmis niloticus using Trichlorfon®, an organophosphate insecticide plant. Keywords: Organophosphorus pesticide, Teleost, Toxicology. Supporting funding: FAPEMIG/CNPq.

C63 INVESTIGATION OF REPRODUCTIVE TOXICITY IN PREGNANT RATS TREATED WITH USNIC ACID OF Cladonia substellata (RADDI). Silva, C.R.1; Silva, T.D.S.2; Barbosa, J.A.P.2; Santos, K.R.P.3; Silva, N.H.4; Santos, N.P.S.3

Introduction: The usnic acid is a dibenzofuran derivative produced by some lichen species. Many potential pharmacological uses of usnic acid have been reported, such as its antimicrobial antiviral, antiinflammatory, antiproliferative and antitumour properties. Although the usnic acid presents interesting pharmacological properties, its use in therapy is limited by hepatotoxicity. The gestation period is one of the most sensitive phases of the reproductive cycle. During this period, most agents readily crosses the placenta and, thus, can result in adverse effects on an organism liability. Objectives: The aim of this was to investigate reproductive toxicity in pregnant rats treated with usnic acid in the period of organogenesis 6th to 14th day of gestation. Methodology: Fertile period was determined. Three groups of rats Wistar (n=18) were orally treated 6th to 14th day of gestation with usnic acid 25 mg/kg, 15mg/kg and saline solution. On the twentieth day of gestation the females were sacrificed and then the livers of females and fetuses were removed. Reproductive indices and the morphologic analysis of liver were determined. Results: The body weight of the females and fetuses were applicable reduction. In the uterus of female occurred increased absorptions in the treated group 5±1.09 and 3.5±0.54, respectively when compared to the control group 1.1±0.4. Also observed were a significant reduction in the number of viable fetuses 8±1.41 and 10.33±1.03 for treated animals with 25mg/kg and 15mg/kg respectively, while the control group...
demonstrated 12.33±0.51. Relative reductions in fetal weights were visualized in the treated animals 3.5±0.13 and 4.1±0.18 grams/body weights, respectively at doses of 25mg/kg and 15mg/kg. On the other hand the control group showed 5.7 ± 0.33 grams/body weights. **Conclusion:** The usnic acid showed toxicity when administered during organogenesis, histological studies are being developed for these results.

Keywords: Lichen, Usnic Acid, Reproductive Toxicity

Supporting funding: FACEPE,CNPQ

**C64 KIDNEY AND VITAMIN D TOXICITY.**
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**Introduction:** Vitamin D3 or calcitriol has important functions on calcium homeostasis, cell differentiation and immune responses. Calcitriol promotes cell differentiation and cell cycle arrest and inhibit cell growth in a number of cancer cell types. Although, in vivo studies for anti-cancer benefits of vitamin D are limited by its dosage requirements that range 100nmol/L, while the serum physiologic concentrations of calcitriol are kept in the order of pmol/L. Hypercalcemia and metastatic and dystrophic calcification impair crucial organs like the kidney. Glucocorticoids decrease the intestinal absorption of calcium and increase calcium urinary excretion but do not decrease the serum level of calcitriol still available to interact with vitamin D receptor.

**Objective:** Evaluate the effects of dexamethasone on rats with toxic dose of vitamin D. Materials and Methods: 15 Sprague dawley female rats with 25 weeks of age were randomly distributed into three groups of 5 animals. The group I had no manipulation. The group II received calcitriol 2.5mg/rat by intramuscular injection (IM) weekly. The group III received calcitriol 2,5mg/rat IM plus dexamethasone 0,1mg/rat IM weekly. All animals were maintained in biotery controlled conditions for 4 weeks. At the end of the experiment the euthanasia was ethically performed followed by a full necropsy. The formalin fixation and the histological routine technique were performed on kidneys. The histopathological preparations were evaluated on light microscopy and the alterations were registered on an enhanced pathology grading system (from 0 to 4). Results: The kidneys of the control group had no pathological changes. Tubular necrosis, tubular mineralization both metastatic and dystrophic and focal and diffuse stromal infiltration with mononuclear inflammatory cells were more pronounced on group II than on group III. Glomerular atrophy, congestion and hemorrhage were more evident on group III than on group II. Conclusions: Dexamethasone administered on anti-inflammatory dose is able to reduce the tubular and stromal damages caused by the overdose of vitamin D3. Dexamethasone is useful on in vivo studies for vitamin D3 anti-cancer effects, since the kidneys are partially protected against calcitriol toxicity.

Keywords: Vitamine D, Dexamethasone, Kidney

**C65 MELANIZATION IN AEDES AEGYPTI LARVAE AFTER EXPOSURE TO A FATTY ACID METHYL ESTER (FAME).**
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Introduction: Aedes aegypti is a blood-sucking mosquitoes capable of transmitting diseases such as yellow fever and dengue, especially in urban areas of tropical and temperate regions they are found under the local clearance. Currently, natural compounds which are effective, ive and non-toxic to associate populations are being researched in order to combine effectiveness, low production cost and minimize the emergence of resistance. Thus, Fatty Acid Methyl Esters (FAMES), obtained by transesterification of plant material, are being tested with some success. However, little is observed in the pathological process in insect larvae. Objective: The present work aims to observe changes in the larvae of Aedes aegypti after exposure to different FAMES. Methodology: A group of 12 larvae were exposed to sub-lethal concentration of FAME for 12 hours. Every hour 3 larvae of the solution was taken for analysis in stereomicroscopy. The control group was exposed to unchlorinated water with 1% DMSO and also observed in stereomicroscopy Results: The pigments promoted the emergence of the larvae the 1 hour exposure. Conclusions: The pigments observed may be part of the process of melanization that is involved in cuticle and immune system of insects. Associated to this process also, there sclerotization with the production of N-acetyl-dopamine (NADA) and N-β-alanil-dopamine (NBDA) and thus exposed larvae to FAME’s may have changed in these processes. However, further studies should be conducted to observe the biochemical, morphological and fisiológicos effects that FAMES can cause on the A. aegypti larvae.

Keywords: FAME, Aedes aegypti, Melanization

Supporting funding: CAPES/FAPEMIG

**C66 METABOLIC ADAPTATIONS TO ARSENIC-INDUCED OXIDATIVE STRESS IN LIVER AND KIDNEYS OF MALE WISTAR RATS.**
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Introduction: Arsenic compounds are ubiquitously distributed natural toxicants. Human exposure to arsenic is associated with cancers, organ injury, and several ill health effects. A proposed mechanism of action in exerting the toxic effects include mitochondrial damage, leading to impairment of tissue respiration, oxidative stress, and tumor promotion. The inorganic trivalent form of arsenic is now thought to be more toxic than the organic species. Objectives: Therefore, this work aimed to evaluate the effect of chronic administration of a fencic, as sodium arsenate and arsenite, on the antioxidant enzyme concentrations in liver and kidneys.

Methodology: Adult Wistar rats were randomly divided into five groups (n = 5 animals/group). The
G1 received saline, while the treated animals were exposed orally to sodium arsenate (G2 and G3) and arsenite (G4 and G5), testing for each chemical form the concentrations of 0.01 mg/L and 10 mg/L. The animals were weighed and euthanized after 57 days of treatment. The liver and kidneys were dissected and weighed. For the analysis of the activities of superoxide dismutase (SOD), and catalase (CAT), samples of 100 mg of organs (frozen) were homogenized in phosphate buffer. Subsequently, Triton x 100 was added to the samples in which the CAT activity was examined. The homogenized samples were then centrifuged, 5 rpm at 4 °C for 10 minutes, and the supernatant used for analysis of CAT and SOD. The results were expressed in nmol g-1. Results: No difference was observed among treatments for the body weight, absolute and relative weights of liver and absolute weight of kidneys (P > 0.05). However, the relative weight of the kidney increased in rats of the G4 when compared to G1. The SOD activity in the liver was reduced on G3, G4, and G5 when compared to G1, especially in animals treated with arsenite. The SOD activity in the kidney at G4 (0.020 ± 0.001) was higher than the G2 (0.010 ± 0.001). Considering CAT enzyme, its activity in the liver at G3 (25.34 ± 4.30) was lower than the G5 (41.80 ± 0.80). In the kidney, this enzyme showed greater activity on G4 and G5 when compared to the other groups. Conclusions: These observations indicated that chronic exposure to arsenic causes induction of oxidative stress and biochemical alterations on liver and kidneys.

Keywords: Catalase; superoxide dismutase; arsenite
Financial support: FAPEMIG

C67 STRUCTURAL AND ULTRASTRUCTURAL HEPATIC CHANGES BY EXPOSITION TO BENZNIDAZOLE.
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Introduction: The reference chemotherapy for the therapeutic management of Chagas’ disease is based on Nifurtimox and Benznidazole, both drugs with marked side effects and poor organic tolerability. Objective: To investigate the effect of acute exposition to Benznidazole on morphological and biochemical parameters of the hepatic tissue. Methodology: Twelve male adult Swiss mices (25.29 ± 2.65g, CV=0.11) were equally randomized into 2 groups: Control group (CG), carboximetilcelulosi (1%); Group Benznidazole (BZ), Benznidazole (100 mg/kg, therapeutic dose). The treatments were administered during five days and the animals were euthanized 24 hours after the last treatment for analyses of tissue content of nitric oxide (NO2-/NO3-), Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) in serum, stereological analysis of glycogen and collagen fibers by bright field microscopy, and ultrastructural analysis of the liver tissue by transmission electron microscopy. Results: Treatment with Benznidazole conducd to a marked increase in NO2-/NO3- (CG, 0.14 ± 0.03 μM/mg protein; vs. BZ, 0.35 ± 0.06 μM/mg protein; p<0.001), as well as AST (CG 53.18 ± 6.11 μM/mg protein; vs. BZ, 145.87 ± 11.39 IU/L; p<0.001) and ALT (CG 169.83 ± 20.50 IU/L; vs. BZ, 392.14 ± 37.26 IU/L; p<0.001) serum levels. The stereological analysis indicated that volume density of cytoplasm glycogen inclusions and collagen fibers were similar in both groups. However, type I collagen fibers presented a fragmented aspect and type III collagen fibers were more evident in the liver tissue from animals receiving BZ. In this group, the ultrastructural analysis of hepatocytes indicated degenerated mitochondria with electron-Lucent matrix, disorganized internal cell membranes (multilamelar bodies) and cell vacuolization compared to CG. Conclusion: The results indicated that acute exposition to Benznidazole induces structural and functional damage of the liver tissue in mice. Pathological changes of the liver parenchyma are clearly evidenced by transmission electron microscopy, but not by conventional light microscopy. These changes are possibly related to the increased production of nitric oxide during Benznidazole metabolism, event widely associated with the cytotoxic and antiparasitic effect of this drug. Keywords: Experimental pathology, Antiparasitic chemotherapy, Toxicology
Supporting funding: CAPES

C68 SUBCHRONIC EXPOSITION TO TRIBUTYL Tin PROMOTES THYROID GLAND HISTOMORPHOLOGICAL CHANGES.
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Introduction: Triorganotins, such as tributyltin (TBT), are environmental contaminants commonly found in antifouling paints that are used on the ships and other vessels. These chemicals are also suspected to cause endocrine-disrupting effects in mammals, due in part to their possible transfer through marine food chains and to the consumption of contaminated seafood. The importance of triorganotins as environmental endocrine disruptors in different animal models is well known; however, the adverse effects on thyroid gland are poorly defined. Objective: In the study we evaluate whether the treatment with TBT induces histological changes on the thyroid gland or thyroid dysfunction. Methodology: Male Wistar rats (8 weeks old, ±250g) were divided into 3 groups: control (vehicle, 0.4% ethanol), TBT1 (100ng.kg-1.day-1) and TBT2 (200 ng.kg-1.day-1) treated daily for 15 days by gavage. Then, the animals were euthanized and thyroid glands were removed and fixed to perform histo logical analysis. Results: Both doses of TBT promoted a follicular disorganization. We observed thyocytes hypertrophy and hyperplasia, congestion of the tissue, as well as increased mast cells
infiltration and collagen deposition in TBT treated animals, when compared with the thyroids of the control group. However, histomorphometric analysis did not show alterations of follicle and colloid areas and epithelial height in the treated group. Finally, we did not observe any changes in plasma T3 and T4 levels, although the thyroid and liver type 1 deiodinase activities were decreased in the TBT treated group. **Conclusion:** we observe that subchronic TBT exposure induce thyroid gland morphophysiological changes, and may correspond to a potential risk factor for thyroid disorders in mammals. 

**Keywords:** tributyltin, endocrine disruptors, thyroid gland
D01 A POTENTIAL LINK BETWEEN INCREASED ACTIVATION OF PERIPHERAL LEUCOCYTES AND KIDNEY DAMAGE IN ANIMALS WITH NEPHROTIC SYNDROME INDUCED BY DOXORUBICIN

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Introduction: The Nephrotic Syndrome (NS) is characterized by proteinuria, hypoalbunemia and generalized edema. Although the pathophysiological mechanisms of NS remain unknown, studies with animal models and patients have associated the NS with changes in immune response. Objectives: The present study investigated the expression of molecules related to cell activation, such as the beta-2 integrin (CD18) and CD80 on peripheral blood leukocytes and renal production of reactive oxygen species in rats with NS induced by Doxorubicin. Methodology: Male Wistar rats, 250-300g, were divided into two groups: animals receiving intravenous injection of doxorubicin (7.5 mg/kg) (DOX, n=32) and control animals that received saline (CON, n=32). The animals were sacrificed at days 7, 14, 21 and 28 after injection, and 24 hour urine and blood samples were collected for biochemical and immunological analyses. The phenotypic analysis of leukocytes was performed by flow cytometry. The expression of CD18 in monocytes, CD4+, CD8+ and NK cells and the expression of CD80 in monocytes were measured. In renal tissue samples, the oxidative activity was evaluated by TBARS production and antioxidant activity of SOD and catalase. Results: The DOX group animals showed significant increase in cellular CD18 expression and in the percentage of cytotoxic T lymphocytes, NK cells and monocytes that expressed CD18 as well as raised CD80 expression on peripheral blood monocytes when compared to the CON group. The increased production of reactive oxygen species in renal tissue of DOX group animals was positively correlated with the CD80 expression on monocytes and serum levels of creatinine. Conclusions: These findings indicate a potential link between increased activation of peripheral monocytes and kidney damage in animals with NS. Additional studies analyzing the effects of the blockade of integrins and co-stimulatory molecules may offer new therapeutic opportunities to treat human NS.
Keywords - Nephrotic syndrome, rat, immunology. Financial Support – CAPES, CNPq, FAPEMIG

D02 ALTERED LEVELS OF INFLAMMATORY CYTOKINES IN PATIENTS WITH FIBROMYALGIA.

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Introduction: Fibromyalgia (FM) is a clinical condition characterized by chronic and widespread pain, associated with hypersensitivity to palpation of at least 11 of 18 tender points already established by American College of Rheumatology. Other symptoms may also be associated with the clinical status of FM as morning stiffness, sleep disturbances, fatigue and cognitive changes. To date, the pathophysiology of FM is not completely understood and therefore, several hypotheses have been used in attempt to explain it. One of these hypotheses suggests that the genesis and evolution of the FM may be related to an immunological changes that result in an imbalance in the production of inflammatory cytokines. Objective: To evaluate the plasma concentrations of some inflammatory cytokines of profile Th1, Th2 and Th17 in patients with FM. Methodology: This is a cohort and observational study with use of a control group. Thus, we used 58 women diagnosed with fibromyalgia and 39 healthy women with similar age and body mass index (BMI). The peripheral blood samples were obtained by puncture in the brachial vein. After centrifugation, plasma was separated and analyzed by flow cytometry, via the technique cytometric bead array (CBA). The cytokines IL-2, IL-4, IL-6, IL-10, TNF, IFN-γ and IL-17A were evaluated. Data analysis was carried out by GraphPad Prism v5.0 and differences were considered significant when p < 0.05. Results: We identified significantly elevated levels of IL-2, IL-4, TNF, IFN and IL-17A in FM patients when compared to healthy controls. The levels of IL-6 and IL-10 were not different between patients and controls. Conclusion: The Results of this study reinforce the hypothesis of the involvement of the immune system in the pathophysiology of FM. The elevated levels of cytokines in these patients, particularly the proinflammatory, may lead clinicians and researchers to consider the use of anti-inflammatory drugs for the treatment of fibromyalgia.
Keywords: Fibromyalgia, Cytokines, Pathophysiology.
Supporting funding: FAPEMIG e CNPq

D03 NEW TYPE OF DELAYED HYPERSENSITIVITY WITH INFILTRATION OF EOSINOPHIL, LYMPHOCYTES AND TH1 PROFILE INDUCED BY LAAG PLUS SAPONIN VACCINE

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Introduction: Leishmania amazonensis is the main agent of anergic diffuse cutaneous leishmaniasis. Our previous studies demonstrated that LaAg vaccine plus Saponin induced a partial protection against L. amazonensis infection observed by partial reduction of lesion growth and parasite...
load. **Objective:** In this study, we investigated the initial immune response after challenge to characterize the protective response. **Methodology:** Prior to footpad infection with L. amazonensis, BALB/c mice were twice vaccinated by the intramuscular route with 25 μg of LaAg containing 100 μg of Saponin. **Results:** We found that vaccinated mice developed delayed hypersensitivity peaking at 15-18 hour similar to Jones-Mote reaction. Histological studies demonstrated a lymphocyte and eosinophil infiltration and reduction of neutrophil, macrophage and mast cells infiltration in comparison to non-vaccinated mice. Eosinophil and lymphocyte (Th1) cells are related to parasite control and Neutrophils and Mast Cells are associated to susceptibility of L. amazonensis infection, suggesting that vaccine induced a cellular infiltrate that corroborate to parasite site control. Indeed, We observed by mRNA quantification in the peak of hypersensitivity an increase of T-bet and GATA-3, but not FOXP3 on popliteal lymph node cells and demonstrated in footpad an increase of IL-4, IL-10, IFN-γ and IL-12 production and TGF-β reduction demonstrating a Th1 and Th2 profile. However, we observed an increase of iNOS levels in the footpad of vaccinated mice. The increase of iNOS in infected footpad is related to Th1 profile and associate with parasite elimination. **Conclusions:** Our result demonstrated a new-type of delayed hypersensitivity associated with Th1 response different of classical DTH reaction and suggests the involvement of this new-type of delayed hypersensitivity to parasite control in vaccinated mice against L. amazonensis infection. Keywords: Vaccine; Leishmania amazonensis; saponin

**D04 INFLUENCE OF LEISHMANNIA (VIANNIA) BRAZILIENSIS INOCULUM LOAD ON PATHOGENESIS OF AMERICAN TEGUMENTARY LEISHMANIASIS (ATL) IN THE GOLDEN HAMSTER MODEL (MEOOCRICTUS AURATUS)**
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**Introduction:** ATL is widely spread in Brazil and represents a major public health problem. In this context, Leishmania (Viannia) braziliensis stands out as the most prevalent species. Studies in experimental model are of paramount importance to evaluate immunopathogenesis and efficacy of vaccine candidates and new drugs. Golden hamster (Mesocricetus auratus) is the most suitable experimental model for L. (V.) braziliensis infection, developing chronic lesions resembling humans in both clinical and histopathological aspects. **Objective:** This study aimed to evaluate the influence of inoculum load of L. (V.) braziliensis in the pathogenesis of experimental cutaneous leishmaniasis. **Methodology:** 45 hamsters from three independent experiments, divided in three groups (n=5), intradermally infected with different inocula: 104, 105 and 106 promastigotes in stationary phase of L. (V.) braziliensis. Fragments of skin of inoculated site and spleen were formalin fixed and prepared for paraffin embedding. Sections of five micrometers were stained with hematoxilin and eosin. **Results:** The main difference between different inocula was the absence of inflammatory infiltrate in dermis of 104 Leishmania inoculated animals which didn’t develop clinical lesions. On the other hand, in all groups, animals with clinical lesions showed granulomatous reaction surrounded by lymphocytes, plasma cells and polymorphonuclear, more localized as lower inoculum and more extensive as higher inoculum; Shaumann’s bodies, as well as parasites inside vacuolated macrophages, were seen more frequently in 106 Leishmania infected animals. Spleens showed areas of granuloma and vacuolated macrophages with parasites, most frequently in 106 inoculated animals, as well as some 105 inoculated ones, indicating visceralization of Leishmania, while 104 inoculated animals showed no signs of visceralization. Shaumann’s bodies were rarely seen in spleen, only in 106 inoculated animals. **Conclusions:** Our data indicate that different intradermal inocula of L. (V.) braziliensis influences the pathogenesis of experimental cutaneous leishmaniasis in the hamster model, represented by differences in histopathological features of inoculated skin and by histopathological evidence of Leishmania spleen visceralization. Keywords: Leishmania (Viannia) braziliensis; Golden hamster; histopathology.

**D05 ANTIMICROBIAL AND ANTIMICOTIC ACTIVITY OF THE ESSENTIAL OIL OF CINNAMOMUM ZEYLANICUM AGAINST STRAINS OF CRYPTOCOCCUS NEOFORMANS.**
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**Introduction:** The search for effective antifungal therapies has motivated many researches to discover new antifungal agents that promote interaction with new targets and inhibits the emergence of resistant strains, especially those related to opportunistic infections such as Cryptococcus neoformans. In this context, essential oils emerge as a promising alternative for being recognized for their antimicrobial potential. **Objectives:** The present study aimed to evaluate the effect of essential oil of Cinnamomum zeylanicum against strains of Cryptococcus neoformans. **Methodology:** Antifungal activity of the essential oil was evaluated by determination of the Minimum Inhibitory Concentration (MIC) and Minimum Fungicidal Concentration (MFC) by microdilution assay. To determine the MIC were used sterile 96-well plates. Initially were added to the wells 100 μL of Sabourad dextrose broth (SDB) doubly concentrated, then 100 μL of suspension of the essential oil in initial concentration 2048 μg/mL (also doubly concentrated) and by serial dilution of two, the concentrations of 1024, 512, 256, 128, 64, 32 and 16 μg/mL were obtained. It was also added 10 μL of each tested microorganism suspension prepared in 0.5 of the McFarland scale (1x106 CFU/mL), approximately. The same test was conducted with the antifungal agent flucytosine under same conditions. The determination of MFC of C. zeylanicium oil was performed.
using aliquots 10 μL removed from each well of the microdilution plate from the lowest concentration capable of inhibiting 1 x 10^6 CFU/mL of B. ovis strain ATCC25840 (wild type) by intraprepuial inoculation containing 1.2 x 10^9 CFU/mL of B. ovis ΔabcAB-infected ram. All plates were incubated at 35 °C for 72 hours for MIC and MFC assays. Results: The essential oils used showed growth inhibitory effects in the assays tested, with variations MIC in the range between 128 - 512 μg/mL and MFC between 256 - >1024 μg/mL for all strains of C. neoformans analyzed. Conclusions: Essential oil of C. zeylanicum showed antifungal activity against the strains of C. neoformans studied. Keywords: Antifungal, Cinnamomum zeylanicum, Cryptococcus neoformans.

**D06 BRUCELLA OVIS LACKING A SPECIES-SPECIFIC PUTATIVE ATP-BINDING CASSETTE TRANSPORTER IS ATTENUATED BUT IMMUNOGENIC IN RAMS.**

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Introduction: Ovine brucellosis caused by Brucella ovis is considered one of the most important reproductive diseases of rams worldwide. Previously, we demonstrated that a B. ovis ΔabcAB strain, which lacks a B. ovis-specific ABC transporter, is strongly attenuated in mice. However, this mouse attenuated B. ovis ΔabcAB strain has never been studied in rams, its natural host. Objectives: The aim of this study is to characterize the kinetics of infection of the B. ovis ΔabcAB strain in rams, evaluating its persistence and immunogenic potential. Methods: Twelve 1-year-old crossbred rams were used. Six rams were challenged with 2 mL of a suspension containing 1.2 x 1010 CFU/mL of B. ovis ΔabcAB strain ATCC25840 (wild type) by intraprepuial inoculation and additional 50 μL in each conjunctival sac of a suspension containing 1.2 x 1010 CFU/mL of the same strain. The other six rams were challenged with an equivalent number of CFU of the mutant strain ΔabcAB B. ovis through the same routes. Serum samples for serology and semen and urine samples for bacteriologic culture and PCR were collected weekly during 24 weeks. At 24 weeks post infection, tissue samples were collected for bacteriologic culture and PCR. Results: All rams inoculated with wild type or the ΔabcAB strain seroconverted at the fourth week post infection, remaining positive up to the 16th week post infection. PCR and bacteriologic demonstrated that only rams inoculated with the wild type strain shed the organism in semen and urine. Lymphocytes from rams inoculated with wild type or ΔabcAB B. ovis had significantly higher proliferation in response to B. ovis antigens when compared with unstimulated controls. Tissue bacteriologic and PCR detected B. ovis in all rams challenged with the wild type strain, whereas only one ΔabcAB-infected ram had a positive iliac lymph node sample by PCR. Conclusions: The ΔabcAB B. ovis strain that lacks a functional species-specific ABC transporter is capable to trigger a profile of humoral and cellular host response that is similar to the wild type strain. Additionally, mutant strain is not recovered in the semen, urine or organs as the wild type strain. Together these results support the hypothesis that this mutant has a high potential as a vaccine strain. Keywords: ovine, brucellosis, epididymitis, vaccine. Keywords: 1: ovine, Brucellosis, epididymitis, vaccine. Supporting funding: CNPq, CAPES, FAPEMIG.

**D07 CHEMOKINE PROFILE IN THE SERA AND URINE OF PATIENTS WITH GLOMERULONEPHRITIS OF VARIED CAUSES.**

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Introduction: Glomerulonephritis (GN) is an important cause of morbidity and mortality in patients of all ages throughout the world. The clinical manifestations usually appear when there is already severe kidney damage and, consequently, early diagnosis would allow early intervention and become the progression of the disease slow. Some studies have proposed the identification of early biomarkers of renal injury. Objective: Investigate the serum and urine levels of chemokines in patients with glomerulopathy of varied causes. Methods: This cross-sectional study was conducted of Minas Gerais in Brazil from October 2008 to July 2010. After informed consent was obtained 65 subjects were enrolled and divided among the following 3 groups: 1) glomerulopathy of varied causes, without schistosomiasis (n=22); 2) hepatosplenic schistosomiasis with renal disease (n=12); and 3) apparently healthy (n=31). All of the participants were submitted to clinical and laboratory examinations. Those patients with microalbuminuria >30mg in 24 hours were considered to have renal disease. Sera and urine samples were obtained from all of the participants and stored at -80°C. The sera and urine chemokines CCL2, CCL3, CCL5, CCL11 and CXCL8 were measured using an enzyme linked immunosorbent assay (ELISA) test and commercial kits. The data were analyzed using the SPSS software. Results: The patients with patients with glomerulopathy caused by other diseases as follows: CCL3 in the urine >14.3 pg/ml, sera CCL3 >61.9 pg/ml, CXCL8 in the sera <1,030 pg/ml, CCL11 in the urine >26.7 pg/ml and sera CCL2 >634.3 pg/ml. A similar profile was observed in the patients with hepatosplenic schistosomiasis and renal disease presented, with the exception of serum CCL2>634.3 pg/ml. Conclusions.In cases sera with CCL2 >634.3 pg/ml, the diagnosis of glomerulopathy of varied causes should be considered. However further studies need to be performed to confirm the profile found. Keywords: albuminuria, chemokine, glomerulonephritis.
Introduction: Visceral leishmaniasis is a parasitic disease that causes throughout the world, more than 500 000 new cases per year. Although the transmission happen mainly by vector, have been described cases of transmission in areas where there is no presence of the vector. The venereal transmission in dogs and humans has been described in the literature. Objectives: This study aims to observe the presence of amastigotes of Leishmania in bladed implants of different tissues of the reproductive tract and compare them with skin and bone marrow. Methodology: This study analyzed 22 dogs seropositive for leishmaniasis by serological tests ELISA and IF and with characteristic clinical signs of visceral leishmaniasis. Stool tests done by affixing material blade (imprint), stained with GEMSA, fragments of testis-epididymis, skin and bone marrow. The analysis was done using the correlation coefficient phi. Results: Thus, we studied the correlation between the frequency shaped amastigostas Leishmania found in each tissue, comparing the reproductive tissues such as testis and epididymis with skin tip ear and bone marrow. The correlations between the frequencies of Leishmania found in the tissues (bone marrow x testicle) (Skin x testicle) (Skin x epididymis) showed no significant difference (GL. 1, D = 5%). However, the frequency of amastigotes found in bone marrow is significantly greater when compared to the epididymis. Conclusions: Further studies are being conducted to determine the parasite load in different organs, but yet we observe a systemic spread of parasites not showing a tropism for certain tissues. Keywords: Visceral Leishmaniasis, Histopathology, dogs.

Supporting funding: Fapemig, CNPq

D09 CORRELATION OF ASPARTIC PROTEASES ACTIVITY OF Candida albicans WITH PHAGOCYES APOPTOSIS AND PRODUCTION OF IL-17.

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Introduction: Pepstatin-A is known for inhibiting the activity of Secreted Aspartic Proteases (SAPs) of Candida albicans. Among the SAPs, specifically two are anchored to the cell wall of Candida playing an important role in the processing of cell surface proteins, as well as proteins that are secreted, being highly relevant to the induction of adhesion and tissue damage. Objectives: This study evaluated the effect of pepstatin in reducing apoptosis of peritoneal cells and the pattern of cytokine production during the course of infection by C. albicans. Methodology: The strain CR15 was used, with or without the addition of pepstatin to the inoculum and tissue damage. Results: The peritoneal exudate was collected with complete RPMI 1640 medium, centrifuged and the supernatant stored for cytokine quantification by ELISA. The cell pellet was added to coverslips for admission of phagocytes. After 0.5h, the cells were stained with Annexin-V-FITC and DAPI, then observed under a fluorescent microscope for the analysis of apoptosis. Results: Pepstatin reduced rate of apoptosis of peritoneal phagocytes from 60% to 12% by CR15 after 30 minutes of infection, being this value similar to the baseline found in the PBS group (9%). The infection induces peaks of IL-1β production at 2h (CR15 82.9 pg / ml ± 8.3; CR15 + Pep 108.1 pg / ml ± 9.8) as well as IL-6 (CR15 287.5 pg / ml ± 6, 4; CR15 + Pep 306.7 pg / ml ± 8.2) and TGF-β (CR15 82.9 pg / ml ± 9.8; CR15 + Pep 110.2 pg / ml ± 9.5), which evidenced an increase of these cytokines in the presence of pepstatin, except IL-6 did not differ between the groups. With 18h infection was possible to observe an increase in IL-17 production only in the absence of pepstatin (CR15 513 pg / ml ± 32,8; CR15 Pep + 61,1 pg / ml ± 7,6), indicating the occurrence of Apoptosis was necessary to produce this cytokine. Conclusions: The Results suggest that the SAPs are directly related to the occurrence of apoptosis of phagocytic cells and the cytokine pattern TH17 during infection with C. albicans strain CR15. Keywords: Candida albicans, apoptosis, IL-17

Supporting funding: Fundação Araucária, Capes, PROPPG Universidade Estadual de Londrina

D10 MODEL OF CUTANEOUS CANDIDIASIS IN BALB/C MICE

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Introduction: Chronic cutaneous candidiasis is a heterogeneous group of syndromes with common features including noninvasive chronic infection by Candida albicans in skin, nails and mucous membranes and associated with endocrine and genetic faults in the immune system. Objectives: In this work, we aimed to create a model to study chronic cutaneous candidiasis. Methodology: We established skin lesions with C. albicans in the right hind paw of BALB / c mice used as control left hind paw inoculated with saline solution (PBS) to calculate the area of edema. Five mice per group received C. albicans 5x106 grown in YPD at 30 °C and differentiated by the pseudo-hyphae growth on YPD with 10% fetal bovine serum for 2 hours at 30 °C. The evaluation period was from time zero to 21 days by measuring the extent of the injury with a caliper and calculating edema (infected paw less control paw). The mice were euthanized with ether after 1, 2, 3, 4, 5, 6, 7, 14 and 21 days of infection. The skin tissue containing the lesion C. albicans and controls without infection over the popliteal lymph nodes were collected, fixed in formalin and embedded in paraffin. 7mm cuts were made and stained with hematoxylin-eosin (HE). Results: Edema, compared with controls (0.00 ± 0.00) was
statistically significant, with p <0.01. After 24 hours of infection, edema calculated was 1.92 mm ± 0.33, being significant until 10 days after this time (p <0.05). The peak of the edema occurred between 6 (2.64 ± 0.57) and 9th day after infection (2.70 ± 0.54 mm). Day 10 (2.57 ± 0.60 mm) until the 15th day (2.09 ± 0.44 mm) decreased edema showing a significant (p <0.05) compared to the ninth day. After the 16th day of infection edema remained virtually the same until day 21 (1.89 ± 0.64 mm and 1.85 mm ± 0.35) suggesting then that there was no resolution of the lesion, thus showing that this remained chronic. The HE stained sections showed infiltration by immune cells in lesions caused by C. albicans. Conclusions: The Results show that the model developed appears to be effective in establishing a chronic skin lesion caused by C. albicans infection. Evaluation by immunohistochemical will differentiate the immune cells that migrate to the site of infection and cytokine will elucidate and confirm if the lesion has a tendency to chronic infection. Keywords: model, chronic lesion, cutaneous candidiasis.

Supporting funding: Fundação Araucária, CAPES, Prö-PPG

D11 FUSARIUM OXYSPORUM MYCOTOXINS INDUCE APOPTOSIS IN KERATINOCYTES AND FIBROBLASTS IN THE SKIN.

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Introduction: Fusarium is an emerging pathogen due to increasing number of severe cases with high morbidity and mortality. Fusarium oxysporum is one of the most pathogenic species for causing infections that compromise the integumentary system. Toxins, produced by this genus, have been studied to elucidate their biological effects. Recently, it was demonstrated the irritant potential effect of crude extract of Fusarium oxysporum produced in vitro, capable of inducing tissue response and programmed cell death in the skin of healthy rats. Objectives: This study investigates morphological changes in the skin and subcutaneous tissue in healthy rats after intradermal injection of a fraction obtained from the extract of Fusarium oxysporum. Methodology: This study was performed in intradermal injection of a fraction obtained from the extract of Fusarium oxysporum. This study was performed in compliance with the Animal Care and Use Committee at State University of Maringá (protocol 005/2010 and 080/2010). The extract was obtained from the fungus cultivation in sterilized Czapek-Dox, by filtration and addition of thimerosal and the fraction isolated by electrophoresis. Were used 40 male Wistar rats, weighing 150-200g. Was performed only one application of the 50mL fraction (0.5 mg / ml) intradermally into the mice skin, which were sacrificed at 3, 6, 12 and 24 hours after inoculation. Skin samples were collected, fixed, embedded in paraffin and immunostained by the TUNEL technique and counterstained with hematoxylin to visualize apoptotic cells. The analyses were performed on Olympus BX41 microscope and immunostaining was comparable qualitatively based on a frequency of labeled cells. Results: Keratinocytes in the basal layer of the epidermis and dermal fibroblasts showed TUNEL-positive immunostaining in all observation periods, being more intense at 12h. At 3, 6 and 12 hours were observed the detachment of the epidermis and balloon-degenerated keratinocytes were observed. The cells of inflammatory infiltrate were not positive for TUNEL in the analyzed periods. Conclusions: These results indicated that a single intradermal application of a fraction isolated from the crude extract of F. oxysporum induced apoptosis in different cells lines indicating the presence of toxic substances in the fraction.

Keywords: Fusarium oxysporum, TUNEL, histopathology.

Supporting funding: Fusarium oxysporum, TUNEL, histopathology.

D12 GLUCOCORTICOID-INDUCED TUMOR NECROSIS FACTOR RECEPTOR EXPRESSION IN PATIENTS WITH CERVICAL HUMAN PA-PILLOMAVIRUS INFECTION.

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Introduction: The progression of human papillomavirus (HPV) infection in the anogenital tract has been associated with the involvement of cells with regulatory properties. Evidence has shown that glucocorticoid-induced tumor necrosis factor receptor (GITR) is an important surface molecule for the characterization of these cells and proposes that GITR ligand may constitute a rational treatment for many cancer types. Objective: To detect the presence of GITR and CD25 in cervical stroma cells with and without pathological changes or HPV infection to better understand the immune response in the infected tissue microenvironment. Methods: We subjected 49 paraffin-embedded cervical tissue samples to HPV DNA detection and histopathological analysis, and subsequently immunohistochemistry to detect GITR and CD25 in lymphocytes. Results: We observed that 76.9% of all samples with high GITR expression were HPV-positive regardless of histopathological findings. High GITR expression (77.8%) was predominant in samples with ≥1,000 RLU/PCB. Of the HPV-positive samples negative for intraepithelial lesion and malignancy, 62.5% had high GITR expression. High GITR expression was observed in both carcinoma and high-grade squamous intraepithelial lesion (HSIL) samples (p = 0.16). CD25 was present in great quantities in all samples. Conclusions: The predominance of high GITR expression in samples with high viral load that were classified as HSIL and carcinoma suggests that GITR+ cells can exhibit regulatory properties and may contribute to the progression of HPV-induced cervical neoplasia, emphasizing the importance of GITR as a potential target for immune therapy of cervical cancer and as a disease evolution biomarker.

Keywords: Human papillomavirus, Immune response, Immunohistochemistry.

Supporting funding: CAPES and FUNDECT-MS
D13 INDUCIBLE NITRIC OXIDE SYNTHASE (iNOS) OF MURINE MACROPHAGES INFECTED BY L. AMAZONENSIS AND TREATED WITH TETRANIA RIPARIA ESSENTIAL OIL.

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Introduction: The inducible nitric oxide synthase (iNOS) can synthesize a huge amount of nitric oxide (NO), which is a leishmanicid substance. On cutaneous Leishmaniasis, the specie L. amazonensis produces NO which subvert this microbicidal activity and escape of the death. The NO leads to resolution of infection. The Tetradenia riparia essential oil (‘falsa mirra’) is as leishmaniostatic agent. However its action on the NO route is unknown. Objectives: We investigated the iNOS mRNA expression and NO production of murine macrophages infected with L. amazonensis and treated with the T. riparia essential oil. Methods: Peritoneal macrophages of BALB/c mice were undergone to different conditions: 1) addition of lipopolysaccharide, positive control; 2) addition of RPMI medium, negative control, 3) infection by promastigote of L. amazonensis; 4) addition of T. riparia essential oil (30ng/ml); 5) infection and treatment with the essential oil. After 3, 6 and 24 hours, the supernatant was removed and was done the quantification of the NO production using the Griess method. The extraction RNA of cellular monolayer (3 e 6h) was done and them expression analysis by transcription reverse PCR (polymerase chain reaction). The glycerol-3-phosphate dehydrogenase mRNA was used as internal control. The expression of mRNA was revelead by agarose gel electrophoresis. Results: iNOS was expressed by Leishmania-infected macrophages and T. riparia-treated macrophages after 3 and 6 hours, but not by negative control. Macrophages infected by Leishmania and treated with T. riparia essential oil not showed iNOS expression. The NO production was not difference in 3 and 6 hours. After 24h, the production of control positive was 5.0μM, control negative (1.5μM), as Leishmania-infected macrophages produced 0.8μM. Conclusion: Macrophages infected by L. amazonensis expressed iNOS, but this infection decreased the nitric oxide production. The T. riparia essential oil alone induced the expression of iNOS and not modified the NO. Therefore, the NO production by Leishmania-infected macrophages was inhibited, and treatment with T. riparia of infected macrophages not modifies the nitric oxide route. Keywords: Leishmania, Tetradenia riparia, nitric oxide.

D14 LICHEN PLANUS AND LICHENOID REACTIONS OF THE ORAL MUCOSA: COMPARATIVE STUDY OF EXPRESSION OF MOLECULES RELATED TO OXIDATIVE STRESS.

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Introduction: Oral lichen planus (OLP) is a chronic inflammatory disease mediated by T cells of unknown cause that affects the skin and mucous membranes. It is more common in middle-aged adults, especially in women. The clinical presentation of the disease may vary among different kinds, main reticular and erosive feature. Histologically, it is characterized by a dense lymphocytic infiltrated subepithelial band, apoptosis of keratinocytes, liquefaction of the basal layer, epithelial hyperplasia and hyperkeratosis. The oral lichenoid reactions (OLR) are very similar to OLP as the clinical and histopathological findings, but it is distinguished by associating specifically with precipitating factor. A current and relevant problem about these lesions is the possibility of malignant transformation, which has been related to the process of oxidative stress. Some molecules are closely linked to the effects of oxidative stress, such as the MSH-2 enzyme and metallothionein protein. Objectives: The aim of this study was to identify possible differences in the expression of MSH2 and metallothionein molecules related to oxidative stress, between lesions of OLP and OLR. Methodology: Immunohistochemical reactions were performed to identify the metallothionein expression and MSH-2 in cases of OLP and OLR, carefully according to the clinical and histological diagnosis. Results: Reactivity for MSH-2 was found in nuclear pattern at the same time as expression for metallothionein was found in both cytoplasmatic and nuclear compartments of the cells. There was no significant difference in the proportion and intensity of reactivity for MSH-2 and metallothionein in between OLP and OLR. On the other hand, significant correlation was observed in the rate of QuickScore for MSH-2 and metallothionein in cells of the basal and parabasal layer of OLRs. Conclusion: The results suggest that the oxidative stress response does not change of intense form between OLP and OLR, but show a possible relationship between the expression of metallothionein and DNA repair molecules such as MSH2, in OLRs. Keywords: Oxidative stress, Immunohistochemistry, Oral lichen planus. Supporting funding: FAPEMIG

D15 PHOTODYNAMIC THERAPY APPLIED AGAINST CANDIDA YEASTS.

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Introduction: Candida yeasts are normal part of human microbiota. However, there are predisposing factors that that create a propitious environment to development of infections by these microorganisms. Photodynamic therapy (PDT) was initially developed by treatment of cancer, but recently it has been considered a promising tool in treatment of microbial infections. In this context, it is very relevant to investigate the efficacy of PDT against Candida yeasts. Methodology: Reference samples (ATCC) of C. albicans, C. krusei were used and incubated for one hour in dark at 37°C. After this period, samples were irradiated...
with LED as light source during 15 minutes. In order to evaluate the effect of the experiment in yeasts growth, 100 microliters each a samples were inoculated in Sabouraud dextrose agar at 37°C for 24 hours for colony counting. Results: MA without irradiation demonstrated a small action in the growth inhibition of Candida yeasts when compared with the Results of PDT. The LED irradiation without MA stimulated yeast growth. However the employment of PDT was successful in the inhibition of Candida growth. Discussion e Conclusion: MA PDT have demonstrated to be effective in the inhibition of Candida species. The treatment arrises as an important alternative to conventional antifungal therapies, as well as it can be employed as a coadjuvant to antifungals. The treatment is non invasive and it can improve the fungal killing and bring more comfort and quick Results to the patient. Keywords: Photodynamic Therapy, Candida yeasts, Methylene blue. Supporting funding: CNPq

D16 PROFILE STUDY ON THE INITIAL IMMUNE RESPONSE OF MACROPHAGES CHALLENGED BY A SYNTHETIC CHALCONE.

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Introduction: The chalcones, or 1,3-diaryl-2-propen-1-ones, are compounds precursors of the flavonoids biosynthetic pathway, which on recent researches demonstrated an expressive leishmanicidal activity. However, its mechanism of action is not yet totally elucidated. Objectives: To evaluate the immunomodulatory mechanisms involved on the initial immune response of murine macrophages against a synthetic chalcone. Methodology: For this, adult female BALB/c peritoneal macrophages obtained by peritoneal lavage were plated in twelve wells culture plates (1.0 x 106 cells/mL/well) in RPMI 1640 medium supplemented with 10% inactivated fetal calf serum and incubated at 37°C with 5% CO2 tension for 1 h for macrophages adherence. Then the cells were washed with RPMI 1640 medium and re-incubated overnight (14-16h) to eliminate any interfering at the protein expression, and subsequently challenged with the synthetic chalcone LH-1 at its 50% cytotoxic concentration (18 µg/mL). As negative control, cells were incubated only with medium. The plates were incubated 37°C with 5% CO2 tension for 3 h and then the supernatant was separated for Nitric Oxide (NO) measurement by the Griess method. After that, total RNA was extracted macrophages monolayer using TRizol Reagent according to manufacturers’ Information and the TNF-α, IL-12, IL-18, IL-1β, IFN-γ; iNOS and GAPDH (PCR internal standard) expression were monitored by reversetranscriptional polymerase chain reaction (RT-PCR) and analyzed by 1.5% agarose gel stained with ethidium bromide. Results: After agarose gel analysis, bands were not observed for the above cytokines, except those related to the PCR internal standard (GAPDH) and positive control of PCR, which guaranteed the reliability of the data. The NO dosage also showed no significant differences compared to control. Conclusions: The results suggest that the synthetic chalcone LH-1 did not induce the expression of cytokines important in the initial immune response. I.e., its mechanism of action would be more related to its direct action on the parasite than its immunomodulation of the microbialic mechanisms of action in the host macrophages. Moreover researches in this area, including also the activity of chalcone LH-1 directly at the leishmaniases parasites, are being conducted to confirm this data. Keywords: immune response, chalcone, peritoneal macrophages

Supporting funding: CNPq e CAPES

D17 REGULATORY ROLE OF CORTISOL ON CYTOKINE CONCENTRATIONS IN PATIENTS WITH FIBROMYALGIA.

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Introduction: Fibromyalgia (FM) is characterized by the presence of chronic and widespread pain, accompanied by hypersensitivity to palpation of 11 of 18 tender points established by the American College of Rheumatology. Other symptoms may also compose the clinical picture of FM. The pathophysiology of FM is not completely understood, but it appears that consensus neuroimmunoendocrine changes, including alterations in the Hypothalamic Pituitary Adrenal (HPA) contribute to the onset or progression of the clinical status of FM. This is due at least in part to the potent immunomodulatory role played by cortisol, a hormone released after activation of the HPA axis. Nevertheless it has been shown that patients with fibromyalgia suffer from hypocortisolism. Objective: To identify possible relationships between plasma levels of inflammatory cytokines and salivary levels of cortisol obtained at three times of day. Methodology: Fifty-eight women diagnosed with FM participated in the study. The plasma levels of the cytokines IL-2, IL-4, IL-6, IL-10, TNF, IFN and IL-17A were measured by Cytometric Bead Array (CBA) technique and salivary levels of cortisol are taken at 8 p.m., 5 p.m. and 10 p.m. were analyzed by enzyme linked immunosorbent assay. Correlation analyzes were performed using the Spearman correlation test in GraphPad Prism Software v5.0 and were considered significant when p ≤ 0.05. Results: Among all the combinations tested, discovered a statistically significant correlation between IL-4 and 5 pm cortisol (p = 0.0212 and r = -0.3284), IL-2 and cortisol 10 pm (p = 0.0017 and r = -0.4414), IL-4 and 10 pm cortisol (p = 0.0166, and r = -0.3408), IL-10 am 10 pm and cortisol (p = 0.0197, and r = -0.3322), and between TNF 10 pm and cortisol (p = 0.0094 and r = -0.3639). Conclusion: The identification of negative correlations between plasma levels of cytokines and salivary cortisol levels supports the immunomodulatory role of cortisol in the pathophysiology of FM. These results may suggest that treatments based on the use of corticosteroids can be considered as a viable alternative for the treatment of symptoms of fibromyalgia. Keywords: Fibromyalgia, Cytokines, Cortisol.

Supporting funding: FAPEMIG e CNPq

D18 ROLE OF THE OBESITY ON THE IMMUNOMODULATION OF THE EXPERIMENTAL INFECTION BY MYCOBACTERIUM BOVIS BCG.

Experimental Pathology and Health Sciences
Introduction: Tuberculosis is a contagious infectious disease, caused by bacteria the Mycobacterium tuberculosis Complex, a resistant intracellular parasite, aerobic and capable of replicate inside phagosomes, that still causes one million four hundred deaths per year around the globe. Obesity is another huge health problem that it is associated with chronic inflammatory response of white adipose tissue due to infiltration of macrophages. Because that, works suggest that obesity may have an influence on the immunological response to innumerable pathogens. Studies show that the obesity apparently helps patients with tuberculosis and HIV infection, because obese patients HIV positive have lower death rate than skinny patients.

The Objective of our study is to investigate the relation between obesity and the infection by Mycobacterium bovis BCG in mice. Methodology: For that, we used C57/B6 mice and divided them in two different die type, one with high sugar content and other with normal chow. After three months on this treatment, the animals were intraperitoneally infected with BCG. The control received saline (Animal ethical approval 109/2012 CEUA/UFJF).

Results: At 24h after infection, we observed an increase in the mass of fat in the high- sugar diet (Mean ± SEM: : 0,112 ± 0,017 in control to 0,219 ± 0,002 in high –sugar group; n=10) and a significant reduction in influx of leukocytes into the pleural cavity (2.05 ± 0.366 in control to 20,100 ± 5,460 in infected on common chow group; : 4,480 ± 0,615 in control to 8,060 ± 2,168 in infected on high –sugar diet group; n=5) as well as the neutrophils and eosinophils migration in obese mice. Also, there was less lipid body formation in obese compared with normal animals (1,200 ± 0,600 in control to 3,920 ± 0,736 in infected group on common chow, 1,713 ± 0,081 in control to 1,460 ± 0,102 in infected on high –sugar diet group).

Conclusions: These Results suggest that, obese animals apparently have a slower development of the infection derived M. bovis BCG that may be beneficial to the host, since the pathological symptoms of the tuberculosis are consequent of an exacerbated immunological response to the bacteria and a slow evolution can represent less tissue damage.

Keywords: Obesity, Lipid Bodies, Mycobacterium bovi.

Supporting funding: FAPEMIG; CNPq and PRO-PESA/UFJF.

D19  SPLEEN APOPTOSIS AFTER PARA-COCCIDIOIDES BRASILIENSIS INFECTION.

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Introduction: The paracoccidioidomycosis (PCM) is a systemic mycosis that has predilection for the lungs and organs of the mononuclear phagocyte system. The spleen is an organ responsible for the contribution of the immune response and the presence of P. brasiliensis (Pb) in this organ can influence the immune system and promotes the fungal establishment. The occurrence of programmed cell death in the spleen has not been described in PCM experimental. Objectives: Identify the occurrence of programmed cell death in the spleen of Swiss mice infected with Pb. Methodology: Were used Swiss mice 32 males, 30 grams, and age between 4 cell death in the spleen of Swiss mice infected with Pb. Methodology: Were used Swiss mice 32 males, 30 grams, and age between 4 and 5 weeks, kept under controlled environmental conditions with free access to water and food. The animals were divided into 4 groups of 8, with 2 control animals. Infection was performed by means of lateral tail vein with 0.1 ml of a suspension of 2 × 106 cellsof yeast Pb18. Controls received 0.1 ml of PBS. The animals were sacrificed one, two, four and eight weeks. The right lobe of the spleen was fixed in 4% paraformaldehyde and processed for paraffin embedding. Sections were made semi- paraffin 5μm and stained with hematoxylin and eosin.

Results: The histological observation showed the presence of yeast, with formation of granulomas loose difficult to visualize distributed mainly in the periphery of the spleen, and the presence of the fungus in the red pulp. The highest frequency of neutrophils was observed in the first and second week with predominance the periphery splenic in portions subcapsular. For the TUNEL technique may be observed marking parenchyma cells and yeast fungus, most often in the second week of infection, where (CFU / g) were also larger. Conclusions: The paracoccidioidomycosis induces apoptosis in the spleen; the highest frequency of apoptotic cells was associated with a greater frequency of inflammatory cells. Keywords: Paracoccidioides brasiliensis. Apoptosis, Neutrophils.

Supporting funding: CNPq and CAPES.

D20  SYNERGIC EFFECT OF PHOTODYNAMIC THERAPY ASSOCIATE TO SURFACTANTS AGAINST C. ALBICANS.

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Introduction: Candida albicans is an opportunistic pathogen which can be responsible for superficial to invasive infections when predisposing and local factors associated to the host are present. The Photodynamic Therapy (PDT) associates a photosensitizer and a light source and recently, several studies have demonstrated its efficacy against microorganisms. Surfactants are amphipathic molecules constituted of a hydrophobic portion and hydrophilic portion. These compounds have been associated to reduction of adhesion of C. albicans to buccal epithelial cells. In this context is very relevant to evaluate the association of PDT and surfactants in C. albicans growth. Methodology: A suspension of C. albicans was prepared and mixed with the surfactants CTAC, SDS, HPS and Triton X-100 in concentrations of 0.03 μg/ml and incubated...
of clinical signs (1.0) total drop tail; (2.0) severe paresis of one or two hind feet, (3.0) paralysis of the hind feet and paresis of one or two forefeet; (4.0) paralysis of hind legs and forefeet, (5.0) death. Results: The score evaluation showed a statistically significant difference between the experimental group and the EAE at day 10, 11 and 12 after induction with p <0.0001. These data are representative of two repeated experiments. Conclusion: Our results indicate that oral tolerance induction in previously immunized animals interfere with the onset of clinical signs of EAE. Keywords: oral tolerance, EAE, ovalbumin, Supporting funding: CAPES CNPq FAPEMIG

D22 THE TETRASPANIN CD63 IS ASSOCIATED WITH SECRETION OF GRANULE-STORED PRODUCTS IN HUMAN EOSINOPHILS.
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Introduction: Eosinophils, leukocytes of the innate immune system, with functions in allergic, inflammatory and immunoregulator responses, are able to release numerous proteins their secretory granules. These granules, also termed secondary or crystalline granules, have a unique morphology and are considered a hallmark of eosinophils. The tetraspanin CD63 (also known as LAMP-3) is associated with intracellular events involved in eosinophil activation. However, the ultrastructural localization of CD63 in eosinophils, as well as its functional activity, during inflammatory responses is not well characterized. Objective: In this work, we used pre-embedding immunonanogold electron microscopy to ascertain the localization of CD63 within human eosinophils stimulated with inflammatory mediators. Methodology: Eosinophils were isolated the blood of healthy donors by negative selection, stimulated with eotaxin-1 (CCL11) or tumor necrosis factor-alpha (TNF-α) or medium alone, fixed, labeled with a pre-embedding immunonanogold electron microscopy technique and processed for transmission electron microscopy (TEM). Results: Eotaxin-stimulated eosinophils showed vesicle-mediated release of their products, a process termed piecemeal degranulation, while TNF-α-stimulated eosinophils exhibited classical granule exocytosis, characterized by granule-granule and granule-plasma membrane fusions. CD63 labeling was densely detected at granules undergoing release of their products within stimulated eosinophils compared to unstimulated cells. Quantitative EM revealed that more than 85% of secretory granules undergoing piecemeal degranulation in eotaxin-stimulated or classical exocytosis in TNF-α-stimulated eosinophils were labeled for CD63. Conclusions: Our results demonstrate that CD63 is highly expressed in eosinophil secretory granules and can be used as a marker for these organelles. Moreover, our results show, for the first time, that CD63 is consistently associated with secretory mechanisms in human eosinophils. This is important because
Introduction: Neuropathic pain results injury to central or peripheral nervous systems. Trigeminal neuropathic pain is an excruciating pain localized to small facial area and its ethiopathological mechanisms have not been completed understood. An experimental model of trigeminal neuropathic pain in rats is produced by a chronic constriction injury (CCI) to the infraorbital nerve. Development of neuropathic pain can arise structural impairment of nervous fibers, glial response to nerve lesion and inflammatory mediators production.

Objectives: The aim of this work was to study the morphological alterations, demyelination, and inflammatory mediators immuno-expression that underlie pain development in the experimental trigeminal neuropathic pain.

Methods: Wistar rats received CCI to the infraorbital nerve (CCI group) or sham operation without nerve ligation (Sham group). Spontaneous and evoked behavior were evaluated at postoperative days 3, 6, 9, 12 and 15. Trigeminal nerves were dissected and processed for optic microscopy.

Results: CCI rats showed an early hyporesponsiveness and a late hyperresponsiveness, suggesting neuropathic pain behavior, as previously described. Histopathological alterations included increased cellularity of nerve fascicles and immunopositivity for glial marker, suggesting proliferative response. Intense demyelination and degenerated myelin sheaths, which have been associated to neuropathic pain states in humans and experimental models, were also observed in distal regions of trigeminal nerve. Additionally, immunoexpression of substance P and interleukin 1β, both implicated in peripheral and central sensitization, was observed in trigeminal ganglion and nerve.

Conclusions: Our results show that pain development is related to glial cell proliferation, demyelination, and expression of important pain mediators in the experimental model of trigeminal neuropathic pain in rats and contribute to clarify cellular mechanisms underlying pain development, as it has been done for extra cephalic neuropathic pains.

Keywords: neuropathic pain, trigeminal nerve, demyelination.
Section E
Neuroscience and Noetic sciences
Anesthesiology, Pain and Acupuncture
Experimental Sport Science

E01 BENEFICIAL EFFECTS OF ACUTE HIGH INTENSITY INTERVAL SWIMMING IN MICE FED HIGH FRUCTOSE AND SUCROSE DIETS.

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Introduction: Chronic consumption of diets rich in fructose and sucrose combined with a sedentary lifestyle has impact on the development of metabolic syndrome (MS). The exercise of short duration and high intensity (HIIT) increases the adherence of the population to training programs and is beneficial to treat comorbidities associated with MS. Objectives: To evaluate the magnitude of the HIIT effect on metabolism of mice fed diets rich in fructose and sucrose. Methodology: Male C57BL6 mice were divided according to the experimental diets: SC (standard chow; 76% of corn starch); HFr (50% of fructose) and HS (50% of sucrose). Body mass (BM) was measured weekly. Oral glucose tolerance test (OGTT) was performed before the exercise protocol. On the fifth week, animals were divided into groups non-trained (SC-NT, HFr-NT and HS-NT) or trained (SC-T, HFr-T and HS-T; n=10/group). HIIT was characterized by a series of 20 seconds of swimming with 10% of body mass, followed by 10 seconds of passive recovery and sacrificed immediately after exercise. All data were analyzed by one-way ANOVA with Holm-Sidak post-hoc test. Results: There was no difference in BM between animals SC-NT, HS-NT, and HFr-NT. Plasma glucose and OGTT were significantly higher in both HFr-NT and HS-NT compared to SC-NT (P<0.001). The plasma lactate was higher in groups SC-T (+45%), HFr-T (+48%), and HS-T (+34%) than their counterparts (P<0.01). HIIT was able to reduce plasma glucose in SC-T (-14%), HFr-T (-29%; P<0.001), and HS-T (-34%; P<0.001) than their sedentary counterparts. Total cholesterol (TC) was higher in HFr-NT (+22%; P<0.05), and HS-NT (+40%; P<0.001) than in SC-NT. HIIT efficiently reduced TC in HFr-Tr (-16%), and in HS-Tr (-12%) than their sedentary counterparts. Triglycerides (TG) were higher in HFrNT (+22%; P<0.05) and HS-NT (+14%; P<0.05) than in SC-NT. In addition, HIIT significantly reduced TG in trained groups: SC-T (-9%), HFr-T (-15%) and HS-T (-14%) than in their sedentary counterparts.

Conclusion: Preliminary data are in favor of the beneficial effects of a session of acute exercise of HIIT in the metabolism of mice fed high-fructose and high-sucrose diets. Keywords: Fructose, Sucrose, Exercise, HIIT. Supporting funding: CAPES, CNPq and FAPERJ.

E02 HIGH-INTENSITY INTERVAL TRAINING REDUCES INSULIN RESISTANCE AND LIVER STEATOSIS IN DIET-INDUCED OBESE MICE.

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Introduction: High fat (HF) diet associated with sedentary lifestyle triggers the development of insulin resistance (IR) and hepatic steatosis, component factors of the Metabolic Syndrome (MS). The short duration high-intensity interval training (HIIT) although not commonly used, promotes beneficial changes in the components of the MS. Objectives: To investigate the changes caused by HIIT in diet-induced obese mice with IR and hepatic steatosis. Methodology: C57BL/6 mice were divided into 2 groups according to the diet: LE (lean, standard chow), and OB (obese, high fat diet). Body mass (BM) was measured weekly. Oral glucose tolerance test (OGTT) was performed before and after the exercise protocol. After 12 weeks on diets, animals were separated into Non-Trained (LE-NT, OB-NT) or Trained (LE-T, OB-T). The exercise consisted of a series of 20 seconds of exercise (swimming pool) to 10 seconds of passive recovery, with 10% of body mass (BM) charged in the tail. For experimental protocol, we used 50% of this maximum number determined in series test. Every two weeks, the percentage of the implement was increased gradually. Moreover, in the 2nd week of each percentage, the number of series was increased gradually. Initially, the data were statistically analyzed with Student T test (LE and OB) and One-way ANOVA with Holm-Sidak post-hoc test (LE-NT, LE-T, OB-NT, OB-T). It was considered statistically significant P<0.05. Results: Since the 1st week on the diets we observed a significant difference on BM between lean and obese groups (P<0.01), lasted until the 12th week. From the 13th week and the beginning of exercise protocol, the BM reduced compared to non-trained counterpart (LE-T: -11%; OB-T: -11%, both P<0.001). Before the exercise protocol, OB group showed increased glycemia by 39% (P<0.003) compared to LE. At the end of the exercise protocol, Trained groups showed decreased OGTT (LE-T: -23% P<0.01; OB-T: -16% P<0.01) compared to NT. OB group showed excessive accumulation of fat in the liver characterizing liver steatosis (+550%, P<0.001) than LE. At the end of the experiment, LE-T showed reduction of liver steatosis by 57% (P<0.05) than LE-NT, and OB-T by 77% (P<0.001) than OB-NT. Conclusion: The results are indicative that short duration HIIT has beneficial effects in improving metabolism and liver steatosis of the animals. Keywords: High-Intensity Interval Training, Insulin Resistance, Liver Steatosis. Supporting funding: CAPES, FAPERJ, CNPQ.

E03 CHROMOGENIC HYBRIZATION OF EGFR ON DUCTAL CARCINOMA IN SITU AT THE CANINE MAMMARY GLAND.

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Introduction: The epidermal growth factor receptor (EGFR), encoded by the proto-oncogene c-erbB-1, belongs to the same family HER-2. Overexpression of epidermal growth factor EGFR and HER-2 in breast carcinomas at the bitch possibly related to tumor development and progression. Objectives: This present study aimed to determine the character of hybridization to EGFR by technique chromogenic hybridization in situ (CISH) in ductal carcinoma in situ on the canine mammary gland.

Methodology: Were reviewed and ed 10 cases of ductal carcinoma in situ was classified in Department of pathology veterinary (SPV) in Universidade Federal de Lavras (UFLA) and Laboratory of pathology compared (LPC) on the ICB/UFMG. For the hybridization was used the detection kit CISH SPOT-Light Chromogenic ISH (Zymed Laboratories Inc) with probe hybridization EGFR (SPOT-Light HER-2 Probe). The interpretation of hybridization was performed according to recommendations supplied in the kit. Results: Histopathology were confirmed the degrees of the respective ductal carcinoma in situ, which 06 cases were included in the high-grade ductal carcinoma in situ, and 04 cases in the low-grade. In all cases have been identified both in normal cells, as the neoplastic cells, two or four points of chromosomal hybridization to EGFR, characterized the absence of gene amplification in this case. The degree of ductal carcinomas no influence the profile of hybridization, since the results varied within a situation of no amplification of the EGFR gene. Conclusions: The absence of gene amplification in canine breast carcinomas in situ by EGFR suggests that the process of neoplastic progression is not directly related to the increase in the number of such genes.

Keywords: Cancer; Dog; Hybridization.

Supporting funding: FAPEMIG; CNPq; CAPES; PRPq-UFMG

E04 EFFECT OF THE INGESTION OF COCONUT WATER IN EXPERIMENTAL WOUNDS REPAIR.

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Introduction: Some studies have shown that the use of coconut water in the treatment of wounds showed positive influence on the healing process.

Objectives: This study aimed to evaluate the process of tissue repair in rabbits treated orally with coconut water in order to understand whether this therapeutic process influences the skin wounds repair.

Methodology: Were used 20 rabbits New Zealand White divided into 4 groups with different oral treatments, which each two groups received antibiotic ointment on the experimental wound (G1: mineral water + ointment; G2: coconut water + ointment; G3: mineral water; G4: coconut water). Four surgical wounds were made on the backs of the animals with a circular scalpel of 6mm. Clinical evaluation were done at the moments 0, 6, 12, 24, 48, 72, 96, 120, 144 and 168 hrs, checking the rectal temperature and the wounds: edema, erythematous halo. The healing time was determined when the whole wound area was replaced by scar tissue. The epithelialization time was considered when all tissue was regenerated. Measurements were made in the four wounds of each animal until the day of healing. Tissue biopsies from the wound-s edges were taken for histological evaluation (hematoxylin and eosin) and observation of collagen fibers (picrosirius) and elastic fibers (Verhoeff).

Results: In all groups, the body temperature remained within the normal range (37.1 to 39.4°C), and the wounds did not present edema, erythematous halo or clinical signs of infection. The healing and epithelialization time, in days, were: G1: 20 and 29; G2: 15 and 17; G3: 7 and 13; G4: 5 and 12. The G4 showed granulation tissue in the 1st biopsy, at 7 days. In the other groups the granulation tissue was observed only after the 2nd biopsy, at 14 days. The G2 and G3 presented the lower intensity of inflammatory infiltrate. Type I collagen was the predominant type in all samples and the ratio of elastic fibers (FE/μm2) did not change over time within the groups. The G4 presented the highest value of elastic fibers. Conclusions: The ingestion of the coconut water influenced the tissue repair process.

The animals that ingest coconut water showed the lowest time of healing and epithelialization. The effect of water into the speed regression wound is influenced by the use or not of ointment. The use of ointment is effective regardless of the water.

Keywords: coconut water, skin, wound, healing.
normal range (37.1 to 39.4°C), and the wounds did not present edema, erythematous halo or clinical signs of infection. The healing and epithelialization time, in days, were: G1: 20 and 29; G2: 13 and 17; G3: 7 and 13; G4: 4 and 10; The G4 showed granulation tissue in the 1st biopsy, at 7 days. In the other groups the granulation tissue was observed only after the 2nd biopsy, at 14 days. The G2 and G4 presented the lower intensity of inflammatory infiltrate. Type I collagen was the predominant type in all samples and the ratio of elastic fibers (FE/μm2) did not change over time within the groups.

Conclusions: The ingestion of the magnetized water influenced the tissue repair process. The animals that ingested magnetized water showed the lowest time of healing and epithelialization. The effect of water into the speed regression wound is influenced by the use or not of ointment. The use of ointment is effective regardless of the water.

Keywords: healing, magnetized water, skin, wound.

E06 MATRIX-PRODUCING TUMORS IN CANINE MAMMARY GLAND—A STUDY OF OCCURRENCE.
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Introduction: Matrix-producing tumor is a group of neoplasias characterized by the presence of foci of cells benign or malignant (epithelial cells and / or mesenchymal cells) that produce myxoid, chondroid and/or bone matrix. Carcinomas in benign mixed tumors, carcinomas and sarcomas are corresponding of mixed malignant tumors in female dogs. In humans, these tumors are rare and have histological features comparable to metaplastic carcinoma, osteosarcoma, chondrosarcoma and carcinosarcoma that are matrix producing tumors.

Objective: The aim of this study is to survey data of number of cases compatible with the histological diagnosis of tumors producing matrix in canine mammary gland. Methodology: Have been selected cases of mammary neoplasms in female dogs diagnosed at the Laboratory of Comparative Pathology (LPC), Institute of Biological Sciences and the Veterinary Hospital of the Federal University of Minas Gerais between 2001 and 2012. The cases were selected according to their histological diagnosis, and were characterized by benign or malignant proliferation of epithelial (luminal and myoepithelial cells) and/or mesenchymal (cartilage, bone, fat in combination with fibrous tissue) components of the mammmary gland. Results: In total, have been analyzed 3723 canine mammary tumors in which, in 1876 (50.39%) are matrix-producing neoplasms. Among the cases, the most frequent histological types are carcinoma in benign mixed tumor (976 cases) and benign mixed tumor (832 cases). Other types such as carcinosarcoma (57 cases), osteosarcoma (4 cases), chondrosarcoma (3 cases), chondroma (1 cases) and sarcoma in mixed tumor (3 cases) are less frequent. Conclusion: The matrix-producing tumors are frequently diagnosed in female dogs. However, the biology character of these tumors is not elucidated yet. Thus, canine matrix-producing tumor can be a good model for the study of these tumors comparing with human model. Understanding the biology of these tumors is necessary to establish new criteria for diagnosis and better treatment of animals and human.

Keywords: mixed tumor, matrix, canine, mammary gland.

Supporting funding: FAPEMIG and CAPES

E07 RELATIONSHIP OF HER-3 EXPRESSION AND HISTOLOGICAL DEGREE IN DUCTAL CARCINOMA IN SITU OF CANINE MAMMARY GLAND.
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Introduction: The receptor for epidermal growth factor (HER) consists of four members: EGFR, HER-2, HER-3 and HER-4. The prognostic value of HER-3 expression in canine breast cancer has been poorly documented and the data are not conclusive. Objectives: The present study aimed to characterize the immunohistochemical expression of the protein HER-3 in ductal carcinoma in situ (DCIS) of the canine mammary gland. Methodology: Were reviewed and ed 26 cases of DCIS diagnosed in the Department of Veterinary Pathology UFLA and the Laboratory of Comparative Pathology ICB / UFMG. The immunohistochemical procedure was performed after antigen retrieval with EDTA solution pH 9.0 at 98 °C, endogenous peroxidase blocking solution in incubation in 3% H202 and identification polymerized secondary antibody (HRP ADVANCE - ready to use - DakoCytomation) after overnight incubation in primary antibody, HER- 3 (H3-DAK-IC; Dako, dilution: 1:50). Immunostaining was analyzed according to the determination of cytostatic score (+ = weak, ++ = moderate and +++ = strong). Results: We identified 16 cases of high-grade DCIS and 10 cases of low grade. Of these, ten cases had no cytoplasmic staining for HER-3, fourteen had low-intensity staining, one moderate and one strong intensity staining. We observed a low mean expression of HER-3 (0.73 ± 0.72) in the cases analyzed. Low-grade carcinomas had a higher average in relation to high-grade carcinomas (0.8 ± 0.9 and 0.6 ± 0.68, respectively; p = 0.7). In the analysis of correlation between tumor grade and expression of HER-3 was identified this loss of expression (r = -0.006), but without statistical significance. Conclusion: High-grade carcinomas in situ showed low expression for HER-3, with a negative relationship with the histological grade. The results suggested that the loss of HER-3 expression is an early event in tumor progression and can be related to increased tumor aggressiveness of canine mammary carcinomas.

Keywords: Cancer, Dog, Immunohistochemistry.

Supporting funding: FAPEMIG; CNPq; CAPES; PRPq-UFMG

E08 ALTERATIONS IN THE MORPHOLOGY OF SKELETAL MUSCLE FIBERS IN MICE WITH...
REduced expression of the vesicular acetylcholine transporter gene.

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Introduction: Skeletal muscle fibers can be divided into four types, named I, IIa, IIx, IIb, from slowest to fastest contracting. In mammals, muscle contraction occurs when acetylcholine binds to nicotinic receptors. The storage of acetylcholine in synaptic vesicles by the vesicular acetylcholine transporter (VACHT) is crucial to muscle function. To assess the functional importance of the VACHT in the cholinergic neurotransmission, Prado and colleagues (2006) generated a strain of mice knockdown for the VACHT gene (VACHT KD). These animals have a 65% reduction in this protein levels and motor performance studies showed a significant deficit in muscle strength. Objectives: The aim of this study is to evaluate the changes of Soleus and Extensor Digitorum Longus (EDL) skeletal muscles from these mice. Methodology: Briefly, Soleus and EDL from at least three pairs of three months old male VACHT KD and WT animals were dissected, fixed in glutaraldehyde 4%, dehydrated in an alcohol ascendant series, embedded and included in Glycol methacrylate. Cross sections (5μm) were cut and stained with toluidine blue. We analyzed the distribution of muscle fibers in fixed intervals of area and perimeter. We applied the Kolmogorov-Smirnov statistical test in a graph of cumulative frequency where we evaluated the area and perimeter of muscle fibers. For both analyzes we had 1187 muscle fibers in Soleus and 2019 muscle fibers in EDL for each genotype. Results: Soleus, a slow twitch muscle presented a higher distribution of its muscle fibers located at higher intervals of area and perimeter in VACHT KDHOM compared to VACHT WT. The graph of cumulative frequency showed that the area in Soleus VACHT KDHOM presented about 80% of the fibers with more than 1500μm² and the same percentage of muscle fibers in VACHT WT measured 160μm whereas in BACHD the perimeter measured 125μm (p<0,0001). Regarding the perimeter analyzes we observed that 80% of the muscle fibers from VACHT WT measured 150μm whereas in VACHT KDHOM their perimeter measured 125μm (p<0,0001). Interestingly, we did not observe any difference in the distribution of muscle fibers in the EDL, a fast twitching muscle, when comparing VACHT WT with VACHT KDHOM animal pairs. Conclusions: Our results so far suggest that reduced expression of VACHT affects only the structure of slow twitch muscles and might explain the significant reduction in muscle function observed in VACHT KDHOM mice. Keywords: Skeletal muscle fibers, Acetylcholine, Vesicular Acetylcholine Transporter Supporting funding: CAPES, CNPq and FAPEMIG

E10 DEPRESSIVE BEHAVIOR IN TRYPANO SOMA CRUZI INFECTION IS SENSITIVE PARASITICIDE AND IMMUNOMODULATORY INTERVENTION IN THE CHRONIC PHASE OF INFECTION.

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Introduction: Huntington’s disease (HD) is a progressive, debilitating, fatal neurological disease caused by a CAG trinucleotide repetition that leads to a polyglutamine expansion in the huntingtin (htt) protein. Its symptoms include chorea, cognitive disturbances and progressive motor decline starting with motor impairment of the muscles of the face and then the muscles of the trunk and limbs. Objectives: The aim of this study is to evaluate the morphological changes of different muscular groups such as sternomastoid (STM), located on the neck and Soleus (SOL), a lower limb muscle, from BACHD mice, a transgenic model for HD. Methodology: Briefly, ETM and SOL skeletal muscles from at least three pairs of three-month-old male BACHD and WT animals were dissected, fixed in glutaraldehyde 4%, dehydrated in an alcohol ascendant series, embedded and included in Glycol methacrylate. Cross sections (5μm) were cut and stained with toluidine blue. We analyzed the distribution of muscle fibers in fixed intervals of area and perimeter. We applied the Kolmogorov-Smirnov statistical test in a graph of cumulative frequency where we evaluated the area and perimeter of muscle fibers. We analyzed 7475 muscle fibers in STM and 1585 muscle fibers in SOL for each genotype. Results: The STM muscle presented more muscle fibers located at higher intervals of area and perimeter in BACHD compared to WT. The cumulative frequency analysis showed that the area in STM from BACHD mice presented about 80% of the fibers with more than 4000μm² whereas in WT mice, the same percentage of the fibers measured 2000μm² (p<0,0001). Regarding the perimeter, we observed that 80% of the muscle fibers from WT measured 160μm whereas in BACHD the perimeter measured 250μm (p<0,001). By contrast, we did not observe any difference in the distribution of muscle fibers in the SOL, a slow twitching muscle, when comparing the genotypes. Conclusion: Our results so far suggest that distinct muscular groups are affected differently in BACHD mice model at the age of three months. We are currently analyzing other muscle groups in order to investigate if slow twitching muscle fibers are more susceptible than fast twitching in BACHD mice. Keywords: Huntington’s disease, Skeletal muscles, Neurological disorders Supporting funding: CAPES, CNPq and FAPEMIG
**Introduction:** Infectious agents and inflammation have emerged as triggers of depressive behavior. Patients chronically infected with Trypanosoma cruzi exhibit behavioral changes such as depression. Objectives: The present study was conducted to test the contribution of T. cruzi-induced CNS inflammation and the parasite strain infecting the host to chronic behavioral alterations. **Methodology:** C3H/He and C57BL/6 mice (10 per group) were infected with the Colombian (type I) and Y (type II) T. cruzi strains and evaluated for depressive behavior (forced swim and tail suspension tests). We tested the ability of the antidepressant iv inhibitor of serotonin reuptake fluoxetine and the parasiticide drug benznidazole (Bz) to ameliorate T. cruzi-induced depression. Immunological unbalance with high TNF plasma levels is a feature of chronic Chagas disease, therefore we investigated the existence of an inflammatory component in T. cruzi-induced depressive-like behavior by targeting TNF. **Results:** In the acute and chronic infection, C3H/He and C57BL/6 mice showed increased time of immobility, arguing that depression in chronic infection is not associated with acute inflammation in the CNS and is not, therefore, a sequel of this process. Fluoxetine therapy improved the depression, suggesting the contribution of a neurochemical component. Depression in chronic phase was induced by the Colombian strain, but not with the Y strain of T. cruzi. Further, Bz therapy in the acute and chronic phases of infection was beneficial reducing the immobility time. In the chronic phase of infection, therapy with pentoxifylline, an immunoregulator acting at TNF circuit, associated or not with suboptimal dose of Bz, reduced the immobility time. **Conclusions:** Depressive behavior in chagasian infection does not only depend on psychological factors, but can reside in a complex network of interactions triggered by the parasite T. cruzi and the immune stressor TNF, which result in a decrease of the neurotransmitter serotonin. Our data show the importance of the administration of trypanocidal therapy, either in the acute or chronic phase of infection. Our findings support the existence of chronic nervous form of Chagas disease and open new avenues for therapeutic interventions, which should be explored in chronic depressive disorders. **Keywords:** Depression, Trypanosoma cruzi, Inflammation Supporting funding: INCTV, CNPq e FAPERJ

**E11 HISTOCHEMICAL ANALYSIS OF THE DENTATE GYRUS HIPPOCAMPUS OF RATS: EFFECTS OF EXERCISE BEFORE AND AFTER CEREBRAL ISCHEMIA.**


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**Introduction:** Cerebral ischemia is characterized by vascular dysfunction in key areas of the brain such as the hippocampus. Objectives: The aim of this study is to do histochemical analysis of the apex of the dentate gyrus of the hippocampus of rats and verify the morphological changes induced by exercise before and after cerebral ischemia. **Methodology:** We used 36 male Wistar rats weighing between 138-400g. The animals were initially divided into two groups: ischemia by transient occlusion of the middle cerebral artery (AI, DI and SI) and control (AC, DC and SC). The animals were subdivided into the following groups: AI and AC: before exercised animals (n = 12); DI and DC: after exercised animals (n = 12); SI and SC: animals that did not exercise (n = 12). The animals of group AI and AC were trained on the treadmill without inclination for six weeks, five days a week for 30 min / day at a speed of 25 m / min. The rats allocated to physical training after cerebral ischemia and surgery (DI and DC) ran on the treadmill at a speed controlled from 10 to 20m/min. Cerebral ischemia was induced by 60 minutes of occlusion of the middle cerebral artery. After six weeks, the brains were removed and sectioned into slices of 1 mm. The sections of -2.80 mm (hippocampus) guided by atlas of Paxinos and Watson were processed in paraffin (10 um) and stained by Nissl method. Then we proceeded to quantify neuronal an area of 50 μm2 apex dentate gyrus. **Results:** Statistical analysis (ANOVA, p <0.05), showed that animals AI (48.33 ± 2.813) had a greater number of neuronal cells in the apex of the dentate gyrus of the left cerebral hemisphere compared to animals of the SI group (40 ± 1.57) and DI (38.33 ± 0.8028). In the control group was also evidenced a significant increase in the number of cells in the group that exercised before cerebral ischemia (p <0.05). **Conclusion:** The physical exercise before ischemia promotes neuroprotection in the dentate gyrus of the hippocampus of rats. **Keywords:** dentate gyrus of the hippocampus, cerebral ischemia, exercise

**E12 HISTOLOGICAL ALTERATIONS IN SOLLEUS AND EXTENSOR DIGITORUM LONGUS MUSCLES IN MIDDLE-AGED MICE WITH CHOLINERGIC DYSFUNCTION.**

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**Introduction:** The mammalian skeletal muscle contraction is triggered by the binding of acetylcholine (ACh) in nicotinic receptors. The storage of ACh in synaptic vesicles by the vesicular ACh transporter (VACHT) is crucial to muscle contraction. To assess the functional importance of the VACHT in the neurotransmission, Prado and colleagues (2006) generated a strain of mice knockdown for the VACHT
gene and these animals have an important deficit of muscle strength. **Objectives:** To understand better the changes during aging process, we investigated the effect of a deficit in the release of ACh in different muscles in middle-age mice. **Methodology:** We performed routine histology analysis in Soleus (SOL) and Extensor Digitorum Longus (EDL). We analyzed the area and perimeter of muscle fibers from EDL and SOL. **Results:** Cumulative frequency analysis showed that the area in SOL from VACHT KDHOM mice presented about 80% of the fibers with more than 1500μm² whereas in the VACHT WT mice, the same percentage of the fibers measured 1000μm² (p<0.0001). Perimeter analysis in SOL, showed that 80% of the fibers measured 150μm in VACHT KDHOM and 125μm in VACHT WT (p<0.0001). Interestingly, the opposite was observed in EDL muscles. VACHT KDHOM presented a considerable reduction in the frequency of muscle fibers with bigger area and perimeter compared to VACHT WT. Cumulative frequency analysis showed that the EDL area in VACHT KDHOM mice has more than 90% of the fibers measuring about 1500um² compared to VACHT WT that measured about 1800μm² (p<0.05). The perimeter in VAChT KDHOM measured 130μm and in VACHT WT 150μm (p<0.05). **Conclusions:** The phenotype of skeletal muscles can be altered by changes in the expression level of the four isoforms of the myosin heavy chain (I, IIA, IIX and IIB). These changes normally observed during aging processes could be modified in cholinergic deficit. Considering the differences presented by these muscles, we suggest that this could be explained by shifts in myosin isoforms expression under reduced ACh release. All experimental procedures were approved by the local animal care committee (CETEA-UFMG).

Keywords: skeletal muscle, neurotransmitter acetylcholine, vesicular acetylcholine transporter, middle-aged mice

Supporting funding: This work was supported by, CNPq, CAPES and FAPEMIG.

**E13 INCREASED END PLATES DENERVATION AND MOTOR IMPAIRMENT IN AGED MICE WITH CHOLINERGIC HYPOFUNCTION.**

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**Introduction:** The Vesicular Acetylcholine Transporter (VACHT) is a protein that is responsible for packing the neurotransmitter acetylcholine (ACh) into synaptic vesicles, thereby playing a central role in cholinergic neurotransmission. In mammals, ACh that is released into synaptic cleft binds to nicotinic receptors. This event is essential for muscle contraction. Studies have shown that in senescent healthy mice, there is a degeneration of both in pre- and post-synaptic elements. Although it has been described many causes for these degenerative changes, none of them were conclusive. **Objectives:** To contribute to a better understanding of neuromuscular degeneration in senescence, we are currently investigating the impact of a reduced release of ACh in middle-age mice. **Methodology:** We are using a mice knockdown for the VACHT gene (VACHT KD HOM) with 65% reduction in the expression of this protein. In order to investigate the effects of the reduced release of ACh on the morphology and function of the neuromuscular junctions, we performed confocal microscopy optical analysis in at least three pairs of 12 to 14 months old male VACHT KD HOM animals and compared with VACHT WT. We performed quantitative analyzes of fluorescence intensity, quantification of the total analyzed area and density of pre and post-synaptic elements. **Results:** Staining for the pre-synaptic synaptic vesicle protein synaptophysin and for post-synaptic nicotinic receptors was similar in both VACHT KDHOM and VACHT WT. However, we observed complete or partial denervation of the postsynaptic ACh receptors sites in VACHT KDHOM mice diaphragm muscle compared to the WT (p<0.05). In addition, behavioral tests (wire hang, rotarod and open field) showed that VACHT KDHOM mice did not perform well compared to VAChT WT (p<0.0001 for wire hanging; p<0.001 for rotarod and open field). **Conclusions:** This study suggest that reduced expression of VACHT in this model mice affects the structure of the end plates compared to healthy mice. As motor performance was also impaired, these findings might explain the significant reduction in muscle function observed in VACHT KDHOM mice. This work should contribute to understanding of the neuromuscular degeneration during aging which is responsible for immobility and the tendency to fall, major causes of disability in the elderly population.

Keywords: end plates, denervation, motor function, cholinergic hypofunction

Supporting funding: This work was supported by, CNPq, CAPES and FAPEMIG.
age ranged from 36 to 80 years with mean and median similar to the general. While among men the age ranged from 51 to 68 years with an average of 61.8 years and a median of 62 years. Given this total n = 6 (35.3%) were caused by the death Ischemic Cerebrovascular accident (CVA-I), n = 7 (41.2%) Hemorrhagic Stroke (CVA-H), 11.8% (two) purulent meningitis, 5.9% (one) Sequel Stroke and 5.9% (one) Intracranial Hypertension / Brain Injury Expansive. The distinction by sex, when the variable was the AVE-I 33.33% were male and 66.66% female with a mean age of 63.5 years, 68.25 years for women and 54 years for men. However when the variable was AVE-H 28.57% were male and 71.42% female and the average age was 61.1 years with an average of 59 years for women and 66.5 years for men. Already purulent meningitis had a mean age of 57.5 years.

Conclusion: Through this analysis we can conclude that the most occurrence with cerebrovascular disease was female and the average age at which this is occurring earlier in men as compared to women.

Keywords: vascular disease, cerebrovascular disorders, mortality

**E15 NEUROPLASTICITY OF PRELIMIC CORTEX ON POTENTIATION AND MAINTENANCE OF NEUROPATHIC PAIN INDUCED BY ISCHIATIC NERVE CHRONIC CONSTRICITION INJURY.**

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Introduction: The prelimbic division (PL) of medial prefrontal cortex (MPFC) is important for the perception of acute and chronic pain. There is evidence that neuropathic pain (NP) leads to rearrangement of the MPFC. PL cortex is therefore intriguing to suggest that the increased spine number and NMDA currents may lead to increased glutamatergic input-mediated calcium influx, which, in turn, may result into glutamate excitotoxicity and neuronal loss. Aim: The role of MPFC is unclear and the aim of the present work was to investigate the involvement of the PL cortex in the elaboration of genesis and maintenance of the neuropathic pain induced by chronic constriction injury (CCI) by placing only one loose ligature around the ischiatic nerve.

Methods: Male Wistar rats (n=6-7 per group) were used. Neuropathic pain was induced by CCI and as control group it was used sham surgery (without CCI). After 2 or 14 days of CCI or sham procedure, a guide cannula was unilaterally implanted (right hemisphere) into the PL cortex through of stereotaxic surgery. PL cortex-treatment with cobalt chloride (1.0mM/200nL) as synapse block or saline (NaCl 0.9%; 200nL) was performed in groups 7, 14, 21, or 28 days after CCI or sham procedure, followed by von Frey’s test (in the left and right paws) during 60 minutes. Results: The present data showed that PL cortex-pretreatment (in the right hemisphere) with cobalt chloride did not increase the mechanical threshold in the von Frey test after 7 and 14 days of CCI (P>0.05). However, cobalt chloride microinjected into the PL cortex (in the right hemisphere) increased the mechanical alldynia threshold after 21 and 28 days of neuropathic pain induced by CCI (P<0.05).

Conclusion: Considering that mechanical alldynia sensations are processed by pathways from spinal cord that send contralateral projections to cerebral cortex these findings suggest that the morphological reorganization of PL cortex occurred and this phenomenon is involved in the potentiation and maintenance of the chronic pain in model of neuropathic pain in rodents.

Keywords: Prelimbic medial prefrontal cortex, Chronic constriction injury, Neuropathic pain, Neurophysiological approaches

Supporting funding: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP)
F01 HYPOXIA CHEMICALLY INDUCED BY COCl2 IN SALIVARY GLAND MALIGNANT NEOPLASM LINEAGE.
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Introduction: The use of neoplastic cell lines to evaluate and understand the biological behavior of neoplastic cell is widely spread, as an example, the hypoxia induction and its influence on neoplastic cell lines. Some studies argue that hypoxia promotes the genetic instability, ability to evade apoptosis, increased aggressiveness, as well as promotes extracellular matrix breakdown, tumor progression and metastasis. For this purpose, we need an in vitro model that takes into account the importance of hypoxia and neoplastic cell biological behavior.

Objective: the present study evaluated the CoCl2 chemically induced hypoxia in malignant neoplasm of salivary glands lineage. Material and Methods: The chemically induced hypoxia conditions were achieved by exposing normoxic cultures of HTB-41 (ATCC) cells to CoCl2. They were then plated (5x105 cells/ml) in Maccoy 5A medium and incubated in the presence of medium alone or 50, 150, 300 μM of CoCl2 for 12 and 24 h at 37°C, 5% CO2. The chemically induced hypoxia was identified and evaluated through the western blotting analysis of Hif1-α, CAIX and Glut1.

Results: The present study demonstrated that HTB-41 (ATCC) supports the CoCl2 stimulus in different concentrations (50, 150, 300 μM). We also observed that Hif1-α, CAIX and Glut1 expression were associated with chemically induced hypoxia and the expression were accentuated at 300 μM CoCl2. In addition, the present study also found two molecular weights for the Hif1-α (93 e 115 KDa).

Conclusion: In summary, our investigation confirms that CoCl2 stimulus can mimic hypoxic microenvironment. Additionally, we have presented some evidence that Hif1-α, CAIX and Glut1 expression was associated with chemically induced hypoxia at different concentrations (50, 150, 300 μM).

Keywords: Hypoxia, Hif1-α, CoCl2.
Supporting funding: Fapesp
Section G
Experimental Veterinary Sciences
Experimental Pathology Teaching
Experimental Models
Animal Facilities and Animal Welfare
Alternative Methods

G01 MORPHOMETRIC, HISTOLOGICAL AND BIOCHEMICAL CHANGES IN RATS WITH DOXORUBICIN-INDUCED NEPHROPATHY
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Introduction: Experimental models have contributed to our understanding of the pathophysiology of nephrotic syndrome (NS). In this regard, in rodents the injection of doxorubicin induced proteinuria and renal lesions which mimic human NS. Objectives: The aim of this study was to describe the morphometric, biochemical and histological changes at different stages of disease progression in rats with doxorubicin-induced nephropathy as a contribution to the characterization of this experimental model. Methodology: Male Wistar rats (250-300g) were divided into two groups: animals injected with intravenous Doxorubicin (7.5 mg/kg) (DOX, n=25) and animals injected with saline (control, CON n=20). Twenty-four hour urine samples were collected at days 7, 14, 21 and 28 after injections. At the same time points, animals were sacrificed and blood samples collected for analysis. After perfusion with phosphate buffered saline (PBS), the organs were removed, weighed and kidneys prepared for histology. Results: Animals of DOX group developed proteinuria, dyslipidemia, biometric and histological changes, consistent with chronic tubulointerstitial inflammatory infiltrate and renal fibrogenic process. In this animal model, some biochemical changes appear early and serve as nonspecific biomarkers for NS, while histological lesions become quite intense from day 21 after the doxorubicin injection. Conclusions: The detailed characterization of this animal model through morphometric, biochemical and histological studies may contribute to better understanding the natural history of NS. Keywords: Nephrotic syndrome, animal model, doxorubicin

G02 A CONTRIBUTION TO THE STUDY OF VARICOSE VEINS IN AN EXPERIMENTAL MODEL.
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Introduction: Despite the knowledge achieved, the cause of varicose veins of the lower limbs is not yet well known, although several possible causes have been identified. It is a common disease and exclusive to the human species. This raises many difficulties in the study and research of the disease. With an animal model it would be easier to understand the aetiology of the disease, its evolution and to study new treatments. Objectives: The aims of this study is to make an experimental model of varicose veins in an animal, producing venous hypertension in a vein in an orthostatic position, and then to make a comparative study between varicose lesions observed in humans and the ones observed in these animals. Methodology: We used 18 Belier-French male rabbits, three months old. In their left ear was surgically produced venous hypertension, by middle ear vein occlusion, and control was carried out with the right ear, not intervened. The evaluation was done until six months after surgery. Results: The results clearly showed significant morphological venous alterations. These changes were not constant over time and had considerable variation, from one animal to another. Conclusions: The proposed model is able to induce morphological and functional changes in the veins of the animal ear, approaching those described in the varicose veins in human species. Keywords: varicose, experimental model, veins.

G03 CALPAIN-1 INHIBITOR PREVENTS LOSS OF DYSTROPHIN IN EXPERIMENTAL SEPTIC CARDIOMYOPATHY INDUCED BY CEAL LIGATION AND PUNCTURE (CLP).
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Introduction: Evidences from our laboratory demonstrated that cytosolic calcium overload caused increased activation of intracellular calcium-dependent proteases, such as calpain-1 resulting in sarcolemal dystrophin disruption in severe sepsis induced by CLP in mice. Objective: This study was designed to determine the hypothesis that N-Acetyl-L-leucyl-L-leucyl-L-norleucinal (ALLN), calpain-1 inhibitor, could attenuate dystrophin disruption and cardiac contractile proteins loss/reduction in experimental sepsis induced by CLP. Material and Methods: Male C57Bl/6 mice were subjected to sham and severe septic injury (SSI) induced by CLP. Half of animals from each group were treated with ALLN (3mg/kg, SSI+ALLN; SH+ALLN) 4hs after surgery. Results: In SSI+ALLN mice reduced amounts of myocardial calpain-1 were associated with increased actin/myosin expression as compared to SSI mice. Additionally, ALLN treatment of septic mice significantly prevented loss of dystrophin and β-dystroglycan as compared to SSI mice. Concurrently, SSI+ALLN mice presented an increased survival rate. Conclusions: Calpain inhibitor, ALLN, suppressed the increased calpain-1 expression and prevented myocardial structural injury caused by experimental severe sepsis. These observations reinforce the concept that calpain-1 activation represents a key target in dystrophin disruption behind cardiac dysfunction in severe sepsis/septic shock. Further studies are needed to elucidate this mechanism that may provide new interventional pathways to prevent septic cardiomyopathy. Keywords: Sepsis, Calpain-1, Dystrophin.
Introduction: The human inflammatory bowel diseases (IBD) are gastrointestinal disorders that represent serious public health problem and its incidence and prevalence has increased in recent decades. The pathophysiological and clinical implications are severe, including hospitalization and morbidity associated to the high cost of the treatment. The maintenance of the inflammation is a continuous reaction against an aggression, trying to repair the damaged tissue, leading to an inflammatory situation in mucosa and across the whole intestinal wall. Experimental models of inflammatory bowel disease are used extensively to study the immune response of the normal and pathological intestine in humans and animals. Some of these models involve the use of animals treated with a combination of dextran sulfate sodium (DSS) and dinitrobenzene sulfonylamine (TNBS), which cause inflammation of the colon and rectum, respectively. The main advantages of these models are the low cost of the treatment, the simplicity of the technique, and the reproducibility of the results. However, these models have limitations, such as the lack of a standardized protocol, the use of different strains of mice, and the absence of a well-defined inflammatory response. The experiments described in this paper were designed to investigate the effects of music intervention on behavior disorders in rats and mice by making a comparative analysis between the two species of rodents at the same age. 

Methods: Forty-five days-old male Wistar rats (n=24) and male Swiss mice (n=24), following approval of the ethical committee for animal care, were divided into three groups, placed in a closed room equipped with a speaker, where taped music (average sound level: 65 dB) was played. Groups were subjected to (1) no music (untreated controls), (2) rock music, and (3) Mozart’s sonata for two pianos (K. 488) repeatedly for 4hs (two sessions of 2hs each) per day over 4 days. Animals were submitted to open field test, elevated plus-maze test, and forced swimming test to evaluate locomotor activity, anxiety- and depression-like behaviors, respectively. 

Results: One way ANOVA followed by Newman–Keuls test showed that in rats, but not in mice, Mozart’s sonata increased the percentage of open arm time at the elevated plus-maze test when compared to the control group [F(2,23)=4.348, p=0.0263], with no alteration at the forced swimming test. Rats reduced the immobility time when compared to the control group [F(2,23)=3.918, p=0.0358]. Behavioral alteration with both types of music was not observed in mice. 

Conclusion: Our results suggest that the effectiveness of music as therapy to treat behavior disorders is dependent on the species of the experimental subject and the type of music. Nevertheless, the precise mechanisms of action and/or neurotransmitters involved in these different responses should be investigated. 

Keywords: Anxiety, Depression, Music therapy. 

Supporting funding: PIBIC/CNPq/UnB

G06 EXPERIMENTAL COLITIS IN RATS INDUCED BY 2,4,6 - TRINITROBENZENESULFONIC ACID (TNBS).

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Introduction: The human inflammatory bowel diseases (IBD) are gastrointestinal disorders that represent serious public health problem and its incidence and prevalence has increased in recent decades. The pathophysiological and clinical implications are severe, including hospitalization and morbidity associated to the high cost of the treatment. The maintenance of the inflammation is a continuous reaction against an aggression, trying to repair the damaged tissue, leading to an inflammatory situation in mucosa and across the whole intestinal wall. Experimental models of inflammation provide valuable information about this pathogenesis and represent important tools for studying new therapies. 

Objectives: In this study we evaluated the effect of TNBS to induce colitis in rats. 

Methodology: Male Wistar rats (n = 50) were divided into three groups to receive single enema...
containing TNBS (20mg), Saline or ethanol (50%). After that, they were evaluated daily for the onset of clinical symptoms and killed after 24 hours and 14 days of induction. Samples of distal colon were prepared for lesion analysis, quantification of myeloperoxidase (MPO) and histological processing with HE and PAS staining for microscopic study of inflammation and morphometry of intestinal tunics.

Results: The enema of TNBS resulted in severe diarrhea significant weight loss and prostration of the animal. The concentration of MPO remained elevated throughout the experimental period. The mucosa showed different degrees of inflammation, presenting regions with hyperemia, ulcers, abscesses, cysts and reduction in the number of goblet cells. The inflammatory infiltrate was transmural, affecting all coats, with the presence of neutrophils cells within the myenteric ganglia. Conclusions: The experimental model of colitis by TNBS in animals proved be efficient in reproducing the characteristics of human disease.

Keywords: experimental colitis, inflammation, intestinal morphology.

Supporting funding: CNPq / FAADCT - PR / UEM.

G07 INDOMETHACIN ATTENUATES THE MECHANICAL ALODYNIA UNTIL FOUR DAYS AFTER THE CHRONIC CONSTRUCTION INJURY IN THE MODEL OF PERIPHERAL NEUROPATHIC PAIN.

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Introduction: Bennett and Xie (1998) developed a model of peripheral mononeuropathy in rats by chronic constriction injury (CCI) by placing four loose ligatures around the ischiatic nerve, which is one of the most commonly employed animal models of neuropathic pain (NP). The underlying NP mechanisms are related to peripheral and central sensitization that originates respectively from the release of inflammatory mediators around peripheral damaged tissue and ectopic discharges from the injured nerve leading to a hyperexcitable state in spinal dorsal horn neurons. Objectives: To clarify the role of cyclooxygenase (COX) in the peripheral nerve on the development and maintenance of NP, the aim of this work is to evaluate in which moment the indomethacin did not decrease significantly mechanical allodynia in seventh and fourteenth day after CCI (P<0.05). Conclusion: It was presented an adaptation NP model developed by Bennett and Xie. These findings suggest that inflammatory mechanisms are involved in induction of NP. However, the indomethacin did not attenuate the NP considering the stages of potentiation and maintenance of chronic pain in NP model in rodents.

Keywords: Indomethacin, Chronic constriction injury, Neuropathic pain.

Supporting funding: Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP).

G08 MECHANISMS OF ACTIONS OF GREEN PROPOLIS IN TISSUE REPAIR AND ITS EFFECTS ON THE ACTIVATION OF SUBPOPULATIONS OF MACROPHAGES.

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Introduction: Propolis is a chemically complex resinous bee products. Objectives: We evaluated the anti-inflammatory activity based on wound healing parameters. Methodology: Mice were subcutaneously implanted with polyester-polyurethane sponge discs, and were orally administered with green propolis (500 mg/kg). Inflammatory angiogenesis were evaluated at 4, 7 and 14 days post-implantation. Blood vessel formation as assessed by hemoglobin content and by morphometric analysis of the implants was reduced by wet weight of sponges compared to the untreated groups. The level of vascular endothelial growth factor increased progressively in the treated group. We also observed a decreased after day 10 in the control group. Neutrophils and macrophages accumulation was determined by measuring myeloperoxidase and N-acetylglucosaminidase activities, respectively. The fibrovascular stroma and extracellular matrix deposition were evaluated by histopathologic and morphometric analyses. Results: In the propolis-treated groups, at days 4 and 7, the inflammatory process was reduced in comparison with control (means ± e.p.m; n=8-10, P<0.001). Neutrophil accumulation was unaffected by propolis, but NAG activity was reduced by the treatment at day 14, (means ± e.p.m; n=8-10, P<0.001). The level TGF-β1 intraimplant increased progressively in both groups but was higher (40%) at day 14 in the control implants. The pro-inflammatory levels of TNF-α peaked at day 7 in the control group, and at day 14 in the propolis-treated group (means ± e.p.m; n=8-10, P<0.05). The progressive increase in cell influx and collagen deposition was observed in control and propolis-treated groups during the whole period. However, these effects were attenuated in the propolis-treated group at days 4 and 7.

Conclusions: Our results indicate that the anti-inflammatory/anti-angiogenic effects of propolis are associated with cytokine modulation.

Supporting funding: FAPEMIG.

G09 MORPHOLOGICAL AND IMMUNOPHENO-NOTICAL CHARACTERIZATION OF MURINE...
MAMMARY CARCINOMA 4T1.
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Introduction: The 4T1 murine mammary carcinoma is an experimental model widely used in assessing and better understanding of tumor biology. It is a highly tumorigenic cell line and invasive, where metastases are observed in various organs. Objectives: This study aims to describe morphological and immunophenotypical aspects of 4T1 mammary carcinoma in mice Balb/c with the aid of the immunohistochemistry. Methodology: Tissues were fixed in formalin and processed using the routine paraffin inclusion technique. Histologic sections (4 µm) were stained through Hematoxylin-Eosin techniquess for morphologic assessments. For immunohistochemical study, we used a panel of 9 (nine) antibodies consist of hormone receptors, receptors for cell proliferation, cytokeratins, vimentin, growth factor receptor and markers of blood vessels. Results: Morphologically, the 4T1 murine mammary carcinoma shows malignant epithelial proliferation arranged in solid, with a proliferation of pleomorphic cells and high mitotic index. In immunohistological analysis, we determined positivity for hormone receptors, cytokeratin AE1/AE3, receptors of cell proliferation and markers of blood vessels. There was negative for vimentin, cytokeratin 5/6, cytokeratin 34βE12 and growth factor receptor. Conclusions: The results show that the characteristics observed in this model are similar to some types of breast cancers found in women like poorly differentiated invasive ductal carcinoma. Thus, the immunophenotypic characterization of mammary carcinoma 4T1 promote a better understanding of the model to the study of new antitumor therapies. Keywords: mouse, mammary gland, 4T1 cells, immunohistochemistry. Supporting funding: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) Conselho Nacional de Desenvolvimento Científico e Tecnológico (FAPESP) Conselho Nacional de Desenvolvimento Científico e Tecnológico (FAPESP) Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG)

G10 MOUSE MODELS TO STUDY MECHANISMS OF EOSINOPHIL SECRETION DURING INFLAMMATORY RESPONSES.
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Introduction: In response to varied stimuli, human eosinophils are recruited the circulation into inflammatory foci, they modulate immune responses through the release of granule-derived products. Eosinophil secretory mechanisms are well characterized in humans, but are still poorly understood in mice. Here we investigated the mechanisms underlying eosinophil degranulation of murine models during inflammatory responses in vivo (asthma model) and in vitro (after stimulation with pro-inflammatory stimuli). Methods: Balb/c mice were immunized with ovalbumin (OVA) as before and lung fragments were processed for light and transmission electron microscopy (TEM). IL-13, IL-4, CCL11 and TGF-β were measured in the lungs. In vitro experiments, eosinophils were isolated the spleen of IL-5 transgenic mice, stimulated with GM-CSF, LPS or medium alone, and processed to TEM. Results: Intense eosinophil infiltration was seen in perivascular and peribronchial spaces of OVA-treated animals. Infiltrating eosinophils showed 36% of granules with degranulation signs associated with piecemeal degranulation (PMD), characterized by reduced electron-density, matrix or core losses, coarse matrix and/or granule enlargement. CCL11, IL-4, IL-13 and EPO levels OVA-stimulated mice were significantly increased compared to controls while the TGF-β levels did not change. Stimulation splenic eosinophils exhibited shape changes and emptying granules. Granule-granule or plasma membrane-granule fusions were not observed. Interestingly, we identified by TEM a distinct electron-dense brim at specific areas of the limiting granule membrane, a novel morphological feature that may be associated with secretion in mice eosinophils. Conclusions: Our data demonstrate that mouse eosinophils are able to degranulate through PMD during inflammatory responses both in vivo and in vitro. Although the morphological features recognized as indicative of PMD are less pronounced in mice compared to those documented in humans, these signs can be detected in mice eosinophils under careful investigation by TEM. Moreover, our study show that other morphological signs, not described for human eosinophils, may be helpful to understand the secretory processes in eosinophils mice models. Keywords: Inflammation, Eosinophil, Mouse, Secretion. Supporting funding: CNPq and Fapemig (Brazil) and NIH R37-AI020241, R01-051645 (USA)

G11 MYENTERIC NEURONAL PLASTICITY AS RESPONSE TO INFLAMMATION IN EXPERIMENTAL MODEL OF COLITIS.
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Introduction: Inflammatory bowel disease (IBD) is a term for various types of chronic diseases of autoimmune nature that affect the gastrointestinal tract (GIT). It is characterized by acute episodes of inflammation interspersed with remission and relapse of disease. The structural integrity and in-
integration of the various functions assigned to GIT as motility, absorption, distribution of blood flow and endocrine activity is attributed to the enteric nervous system (ENS). Failures at this integration and control are involved in triggering of IBD, and during the active phase of the disease, the neurons can turn out to be direct or indirect targets of the immune attack resulting in irreversible damage, neuronal death. Objectives: The objective of this work was to reproduce the experimental colitis in rats and evaluate its effects on the quantitative and morphological parameters in the neuronal population HuC / HuD and nitricergic (nNOS) of the myenteric plexus. Methodology: Wistar rats, adults (n = 36) were divided into three groups which received a single enema of saline, ethanol and TNBS and then killed with 7 and 28 days of induction. Samples of distal colon were used for macroscopic and microscopic assessment, and measurement of myeloperoxidase for evidence of inflammation. Immunofluorescence for neurons HuC / HuD and nNOS were performed by whole mount preparations at 28 days of induction. Results: Grossly, the lesions were characterized by hyperemia, edema and necrosis of the mucosa, more intense in the TNBS group at 7 days. The myenteric ganglia showed reduction in the number of neurons HuC / HuD nNOS with a concomitant increase in the area of the cell body in the TNBS group. Conclusions: The colitis changed the morphology of the intestine and caused serious insult to the myenteric neurons, leading these populations to morphological changes due neuronal loss, which reflects the plasticity of the ENS as an adaptation to ensure homeostasis of the intestinal functions.

Keywords: experimental colitis, TNBS, myenteric neurons.

Supporting funding: CNPq / CAPES/FAADCT - PR / UEM

G12 PROBIOTIC POTENTIAL OF LACTOBACILLUS CRISPATUS IN MURINE MODEL OF BACTERIAL VAGINOSIS.

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Introduction: Bacterial vaginosis (BV) is the most common type of vagINITis among women of reproductive age. The lack of a model for the study of BV and its need for assessment of new therapeutic options are the motivation of this work. Objectives: To propose an animal model for the study of the BV and evaluate the probiotic properties of Lactobacillus crispatus isolated the vaginal ecosystem of healthy women in menacme. Methodology: Twenty-eight conventional NIH female mice (28 days old) were distributed among the control group (NaCl 0.9%, w/v) and experimental groups (L. crispatus 04, G. vaginalis ATCC 14018 + L. crispatus 04 and G. vaginalis ATCC 14018). All animals subcutaneously received 0.5mg of estradiol 3 days prior to the intravaginal administration of lactobacilli (109 CFU/mL) and the challenge with the pathogen (107 CFU/mL). Vaginal tissue samples were subjected to anatomopathological examination. Results: In the group of control animals it was found that the appearance of the vaginal tissue was preserved and not inflamed. The wall was not hyperemic and the epithelial surface was preserved. The group infected with G. vaginalis showed nonspecific changes in epithelial and dermal region. Furthermore, it was observed a thickening of the epithelium with intercellular edema, and reactive basal hyperplasia and cell vacuolation, presence of moderate inflammatory infiltrate in the dermal region, marked mainly by lymphocytes, neutrophils and eosinophils. Treatment with L. crispatus 04 reduced most of the signs and only a mild inflammatory infiltrate was verified. Additionally, treatment with lactobacilli, without experimental infection by G. vaginalis, did not alter the histological tissue analysis. Conclusion: The use of the BV murine model in conventional mice of the strain NIH showed that L. crispatus 04 protected against the experimental infection with G. vaginalis, which was evidenced by the reduction in histopathological lesions caused by this bacterium.

Keywords: Bacterial vaginosis, Experimental model, Lactobacillus crispatus, Probiotics

Supporting funding: Fundação de Amparo à Pesquisa do Estado de Minas Gerais – FAPEMIG

G13 THE EFFECTIVENESS OF DESMOPRESSIN IN MEMORY OF RECOGNITION IN ANIMAL MODEL OF AUTISM.

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Introduction: Autistic spectrum disorder develops in early childhood, it’s more common in men and its prevalence has been increasing over the years. The etiology of this disorder remains unknown, then there’s no effective and curative treatment for this disorder. Therefore many studies have been conducted based on neuropeptides changes presented by patients, they show that some neuropeptides have increased and others decreased, like the decreased of serum vasopressin detected in autistic children. Although it’s more known for its antiuretic action, studies have showed that vasopressin is associated with human behavior, being related to anxiety, confidence, memory and cognition. Objective: Evaluate the efficacy of treatment with desmopressin in recognition memory of Wistar rats exposed to valproic acid in prenatal period.

Method: Desmopressin, a synthetic analogue of vasopressin, was administrated for seven consecutive days in young Wistar rats exposed to valproic acid, autism group, or saline, non-autistic group, in the prenatal period. The animals were subjected to the stereotyping, motor activity, exploratory activity and object recognition tests, 24 hours after the last drug administration. Results: This research demonstrated that the use of desmopressin significantly reversed stereotypy condition induced by prenatal administration of sodium valproate, increased the time of exploration at open field, and increased the object recognition index. Conclusion: the results, it is suggested that desmopressin may be considered a study object possibly being used for the treatment of autism symptoms.

Keywords: Desmopressin, Autism, Recognition memory.
Introduction: Hydrocephalus is a syndrome resulting from the current unbalance between formation and absorption of cerebrospinal fluid (CSF), and consequent accumulation within the cerebral ventricles. Clinically, hydrocephalic children can present several neurological disorders, not always reversed with treatment, even after the development of more and more sophisticated shunt systems. In order to establish what changes are reversible with the relief or restoration of cerebrospinal fluid circulation, and the point of hydrocephalus evolution in which the functional recovery of nervous tissue is still possible, additional studies are needed. Objectives: The goal of this work was to develop a shunt system to reverse hydrocephalus and study the lesions to brain structures around the ventricles and compensate additional studies are needed. Objectives: the goal of this work was to develop a shunt system to hydrocephalic rats, and study the lesions to brain reversed with treatment. Methodology: hydrocephalus was induced in pup rats by injecting kaolin into the cisterna magna. Ventricular size was assessed by magnetic resonance imaging. Some hydrocephalic rats were treated by diversionary shunting of cerebrospinal fluid. The animals were daily weighed, assessed by repeated behavioral testing and a second magnetic resonance. All animals were sacrificed 14 days after hydrocephalus induction and the brains processed for histological examination, including the solochrome-cyanine method to stain myelin. Results: behavioral testing and weight gain were good indicative of the ventriculomegaly: the worse performance and weight gain, the bigger ventricular size. Behavioral testing and weight gain were compromised by hydrocephalus and improved with treatment with diversionary shunting of cerebrospinal fluid. The magnetization transfer provided information on the severity of hydrocephalus, as well as the success of treatment by shunting. Already the histological assessment by solochrome-cyanin reinforced the idea that the hydrocephalus affects myelination of the structures around the ventricles and compensatory myelination can be possible with treatment with shunts. Conclusion: treatment of hydrocephalic rats 1 week after induction can partially restore myelin showed by magnetization transfer ratio and histology.
Introduction: Melissa officinalis (MO), popularly known as lemon balm, has been used predominately in brain-related disorders. Notably, different presentations of this herb are reported to reduce stress and anxiety levels in experimental models.

Objectives: This study analyzed the behavioral and cognitive effects of MO ethanolic extract in sepsis surviving rats models. Methodology: Male Wistar rats (n=8 animals/group) were used with the recommendations of ethical committee for animal care. The animals were anesthetized i.p., using a mixture of ketamine (80 mg/kg) and xylazine (10 mg/kg), to allow exposure of the cecum, which was subsequently removed to count of feces from the perforation site, which was later placed back into the peritoneal cavity. All animals were returned to their cages after administration of ceftriaxone (30 mg/kg) + clindamycin (25 mg/kg). MO ethanolic extract (30 or 100 mg/kg) was administered by gavage, for one week after sepsis induction. On the last day, one hour after MO administration, the animals were submitted to the open field (OF), elevated plus-maze (EPM), forced swimming (FS), and step-down inhibitory avoidance tests. Results: ANOVA, followed by Newman Keuls test, showed that the locomotion (OF test) and immobility time (FS test) was not significantly altered by treatments. In the EPM test, the percentage of open arm time in the EPM of rats that received subchronic MO extract were significantly higher than sham-operated animals treated with vehicle, and the response levels were similar to those of the diazepam group. Kruskal-Wallis test revealed a significant effect of treatment with Melissa officinalis extract (100 mg/kg) in the animal’s latencies during the short- and long-term memory of the retention test session. The Mann-Whitney test indicated that the sepsis group significantly decreased the animals latencies during the short- and long-term memory, when compared to the sham-operated animals. Conclusions: Based in these results, the investigated extract of MO possesses anxiolytic-like properties and mnemonic response. These psychoactive properties, along with the safety profile of the lemon balm, may provide a pharmacological alternative for specific neurologic disorders.

Keywords: Melissa officinalis, Rats, Sepsis.

Supporting funding: CAPES & PIBIC/CNPq/UnB.

H05 MONITORING OF TEMPERATURE AND HUMIDITY IN SMALL ANIMAL CAGES.
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Introduction: Monitoring of small animals cages environmental variables is a major concern in animal experimentation. The access to information like temperature and humidity of the cage allows the researcher to have more information over animal welfare during an experimentation and can help improve the environmental conditions. Objective: The purpose of this work is to develop a microsystem for monitoring the environment in the animal’s cage, using temperature and humidity sensors connected to a microprocessor. Methodology: Using an array of sensors in each cage one can monitor the environment inside the cage individually, managing the information from each sensor and processing that information in real-time. We used three temperature sensors and three humidity sensors. Two humidity and two temperature sensors were inside the cage three centimeters above the base and the other sensors were three centimeters below the top of the cage. In this study there were three test groups: in the first group was measure the tempe-
nature and humidity without rats inside the cage; in the second group were used one rat inside the cage; in the third group were used two rats inside the cage. **Results:** The results show that the temperature inside the cage it was constant, between 23º-24º C during the tests for all groups. The humidity, in the first group was almost constant during the test, oscillating between 53%-63%. In the second group, the humidity was between 55%-85% and in the third group the humidity was between 60%-86%. **Conclusions:** The results suggest that the quality of the environment inside the cage depends on the number of rats per cage, and the humidity levels increase rapidly with the numbers of rats.

Keywords: Monitorization, Sensors, Cage.

**H06 EXTRACTION AND ANALYSIS OF PROTEINS BY WESTERN BLOT FROM FORMALIN FIXED AND PARAFFIN-EMBEDDED SAMPLES.**

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**Introduction:** Formaldehyde fixation and paraffin embedded (FFPE) is the gold standard technique used for preservation and storage of tissue specimens for pathologic analysis. **Objective:** This study was conducted to describe and analyze the potential usefulness of protein extraction protocol from paraffin collected material in different periods. **Materials and Methods:** Paraffin was removed from tissue samples 5-10 micrometers thick sections with an organic solvent, followed by protein extraction using a modified RIPA lysis buffer and solubilization in 5% SDS. The resulting protein extract was used for SDS-PAGE and immunoblot analysis. **Results:** Our results showed that both SDS-PAGE and immunoblot presented a quantity of workable protein. However, FFPE-tissues conserved for 10 years presented less workable proteins than FFPE-samples preserved for 2 years. In addition, protein extraction from tissue with higher density of cells provided more workable protein than the lower density tissues. **Conclusion:** In summary, our investigation presented evidence that the evaluation of protein extraction in FFPE-samples can be used to provide information about protein expression and, some factors such as kind and time preservation of FFPE-samples may influence the quality and quantity workable proteins.

Keywords: FFPE, Western blotting, protein extraction

Supporting funding: FAPESP

**H07 THE IMPORTANCE OF BIOSAFETY IN THE EXPERIMENTAL RESEARCH AND PROPOSAL FOR SAFETY INSPECTION CHECK LIST.**

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**Introduction:** In research laboratories there are numerous procedures, each showing features and specific risks. In order to minimize or even eliminate such risks, the Biosafety has developed techniques and protocols, but in Brazil the adherence to these measures is not yet satisfactory levels for an efficient prevention of risks to health and the environment. **Objectives:** this study aims to promote discussion of specific aspects of biosafety in research laboratories, as well as propose a check list for safety inspection in these laboratories and report the experience of an institution of higher education after establishing an effective biosecurity program. **Methodology:** for the preparation of the check list to help in the management of biosafety research laboratories specific studies had been raised through a search in the database of the Internet in the following web-pages: sciencedirect, webofscience, periodicoscapes, scielor, and also considered the regulatory norms of the Ministry of labor and employment, requirements of national health surveillance agency (ANVISA), environmental agencies, under the guidance of bio-security consultant with professional experience on the appropriateness of the standards in numerous laboratories and health care environments. **Results:** a program of strategies of biosafety actions puts into practice the knowledge about the topic in an integrated manner in order to preserve the quality of life of the academic community and the region. Is an inspection check list, subdivided into thematic axes in the order: 1. physical facilities; 2. management of collective protection equipment (CPE’s) and personal protective equipment (PPE’s); 3. ergonomic Aspects in the work environment; 4. Prevention of fires; 5. Safety Signs; 6. hygiene Measures; and 7. Ocupationalionion, that can be used in the implementation and maintenance of secure actions in research laboratories. **Conclusions:** A committed Research Laboratory biosecurity not only promotes the maintenance of health researchers, trainees, employees and users, preserves the environment, as well as raises the quality and reliability of search results. Keywords: Biosecurity, Prevention, Quality, Experimental research