Seminário Anual do PCF

2021

III ANNUAL MEETING OF THE POSTGRADUATE PROGRAM IN PHARMACEUTICAL SCIENCES

State University of Maringa - UEM

ABSTRACT BOOK

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STATE UNIVERSITY OF MARINGA (UEM)
Health Sciences Center
Department of Pharmacy
Postgraduate Program in Pharmaceutical Sciences

XI ANNUAL MEETING OF THE POSTGRADUATE PROGRAM IN PHARMACEUTICAL SCIENCES

Abstract Book

Maringa, PR, Brazil

2021

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SPEAKERS

Prof. Dr. Ramesh LahiruWalpola
University of New South Wales (Australia)
Title: Medicines safety in vulnerable populations

Prof. Dr. Jackson Roberto Guedes da Silva Almeida
UNIVASF (PE, Brasil)
Title: Chemical and pharmacological study of Passiflora species from the Sao Francisco Valley

Prof. Dr. Paulo Correia de Sá
Instituto de Ciências Biomédicas de Abel Salazar (ICBAS), Universidade do Porto (Portugal)
Title: Fine-tuning modulation of purinergic signaling dynamics: What about human diseases?

Prof. Dr. Flávio Augusto Vicente Seixas
Universidade Estadual de Maringá (PR, Brasil)
Title: "Computational biochemistry and biophysics applied to drug development"

Prof. Dr. Mariana Kiomy Osako
Faculdade de Medicina de Ribeirão Preto (USP, Brasil)
Title: Be a mom and perish?

Prof. Dr. Benedito do Prado Dias Filho
Universidade Estadual de Maringá (PR, Brasil)
Title: Pharmacology of natural and synthetic products: Microbiology 30 years

Prof. Dr. GracietteMatioli
Universidade Estadual de Maringá (PR, Brasil)
Title: Example of a professional journey in the University environment: life learning and knowledge transfer

Oral presentation of research works

Ana CarolinaGuidi – *Poincianellapluviosa* inhibits cytokine production by activated macrophages

Clara Beatriz de Lima – *Evaluation of cytotoxicity of pyrostegiavenusta flowers in l929 cell line*

Sharize Betoni Galende – *In vivo experimental studies and histological analysis: methodological aspects*

Éverton da Silva Santos – *A green strategy for enhanced phenolic compounds extraction from Cereus hildmannianus by elicitation with salicylic and jasmonic acids*

Rafaela Said dos Santos – *Mechanical properties of emulsion systems composed of Carbomer 934P, 974P or polycarbophil, natural oils and Natural bioactive agentes"*

Thaila Fernanda Oliveira da Silva – *Characterization of phenolics compounds from leaf waste of Stevia rebaudiana*

Elisa Parcero Hernandes – *Doxorubicin-loaded iron oxide nanoparticles effect in breast cancer cells*

Karina Miyu Retamiro – *Antitumor activity in vitro of menadione against cervical câncer*

Ludmila Pini Simões – *Inclusion of alginate microparticles containing berberine and fluconazole in pharmaceutical form cream and artificial saliva for the treatment of vulvovaginal and oral candidiasis*

Guilherme Godoy – *Decreased docosahexaenoic acid levels in serum of HIV carrier patients*

Erika Meyer – *Cannabidiol reduces neurological deficits and glial cells activation after middle cerebral artery occlusion in mice*

Lucas Casagrande – *Microencapsulated quercetin and Bifidobacterium animalis protect huc/d-ir neurons in the submucous plexus of wistar rats chemically induced to colorectal carcinogenesis*

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Prof. Dr. Lívia Bracht (Chair)

Dr. Andressa Blainski Pinha – Finzelberg, Germany

Dr. Gisely Cristiny Lopes – Post-doc, State University of Maringá

Dr. Larissa Lachi Silva – Novartis, USA

Dr. Rafael Pazinatto Aguiar - Boehringer Ingelheim Pharma GmbH - Germany

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EVALUATION OF LIVER MORPHOLOGY AND GLYCOGEN RESERVE IN THE LIVER OF WALKER-256 TUMOR MICE AND THE EFFECTS OF SUPPLEMENTATION WITH 1% L-GLUTATIONE

Carla Cristina de Oliveira Bernardo[a]; Lucas Casagrande[a]; Lídia Rodrigues Cicero[a]; Mariana Rodrigues Sanches[a]; João Victor Kuller[a], Jacqueline Nelisis Zanoni[a], Juliana Vanessa Colombo Martins Perles[a].

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Keywords: histopathological evaluation, liver morphology, glycogen.

Introduction: Oxidative stress is closely related to the process of cancer initiation and development. One of the main aggravating factors of cancer is cachexia syndrome, in which the tumor competes with the host for nutrients, and is characterized by great weight loss, anemia and weakness 1, aggravating the body's weakness. One of the pathogenesis also associated with cancer are the modifications generated by oxidative metabolism 2. Due to their potential damage, free radicals must be rapidly eliminated from the cell's environment. Therefore, different organisms have an endogenous antioxidant system composed of several enzymes and components to maintain the redox balance. Reduced glutathione, a tripeptide found in most tissues, especially in high concentrations in the liver, has an important antioxidant potential 3,4. **Aim:** Evaluate the liver morphology, possible biochemical changes and the effects of 1% L-glutathione supplementation in the liver of rats submitted to the Walker-256 experimental tumor model. Methods:All protocol was approved by CEUA8617130120. Adult male rats (48 days old) of the Wistar lineage (Rattus norvegicus) were used, randomly divided into 4 groups (n=6): control (C), control supplemented with 1% L-Glutathione (CGT), tumor of Walker-256 (TW) and Walker-256 tumor supplemented with 1% L-Glutathione (TWGT). After the 15-day experimental period, the animals were euthanized and the liver collected. After processing the material and performing staining techniques with HE and PAS, hepatocyte morphometry and glycogen quantification were performed on 10 and 30 images per animal, respectively, followed by statistical analysis using the block design test followed by post Fisher's test. **Results:** The animals in the TW group showed histopathological changes, such as foci with infiltration of inflammatory cells and apoptotic bodies, changes that were not reversed by supplementation with 1% glutathione. These animals also showed a marked reduction in glycogen stores. On the other hand, we observed an increase, albeit a slight one, in the glycogen reserve in animals from the TWGT group. A reduction in the size of hepatocytes was also observed, detected in the morphometric evaluation in animals from the CGT group. **Conclusion:** Despite not preventing histopathological changes, L-glutathione was effective in preventing glycogen reduction.

Acknowledgments: CNPq - Conselho Nacional de Desenvolvimento Científico e Tecnológico.

References:

(1) Kowata CH, Benedetti GV, Travaglia T et al.Fisiopatologia da caquexia no câncer: Uma revisão Pathophysiology of cancer cachexia: A Review. Arq. Ciênc. Saúde UNIPAR. 2009;267–272:13.
(2) Maia FMM, Santos EB, Reis GE. Oxidative stress and plasma lipoproteins in cancer patients. Einstein. 2014;480–484:12.
(3) Hatem E, Banna NE, Huang M-E. Multifaceted Roles of Glutathione and Glutathione-Based Systems in Carcinogenesis and Anticancer Drug Resistance. Antioxidants & Redox Signaling. 2017; 1217–1234:27.
(4) Lu SC. Glutathione synthesis. Biochimica et Biophysica Acta (BBA) - General Subjects. 2013;3143–3153:1830.

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Post-ischemia administration of a Trichilia catigua lyophilized extract prevents oxidative stress, stimulates dendritic/synaptic plasticity, and restores memory loss after global cerebral ischemia

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Keywords: Cerebral ischemia, memory, oxidative stress, neuroprotection

Introduction:. We originally reported that the administration of an ethyl-acetate fraction of Trichilia *catigua* (EAF-TC) that was initiated prior to transient, global cerebral ischemia (TGCI) reduced both memory deficit, oxidative stress and neuroinflammation in rats. **Aim:** To search whether the EAF-TC treatment that was initiated after TGCI maintains its neuroprotective properties. **Methods:** TGCI was induced for 15 min according the 4-VO model. The EAF-TC treatment was initiated at 1, 4 or 6 hours after TGCI and continued for 7 days consecutively. Oxidative stress was measured 24 h after TGCI and memory performance was assessed in the radial maze test from 10 to 24 days after TGCI. At the end of the behavioral test, the brains were examined for neurodegeneration and dendritic changes in the surviving neurons. Additionally, whether EAF-TC stimulates synaptic plasticity was evaluated. This protocol had the approval of internal Ethical Committee (CEUA nº 7481261017). **Results:** TGCI caused the rats to spend more time to complete the task, and to commit more reference and working memory errors, indicating they forgot the task that was learned prior to ischemia. The EAF-TC treatment initiated 1, 4 or 6 h post-ischemia alleviated the oxidative stress caused by ischemia, as measured by the levels of antioxidant enzymes and protein carbonylation. The memory loss (amnesia) caused by ischemia was greatly reduced by EAF-TC initiated at 4 or 6 h post-ischemia. This treatment did not prevent neurodegeneration, but alleviated the changes in dendritic morphometry and the loss of some synaptic-related proteins. **Conclusion:** The data indicate that the treatment with the EAF preparation of T. *catigua* that was initiated up to 6 h postischemia maintained its neuroprotective properties, expressed by improved memory recovery, reduction of oxidative stress and protection and/or stimulation of dendritic plasticity.

Acknowledgments: Financial support CAPES and CNPq.

References:

(1) KANDEM, J.P.et al. Catuaba (Trichilia catigua) prevents against oxidative damage induced by in vitro ischemia-reperfusion in rat hippocampal slices. Neurochemical Research. vol. 37(12): 2012; 2826-35.
(2) GODINHO, J. et al. Ethyl-acetate fraction of Trichilia catigua restores long-term retrograde memory and reduces oxidative stress and inflammation after global cerebral ischemia in rats. Behav Brain Res, v. 337, p. 173-182, Jan 30 2018. ISSN 1872-7549

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CARBOHYDRATE-RICH DIET EFFECTS ON SWISS MICE'S JEJUNUM AND PROXIMAL COLON

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Keywords: carbohydrate-rich diet, linseed oil, colon, jejunum**.**

Introduction: A hypercaloric diet associated with genetic factors and physical inactivity favors obesity, diabetes, and non-alcoholic hepatic steatosis. The main function of the intestinal mucosa is food absorption, however, its barrier may be impaired by the diet. **Aim:** Verify the effects of the carbohydrate-rich diet and the lipidic font replacement by linseed oil on colonic intestinal mucosa. **Methods:** 40 male Swiss mice were randomly distributed in four groups: Standard Feed (SF), carbohydrate-rich diet (CRD), Diet with 10% substitution of lipidic font by linseed oil (DL-10), and Diet with 100% substitution of lipidic font by linseed oil (DL-100). At 112 days old, the animals were euthanized, the jejunum and proximal colon were collected and processed into histological techniques with HE (Hematoxylin and Eosin). The heights of mucosa, submucosa, muscle layer and total intestinal wall were measured and the results submitted to Block's Design followed by Fisher's post-hoc with a 5% significance level. **Results:** CRD group jejunum presented a 15,74% reduction in the submucosa (vs. SF). In opposition, this group's colon presented raises in the intestinal wall, mucosa, and muscular (15,83%, 8,47% and 13,47%, vs. SF). The jejunum's DL-10 group diminished in the intestinal wall and mucosa, respectively, and raised 4,43% in the muscular (vs. SF.) There were expressive raises in all parameters in the colon (CRD vs. SF). Similarly, the DL-100's colon yielded a 6,43% raise in the intestinal wall, 15,55% in the mucosa and 74,63% in the submucosa whilst the jejunum reduced 6% in the intestinal wall and mucosa (vs. SF). DL-10 showed a 3% and 5,87% reduction in the jejunum's intestinal wall and mucosa, respectively (vs. CRD). The muscular and submucosa displayed a 6,46% and 18,86% raise, sequentially. Also, all the colonic measurements were expressively different in this group (vs. CRD). The DL-100 demonstrated an increase in jejunal and colonic submucosa, and a reduction in the colonic and jejunal intestinal wall (vs. CRD). Finally, the jejunum mucosa diminished whilst the colon mucosa and muscle layer were increased in these animals. **Conclusion:** The Carbohydrate-rich diet affects both intestinal segments in different ways with a higher impact on colon. The diet with 100% substitution of lipidic font by linseed oil prevented the morphometric alterations found in both segments.

Acknowledgments: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - CAPES.

References:

(1) Adefegha SA. Functional foods and nutraceuticals as dietary intervention in chronic diseases; Novel perspectives for health promotion and disease prevention. J. Diet Suppl. 2017:1-33
(2) Barker N, Oudenaarden AV, Clevers H. Identifying the stem cell of the intestinal crypt: Strategies and pitfalls. Cell Stem Cell. 2012;11:452–460
(3) BarrenaHC, Schiavon FPM, Cararra MA et al. Effect of linseed oil and macadamia oil on metabolic changes induced by high-fat diet in mice. Cell Biochemistry and Function. 2014; 32:333–340.
(4) Bray GA. Is dietary fat important? American Journal of Clinical Nutrition. 2011;93:481–482.

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ASSESSING IVIVR OF LAMOTRIGINE IR USING PBBM APPROACH

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Introduction: Physiologically based biopharmaceutics modeling (PBBM) is a viable tool to establish links between bio-predictive *in vitro* dissolution testing and mechanistic oral absorption modeling (1). *In vitro* and *in vivo* parameters are implemented into a physiologically-based pharmacokinetic (PBPK) model and can build an *in vitro-in vivo* relationship (IVIVR) (2). Common applications of PBBM have been mainly for low solubility drugs (3) as lamotrigine (LTG), an antiepileptic drug (4). Thereby, the **aim** of the present work was to develop a PBPK model of LTG and evaluate the IVIVR. **Methods:** The dissolution data were obtained from paddle and flow-through cell apparatus using compendial and biorelevant medium. An oral PBPK was developed using GastroPlus™ software. The predicted PK profiles and parameters were compared with the observed ones to assess the predictability of the model. IVIVR was evaluated and dissolution profiles were incorporated into the model. LTG PBPK was verified with Cmax and AUC predicted/observed ratio in the range of 0.76 and 1.20. **Results:** The IVIVR of LTG using flow-through cell apparatus with biorelevant media (FaSSIF-V1) showed a linear correlation and slope of 0.95. After that, the LTG dissolution data incorporated as a z-factor model, improve the PK prediction with Tmax predicted/observed ratio from 0.48 to 0.87. **Conclusion:** It was possible to verify that the in vitro method that best explains the dissolution of LTG in vivo was flow-through cell apparatus using FaSSIF-V1. The oral model improvement with the incorporation of dissolution data suggests that dissolution is an important step in the LTG absorption.
Keywords: **Lamotrigine, dissolution, biopharmaceuticals**

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Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

References:
(1) Mitra A, Suarez-Sharp S, Pepin SJH et al. Applications of Physiologically Based Biopharmaceutics Modeling (PBBM) to support Drug Product Quality: A Workshop Summary Report. J Pharm Sci. 2021; 110:594-609.
(2) Parrott N, Suarez-Sharp S, Kesisoglou F et al. Best Practices in the Development and Validation of Physiologically Based Biopharmaceutics Modeling. A Workshop Summary Report. J Pharm Sci. 2021; 110:584-593.
(3) Pepin S, Dressman J, Parrott, N et al. In Vitro Biopredictive Methods: A Workshop Summary Report. J Pharm Sci. 2021; 110:567-583.
(4) GlaxoSmithKline (GSK). Lamictal® Tablets Prescribing Information, 2020.

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CANNABIDIOL REDUCES NEUROLOGICAL DEFICITS AND GLIAL CELLS ACTIVATION AFTER MIDDLE CEREBRAL ARTERY OCCLUSION IN MICE

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Introduction: Neuroprotective agents that limit secondary tissue loss and/or improve functional outcomes after stroke are still limited (1). Cannabidiol, the major non-psychoactive component of *Cannabis sativa*, has been proposed as a neuroprotective agent against experimental focal cerebral ischemia (2). The effects of cannabidiol have generally been related to the modulation of neuroinflammation, including the control of glial activation and the toxicity exerted by pro-inflammatory mediators (3). However, so far, most information concerning cannabidiol neuroprotective effects was obtained from immunohistochemical and biochemical post-mortem assays.**Aim:**To test if cannabidiol promotes neuroprotection and whether its effects on glial cells can be also detected *in vivo*. **Methods:**C57BL/6N mice underwent either sham or transient middle cerebral artery occlusion surgery. The animals received intraperitoneal injection of vehicle or cannabidiol (10 mg/kg) 30 min, 24 h, and 48 h after surgery. One day later the neurological score test was performed. Brain tissue was processed to evaluate neuronal loss and microglial activation. Transgenic mice with microglia expression of enhanced green fluorescent protein and astrocyte-specific expression of the calcium sensor GCaMP3 were used to access *in vivo* microglial activity and astrocytic calcium signaling, respectively. For this purpose,we performed time-lapse imaging of microglial activity and astrocytic calcium signaling in the subacute phase of stroke using two-photon laser-scanning microscopy.The animals were submitted to the same experimental design described above and to imaging sessions before, 30 min, 24 h and, 48 h after surgery. Astrocytic calcium signaling was also assessed in acutely isolated slices 5 h after transient middle cerebral artery occlusion surgery in the presence of perfusion or cannabidiol solution.**Results:**Cannabidiol prevented ischemia-induced neurological impairments as well as protected against neuronal loss in ischemic mice. Cannabidiol also reduced ischemia-induced microglial activation, as demonstrated in fixed tissue as well in *in vivo* conditions. No difference in the amplitude and duration of astrocytic calcium signals was detected before and after the middle cerebral artery occlusion *in vivo*. Similarly, no significant difference was found in the astrocytic calcium signals between contra and ipsilateral side of acutely isolated brain slices. **Conclusion:**The present results suggest that the neuroprotective effects of cannabidiol after stroke may occur in the subacute phase of ischemia and reinforces the strong anti-inflammatory property of this compound.

Keywords: **cannabidiol, stroke, neuroprotection**

Acknowledgments: This work was supported by CAPES and CNPq.

References:

(1) Karsy M, Brock A, Guan J et al. Neuroprotective strategiesandtheunderlying molecular basis of cerebrovascular stroke. Neurosurg focus.2017;42:E3.
(2)Hayakawa K, Irie K, Sano K et al. Therapeutic time window of cannabidiol treatment on delayed ischemic damage via high-mobilitygroupbox1-inhibiting mechanism. Biol Pharm Bull. 2009;32:1538-1544.
(3)Ceprián M, Jiménez-Sánchez L, Vargas C et al. Cannabidiol reduces brain damage and improves functional recovery in a neonatal rat model of arterial ischemic stroke. Neuropharmacology.2017;116:151-159.

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DEVELOPMENT OF GASTRORETENTIVE SYSTEMS FOR SILDENAFIL CITRATE RELEASE

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Keywords: sildenafilcitrate, floating gastroretentive system, experimental design, dissolution.

Introduction: Gastroretentive systems are modified release pharmaceutical forms designed to prolong their residence time in the upper gastrointestinal tract. These systems emerged to fill gaps presented by conventional modified release systems. Conventional controlled release systems are not designed to deliver drugs that are preferentially absorbed in the upper part of the digestive system. This inability of conventional controlled-release forms to remain retained in the stomach results in inefficient absorption of the active substance, with a consequent reduction in bioavailability. Therefore, gastroretentive systems allow an improvement in the pharmacokinetics, bioavailability and efficacy of these drugs[1,2,3]. **Aim:** This work aimed to develop gastroretentive delivery systems using sildenafil citrate as a model.**Methods:** Formulations described by a 2^2 factorial design with a central point were proposed, with the aid of the Design Expert software, which were produced and characterized by means of physicochemical tests, including some specific tests for this release system, such as the buoyancy index; determination of potency, purity and evaluation of release kinetics through dissolution tests. **Results:** The formulations with different amounts of HPMC generated different dissolution profiles, actually proving to be the modulator of drug release kinetic.Based on the desired quality attributes and the results obtained, it was found that the formulation containing 90 mg of HPMC K100CR: HPMC K4M (9:2) and 40 mg of sodium bicarbonate showed more promising values. **Conclusion:** It is concluded that gastroretentive systems are a promising alternative to control the dose release of drugs such as sildenafil, which have a narrow absorption window due to their solubility characteristics and may be an interesting way to reduce doses and increase bioavailability of the drug. A pharmacokinetic study is necessary to confirm the proposed theory.

Acknowledgments: Prati-Donaduzzi Pharmaceutical Industry.

References:

(1)Kotreka UK, Adeyeye MC et al. Gastroretentive floating drug-delivery systems: a critical review. Crit Rev Ther Drug Carrier Syst. 2011;28(1):47–99.
(2)Khristi AP, Soni T, Suhugia BN et al. Development, characterization and evaluation of sildenafil aspirin co-crystals. Indo Am J Pharm Res. 2015;5(7):2700-2708.
(3)Wang Y, Chow MSS, Zuo Z et al. Mechanistic analysis of pH-dependent solubility and trans-membrane permeability of amphoteric compounds: Application to sildenafil. Int J Pharmaceut. 2008;352(1):217-224.

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B-MYRCENE PREVENTS ACUTE LIVER INJURY

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Keywords:**β-myrcene; Oxidative Stress; Antioxidant; hepatoprotective.**

Introduction: Liver diseases are responsible for millions of deaths each year, and one of the leading causes of liver damage is drug-induced hepatotoxicity(Asrani et al., 2019). A clinically effective drug that has been linked to liver damage is acetaminophen(Subramanya et al., 2018). Occasionally, liver transplantation is the only treatment option for patient survival(Tezcan et al., 2018). Therefore, there is a clinical need for effective treatments to reduce or reverse acute hepatotoxicity. Within this context, we chose to study β-myrcene (MYR), a very promising monoterpene found in various plant extracts such as lemon balm, rosemary, lemongrass, among others. It has several pharmacological properties, such as antioxidant and anti-inflammatory, in addition to analgesic, sedative, antimicrobial and antinociceptive effects(Hoseini et al., 2019; Rufino et al., 2015). **Aim:** To evaluate the hepatoprotective effects of β-myrcene on paracetamol-induced hepatotoxicity. **Methods:** A total of 40 Balb/c mice were randomly divided into five groups as follows: 1) Normal control group; 2) APAP Group; 3) Silymarin Group; 4 and 5) pretreatment groups, mice were treated with 100 or 200 mg/kg/day of MYR. Animals were treated orally for seven days. On the seventh day, the animals received an overdose of Paracetamol (250mg/kg). Liver samples were collected for analysis of oxidative stress markers. **Results:** Our results showed that MYR pretreatment attenuated liver damage. Furthermore, MYR pretreatment demonstrated significant antioxidant activity by decreasing malondialdehyde (MDA), reactive oxygen species (ROS) and reduced glutathione (GSH) levels. Furthermore, it restored the hepatic level of superoxide dismutase (SOD), catalase (CAT) and oxidized glutathione (GSSG). **Conclusion:** Our results showed that MYR treatment significantly improved liver function, reducing paracetamol-induced oxidative stress.

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References:

(1) Asrani, S. K., Devarbhavi, H., Eaton, J., & Kamath, P. S. Burden of liver diseases in the world. *Journal of Hepatology*, 2019; *70:*151–171.
(2) Hoseini, S. M., Taheri Mirghaed, A., &Yousefi, M. Application of herbal anaesthetics in aquaculture. *Reviews in Aquaculture*, 2019; *11*:550–564.
(3) Rufino, A. T., Ribeiro, M., Sousa, C., Judas, F., Salgueiro, L., Cavaleiro, C., & Mendes, A. F. Evaluation of the anti-inflammatory, anti-catabolic and pro-anabolic effects of E-caryophyllene, myrcene and limonene in a cell model of osteoarthritis. *European Journal of Pharmacology*, 2015; *750*:141–150.
(4) Subramanya, S., Venkataraman, B., Meeran, M., Goyal, S., Patil, C., & Ojha, S. Therapeutic Potential of Plants and Plant Derived Phytochemicals against Acetaminophen-Induced Liver Injury. *International Journal of Molecular Sciences*, 2018; *19:* 3776.
(5) Tezcan, A. H., Ozturk, O., Ustebay, S., Adali, Y., &Yagmurdur, H.The beneficial effects of ozone therapy in acetaminophen-induced hepatotoxicity in mice. *Pharmacological Reports*, 2018;*70*: 340–345.

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DECREASED DOCOSAHEXAENOIC ACID LEVELS IN SERUM OF HIV CARRIER PATIENTS

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Keywords: **AIDS; Fatty acids; inflammation; omega 3 fatty acids.**

Introduction: The mortality caused by HIV has decreased since the antiretroviral therapy introduction (1). There is no cure for AIDS yet. Increased inflammation and a higher risk of cardiovascular (CV) complications in HIV carrier patients have been reported (2,3). This condition is comparable with that of obese non-HIV carrier patients wherein chronic inflammatory and CV disease is associated with increased blood levels of proinflammatory fatty acids (FA) and decreased anti-inflammatory FA (4). The docosahexaenoic acid (DHA) belongs to the omega 3 (n-3) polyunsaturated FA (PUFA) and its nutraceutical properties have been described over the years **Aim:** Considering that there are only a few studies measuring the serum FA composition, that is, saturated FA (SFA); monounsaturated FA (MUFA), and PUFA in HIV carrier patients.Herein, we compared the serum FA composition in HIV carrier patients with non-HIV carrier patients. **Methods:** The Ethics Committee (COPEP 1.166.674) and the Brazilian Clinical Trials Register (RBR843tnq) approved clinical protocol. Inclusion criteria: HIV infection diagnosis, CD4+ T cells count <500 cells/mm3, and unchanged antiretroviral therapy for at least 1 year. Exclusion criteria: pregnant women and patients with nephropathies and/or hepatopathies. Serum from all HIV carrier patients (n = 11) from our previous study (3) stored and frozen at -80C in our laboratory was used. Two samples were excluded since the patients were hyperlipidemic. For comparison, serum samples from 9 normolipidemic non-HIV carrier patients from the serum bank of Immunogenetics laboratory of our university were selected based on: age and a similar proportion of men/women in comparison with HIV carrier patients. To measure the serum FA composition we used the method described by Figueiredo et al. (9) to directly transesterify the FA into FA methyl esters (FAME). The FAME was separated, identified, and measured by gas chromatography. The Student's t-test compared the results expressed as the mean ± standard error of three analyses of FA**Results:**We measured eleven FA. The SFA: myristic acid, palmitic acid, stearic acid, and docosanoic acid. The MUFA: 7-hexadecenoic acid, palmitoleic acid, oleic acid, and vaccenic acid; and the PUFA: linoleic acid, dihomo-linolenic acid, and DHA. Excluding the DHA concentration, the serum FA compositions were not different between groups. We observed decreased ($P < 0.05$) DHA levels (by 40%) in HIV carrier patients.**Conclusion:**DHA has a pivotal role as a precursor of anti-inflammatory molecules with beneficial effects on metabolism, CV system, and immunological system. Even though most clinical studies reported beneficial effects of DHA supplementation in HIV carrier patients, this issue remains under debate. Further investigations are then required to fully clarify the role of DHA in preventing or alleviating the comorbidities associated with HIV infection.

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References:

(1) Broder S. The development of antiretroviral therapy and its impact on the HIV-1/AIDS pandemic. Antiretroviral Res. 2010;85:1-18.
(4) Pierre S, Seo G, Rivera VR *et al*. Prevalence of hypertension and cardiovascular risk factors among long-term AIDS survivors: A report from the field. J Clin Hypertens. 2019;21:1558-1566
(3) Krupek T, Ferrari BJW, Silva MARCP et al. Effervescent glutamine formulation improves the beneficial effects of antiretroviral therapies on immune function in HIV/AIDS carrier patients. J Med Food. 2020;23:485-490.

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EFFECT OF ROFLUMILAST ON THE INTEGRITY OF THE BLOOD BRAIN BARRIER AND INJURY OF THE WHITE MATTER RESULTING FROM GLOBAL AND TRANSIENT CEREBRAL ISCHEMIA

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Keywords: **Phosphodiesterase-4 inhibitor, Roflumilast, global andtransient cerebral ischemia, blood-brainbarrier.**

Introduction: Roflumilast is a Phosphodiesterase 4 inhibitors (PDE4-I) clinically approved as an anti-inflammatory for the treatment of chronic obstructive pulmonary disease. Roflumilasthas beneficial effects on ischemic brain injurydue to its ability to improve cognitionand target different phases and mechanisms of cerebral ischemia, includingapoptosis, neurogenesis, angiogenesis and inflammation (1,2,3). However, no studies are indicating the effec to froflumilast concerning the damage in blood-brainbarrier (BBB) and white matter induced by transient global cerebral ischemia (TGCI). **Aim:** The presentstudyinvestigatedwhethertreatmentwithroflumilastaffectstheBBB and White matter integrityadter TGCI. **Methods:** Wistar rats were subjected to the 4-vessel occlusion model of TGCI (EthicsCommittee approval5561250919). In the experiment I, one hour after reperfusion, the vehicle was administrated (i.p.) and treatment continued daily for 24 h, 72 h, or 7 days.These animals were injected with Evans blue dye (EB) (i.v.) to assess the permeability of the BBB. In experiment II, one hour after reperfusion vehicle or roflumilast (0.003 mg/kg or 0.01 mg/kg) was administered (i.p) and treatment continued daily for 3 days. The animals were sacrificed and the brains were removed and processed to assess the permeability of the BBBand white matter integrity. Protein levels of Bcl-2 (anti-apoptosis marker), eNOS (marker for vascular reactivity and NG2 (a marker for immature oligodentrocytes) were assessed in the hippocampal CA1 subfield by Western blot. To verify the integrity of the white matter in the corpus callosum and optic tract, the Kluver Barrera staining technique was performed. **Results:** The TGCI caused BBB disruption 72 h after reperfusion. This injury was not accompanied by damage to the oligodendrocytes present in subfield CA1 of the hippocampu sorby changes in the structural composition of the White matter. Roflumilast (0.003 mg/kg) attenuated BBB permeability caused by TGCI and increased the expression of eNOSand Bcl-2 in the CA1 subfield of the hippocampus. **Conclusion:** Roflumilast treatment decreased BBB permeability caused by ICGT and increased eNOS and Bcl-2 expression in the hippocampal CA1 subfield, which may be related to neuroprotective mechanisms of the drug in cerebral ischemic conditions.

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References:

(1) Bonato JM, Meyer E, Mendonça PSB, Milani H, Prickaerts J, de Oliveira RMMW. Roflumilastprotectsagainstspatialmemoryimpairmentsandexertsanti-inflammatoryeffectsaftertransient global cerebral ischemia. EuropeanJournalofNeuroscience. 2021;53(4):1171-1188.

(2) Santiago A, Soares LM, Schepers M, Milani H, Vanmierlo T, Prickaerts J, Weffort de Oliveira R.M. Roflumilast promotes memory recovery and attenuates white matter injury in aged rats subjected to chronic cerebral hypoperfusion. Neuropharmacology 2018;138:360-370.

(3) Wu Q, Qi L, Li H, Mao L, Yang M, Xie R, Yang X, Wang J, Zhang Z, Kong J, Sun B. RoflumilastReduces Cerebral Inflammation in a Rat Model of Experimental SubarachnoidHemorrhage. Inflammation. 2017 Aug;40(4):1245-1253.

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EFFECT OF TREATMENT WITH QUERCETIN-LOADED-MICROCAPSULES ON THE PHISYOLOGICAL PARAMETERS AND POPULATION OF MAST CELLS IN THE JEJUNAL MUCOSA OFARTHRITICRATS

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Keywords:**Inflammation;arthritis; mastcells; quercetin.**

Introduction: Rheumatoid arthritis (RA) is a chronic inflammatory disease that affects mainly bonesandjoints[1]. By causing severe systemic inflammation, is possible that the population of mast cells present in the intestine is altered during the development of the disease.The most common treatment for RA is non-steroidalanti-inflammatorydrugs. Nonetheless, their use isalsolimitedbythepresenceof adverse effects[2].Due to this, natural substances have been researched as an alternative treatment for the disease, such as quercetin, a flavonoid with potential anti-inflammatory and antioxidant activities[2]. **Aim:**Evaluatetheweight, volume ofthelefthindpaw edema anddensityofmastcellsin the jejunum mucosa of arthritic rats treated with quercetin-loadedmicrocapsules.**Methods:**Thirty male Holtzman rats were used (CEUA-UEM protocol 4462180216) and divided in 5 groups: C (control), control treated with quercetin (CQ), arthritic (AIA), arthritic treated with ibuprofen 17.5mg/kg (AI) and arthritic treated with quercetin-loaded microcapsules 10mg/kg (AQ). Arthritic animals were induced by intradermal administration of Freund's complete adjuvant. The animals were weighed at onset and the end of the experiment, and the paw volume evaluated on days 0,1,2, and 6. The experimental period lasted 60 days with daily treatments by gavage. After this period, the animals were euthanized, the jejunum was collected and preparedto perform histological technique with subsequent staining in Giemsa.Quantificationofmastcellswasperformedat 30 villi per animal using Image Pro Plus 4® program[3].**Results:**The body weightatonsetoftheexperimentdisplayed no differencebetweengroups. Inthe final weight, onlythe AIA groupshowedareductionof 23% ($p<0.05$) comparedtoC. The othergroupswerenotsignificant.Consideringthe volume ofpaw edema, AIA groupdisplayed a151%increase (day 1), 128% (day 3) and 153% (day 6). In thetreatedgroups, therewere no significantdiferences.Inthemastcellsanalysis, asignificantraise in celldensitywasobserved in AIA (115%, vs. C).The treatedgroupspresented a significantreduction in themastcelldensity, of 96% in AI and 92% in AQ (vs. AIA). **Conclusion:** RAcausedweightloss, paw edema andincreased intestinal mastcellpopulation. Quercetintreatmentwaseffective in reducingthedensityofmastcells, action similar toibuprofen. However, neithertreatmentwasabletopreventweightlossandpaw edema.

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References:

(1) Smolen JS, Aletaha D, Barton A, Burmester GR, Emery P, Firestein G.S., Kavanaugh, A, McInnes IB, Solomon DH, Strand V, Yamamoto K, 2018. RhematoidArthritis. 2018. Nature Reviews Disease Primers. 18001.
(2) Khan MA, Khan MJ. Nano-golddisplayedanti-inflammatoryproperty via NF-kBpathwaysbysuppressing COX-2 activity. ArtifCellsNanomedBiotechnol. 2018;46:1149-1158.
(3) PiovezanaBossolani GD, Silva BT, Colombo Martins Perles JV, Lima, M.M., Vieira Frez, F.C., Garcia de Souza, S.R., Sehaber-Sierakowski, C.C., Bersani-Amado, C. A., Zanoni, J.N., 2019. Rheumatoidarthritisinducesentericneurodegenerationand jejunal inflammation, andquercetinpromotesneuroprotectiveandanti-inflammatoryactions. Life Sciences. 238,116956.

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COMPARATIVE ANTIINFLAMMATORY AND ANTIOXIDANT EFFECTS OF THE COUMARINS 1,2-BENZOPYRONONE, UMBELLIFERONE AND ESCULETIN

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Keywords: Pleurisy; acute inflammation; radical scavenging.

Introduction: Coumarins are an important class of plant secondary metabolites and have some beneficial effects both as anti-inflammatories and antioxidants (2,3,4), although comparative studies are lacking.**Aim:** To compare the anti-inflammatory and the antioxidant effects of the three coumarins: 1,2-benzopyrone, umbelliferone and esculetin. Additionally, this study aimed to evaluate the potential effects of the combination coumarin + dexamethasone on a rat model of pleurisy. **Methods:** Pleurisy was induced by the injection of carrageenan in the pleural cavity of Wistar rats. Rats were treated with the coumarins or coumarin-dexamethasone combination 1 hour before carrageenan injection. Four hours after pleurisy induction, the animals were anesthetized for the pleural exudate collection.The experimental protocol was previously approved by the Ethical Committee on Animal Experimentation of UEM (#3183190121).The antioxidant capacity of coumarins was tested against mitochondrial ROS generation, DPPH radical scavenging, brain lipid peroxidation, and ABTS radical formation.**Results:**From all three coumarins studied, 1,2-benzopyrone was the most efficient to counteract pleural inflammation. There was a dose dependent effect on both the reduction of pleural exudate and leukocyte recruitment. The volume of pleural exudate was inhibited by 73%, 66% and 27% at the doses of 200,100 and 50 mg/Kg, respectively. The same doses of 1,2-benzopyronone caused a reduction on leukocyte recruitmentby 62%, 50% and 25%, respectively, and bothmononuclear (MN) and polymorphonuclear (PMN) leukocytes was decreased. Umbelliferone and esculetin treatments failed to reduce the volume of pleural exudate, even in higher doses (200 and 400 mg/Kg). However, treatment with umbelliferone caused a slight reduction on MN (39%) and PMN (23%) leukocytes at the dose of 400 mg/Kg. The combination 1,2-benzopyrone (50 mg/Kg) + dexamethasone (0.01 mg/Kg) potentiatedthe anti-inflammatory effectsof these two substances alone.This combination decreased the volume of pleural exudate by 74% and the number of total leukocytes by 58%, an effect almost 3 times higher than when these substances were used in monotherapy. As for the antioxidant capacity, esculetin was the most efficient as proved by the assays of DPPH, ABTS and mitochondrial ROS generation. In the latter, it almost completely suppressed the mitochondrial generation of ROS in a wide range of concentrations(1 µM to 500 µM). Lipid peroxidation, on the other hand, was greatly reduced in the presence of all three coumarins used. **Conclusion:** The coumarin1,2-benzopyrone presented the highest anti-inflammatory activity in the rat pleurisy model. However, regarding the antioxidant activity, esculetin was the most efficient. Additionally, the use of 1,2-benzopyrone in combination with dexamethasonepotentiated the effects of monotherapy with these substances.

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References

(1) Piller NB. A comparison of the effectiveness of some anti inflammatory drugs on thermal oedema. Br J Exp Pathol. 1975;56:554–560.
(2) Subramaniam SR, Ellis, EM. Umbelliferone and esculetin protect against Nnitrosodiethylamine-induced hepatotoxicity in rats. Cell Biol Int.2016;40:761–769.
(3) He Y, Li C, Ma Q et al. Esculetin inhibits oxidative stress and apoptosis in H9c2 cardiomyocytes following hypoxia/reoxygenation injury. BiochemBiophys Res Commun. 2018;501:139–144.

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THE EFFECTS OF A TRICHILIA CATIGUA MICROEMULSIONAFTER CEREBRAL ISCHEMIA IN RATS.

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Keywords: Cerebral ischemia, Memory, Neuroplasticity, Neuroinflammation.

Introduction: Previousstudiesdemonstratedthe neuroprotective effects of an ethyl-acetate fraction(EAF) of Trichilia*catigua*aftertransient,global cerebral ischemia (TGCI) in rats. **Aim:**To investigate whether a microemulsion of the EAF(EAF-ME) maintains the neuroprotective effects observed previously with the EAF formulation of Trichilia*catigua*. **Methods:** Naive rats were trained to learnthe aversive radial maze (AvRM) task, and then subjected to TGCI (4-VO model). The EAF-ME(100mg/kg) or vehicle was administeredorally4h after TGCI, and continued once per day for 7 days consecutive. On days 8, 15 and 22 after ischemia, the rats were tested for their ability to remember the task that was learned during training (i.e., retrograde memory). At the end of the behavioral test, the brains were examined for dendritic changes in the surviving neurons in the hippocampus (Hip) and prefrontal cortex (PFC).In another groups, the effects of the EAF-ME on ischemia-induced neuroinflammationwas evaluated at 5 days post-ischemia. This protocol had the approval of the internal EthicalCommittee (CEUA nº 2102271119). **Results:**Compared to sham-operation, TGCI caused persistent loss of memory. Thisamnesic effect of ischemia was alleviated by the EAF-MEtreatment, but not at the 5% level of statistical significance.The EAF-ME treatment also alleviated significantly the loss of dendritic spines caused by ischemia in the Hip, but not in PFC. Dendritic ramification was reduced by ischemia in PFC, an effect also alleviated by the EAF-ME. The TGCI-induced inflammatory response was not modified by the EAF-ME.**Conclusion:**Compared with previous studies using the EAF formulationof T. *catigua*, the neuroprotective effects of the EAF-ME after TGCI were modest, or absent. Whether these results were due to the microemulsion formulation by itself, or the small dose used (100 mg/kg EAF-ME *vs.* 400 mg/kg EAF) needs additional studies.

Acknowledgments: Financial support CAPES and CNPq.

References:

(1)Godinho J, Oliveira RMW, Sa-Nakanishi AB et al. Ethyl-acetate fraction of Trichiliacatigua restores long-term retrogrademory and reduces oxidative stress andinflammation after global cerebralischemia in rats. Behav Brain Res. 2018;337:173-182.

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MICROENCAPSULATED QUERCETIN AND *Bifidobacterium animalis* PROTECT HUC/D-IR NEURONS IN THE SUBMUCOUS PLEXUS OF WISTAR RATS CHEMICALLY INDUCED TO COLORECTAL CARCINOGENESIS

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Keywords:**Colorectal cancer; enteric nervous system; and probiotic.**

Introduction: Colorectalcanceris characterized by sequential evolution starting from aberrant crypt foci, originating adenoma, and then forming carcinoma. Aberrant crypts are preneoplastic lesions that consist of crypts with larger, thicker epithelium compared to normal crypts, often clustered in foci.[1]. Quercetin is a flavonoid present in several foods that has a widely studied antioxidant function. In addition to its antioxidant function, this compound has an anti-inflammatory and antitumor effect[2]. *Bifidobacterium* strains have protective and preventive effects on the composition of the microbiota and may influence the regulation of CCR epigenetics[3].**Aim:**The objective was to quantify and measure the HuC/D immunoreactive neurons (HuC/D-IR) in the submucosal plexus of the jejunum of rats induced to colorectal carcinogenesis (CCR) treated with microencapsulated quercetin, probiotic *Bifidobacterium animalis* subtype latis and the association of both. **Methods:**After approval by CEUA (n°1126010419) the animals were randomly distributed into 5 experimental groups (N=8): control(C), CCR(CR), CCR administered with probiotic *Bifidobacterium animalis*($5x10^{-7}$UFC) (CP), CCR administered with microencapsulated quercetin (10mg/Kg)(CQ) and CCR administered with microencapsulated quercetin and probiotic *Bifidobacterium animalis* (CQP). The CCR was induced by DHM (40mg/Kg) intraperitoneal injection twice a week for 2 weeks. Then, after 14 weeks, the animals were sacrificed, and the jejunum was collected. After processing the material and performing immunohistochemical techniques for HuC/D, quantification of HuC/D-IR neurons was performed in 32 images per animal, the morphometric was performed in 100 cells HUC/D-IR per animal[4], followed by statistical analysis. **Results:**Our data showed a significant reduction of 18.32% in the mean number of HuC/D-IR neurons in the CR group compared to the C group. Among the different administrations used, only CQ showed a significant difference compared to the CR group, with an increase in 20% mean HuC/D-IR neurons. The CP and CQP groups did not present significant differences when compared to the CR group. By analyzing the morphometry of HUC/D-IR cells, we found a reduction of 23.18% in neurons in the CR group compared to the C group. When comparing the CQ, CP and CQP groups to the CR group, we showed an increase of 26.07, 34.17 and 17,85%, respectively.**Conclusion:**Colorectal carcinogenesis reduced the size and number of HUC/D-IR cells in the submucosal plexus of the rat jejunum. Treatment with microencapsulated quercetin and *Bifidobacterium animalis* subtype latis was able to preserve the size of enteric neurons. Furthermore, treatment with microencapsulated quercetin was able to preserve the number of HUC/D-IR neurons of the submucosal plexus in the jejunum of rats chemically induced to colorectal carcinogenesis.

Acknowledgments: Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (Capes).

References:

(1) Lucas, C.; Barnich, N.; Nguyen, H. T. T. Microbiota, Inflammation and Colorectal Cancer. *Int. J. Mol. Sci.***2017**, *18* (6).
(2) Darband, S. G.; Kaviani, M.; Yousefi, B.; Sadighparvar, S.; Pakdel, F. G.; Attari, J. A.; Mohebbi, I.; Naderi, S.; Majidinia, M. Quercetin: A Functional Dietary Flavonoid with Potential Chemo-Preventive Properties in Colorectal Cancer. *J. Cell. Physiol.***2018**, *233* (9), 6544–6560.
(3) Bozkurt, H. S.; Quigley, E. M.; Kara, B. *Bifidobacterium Animalis* Subspecies *Lactis* Engineered to Produce Mycosporin-like Amino Acids in Colorectal Cancer Prevention. *SAGE Open Med.***2019**, *7*, 205031211982578.

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TIME COURSE OF THE BLOOD-BRAIN BARRIER PERMEABILITY IN ISCHEMIC MICE

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Keywords: Cerebral ischemia,blood-brain barrier, mice

Introduction: Cerebral ischemia (CI) is one of the major causes of morbidity and mortality in the world. Several mechanisms are involved in the pathophysiology of IC including neuroinflammation and blood-brain barrier (BBB) desintegrity. The interruption of blood flow causes the breakdown of the BBB integrity, leading to an increase in permeability and consequent cerebral edema, neuroinflammation, and neuronal damage. The BBB is the first structure to be injured after CI. BBB disruption can occur within minutes to hours after CI, characterized by proteolytic degradation of tight junctions and basement membranes, endothelial bonded junctions, and increased permeability of blood-borne cell chemicals across the BBB leading to progression of additional brain damage. The underlying events of CI can affect all components of the BBB, including the loss of endothelial cells, astrocytes, pericytes, and the extracellular matrix. Thus, to study drugs that can prevent or reduce damage to the BBB, it is first necessary to carry out a time course and thus verify the period in which the damage to the BBB occurs. **Aim**:To evaluate the time course of the blood-brain barrier permeability in ischemic mice**Methods**:Male C57BL/6J mice, 2 and 3 months were used. Transient global cerebral ischemia was induced by bilateral occlusion of the common carotid arteries (BCCAO) for 20 minutes. To determine the period of BBB break, the animals were randomly divided into 5 groups: sham, ischemic 24, 48, 72, or 168 hours. Eight hours before sacrifice the animals were injected i.p. with a 2% solution of Evans Blue dye (EA, 0.2 mg/kg, 1 ml/kg) to observe the BBB integrity. The analysis to determine the extravasationof the BBB was performed bythe Elisa technique. The results were analyzed by ANOVA followed by the Tukey post hoc test.**Results**:In all ischemic groups analyzed, there was Evans Blue extravasation, indicating an increase in the permeability of the BBB of ischemic mice ($F_{4,58}$=28,9, p<0.0001). Among the ischemic animals, the greatest increase in EB extravasation was detected in BCCAO mice after 168h ($p < 0.001$). **Conclusion:** The results show that 168 hours isthe best timefor evaluating neuroprotective drugs aimed to reduce damage to the BBBafter BCCAO.

Acknowledgments: Financial support CAPES and CNPq.

References:

(1) Abdullahi, W., Tripathi, D., &Ronaldson, P. T. (2018). Blood-brainbarrierdysfunction in ischemicstroke: targetingtightjunctionsandtransporters for vascular protection. *American JournalofPhysiology-CellPhysiology*, *315*(3), C343-C356.
(2) Fan, F., Yang, J., Xu, Y., &Guan, S. (2018). MiR-539 targets MMP-9 toregulatethepermeabilityofblood–brainbarrier in ischemia/reperfusioninjuryofbrain. *Neurochemicalresearch*, *43*(12), 2260-2267.

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MICROENCAPSULATED QUERCETIN'S EFFECTS ON COLONIC MUCOSA IN AN EXPERIMENTAL MODEL OF RHEUMATOID ARTHRITIS

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Keywords:rheumatoid arthritis, quercetin, hematoxylin eosin, goblet cells.

Introduction: Rheumatoid arthritis (RA) is a systemic inflammatory disease, affecting the joints and also organs like the intestine. The conventional RA treatment consists in non-steroidal anti-inflammatory drugs, but they are known to cause harmful side effects. Quercetin is a natural compost with antioxidant properties and potential to treat RA. **Aim:** evaluate the effects of the quercetin-loaded microcapsules on the colonic mucosa of arthritic rats. **Methods:** Thirty-three 50 days old male Holtzman rats were randomly distributed in five groups: control (C), quercetin-treated control (CQ), arthritics (AIA), ibuprofen-treated arthritics (AI), and quercetin-treated arthritics (AQ). The RA was induced by an intradermic injection of Freund's complete adjuvant containing 0,1 mL of a 5% *Mycobacterium tuberculosis* suspension, in the plantar region of the left hind paw. The CQ and AQ received the quercetin-loaded microcapsules by gavage at the dose of 10 mg/kg, whilst the AI group received ibuprofen at the dose of 17,5 mg/kg also by gavage, during sixty days. After the euthanasia, the colon was collected, processed and colored with Schiff's periodic acid and Hematoxylin and Eosin for mucosa and intestinal wall morphometric analyses. **Results:**In the morphometric analysis of HE, the AIA group significantly reduced the total wall -12.6%, the tunica mucosa -8.3% and the crypt depth -13.3% compared to C. AQ showed a significant increase compared to AIA, while AI significantly reduced -15.6 % in total wall, tunica mucosa and crypt depth compared to AIA. CQ behaved similarly to AQ, showing a significant 11.4% increase compared to C, however, in the crypt depth the CQ was not significant compared to group C. In the quantification analysis of PAS+ cells, a significant reduction was detected - 29.5% in the number of PAS+ cells in the AIA group compared to C. AQ and AI showed a significant 17% increase compared to AIA, but in AQ this increase was greater than in the AI group. The CQ group showed a significant -30.1% reduction in PAS+ cells compared to C.**Conclusion:** Quercetin loaded-microcapsules prevented the reductions of the total intestinal wall, mucosa height, crypt depth, and PAS+-goblet cells of arthritic rats. Furthermore, quercetin treatment was more effective that the ibuprofen treatment.

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References:

(1) Torigoe DY,Laurindo IMM. Artrite reumatóide e doenças cardiovasculares. Revista Brasileira de Reumatologia. 2006;46:60–66.
(2) Hollman PC,Katan MB. Absorption, metabolism and health effects of dietary flavonoids in man. Biomedicine & pharmacotherapy = Biomedecine &pharmacotherapie. 1997;51:305–10.
(3) Pearson CM,Wood FD. Studies of arthritis and other lesions induced in rats by the injection of mycobacterial adjuvant: VII. Pathologic details of the arthritis and spondylitis. The American journal of pathology.1963;42:73.

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THE MYRISTIC ACID: DHA RATIO VERSUS THE n-6 PUFA: n-3 PUFA RATIO AS NONALCOHOLIC FATTY LIVER DISEASE BIOMARKERS

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Keywords: Liver disease; Liver inflammation; Steatosis; Fatty acids.

Introduction: It is well established that diets containing an increased omega-6 polyunsaturated fatty acid (n-6 PUFA) to omega-3 polyunsaturated fatty acid (n-3 PUFA) ratios are linked to inflammation and chronic diseases like nonalcoholic fatty liver disease (NAFLD). However, the influence of an elevated n-6 PUFA: n-3 PUFA ratio in the tissues requires clarification, and other fatty acid ratios in the tissues could be more associated with inflammation and NAFLD. In previous experimental studies we found that myristic acid (Myr): docosahexaenoic acid (DHA) ishigherthanthe n-6: n-3 ratio in thegroupthatpresentedhigherfattyacidaccumulationandinflammation in liver. **Aim:** I this way, we sought to identify primary publications that allowed us to evaluate the Myr: DHA and n-6 PUFA: n-3 PUFA ratios as potential biomarkers of chronic liver disease in animal models and humans. **Methods:** We searched for primary experimental and clinical studies in the PubMed and EMBASE databases with the following terms: ("fatty acid composition and steatosis", "fatty acid profile and inflammation", "lipid composition and fatty liver", "lipid composition and inflammation"). We also evaluated the references of the publications to the identified publications looking for additional articles that could be included in this study. Articles were included if quantitative values of n-6 PUFA, n-3 PUFA, Myr, DHA in the liver, serum, or plasma, and information about liver inflammation or liver disease progression parameters were provided. We recorded the authors' names, year of publication, liver and/or serum evaluation, lipid fraction (total lipid or triacylglycerol), percent of changes of n-6 PUFA: n-3 PUFA ratio and Myr: DHA ratio, markers of inflammation and/or liver diseases, and comparison of n-6 PUFA: n-3 PUFA *vs.*Myr: DHA ratios. Also, we recorded animal age and/or weight, duration of the experiments for the articles that utilized animal models and, patients (number, mean age and gender) for clinical studies. **Results:** Overall, most experimental (91.6%) and clinical studies (87.5%) reported higher Myr: DHA ratios associated with inflammation and/or NAFLD progression than the n-6 PUFA: n-3 PUFA ratio. **Conclusion:** We conclude that the Myr: DHA ratio represents a better biomarker of NAFLD than the n-6 PUFA: n-3 PUFA ratio. Future studies are necessary for verifying this observation.

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References:

(1) Bruce CR, Thrush AB, Mertz VA et al. Endurance training in obese humans improves glucose tolerance and mitochondrial fatty acid oxidation and alters muscle lipid content. Am J Physiol Endocrinol Metab. 2010;291:99–107.

(1) Rinella ME. Nonalcoholic Fatty Liver Disease: A Systematic Review. ClinRev Educ. 2015;313: 2263–73.

(2) da Silva-Santi LG, Antunes MM, Caparroz-Assef SM, Carbonera F, Masi LN, Curi R, et al. Liver fatty acid composition and inflammation in mice fed with high-carbohydrate diet or high-fat diet. Nutrients. 2016; 8:682.

(3) Wang X, Cao Y, Fu Y, Guo G, Zhang X. Liver fatty acid composition in mice with or without nonalcoholic fatty liver disease. Lipids Health Dis. 2011; 10:1–7.

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MICROENCAPSULATED QUERCETIN'S EFFECTS ON ILEUM'S VILLI IN AN EXPERIMENTAL MODEL OF RHEUMATOID ARTHRITIS

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Keywords: **Ileum mucosa. Villi. Crypt.**

Introduction: Rheumatoid arthritis (RA) is a disease that affects 1% of the global population. It is characterized by oxidative stress that triggers a systemic inflammation that affects the gastrointestinal tract (GIT). Among the drugs used in the usual treatment of RA there are nonsteroidal anti-inflammatories that are known to have harmful side effects to the organism, including the GIT. Quercetin is an antioxidant that has been showing anti-inflammatory activity, making it a possible RA treatment. **Aim:** Evaluate the quercetin microencapsulated's effects on ileum's villi in arthritic rats. **Methods:** Thirty 56 days old male Holtzmann rats were used and randomly distributed in 5 groups: Control (C), Control treated with microencapsulated quercetin (CQ), arthritics (AIA) arthritics treated with microencapsulated quercetin (AQ) and arthritics treated with Ibuprofen (AI). The arthritis was induced by Freund's Complete adjuvant and the treatments were given by gavage in a dose of 10 mg/Kg for quercetin and 17,5 mg/Kg for Ibuprofen. After the experimental period, the animals were euthanized, the ileums were collected, processed and the slides were made in a microtome and colored with HE (Hematoxylin and Eosin). Villi's height and width, and crypt depth were measured and the results submitted to statistical analyses by Block's Design followed by Fisher's post-hoc with a 5% significance level. **Results:** AIA had an expressive 29,05% reduction in the villi's height jointly with a 8,44% reduction in the villi's width, and a decrease of 8,20% in crypt depth when compared to the C group. The AI group yielded 12,97% raise in the villi's height compared to AIA group, similarly to AQ animals that had a 6,84% growth in the villi's height compared to AIA. The AQ group presented a 5,10% thickening in the villi's width. The depth crypt did not demonstrate significant differences in AI and AQ groups. Finally, the CQ group presented a 7,50% raise in the villi's width and an expressive reduction of 17,84% in the villi's height when compared to the C group, there was not a significant difference in the crypt depth in the CQ group. **Conclusion:** The RA affected the GIT and the Ibuprofen restored only the villi's height whilst the quercetin repaired both the villi height and width. However, in healthy animals, the quercetin demonstrated a toxic effect.

References:

(1) Bossolani GDP, Silva BT, Perles JVCM et al. Rheumatoid arthritis induces enteric neurodegeneration and jejunal inflammation, and quercetin promotes neuroprotective and anti-inflammatory actions. Life Sciences. 2019;238.
(2) D'Andrea G. Quercetin: a flavonol with multifaceted therapeutic applications?.Fitoterapia. 2015;106:256-271.
(3) Goeldner I, Skare TL, Reason ITM et al. Artrite reumatoide: uma visão atual. Bras Patol Med Lab. 2011;47:495-503.
(4) Pearson CM, Wood FD. Studies of arthritis and other lesions induced in rats by the injection of mycobacterial adjuvant: VII. Pathologic details of the arthritis and spondylitis. The American journal of pathology, 1963;42:73.

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DEVELOPMENT OF PHYSIOLOGICALLY- BASED PHARMACOKINETICS (PBPK) MODEL FOR R-KETAMINE.

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Keywords: Analgesia; Pharmacometrics; Simulation.

Introduction: Ketamine (KET) is aracemic drug used as a dissociative anesthetic used in clinical practice for decades. Low dose of this drug has been used for analgesia because of the antagonistic effect on N-methyl-D-aspartate (NMDA) receptors. In humans, analgesia studies with KET used intravenous route (IV). Pharmacometry has been used to assess dose adequacy for drugs. Thus, the development of a R-Ketamine (R-KET) IV Physiologically- based pharmacokinetics (PBPK) model will help to predict the doses required to use KET as an analgesic in different populations.**Aim:** To develop R-KET IV PBPK model. **Methods:**R-KET plasma data was acquired from literature, as well as the molecular chemical descriptors of R-KET used to establish the PBPK model. The Simcyp® Version 19 (SV) simulator was used to generate the model. The population selected in SV was same used in each studied used to make the model. The performance of simulations was assessed by the mean fold error (MFE) (MFE = Pharmacokinetic(PK) parameter predicted mean/PK parameter observed mean) for PK parameters area under the curve (AUC) and Cmax extracted from SV. The model was accepted if all predicted PK parameters has MFE between 0.5–2.0.**Results:**The parameters assumed to obtain the PBPK model IV that best fit the observed data were: molecular weight (237.72 g/mol), monoprotic base, log P (2.18), pKa (7.31), blood/plasma ratio (0.73), hematocrit (45%), fraction unbound (0.53), CYP2B6rCYP system Lymph B (Clint 0.526 µL/min/pmol), CYP2C9rCYP system Lymph B (Clint 0.057 µL/min/pmol), CYP3A4rCYP system Lymph B (Clint 0.076 µL/min/pmol), additional Cl HLM (125,105 µL/min/mg). The full PBPK method was assumed. The steady state distribution volume value was predicted by method 1 (1). The PBPK model successfully predicted the R-KET disposition, where MFE for AUC and Cmax were 0,57 - 1,38 and 0,63 - 1,37 respectively.**Conclusion:**The R-KET PBPK model developed for healthy volunteers was predictive and could be extrapolated to estimate the plasma exposure of KET to different populations in order to explore dose adequacy in analgesic protocols.

Acknowledgments: CAPES/CNPq.

References:

1. Poulin P, Ekins S, Theil FP. A hybrid approach to advancing quantitative prediction of tissue distribution of basic drugs in human. Toxicol Appl Pharmacol [Internet]. 2011;250(2):194–212. Available from: http://dx.doi.org/10.1016/j.taap.2010.10.014

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EVALUATION OF THE INTESTINAL MUCOSA OF RATS CHEMICALLY INDUCED TO COLORECTAL CARCINOGENESIS ADMINISTERED WITH MICROENCAPSULATED QUERCETIN AND *Bifidobacterium animalis*

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Keywords: Jejunum. Oxidative stress. Antioxidant. Probiotic.

Introduction: According to the National Cancer Institute, the number of people with colorectal cancer has increased each year, with 20,540 new cases diagnosed in men and 20,470 in women in the year 2020[1]. And among the systemic consequences generated by cancer, we can highlight the oxidative stress[2] that causes cellular apoptosis and can affect the gastrointestinal tract. **Aim:** The aim of our study was to evaluate the morphometry of intestinal mucosa of chemically induced rats to colorectal carcinogenesis administered with microencapsulated quercetin and *Bifidobacterium animalis*. **Methods:**Thirty-five50-day-old male Wistar rats, randomly distributed in five groups with 7 animals each: control (C); colorectal cancer (CR); CR + quercetin(CQ),CR + probiotic(PC); CR + probiotic +quercetin (CQP); CR was chemically induced by DMH administration (40 mg / kg)[3]. The treated animals received microencapsulated quercetin 10 mg / kg and *Bifidobacterium animalis* $5{\cdot}10^7$ UFC daily by gavage. At the end of the experimental period of 112 days, the animals were euthanatizedand the jejunum was collected and intended for the standard histological technique (fixation, embedded in paraffin and cuts) and posterior staining with hematoxylin and eosin. Forty measurements of villus height,crypt depth and mucosal height and total wall per animal were analyzed through Image-Pro Plus software.The results were discovered through statistical analysis and the significance level was 5%**Results:**The results showed a reduction of 6,5%, 6,1% and 4,5% in the height of the villi, mucosa and total wall, respectivelyin the animals of the CR group compared to the control ($p<0.0002$). The treated animals (CQ, CQP and CP) also showed a reduction in the height of the villi, mucosa and total wall compared to the CR group ($p<0.0001$). These parameters were not recovered in the CQ, CQP and CP groupswhen compared to the CR group.**Conclusion:**The changes in the morphometry of the intestinal mucosa demonstrate the possible damage caused by oxidative stress generated by cancer, the administration of antioxidant quercetin and probiotic were not able to reverse these damages. More studies are needed to actually observe the reasons that led to these changes, whether it is decreased cell proliferation, enhanced apoptosis or both.

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References:

(1) INCA. Instituto Nacional do Cancêr.Estatísticas de câncer. Ministério da Saúde. Rio de Janeiro, 2020. Disponível em: <https://www.inca.gov.br/numeros-de-cancer>. Acessoem: 07nov. 2021.

(2) Schumacker PT. Reactive oxygen species in cancer: a dance with the devil. Cancer cell. 2015; *27*(2): 156-157.

(3)Fukui M, FujinoT, Tsutsui K et al. The tumor-preventing effect of a mixture of several lactic acid bacteria on 1, 2-dimethylhydrazine-induced colon carcinogenesis in mice. Oncology reports. 2001; *8*(5): 1073-1078.

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Hepatoprotective effect of (-)-α-bisabolol in an experimental model of paracetamol-induced injury

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Keywords: (-)-α-BISA; Hepatoprotective; Acetaminophen; Antioxidant activities.

Introduction: The increase in morbidity and mortality due to acute liver failure worldwide has sparked research to identify the etiopathogenesis involved. Among the causes is the use of non-steroidal anti-inflammatory drugs (AINEs). Paracetamol (APAP) is the AINEs responsible for more than 40% of cases of drug-induced liver disease (DILI). Its active metabolite causes lipid peroxidation, inflammation and damage to hepatocytes. Alpha-bisabolol (BISA), a component of chamomile essential oil, has anti-inflammatory and antioxidant activities, among others.Aim:The intention of the work was to investigate the hepatoprotection of BISA in mice.**Methods**: The animals were treated with doses of 50, 100 and 200 mg / kg of BISA (via gavage), for 7 days before induction of DILI by APAP. The release of AST, ALT, FA, GGT, the production of TBARS, NO, MPO activity and histopathological examination were analyzed.**Results:**Our results focused on the reduction of markers of liver injury, decrease in NO levels, improvement in TBARS and MPO activity in all doses used of BISA, when compared to APAP.The data were confirmed by histopathological analysis, which showed less cell damage and inflammatory infiltrate in animals treated with BISA.**Conclusion:**Our results obtained that the effect of BISA as hepatoprotective is promising and can be used as a useful tool in DILI.

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References:

(1) Wong A, Graudins A. Risk prediction of hepatotoxicity in paracetamol poisoning. ClinToxicol (Phila). 2017; 55(8), pp. 879-892.
(2) Rocha NR, Rios ERV, Carvalho AMR et al. Anti-nociceptiveand anti-inflammatory activitiesof (-)-α - bisabolol inrodents. NaunynSchmiedebergsArchPharmacol. 2011; 384 (6), pp. 525-533.
(3) Ortega-Alonso A, Andrade RJ. Chronic liver injury induced by drugs and toxins. J Dig Dis. 2018; 19 (9), pp. 514-521.
(4) Zhang Y, Dai M, Yuan Z. Methods for Detection of Reactive Oxygen Species. AnalyticalMethods. 2018; 10 (38), pp. 1-17.
(5) Leite GO, Ecker A, Seeger RL, Krum BN, Lugokenski TH, Fachinetto R, Sudati JH, Barbosa NV, Wagner C. Protective effect of (−)-α-bisabolol on rotenone-induced toxicity in Drosophila melanogaster. Canadian Journal of Physiology and Pharmacology. 2018; 96 (4), pp. 359-365.

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TECHNOLOGY AND BIOTECHNOLOGY

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DEVELOPMENT OF A THERMORESPONSIVE BIOADHESIVE SYSTEM FOR THE DELIVERY OF EPRINOMECTIN IN BOVINES

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Keywords:**eprinomectin, semi-solid, bioadhesive, thermoresponsive system.**

Introduction: Eprinomectin is a semi-synthetic derivative of the avermectin group and is highly effective against endo and ectoparasites, being safe for use inlactatinganimals.This drug is available in the pharmaceutical market as parenteral and topicaladministration formulations. Butits efficacy can be compromised with the possible losses during the administration and dosing error. Moreover, traditional formulations can be not efficacious when transposing skin barrier to the drug delivery in the animal organism. However, the use of improvedpharmaceutical platforms can guarantee a safe and effective treatment. Polymers with bioadhesive properties, such as carbomers, and thermoresponsive such aspolaxamers, can been applied to develop pharmaceutical platforms to control the drug delivery, as well as to improve its availability at the place of application and promote absorption. **Aim:**Development of a thermoresponsivebioadhesive system for the deliveryof eprinomectin in bovines from topical administration.**Methods:**Aanalytical method was developed and validated for the quantitationof eprinomectin by high performance liquid chromatography. A factorial design was performed varying Carbopol 934P and 974P, poloxamer 407 and isopropanol. Five groups with 10 formulations each were formed and evaluated as toprecipitationand sol-gel transition temperature ($T_{sol-gel}$). **Results:**The analytical method was validated. With the study of the formulations, it was observed that isopropanol has a more significant influence on the system, with an amount greater than 15% of which there is no gel formation and a concentration lower than this there was drug precipitation. **Conclusion:**Abioadhesivethermoresponsiveformulationcouldbe obtained using isopropanol as a solvent of drug, being used 0.5% (w/w) of eprinomectin and 15% (v/w) of isopropanol. The amounts of Carbopol and poloxamer 407 for the best formulations were 0.2-0.3% (w/w) and 15-17.5% (w/w), respectively.

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References:

ICH- International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for human use (ICH). Validation of Analytical Procedures: Text and Methodology, 2015.
MAO, Y.; CHEN, X.; XU, B.; SHEN, Y. *et al.* Eprinomectin nanoemulgel for transdermal delivery against endoparasites and ectoparasites: preparation. Drug Delivery. 2019; 26:1104-1114.

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TECHNOLOGICAL DEVELOPMENT OF BIOADHESIVE TERMORESPONSIVE EMULGELS CONTAINING ANDIROBA OIL (*Carapaguianensis*Aublet).

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Keywords: **Emulgels; bioadhesion; Andiroba oil; poloxamer 407.**

Introduction: Emulgels are bi-compartmentalized systems with a hydrophilic phase, in which a polymer is dispersed and a hydrophobic oily portion. This type of dosage form allows better stabilization of the emulsion. The use of bioadhesiveandthermoresponsivepolymerslead to an emulgel with *in situ* gelling capacity which improves retention time on the biological surface.Andiroba oil has been reported as an excellent pharmacologically molecule, due to its extensive pharmacological activities, such as antibacterial, antifungal, antimalarial, insecticide, antiallergic, anti-inflammatory, anticancer, and neuroprotective. Its use is widespread in local medicine, mainly on riverside Amazonian people.**Aim:** The technological development of emulsion systems based on Andiroba oil was proposed in this work. A factorial design with three levels and four factors was used, and the factors analyzed were the types of polymers (Carbopol 974P, Policarbofil, and Poly(methyl vinyl ether-alt-maleic anhydride) and the proportions of bioadhesive polymer, poloxamer 407 and andiroba oil. **Methods:**The systems were evaluated by advanced mechanical, rheological, and microscopy characterization techniques aiming to find the best formulation for pharmaceutical application.**Results:**The system's preliminary stability screening selected the most stable formulations. According to the texture profile and softness analysis, the selected samples were mainly influenced by the increase of andiroba oil and poloxamer, showing viscoelastic behavior for temperatures above the sol-gel transition, which ranged from 18 °C to 24 °C. Bioadhesion assays in porcine skin pointed the formulations as being equally bioadhesive. Transmission electron cryomicroscopy (Cryo-TEM) images showed well-structured micelles, with a size <200 nm. **Conclusion:**A broad understanding of the emulgels structuring was obtained. They were shown to be physicochemically stable and with good mechanical and rheological characteristics.

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References:

(1) Ferreira, SBS, da Silva JB, Junqueira MV et al. The importance of the relationship between mechanical analyses and rheometry of mucoadhesive thermoresponsive polymeric materials for biomedical applications.Journal of the mechanical behavior of biomedical materials. 2017; 74; 142-153.

(2) Ferreira, SBS, Bruschi ML. Investigation of the physicochemical stability of emulgels composed of poloxamer 407 and different oil phases using the Quality by Design approach.Journal of Molecular Liquids. 2021; 332; 115856.

(3) Pastor, Y, Ting I, Martínez AL et al. Intranasal delivery system of bacterial antigen using thermosensitive hydrogels based on a Pluronic-Gantrez conjugate. International journal of pharmaceutics. 2020. 579; 119154.

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COMPUTER-AIDED DRUG DESIGN OF PYRAZOLE DERIVATIVESAS POTENTIAL INHIBITORS OF SARS-CoV-2 MAIN PROTEASE

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Keywords: SARS-CoV-2, Pyrazole, M^{pro}, Molecular Docking.

Introduction: Covid-19 has become a global emergency since the pandemic state was declared in march 2020.Since then, efforts are being made to discover new antiviral molecules specific for SARS-CoV-2 (1). Pyrazole-containing compounds present a wide range of pharmacological activities (2), including antiviral, making it an important building block for bioactive molecules targeting coronavirus. Main Protease (M^{pro}) is a crucial enzyme for viral replication and became an important target for SARS-CoV-2, allowing researchers worldwide to study many drug candidates for it (3). **Aim:**Design of pyrazole analogues of antiviral **X77** targeting M^{pro} using Molecular Dockingsimulations (MDS) and Molecular Dynamics (MD).**Methods:**M^{pro} crystal structure was taken from Protein Data Bank (code 6w63). Molecular design was done from analyses of **X77** structure and medicinal chemistry approaches. MDS were made with GOLD software. **Results:**720 analogues (numerated from **1aaa** to **5ehr**) were projected and ranked by their score values from GOLD. 40 molecules with score higher than that for **X77** were analyzed. Spatial orientation within M^{pro} active site was dependent on the size of the molecular fragments on pyrazole scaffold and all 40 molecules blocked the enzyme catalytic dyad (His41 and Cys145). Some structural features showed to improve affinity for M^{pro}: cycloalkanes, 2- and4-substituted benzene rings and aza-heterocycles. Compounds **1aac** and **1acc** showed to have a very similar van der Waals surfaces and steric complementarity as **X77**and were submitted to 100 ns MD studies. It has revealed that His41, Met49, Cys145 and Met165 as important residues for maintaining stability of the compounds bound to M^{pro}. **Conclusion:** MDS allowed identification of 40 analogues of **X77** as promising inhibitors of M^{pro}, being 2 of them validated by MD. Further synthetic obtainment and *in vitro* studies will assess their affinity over M^{pro}.

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References:

(1) Vuong W et al. Nat Commun. 2020; 11:4282
(2) Camargo JNA et al. Front Pharmacol. 2020; 11:591570
(3) Zanetti-Polzi L et al. J Phys Chem Lett. 2021; 12:4195-4202

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PREPARATION OFMICRONEEDLES CONTAINING GLYCOLIC ORETHANOLIC EXTRACT OF PROPOLIS: COMPRESSION STRENGTH AND POLYPHENOL CONTENT

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Keywords: **microneedles; propolis, development.**

Introduction: Microneedles (MN) are an alternative technological strategy to break the skin's protective barrier in a minimally invasive way and they have been widely used for dermal administration of different drugs in a non-invasive way (1). Propolis (PRP) is a highly adhesive gum-resin widely used in therapeutics due to its antimicrobial, immune system strengthener and anticancer properties.Recognizing the therapeutic concept of PRP and its challenges in terms of improving the bioavailability of its biologically active substances, polymeric systems have been developed in the form of MNfordeliveryof PRP extract.**Aim:**The aim of this work was to prepareMNcontainingPRPextract and evaluate their mechanical strength and PRPcontent. **Methods:**The formulations were prepared using a polymeric matrix composed of polyvinyl alcohol (PVA), polyvinylpyrrolidone (PVP) and poloxamer 407 (P407). Three different concentrations of P407 (1, 2 and 3%, w/w) and glycolic extract of PRP (EGPRP) (2, 4 and 8%, w/w) or of ethanolic extract (EEPRP) (4, 8 and 16% w/w) were used in the fixed binary mixture of PVA polymer: PVP (ratio 1:1) A 3^2 factorial design was used, totaling nine formulations for each type of extract. The effect of applying a known compressive load to the obtained polymeric MN wereevaluated using a TA-XTplustexturometer (2). The best formulations were also evaluated for the content of total polyphenols (PFC). **Results:**The MN containing the highest concentrations of EGPRP were more difficult to obtain due to the large amount of propylene glycol. The results of the analysis of the compression force showed that the highest values of applied force were in the formulations containing 4% (w/w) of EGPRP, being the highest in the system containing P407 (3%, w/w). Thus, the G6 formulation (P407 - 3% and EGPRP - 4%) was chosen as the best formulation of the glycolic extract. As for the ethanol extract, all formulations were obtained successfully, with higher compression force in MN containing 3% P407. For this extract, threeformulations were chosen: E3, E6 and E9, containing 4, 8 and 12% of ethanol extract, respectively, all containing 3% of P407.For the PFC in the best formulations, the results obtained were: 0.44; 0.89; 1.14 and 0.90 g of PFT in 100 g MN, for formulations E3, E6, E9 and G6 respectively.**Conclusion:** The polymeric MN containing extract of PRP showed to be a viable alternative for topical application of PRP and more studies of characterization should be acomplished.

Acknowledgments: CAPES, CNPQ, FINEP, Araucaria Foundation.

References:

(1)Radhika C, Gnanavel BK. Finite element analysis of polymer microneedle for transdermal drug delivery. Materials Today: Proceedings. 2021;39:1538–42.

(2)Donnelly RF, Majithiya R, Singh TRR, Morrow DIJ, Garland MJ, Demir YK, et al. Design, optimization and characterisation of polymeric microneedle arrays prepared by a novel laser-based micromoulding technique. Pharmaceutical Research. 2011;28(1):41–57.

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A GREEN STRATEGY FOR ENHANCED PHENOLIC COMPOUNDS EXTRACTION FROM *Cereus hildmannianus* BY ELICITATION WITH SALICYLIC AND JASMONIC ACIDS

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Keywords: Elicitors; Cactus; Phytochemicals

Introduction:*Cereushildmannianus*(K.) Schum. (Cactaceae) is a species of cactus with various medicinal and industrial applications(1,2). Plant biotechnology is used for producing metabolites under controlled conditions, in addition, the elicitation process is considered a highly effective technique for increasing the production of pharmaceutically active(3).**Aim:**In this regard, the present study investigated the effects of elicitors salicylic and jasmonicacidsin the production of phenolic compounds and antioxidant activity.**Methods:**Callus culture were cultivated*invitro*inMurashige and Skoog medium at 32 ºC with a photo-period of 16 h (control), after were sub-cultured in medium with elicitors salicylic acid (SA, 50–200 µmol), and jasmonic acid (JA, 50–200 µmol), for 45 days. The total phenolics and total flavonoids contents, and antioxidant activity (FRAP, DPPH, and ABTS) were evaluated after extractions by maceration.**Results:**The most concentration of total phenolics were detected in callus tissues induced with 100 µmol JA (390.8µg mg^{-1} DW), and 200 µmol SA (355.5 µg mg^{-1} DW), andthe total flavonoids in 200 µmol SA (89.8 µg mg^{-1} DW). The callus tissues induced with 100 µmol JA (115.2; 83.7 and 57.1 µmol Trolox mg^{-1} DW by FRAP, DPPH and ABTS, respectively), and 200 µmol SA (124.1; 67.1 and 56.9µmol Trolox mg^{-1}DWby FRAP, DPPH and ABTS, respectively) showed the highest antioxidant activity.**Conclusion:**The elicitors were able to increase the production of phenolic compounds in callus cultures of *C. hildmannianus*and antioxidant activity. These results are promising for the clean and sustainable biotechnological production of bioactive compounds.

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References:

(1) Santos ÉS, et al. *Cereus hildmannianus*(K.) Schum. (Cactaceae): Ethnomedical uses, phytochemistry and biological activities. J Ethnopharmacol. 2021; 264:113339.
(2) Jacomini D, et al. Lipid profile and antiproliferative activity of callus cultures of *Cereus peruvianus* Mill. Ind Crops Prod. 2015; 69:408-414.
(3) Ramirez-Estrada K, et al. Elicitation, an effective strategy for the biotechnological production of bioactive high-added value compounds in plant cell factories. Molecules. *2016;* 21:1-24.

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FATTY ACID COMPOSITION OF *Lentinus crinitus* FRUITING BODIES BY GC-FID

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Keywords: *Lentinus crinitus*; Fatty acids; Gas chromatography

Introduction: *Lentinus crinitus*(L.) Fr. produce edible fruiting bodies (Basidiomycota, Polyporaceae) with medicinal, nutritional, and biotechnological applications. The compounds from fungal fruiting bodies can be applied in the preparation of products in the food, cosmetic, biomedical, or pharmaceutical indutry[1]. **Aim:** The objective of this study was to identify and quantify the fatty acids in *L. crinitus*fruiting bodies. **Methods:** The fruiting bodies of *L. crinitus*were cultivated in autoclaved sugarcane bagasse and rice husks (1:1, v:v). The lipids were extracted by maceration with isopropanol for 24h, followed by re-extraction with a solution of chloroform:methanol (2:1, v:v), under the same conditions, resulting in the crude lipid extract (EBL)[2]. The lipids were converted to fatty acid methyl esters by transesterification and analyzed by gas chromatography–flame ionization detector (GC-FID). The identification of the different fatty acids was made by comparison of the relative retention time of fatty acid methyl esters (FAME) peaks from samples with standards. The experiments were performed in triplicate and expressed as mean ± standard deviation (SD). Using the peaks integration data, it was possible to estimate the relative amount of saturated fatty acids (SFA) and unsatured fatty acids (UFA). **Results:** The fatty acids identified in the lipid fraction had a predominance of UFA (81.16 ± 0.77%) compared to SFA (18.84 ± 0.71%). The major chemical compounds were linoleic (69.32 ± 0.84%), palmitic (12.19 ± 0.24 %), and oleic (8.09 % ± 0.06) acids. **Conclusion:** From lipid analysis of *L. crinitus*fruiting bodies, it was found important fatty acid types such as polyunsaturated fatty acids (PUFA), represented by the linoleic acid, and monounsaturated fatty acids (MUFA), with oleic acid as major compound. The predominance of linoleic acid (omega-6) is very interesting as it is considered an essential fatty acid that cannot be synthesized by the human body and is a precursor of arachidonic acid, which plays an important role in inflammatory processes, being significant economically from a nutritional and technological point of view.

Acknowledgments: CNPq; CAPES; UNICESUMAR; UFBA; UEM

References:

(1) Niebisch, CH, Malinowski, AK, Schadeck et al. Decolorization and biodegradation of reactive blue 220 textile dye by *Lentinus crinitus* extracellular extract. J Hazard Mater. 2010;180: 316-322.
(2) Machado, FAPS, Capelasso, M, Oliveira, AJB et al. Alkaloid production and isozymes expression from cell suspension culture of *Cereus peruvianus* Mill. (Cactaceae). Int J Plant Sci. 2006; 69: 324-331.

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COMPLEXATION OF ESSENTIAL OIL FROM *Cymbopogon winterianus* WITH BETA-CYCLODEXTRIN AND EVALUATION OF ANTIOXIDANT

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Keywords: ***Cymbopogon winterianus*, inclusion complex, beta-cyclodextrin, antioxidant activity.**
Introduction: *Cymbopogon winterianus*(citronella) is an aromatic plant widely found in India and Brazil.Its essential oil (EO) has repellent properties, anti-inflammatory, antioxidant, antimicrobial, antiparasitic activities (1). The use of EO is limited due to their low solubility and instability against heat, light, temperature, among others. An alternative to improve its application is microencapsulation using cyclodextrins (CDs) (2). **Aim:** Microencapsulate citronella EO with beta-CD and evaluate antioxidant stability at high temperature. **Methods: Essential oil characterization –** The EO was characterized by GC-MS and the compounds were identified by comparison using the NIST 2.0 library (3). **Complex formation** – The complexes were formed by the methodologies of physical mixture, co-precipitation and kneading (3). The physical mixture methodology was used as a standard in the analysis**. Antioxidant activity** – The antioxidant activity was determined by the methodology of Li, Du and Ma (2011) (4) and the result was expressed in Trolox equivalent. **Antioxidant stability** – The stability of EO and its complexes was based on the methodology of Tomaino et al. (2005) (5). **Results:Essential oil characterization –** The major compound of citronella EO was citronellal, with a retention time of 14.9 min. **Antioxidant activity** – The EO had an activity equivalent to 121.333 ± 16.499 µmol/mg/mL of Trolox at room temperature. **Antioxidant stability** – The EO, the physical mixture and the complexes formed by kneading and co-precipitation showed an activity equivalent to 121.333 ± 16.499 µmol/mg/mL of Trolox, 111.333 ± 28.674 µmol/mg/mL of Trolox, 90.500 ± 2.500 µmol/mg/ mL of Trolox and 95.500 ± 2.500 µmol/mg/mL of Trolox, respectively, at room temperature. After exposure to 140 °C for 3 h, the DPPH free radical scavenging activity for the EO and the physical mixture was 58.000 ± 10.000 µmol/mg/mL of Trolox and 25.500 ± 2.500 µmol/mg/mL of Trolox, respectively.For the complexes, the activity remained high, with values of 111,333 ± 16.499 µmol/mg/mL of Trolox for kneading, and 118,000 ± 5.000 µmol/mg/mL of Trolox for co-precipitation. **Conclusion:** Through the methodology of the DPPH free radical scavenging activity it was possible to verify that the citronella EO presented an antioxidant activity. The inclusion complexes between OE and beta-CD were shown to be favorable for the protection of OE, ensuring that its antioxidant activity remains stable at high temperatures.
Acknowledgments: CAPES, CNPq, FundaçãoAraucária and FINEP.

References:
(1) Leite, BLS et al. Assessment of antinociceptive, anti-inflammatory and antioxidant properties of *Cymbopogon winterianus* leaf essential oil. Pharm. Biol. 2010;48:1164–1169.
(2) Carneiro SB, Duarte FIC, Heimfarth L, Quintans JSS et al. Cyclodextrin–Drug Inclusion Complexes: In Vivo and In Vitro Approaches Int. J. Mol. Sci. 2019;20:642.
(3) Galvão JG, Silva VF, Ferreira SG et al. β-cyclodextrin inclusion complexes containing *Citrus sinensis* (L.) Osbeck essential oil: An alternative to control *Aedes aegypti larvae*. Thermochim. Acta. 2015;608:14–19.
(4) Li PM, Du GR, Ma FW. Phenolics concentration and antioxidant capacity of different fruit tissues of astringent versus non-astringent persimmons. Sci. Hortic. 2011;129:710–714.
(5) Tomaino A, Cimino F, Zimbalatti V et al. Influence of heating on antioxidant activity and the chemical composition of some spice essential oils. Food Chem. 2005;89:549–554.

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PREPARATION OF EMULSIFYING SYSTEM CONTANING COPAIBA OIL FOR ORAL ADMINISTRATION AIMING THE LEISHMANIASIS TREATMENT

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Keywords: **drug delivery system; copaiba oil; leishmaniasis**

Introduction: Leishmaniasis has been considered a neglected tropical disease, being endemic in Brazil. Its current treatment has some challenges such as drug toxicity, resistant strains and parenteral administration[1]. The developmentof a new formulation for oral administration is necessary. Copaiba oil (CO) is an oleoresin displayingsignificant anti-leishmanial activity. However, CO is a poor water-soluble compound, so when administered orally, has poor bioavailability[2]. When orally administrated, emulsifying systems (ES) have the capacity to increase the rate of drug absorption due tro the improvement of solubility[3]. **Aim:**This work has as aim the preparation and physicochemical evaluation of ES containing CO for oral administration aiming the treatment of leishmaniasis.**Methods:**Ternary phase diagrams (TPD)was prepared to select the better proportions of components. Afterwards, the preliminary physicochemical stability evaluation was acomplished. The stable formulations were physicochemically characterized by light microscopy morphology analysis, *in vitro* anti-leishmania biological activity, cytotoxicity, evaluation of the emulsification process in simulated intestinal (SI) and gastric fluid (SG)and dispersion time in water,SI and SG at 37 °C. The better formulations were evaluated as their drug dissolution profile byHPLC using a method previsouly validated. These formulations were also had their morphological and granulometric analysis performed by transmission cryomicroscopy (Cryo-TEM) and dynamic light scattering (DLS), as well as mechanical characterization by texture profile analysis (TPA).**Results:**The TPD were developed to show that components proportions were decisive. It was observed that ES were obtained when it had a greater amount of water and co-surfactant and lower proportions of CO. Was selected 36 ES and submitted to the preliminary stability test. The stable ESwereinvestigatedasemulsifing time in aqueous media andsome ES showed the ability to emulsify in any proportions of SG and SI. Also, analyzing by light microscopy, it demonstrated the presence of droplets in these systems. The ES with lower amounts of CO demonstrated better performance in the *in vitro* biological activity against *L.infantum* and *L.amazonenses* and lower cytotoxicity. The dispersion time of the formulations was less than one hour in the SG and in the water, but, in the SI, this time was more than 8 h due to the sol-gel temperature. TPA analysis enabled the characterization of hardness, compressibility, elasticity, cohesiveness and adhesiveness of selected formulations. Morphological analysis by Cryo-TEM and DLS demonstrated that ES are nanostructured. **Conclusion:** The developed emulsion systems displayed suitable physicochemical characteristics for oral administration. Moreover, the biological analysis indicated the selected formulations can be used for the development of a newmedicinefor the treatment of leishmaniasis.

Acknowledgments: CNPq/MS/SCTIE/Decit, PPSUS,CAPES, CNPq and FINEP.

References:

(1) World Health Organization. Control of the leishmaniases. WHO Technical Report Series. 2010.
(2) Herrero-JáureguiC,Casado MA, ZoghbiMGB et al. Chemical variability of *Copaiferareticulata*Ducke oleoresin. Chem. Biodivers. 2011.
(3) Amidon GL, Lennernäs H, Shah VP, Crison JR. A Theoretical Basis for a Biopharmaceutic Drug Classification: The Correlation of in Vitro Drug Product Dissolution and in Vivo Bioavailability. Pharm. Res. 1995.

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MECHANICAL PROPERTIES OF EMULSION SYSTEMS COMPOSED OF CARBOMER 934P, 974P OR POLYCARBOPHIL, NATURAL OILS AND NATURAL BIOACTIVE AGENTS

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Keywords: **Gels, physicochemical properties, mechanical properties.**

Introduction: Emulgels can be by trapping an organic phase within a three-dimensional network constructed by hydrophilic molecules. Polymers based on cross-linked poly (acrylic acid) have been used as gel matrices, improving adhesion, rheological and mechanical properties. Propolis (PRP) produced by bees *Apis mellifera* L. displays a wide range of biological activities and, together with curcumin (CUR), offers a synergistic anti-inflammatory, antioxidant, and antimicrobial activity. **Aim:** This work investigated the effect of different vegetable oils (sweet almond, andiroba, and passion fruit) on the physicochemical properties of emulsions composed of Carbopol 934P®, Carbopol 974P® or polycarbophil for delivery of CUR and PRP. **Methods:** Mechanical textural analysis was performed using a TA-XTplus texture analyzer (Stable Micro Systems, Surrey, UK), in compression mode. The samples were subjected to double compression by an analytical probe of delrin, at temperatures of 25 °C and 34 °C. It was evaluated the parameters hardness, compressibility, adhesiveness, elasticity and cohesiveness [1,2]. **Results:** The formulations showed hardness values lower than 0.55 N, good spreadability, high adhesiveness, allowing a longer contact time with the application site, and all formulations displayed to be elastic [3,4]. **Conclusion:** The systems containing andiroba oil and Carbopol 974® or polycarbophil showed to be promising systems for further investigation as platforms for CUR and or PRP delivery on the skin and mucous membranes. SISGEN registration Nº. A098049 (andiroba) and N°. AC7A2F5 (PRP).

Acknowledgments: CNPq, CAPES, Araucaria Foundation, and FINEP.

References:

[1] S.B. De Souza Ferreira, T.D. Moço, F.B. Borghi-Pangoni, M.V. Junqueira, M.L. Bruschi, Rheological, mucoadhesive and textural properties of thermoresponsive polymer blends for biomedical applications, J. Mech. Behav. Biomed. Mater. 65 (2015) 164–178. https://doi.org/10.1016/j.jmbbm.2015.10.026.
[2] M.L. Bruschi, D.S. Jones, H. Panzeri, M.P.D. Gremião, O. De Freitas, E.H.G. Lara, Semisolid systems containing propolis for the treatment of periodontal disease: In vitro release kinetics, syringeability, rheological, textural, and mucoadhesive properties, J. Pharm. Sci. 96 (2007) 2074–2089. https://doi.org/10.1002/jps.20843.
[3] R. Said dos Santos, H.C. Rosseto, J. Bassi da Silva, C.F. Vecchi, W. Caetano, M.L. Bruschi, The effect of carbomer 934P and different vegetable oils on physical stability, mechanical and rheological properties of emulsion-based systems containing propolis, J. Mol. Liq. 307 (2020). https://doi.org/10.1016/j.molliq.2020.112969.
[4] J.B. da Silva, S.B. de S. Ferreira, A.V. Reis, M.T. Cook, M.L. Bruschi, Assessing mucoadhesion in polymer gels: The effect of method type and instrument variables, Polymers (Basel). 10 (2018). https://doi.org/10.3390/polym10030254.

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LIPID PROFILE OF *IN VITRO* AND *EX VITRO* CULTIVATED *Pfaffia glomerata* LEAVES

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Keywords: ***Pfaffia glomerata*, fatty acids, direct methylation.**

Introduction: *Pfaffia glomerata* is a species that belong to the *Amaranthaceae*, popularly known as "Brasilian gingeng"[1]. It is native to the Central-South region of Brazil and its roots are widely commercialized and used in traditional medicine[2]. Micropropagation represents a viable alternative for large scale cultivation of medicinally important plants, with controlled environmental conditions, independent of seasonalitywhose aerial part harvest is causing the depletion of natural resources[3].However there is a lack of studies regarding the obtainment of interest metabolites, such as fatty acids in the aerial parts of this plant. **Aim:**The aim of the study was to evaluate the lipid profile of the *in vitro* and *ex vitro* leaves of *P. glomerata* by Gas Chromatography coupled with Mass Spectrometry (GC/MS).**Methods:** The analytical method of direct derivatization of fatty acids was performed on leaves cultivated *in vitro* (L-IV) and *ex vitro* (L-EV) of *P. glomerata* and identified by GC/MS analysis[4]. Fatty acid methyl esters were identified by comparison with mass spectra from the *NIST* 11.0 database (*National Institute of Standards and Technology*).**Results:**Five saturated fatty acids were identified in L-EV and L-IV: myristic acid (C14:0), pentadecanoic acid (C15:0), palmitic acid (C16:0), heptadecanoic acid (C17:0) and arachidic acid (C20:0). An important unsaturated fatty acid was also identified in both samples: oleic acid (C18 :1n9c). Only in L-IV were identifiedthe unsaturated α-linoleic acid (C18:3n3,omega-3) and monounsaturated palmitoleic (C16:1), and in L-EV the polyunsaturated linoleic acid (C18:2n6c, omega-6) and saturated lignoceric acid (C24:0). Direct methylation showed satisfactory results for the samples since previous extraction of lipids is not necessary, causing reduction in the amount of solvent and sample, in addition to being fast with less environmental impact when compared to conventional methodologies**Conclusion:**It was possible to obtain the lipid profile by GC/MS and the essential and rare unsaturated fatty acids identified in the samples are important, as they have wide pharmacological and nutritional application and the use of *P. glomerata* leaves represents a potential and sustainable source for obtaining these primaries metabolites of interest.

Acknowledgments: UEM, CAPES, CNPq and ICETI.

References:

(1) DiasFCR, Gomes MLM, Melo FCSA et al. *Pfaffia glomerata* hydroalcoholic extract stimulates penile tissue in adult Swiss mice. J. Ethnopharmacol.2020; 261, 113182.
(2) Franco RR, Almeida TL, Chagas K et al. A 20-hydroxyecdysone-enriched fraction from *Pfaffia glomerata* (Spreng.) pedersen roots alleviates stress, anxiety, and depression in mice. J. Ethnopharmacol.2021; 267, 113599.
(3)Jacomini D, Sinzker RC, Mangolin CA et al. Lipid profile and antiproliferative activity of callus cultures of *Cereus peruvianus* Mill. IndCrops Prod. 2015; 69,408-414.
(4) Santos PD, Silveira R, Reis NV et al. Analytical method development for fatty acid direct methylation in fruits. J. Braz. Chem. Soc. 2018; 29, 1645-165.

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CHARACTERIZATION OF PHENOLICS COMPOUNDS FROM LEAF WASTE OF *Steviarebaudiana*

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Keywords:*Steviarebaudiana*, industrial waste, phenolics compounds.

Introduction:*Steviarebaudiana* (Bertoni) (Asteraceae) is a perennial plant, native to South America[1].This plant has commercial interest due to the presence of stevioside in leaves, and throughout the production of sweetener, a lot of waste is generated and discarded[2]. The use leaf waste of *S. rebaudiana* is an alternative to obtain biologically active compounds. Polyphenols and flavonoids are metabolites of commercial interest and have potential antioxidant activity[3].**Aim:**The aim of this study was to chemical characterization by UHPLC-QTOF-ESI ofphenolicscompoundsfromleaf waste of *S. rebaudiana*.**Methods:**Theleaf waste of *S. rebaudiana*utilized in this study were provided by Stevia Soul sweetener industry. The leaf waste of *S. rebaudiana*(24.0 g) were extracted with deionized water (300 mL) under reflux conditionsfor 4 h (3x). The aqueous extract was separated and filtered. The crude aqueous extract wasthrough by ultrafiltration process and the permeate coming from such step was precipitated with EtOH, resulting in two fractions: supernatant (FAES-U) and precipitate (FAEP-U). The extracts obtained were evaluationin relation to the content of total phenolic (TPC)[4] and total flavonoid (TFC)[5]by colorimetric method.All experiments were performed in triplicate The result was expressed in µg gallic acid equivalente (GAE) per mg of dry extract and µg quercetin equivalente (QE) per mg of dry extract, respectively.The chemical characterization of the compounds was using byUHPLC-QTOF-ESI and mass spectrometry were obtained in the negative ionization mode. **Results:**Qualitative assays indicated the TPC of 136.97 in FAES-U and 26.5 µgGAEmg^{-1} in FAEP-U. The presence of TFC in FAES-U was 7.56 and in FAEP was 13.04 µgQEmg^{-1}.It was possible identify in FAES-U the compounds hydroxybenzaldehyde, 4-methylcatechol, quinic acid, derivative catechin, apigenin-7-*O*-glucoside, rutine, quercetin, kaempferol 7-*O*-neohesperidosideo and kaempferol-3-*O*-rutinosideo by UHPLC-MS/MS. In the fraction FAEP-U were identified4-methylcatechol, kaempferol andapigeninaby UHPLC-MS/MS. **Conclusion:** The chemical characterization of the samples by UHPLC-MS/MS showed that it was possible to obtain polyphenols and flavonoid from leaf waste of *S.rebaudiana*. In conclusion, the leaf waste of sweetener industry represents an alternative for obtaining these bioactive compoundswith pharmaceutical and nutritional applications.

Acknowledgments: UEM, CAPES, CNPq and Stevia Soul Industry.

References:

(1) Ahmad J, Khan I, Blundell R et al.*Steviarebaudiana*Bertoni.: An updated review of its health benefits, industrial applications and safety.Trends in Food Science & Technology. 2020; 100:177-189.
(2)Lopes SMS, Krausová G, Carneiro JWP et al. A new natural source for obtainment of inulin and fructo-oligosaccharides from industrial waste of *Stevia rebaudiana*Bertoni. Food Chemistry. 2017;225:154-161.
(3) Ciulu M, Quirantes-Piné R, Spano N. et al. Evaluation of new extraction approaches to obtain phenolic compound-rich extracts from *Stevia rebaudiana*Bertoni leaves. Industrial Crops and Products. 2017; 108:106-112.

PHYTOCHEMISTRY

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Poincianella pluviosa INHIBITS CYTOKINE PRODUCTION BY ACTIVATED MACROPHAGES

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Keywords: sibipiruna, immunomodulatory, interleukin-6.

Introduction: *Poincianellapluviosa* (DC.) L.P. Queiroz is a tree popularly known as "sibipiruna" and widely used in urban afforestation. Some pharmacological activities were attributed to the plant and one of them is the wound healing activity [1]. During the inflammatory phase of healing, in response to tissue damage, macrophages migrate and increase the release of inflammatory mediators and cytokines, such as interleukin-6 (IL-6) [2].**Aim:**To evaluate the *in vitro* immunomodulatory effect of the ethyl-acetate fraction of *Poincianellapluviosa* (EAF) in decreasing IL-6.**Methods:** For the test, murine macrophages of lineage J774A.1 (ATCC® TIB-67 ™) were used, distributed in 24-well plates at the approximate concentration of $5x10^6$ viable cells/ml of supplemented DMEM medium. The plates were incubated for 2 h (37.0 ºC and 5.0% CO_2) for cell adhesion. After this period, the supernatant was discarded and the cells were stimulated with *Escherichia coli* lipopolysaccharide (LPS) at a concentration of 1 µg/mL, also receiving different concentrations of EAF (6.25; 12.5, and 25 µg/mL). After this period, culture supernatants were collected for detection and quantification of IL-6 by immunoenzymatic method (Invitrogen, USA). Each sample was tested in duplicate. The results were expressed in IL-6 concentration (pg/ml) from a calibration curve.Statistical analyzes were conducted using GraphPad Prism 9.0 software, using one-way analysis of variance (ANOVA) and a Tukey's test with $p<0.05$ to determine differences between the treatments.**Results:** All results were comparable and significantly different from the negative control (-16.1552 pg IL-6/mL), in which there was no LPS stimulation for cytokine production. The highest level of IL-6 was obtained in the LPS control (60.74138 pg IL-6/mL) as a response to the stimulus. For the three concentrations of EAF used, 6.25; 12.5, and 25 µg/ml, IL-6 concentration was 61.34483; 28.41379, and 21.17241 pg IL-6/ml, respectively. EAF was able to significantly inhibit IL-6 expression at concentrations of 12.5 and 25 µg/mL compared to LPS control (IC_{50}: 21.67807 µg/mL of EAF). **Conclusion:** IL-6 is a cytokine that causes an acute inflammatory response and plays an essential role in the pathogenesis of inflammatory diseases. Inhibition of IL-6 cytokine production may be one of the mechanisms to accelerate wound healing of the semipurified fraction of *P. pluviosa*.

Acknowledgments: Thanks to CAPES for financial support.

References:

(1) Guidi AC, de Paula MN, Mosela M et al. Stem bark extract of *Poincianellapluviosa* incorporated in polymer film: evaluation of wound healing and antistaphylococcal activities. Injury 2020;51:840–849.
(2) Aulanni'amA, Ora KM, Ariandini NA et al. Wound healing properties of *Gliricidiasepium* leaves from Indonesia and the Philippines in rats (*Rattus norvegicus*).Veterinary World 2021; 14:820-824.

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EVALUATION OF CYTOTOXICITY OF PYROSTEGIA VENUSTA FLOWERS IN L929 CELL LINE

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Keywords: Cell viability, *Pyrostegiavenusta*,sun protection**.**

Introduction:*Pyrostegiavenusta* popularly known as "São João" flower, has proven activity for leucoderma, vitiligo, and potent healing activity [1,2]. **Aim:**This work aims to evaluate cell viability in the L929 fibroblast lineage exposed to the extract and fractions of *P. venusta* flowers. **Methods:** The registered plant material is registered bySisGen#A2C637E. The crude extract (CE) was produced with dried flowers, without floral peduncle, using 50 ºGL alcohol, by turbolisis. The CE was partitioned with a mixture of ethyl acetate and *n*-butanol in different proportions, obtaining 3 fractions. Cell viability was assessed in L929 fibroblasts by neutral red assay. L929 cells were exposed to ethyl-acetate: *n*-butanol fractions (1.5625–200 µg/mL), crude extract, and crude extract with a mixture of one of the fractions (6.25–400 µg/mL) for 24 h. Afterwards, a spectrophotometer reading at 540 nm was performed.**Results:** Cytotoxicity with the L929 cell line of fractions showed cytotoxicity at concentration of 200 µg/mL and the crude extract, and crude extract with fraction 6:1 showed cytotoxicity at concentration of 400 µg/mL.**Conclusion:**We can observe the cytotoxic profile of L929 cells when exposed to different fractions and extracts of *P. venusta* flowers. This information is important and can be used in future studies.

Acknowledgments: PALAFITO group and CAPES.

References:

(1) Ferreira DT. et al.(2000)Chemical constituents from roots of *Pyrostegiavenusta* and considerations about its medicinal importance, Química Nova.23:42-46.
(2) Roy P.et al. (2012) In vivo antioxidative property, antimicrobial and wound healing activity of flower extracts of *Pyrostegiavenusta* (Ker Gawl) Miers, Journal of Ethnopharmacology. 140:186-192

YIELD OF DIFFERENT EXTRACTS AND SEMIPURIFIED FRACTIONS OF *Abaremacochliacarpos*.

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Keywords: *Mimosa cochliacarpos*, Liquid-Liquid Extraction, Plant Extracts**.**

Introduction: *Abaremacochliacarpos*(Gomes) Barneby J.W. Grimes(Fabaceae) known as "barbatimão" is a plant native to Brazil, which can be found in the southeast and northeast regions of the country(1). Some of the medicinal properties it's healing action, antioxidant, antibacterial, among others (2).**Aim:**It was to carry out the production of extract and semipurified fractions to isolate compounds using column chromatography. **Methods:** The barks of *A. cochliacarpos*were collected in January 2020, in Itaporangad'Ajuda, Sergipe (11º 4' 41" S, 37º 17' 23" W).A voucher specimenwas prepared, identified, and deposited at Herbarium of Tiradentes University under registration number HUT 815. The species is registered in the National System for the Management of Genetic Heritage and Associated Traditional Knowledge - SisGen under number A1CF45D. The extract acetone:water (7:3; v/v; CE1), 70% ethanol (CE2), and 50% ethanol (CE3) were prepared by turbo extraction for 20 min with intervals of 5 min. The semipurified fractions were prepared by liquid-liquid partition with ethyl acetate, and yielded ethyl-acetate fraction (EAF) and aqueous fraction (AQF). **Results:** The results yieldedforCE1, CE2, and CE3 were34.62%, 30.34%, and 31.91%, respectively. The yields of EAF1, EAF2,and EAF3 were41.34%, 40.64%, and 39.56%, respectively. The AQF ofCE1, CE2,and CE3 were 44.49%, 47.89%, and 46.85%, respectively. **Conclusion:** It was possible to observe that all extracts had a high yield. The CE1presented the highest yield and also ofthe EAF1. The AQF it was the opposite, showing a higher performance for CE3 and the lower performance for CE1.

Acknowledgments: CNPq and CAPES.

References:

(1) Santos, ALS. Interação de comunidades rurais com recursos vegetais: o caso dos remanescentes de floresta estacional do Município de Junqueiro. Tese (Programa de Pós-graduação em Geografia), Universidade Federal de Pernambuco. 2008.

(2) Fonseca SAU, Costa JRM, Gomes NRS et al. Antioxidantandcicatrizingactivityofthespecies*Abaremacochliacarpos* (Gomes) BarnebyJ.W.Grimes.African Journal of Pharmacy and Pharmacology. 2019;13:14:170-180.

AVAILABLE DATA OF EPICATECHIN RESEARCHFOR BLOOD PRESSURE

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Keywords: polyphenol, hypertension, systematic review

Introduction: It has been more than 35 years since the search for drug alternatives for high blood pressure from plants has been intensified [1]. Sometradicional herbs such as *Camellia sinensis* (green tea), *Allium sativum* (garlic), and *Theobroma cacao* (cocoa) have proven antihypertensive properties [2], probably due the presence of polyphenol epicatechin (EPI), which is strongly associated with the effect of lowering blood pressure, and another molecules.Even this hypothesis,they have not systematic reviews evaluating its use alone, only as part of foods rich in polyphenols, and there may be a summation or synergistic effect with other biologically active molecules. **Aim:**Thus, this study aimed to summarize the available evidence on the effects of pure epicatechin on blood pressure by means of a broad systematic review.**Methods:**The data collection was assessed with recommendations of the PRISMA [3], Cochrane [4], and JBI methodology [5]for systematic review, with Open Science Framework 54 (OSF) register DOI: 10.17605/OSF.IO/R6KB3. A systematic search was performed in the PubMed, Scopus, and DOAJ electronic databases, with no time or language limits. A manual search in the reference lists of the included studies was also performed.**Results:**The search strategy yielded 702 articles after excluding duplicates. During the screening process, 165 records were selected for full-text reading of which 48 were included for analyses. The reasons for exclusion were population for 79.49%, outcomes for 7.69%, and study design for 12.82% of the studies in full-text reading. Fourteen studies (29.17%) were *ex vivo* analyses, four (8.33%) were clinical trials, twelve (25.00%) were *in vitro* analyses and eighteen (37.50%) were *in vivo* (animal models). Studies were conducted in different countries, mostly in USA (n= 9, 18.75%), and published between 1993 and 2019. The preclinical studies will be capable to establish the hypothesis mechanisms of action and the clinical trials if EPI have any effect in hypertension.**Conclusion:** This systematic review allowed summarize the studies who evaluated EPI for blood pressure and will allow understand thiseffect.

Acknowledgments: CNPq and CAPES.

References:

(1) HigginsJPT, Thomas J, Chandler J et al. (2019) Cochrane handbook for systematic reviews of interventions. John Wiley & Sons.
(2) Page MJ, McKenzie JE, Bossuyt PM et al. (2021) Updating guidance for reporting systematic reviews: development of the PRISMA 2020 statement. Journal of Clinical Epidemiology 134, 103–112.
(3) Peters MDJ, GodfreyCM, McInerneyP et al. (2015) The Joanna Briggs Institute reviewers' manual 2015: methodology for JBI scoping reviews.
(4) Sato T and Miyata G (2000) The nutraceutical benefit. Nutrition 16(9), 787–788.
(5) Tabassum N and Ahmad F (2011) Role of natural herbs in the treatment of hypertension. Pharmacognosy Reviews 5(9), 30–40.

Cell viability of *Maytenusilicifolia*semipurified extracts on three different cell lines

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Keywords: Espinheira-Santa, AGS cells,mouse macrophage, fibroblast.

Introduction: Leaves of *Maytenusilicifolia*Mart. Ex Reissek (Celastraceae) are popularly known as *espinheira-santa* in South America. In Brazil the leaves are widely used to treat gastric ulcers, but indigenous and rural communitiesuse it as antitumor, antiulcerogenic and heling (1, 2).**Aim:**The aim of this work was evaluating the toxicity of semipurifiedextracts obtained from the leaves of *M.ilicifolia*, inthree different cell lines. **Methods:**The crude extract was obtained from the leaves by extraction withethanol:water (1:1, v/v, CE2) andacetone:water (7:3, v/v, CE5) and lyophilisation. The crude extracts were separately partitioned resulting in the ethyl-acetate fraction and aqueous fraction (AQF2 and AQF5). For evaluation of functionality the influence of the extract on the cell viability of human stomach AGS cells (ATCC CRL 1739), mouse macrophage (ATCC J774A.1) and fibroblast cells (ATCC L-929) was tested by MTT Assay(3). **Results:**AGS cell viability was influenced significantly by the crude extractsand aqueous fractions from 50 µg/mL. The semipurified extracts influenced significantly in the mouse macrophage viability from 100 µg/mL for CE2 and from 50 µg/mL for CE5 and from 250 µg/mL for AQF2 and from 50 µg/mL for AQF5. The fibroblast viability was not influenced significantly by the crude extracts and aqueous fractions (1000 to 1 µg/mL).**Conclusion:**For the highest concentration the crude extracts and fractions reduced the AGS and mouse macrophage cell viability, but these extracts and fractions did not influence negatively in the fibroblast viability.

Acknowledgments: CAPES, PCF-UEM.

References:

(1)Crestani S et al.A potent and nitric oxide-dependent hypotensive effect induced in rats by semi-purified fractions from *Maytenusilicifolia*.Vasc.Pharmacol.2009;51(1): 57-63.
(2)Mossi A et al. Chemical variation of tannins and triterpenes in Brazilian populations of *Maytenusilicifolia* Mart. Ex Reiss. Braz. J. Biol.2009;69(2): 339-345.
(3) Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Methods 1983;65(1-2), 55-63.

CHEMICAL CHARACTERIZATION OF PHENOLIC COMPOUNDS OF *Limoniumbrasiliense*BYUHPLC-ESI-QTOF-MS/MS

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Keywords: Chromatography, Natural products, Phytochemistry, Plumbaginaceae

Introduction:*Limoniumbrasiliense*(Boiss.) Kuntze, Plumbaginaceae, is a terrestrial herb popularly known as "*baicuru, guaicuru or guaicurá*", unique native species of *Limonium*Mill. present of Southeast (Rio de Janeiro) and South States (Paraná, Rio Grande do Sul, and Santa Catarina), and in the Atlantic Rainforest from Brazil [(1)]. Its rhizomes are employed in folk medicine for the treatment of menstrual disorders and genitourinary infections and have been demonstrated antioxidant, neuroprotection, and antibacterial potential.**Aim:** This study aimed to identify the main compounds present in ethyl-acetate fraction (EAF) of *L. brasiliense*rizhome by chromatographic methods. **Methods:**Dried and powdered rhizomes were extracted with 70% acetone (1:10, w/v) by turbolysis, the organic phase was removed in a rotavapor and the residue was lyophilised, obtained the crude extract (CE). The CE was partitioned with ethyl acetate (1:10, w/v), resulting in the EAF. The EAF (15.0 g) was fractionated by chromatography column (CC) on Sephadex LH-20 with the following solvents: ethanol, methanol and 70% acetone, and monitored by thin-layer chromatography. The purified subfractions(F#10, 11, 16, 17, and 18) were analysed by ultra-high-performance liquid chromatography coupled withelectrosprayionizationquadrupole-time-of-flightmass spectrometry (UHPLC-ESI-QTOF-MS/MS). **Results:** The crude extract and the EAF had a yieldof 31.25% and 9.43%, respectively. The CC yielded 27subfractions. The analyses by UHPLC-MS/MS showed the peak at 7 min and protonated ions at *m/z* 459.0922 $[M+H]^+$ and 481.0714 $[M+Na]^+$ compatible with $C_{22}H_{19}O_{11}^+$ (error: -4.4 and 1.7 ppm, respectively), suggesting the presence of (epi)gallocatechin-3-*O*-gallate in the F#10and F#11.The peaks at 1.96 and 1.95 min, and protonated ions at *m/z* 763.1459, 763.1457, and 763.1458 $[M+H]^+$were compatible with$C_{37}H_{31}O_{18}^+$ (error: 1.7, 1.4, and 1.6 ppm, respectively), suggesting the compound prodelphinidin-prodelphinidin-B-gallate in the F#16, 17, and 18. The peak at 5.79 min and the protonated ion at *m/z* 761,1323 $[M+H]^+$was compatible with $C_{37}H_{29}O_{18}^+$ (error: 4.5 ppm) and suggested the compound samaragenin A (F#16). The peaks at 7.49, 7.34, and 7.58 and the protonated ions at *m/z* 913.1404, 913.1392 and 913.1405$[M+H]^+$were compatible with $C_{44}H_{33}O_{22}^+$ (error: 0.5, -0.8, and 0.7, respectively), suggesting the compound samaragenin B (F#16, 17, and 18).**Conclusion:** The results were consistent with the fragmentation and molecular mass profiles of the isolated and identified phenolic compounds in previous works of our research group and data literature.

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References:(1)Funez LA. 2020. Plumbaginaceae. *In*: Flora do Brasil 2020. Jardim Botânico do Rio de Janeiro. Availableat:<http://floradobrasil.jbrj.gov.br/reflora/floradobrasil/FB12938>. Accessed on: 08 Nov. 2021.

*Limonium brasilisense*FRACTION INHIBITS THE VIRULENCE FACTOR OF *Porphyromonasgingivalis*

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Keywords: *Limoniumbrasiliense, Porphyromonasgingivalis*,gingipain

Introduction:*Limoniumbrasiliense* (Boiss.) Kuntze (Plumbaginaceae), popularly known as baicuru [1], is used in Brazil for the treatment of menstrual disorders [2]. The crude extract has specific inhibitory activity against *Porphyromonasgingivalis* (*Pg*) [3]. *Pg* is a pathogen strongly involved in chronic and aggressive forms of periodontitis, that is a complex microorganism-induced inflammation of periodontal tissue, and if left untreated leads to the destruction of the tooth supporting system with eventually tooth loss, and this microorganism is also related with Alzheimer disease [4], cardiovascular disease [5], and other systemic problems. **Aim:**to investigate the influence of ethyl-acetate fraction (EAF) on the major adhesins of *Pg*, the arginin-specific gingipain activities were monitored during incubation with this fraction. Gingipain is an enzyme and the most important virulence factor of *Pg*, because is responsible to the adhesion of bacteria in oral tissues.**Methods:**The crude extract of the rhizomes was obtained by turbo-extraction with acetone:water 70:30 and was partitioned with water and ethyl acetate (EAF). A 3 day agar culture of *Pg*was harvested and resuspended inbuffer.The $OD_{660}$was adjusted to 0.006 for Rgp (arginin-gingipain) activityand the suspensions were transferred to a 96-wellplate. Solutions, containing the respective test compounds (EAF and leupeptin, a specific inhibitor for Rgp)were added and the plate was incubated at room temperature for10 min. The substrates BAPNA(Nα-benzoyl-D,L-arginine 4-nitroanilide hydrochloride) for determination of Rgpactivitywas added at 0.5 M and the absorbancewas read at 405 nmover 30min in eachminute in amicroplate reader. Proteaseactivities were related to the untreated control (=100%). **Results:**EAF inhibited Arg-gingipain in a concentration-dependent manner (the concentrations of 100, 50, 25, and 12.5 µg/mL were tested). **Conclusion:**EAF of *L. brasiliense* could have an impact for the development of oral care products against periodontitis because of its specific inhibitory activity against the virulence fator of *Pg*.

Acknowledgments: CNPq, Fundação Araucária, UEM, WWU-Münster

References:

(1) Correa M and Penna LdA.Dicionário das plantas úteis do Brasil e das exóticas cultivadas: volume 5. MR. Rio de Janeiro: Instituto Brasileiro de Desenvolvimento Florestal. 1974; 687.
(2) Lifchitz A. Plantas medicinales: guia práctica de botânica medicinal. 1981.
(3) De Oliveira Caleare A, Hensel A, Mello JCP et al., Flavan-3-ols and proanthocyanidins from *Limoniumbrasiliense* inhibit the adhesion of *Porphyromonasgingivalis* to epithelial host cells by interaction with gingipains. Fitoterapia. 2017; 118: 87-93.
(4) Dominy SS, Lynch C, Ermini F et al., *Porphyromonasgingivalis* in Alzheimer's disease brains: Evidence for disease causation and treatment with small-molecule inhibitors. Science Advances 2019; 5.
(5) Liccardo D, Cannavo A, Spagnuolo G et al. Periodontal disease: A risk factor for diabetes and cardiovascular disease. International Journal of Molecular Sciences 2019; 20: 1414.

IN VIVO EXPERIMENTAL STUDIES AND HISTOLOGICAL ANALYSIS: METHODOLOGICAL ASPECTS

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Keywords: hair growth, methodological parameters, plant extracts.

Introduction: Alopecia is one of the most frequent dermatological disorders with universal prevalence that significantly influences the patient's psychology and quality of life (1). Minoxidil and finasteride are the only drugs approved by the US Food and Drug Administration to treat hair loss. However, both these chemicals have serious adverse effects (2). Therefore, the search for natural herbal products has been widely promoted. Several plant extracts have been researched for the development of new agents against hair loss. **Aim:** This study aimed to evaluate methodological aspects of histological analysis in animal models for hair growth using substances of plant origin. **Methods:** The systematic search was performed in the Medline, Scopus, Web of Science, and Scielo electronic databases. *In vivo* experimental studies were included, which used histological analysis to investigate plant species as a possible therapeutic option for hair growth. The survey was conducted in accordance with the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (3) and the Cochrane Handbook for Systematic Reviews of Interventions (4).**Results:** Initially, 1021 studies were retrieved from the databases, 226 studies for full reading, 123 studies included for data extraction. From these, 72 studies performed the histological analysis. In the studies, the histological analysis evaluated the following parameters: number, size, depth, length, and diameter of hair follicles; distance between the hair follicle and the dermis; the proportion of hair follicles in different cyclic phases (anagen/telogen ratio); hair follicle cycle progression; follicular density; skin thickness, dermis; skin thickness from the epidermis to the subcutaneous layer; number of basal cells in the hair root and hair folliculogram. **Conclusion**: With the synthesis of the data, it was possible to verify the methodological aspects of the histological analysis as a research model for the evaluation of hair growth in animals using plant extracts as possible therapeutic options for hair loss.

Acknowledgments: CAPES e CNPq.

References:

(1) Fricke ACS, Miteva M. Epidemiology and burden of alopecia areata: a systematic review. ClinCosmetInvestigDermatol. 2015;8: 397–403.
(2) Boisvert WA, Yu M, Choi Y et al. Hair growth-promoting effect of Geranium sibiricum extract in human dermal papilla cells and C57BL/6 mice. BMC Complementary and Alternative Medicine. 2017; 17:109.
(3) Higgins, JPT, Green S. Cochrane Handbook for Systematic Reviews of Interventions 5.1.0. 2011; Oxford, England.
(4) Liberati A, Altman DG, Tetzlaff J et al. The PRISMA statement for reporting systematic reviews and meta-analyses of studies that evaluate health care interventions: Explanation and elaboration. BMJ. 2009; v. 339.

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EFFECT OF SEMIPURIFIED EXTRACT OF *LIMONIUM BRASILIENSE* IN MODEL ROTENONE-INDUCTED OF PARKINSON'S DISEASE ON NEUROBLASTOMA SH-SY5Y CELLS

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Keywords: **cellculture, neuronal cell, plant.**

Introduction: The Parkinson'sdiseases (PD) isthesecondneurodegenerative, chronicandprogressivedisease, thatmostaffectspeople, justbehindAlzheimer'sdiseases. Treatment for PD includes drugsthatdelaythediseasebut do not cure it, actingonlyonthesymptoms (1). Whatisknownaboutthephysiopathologyof PD isthelossdopaminergicneurons, mostly in thesubstantianigra*pars compacta.*Nevertheless, thetreatmentbasically includes drugsthatonlytreatthesymptoms. Thus, the use of medicinal plantsis a potential in thesearch for new drugsor help in thetreatmentof PD. **Aim:** It wastestedtheprotectiveeffectofethyl-acetatefraction (EAF) oftherhizomeof*Limonium brasiliense* (Boiss.) Kuntze (Plumbaginaceae) onrotenone-induceddamage, usinghumanneuroblastoma SH-SY5Y cells as a PD model. **Methods:** Rhizomesof*L. brasiliense*werecollected in February 2013, in Rio Grande, Rio Grande do Sul, Brazil, withpermissionfrom IBAMA-SISBIO (nº. 11995-3, November 2, 2010, authenticationcode 46367613). Access tothebotanical material wasauthorizedandlicensedbythe Conselho Nacional de Desenvolvimento Científico e Tecnológico, registeredunder #010252/2015-0. The EAF wasprepared as previouslydescribed (2). Humanneuroblastoma SH-SY5Y cells (ATCC® CRL-2266™) weregrown in cultureflaskswith DMEM medium in pH 7.2, supplementedwith 10.0% FBS, 1.0% penicillin-streptomycinsolution, and 3.7 g sodiumbicarbonateunderincubationconditionsof 37.0 °C in a humidatmospherewith 5.0% CO_2. The rotenone-inducedamagewasevaluatedatconcentrationsfrom 500, 1000, 1500, and 2000 nM, bythe MTT assay (3), in exposure time at 24 h. For cytoprotectionassaythecellsweretreatedwith EAF atconcentrationsof 1.95, 3.9, 7.81, and 15.63 µg/mL, 1 h beforerotenoneexposureatconcentrationthatwascytotoxic for 50% ofcells (CC_{50}). Threeindependentassayswereperformed for each sample. Statisticalanalysiswasperformedusing GraphPad Prism® 6 software. **Results:** For the MTT assay, CC_{50}valuewas 1702.11 nM ± 2.51. Thisassayshowed a concentration-dependentactivityofrotenoneonneuroblastoma SH-SY5Y cellsviability, whilecomparedtocontrolconditions. The protectionatconcentrations 1.95, 3.9, and 7.81 of EAF were 24.59%, 35.12%, and 32.17%, respectively. The cytoprotectionassaysdemonstratedthatpre-treatmentwith EAF in theculturesignificantlyreducedrotenone-inducedcell death. Regardingthe positive control (rotenone), therewas a significantstatisticaldifference in eachconcentration. **Conclusion:**It'spossibletosuggestedthattheethyl-acetatefractionof*L. brasilienses*,attestedconcentrations, wasabletoprotects SH-SY5Y celllineagainstrotenonedamagewithpotentialpromising for furtherinvestigationson neuronal cells.

Acknowledgments: PALAFITO Laboratory and CAPES.

References:

(1) Tysnes OB, Storstein A. EpidemiologyofParkinson'sdisease. Journalof Neural Transmission. 2017; 24(8), 901–905.

(2) Blainski A, Gionco B, Oliveira AG, Andrade G, Scarminio IS, Silva DB, Lopes NP, Mello JCP. Antibacterialactivityof*Limonium brasiliense* (Baicuru) againstmultidrugresistantbacteriausing a statisticalmixture design. JournalofEthnopharmacology. 2017; 198, 313-323.

(3) Mosmann T. Rapidcolorimetricassay for cellulargrowthandsurvival: applicationtoproliferationandcytotoxicityassays. JournalofImmunologicalMethods. 1983.; 65, 55-63.

MICROBIOLOGY

ANTITUMORAL ACTIVITY OF A DIBENZYLIDENEACETONE DERIVATIVE (A3K2A3) AGAINST CERVICAL CANCER CELLS IMMORTALIZED BY HPV 16 AND 18

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Keywords:Cervical cancer, HPV 16 and 18, Anti-tumoractivity

Introduction: Cervical cancer is the fourth most common type of cancer in women worldwide (1). The main risk factor for the development of cervical cancer is the infection with the human papilloma virus (HPV) in its high-risk forms, HPV16 and HPV18. Considering the limitations of anticancer chemotherapy, research is still needed to find drugs with effective and less toxic antitumor activity. The antitumor potential of dibenzylideneacetonesand its derivatives had been already described, reinforcing the need of further studies with the substance **A3K2A3**(2).**Aim:**Thus, the aim of this work was to investigate the *invitro* antitumor activity of **A3K2A3**, a new synthetic dibenzylideneacetonederived from 1,5-diaryl-3-oxo-1,4-pentadienyl, against cervical cancer cells immortalized by HPV 16 and 18 (SiHa andHeLa respectively). **Methods:**For this, cells were plated ($2.5x10^5$ cells/mL) and treated with **A3K2A3** (1, 10, 50 and 100 µM) for 24 and 48 h at 37 °C. Subsequently, cell viability was determined by MTT assay. Inhibitory concentration for 50% of cells (IC_{50}) was determined by linear regression. For morphology evaluation, cells were treated with concentrations of **A3K2A3**(IC_{50} and twice the IC_{50}) and images were obtained after 24 and 48 h of incubation (Olympus CKX41). To investigate ROS production, HeLa and SiHacells were treated and, after 24 h, labeled with H2DCFDA (10 µM). In addition, the mitochondrial membrane potential was assessed by TMRE (25 nM). In both experiments, fluorescence was quantified in a spectrofluorimeter (VICTORX3, PerkinElmer). Finally, cell migration was investigated by the wound healing assay. Briefly, after 24 h of plating, a wound was made with a tip, then cells were treated and observed after 24 and 48 h in an inverted phase contrast microscope (OlympusCKX41; 4x magnification). **Results:A3K2A3** showed antitumoralactivity against HeLa and SiHa with IC_{50} of 18.9 µM and 17.8 µM, respectively, and also induced morphological changes such as irregular shape and cell detachment. Furthermore, there was an increase in ROS levels and mitochondrial depolarization was observed in treated cells, indicating oxidative damage. The substance also induced significant inhibition of cell migration when compared to the negative control. **Conclusion:**Given these results, **A3K2A3** has shown promise as a possible alternative for the treatment of cervical cancer, requiring further studies to better understand its effect against these cancer cells.

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References:

(1)INCA. Estimativa 2020: incidência de câncer no Brasil / Instituto Nacional de Câncer José Alencar Gomes da Silva. – Rio de Janeiro (2019).
(2)M. M. A.Hawash et al.Eur. J. Med. Chem., v. 129 (2017) p. 12–26.

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IN VITRO TRIPANOCIDAL ACTIVITY OF THE COMBINATION OF QUINONES AND ASCORBIC ACID AGAINST TRIPOMASTIGOTES FORMS OF *Trypanosoma cruzi*

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Keywords: **Chagas disease; Quinones; Ascorbicacid**

Introduction: Chagas disease is an infection caused by the protozoan *Trypanosoma cruzi*.Currently, two drugs are available for treatment of Chagas disease:benznidazole and nifurtimox, however,they have several toxiceffects(1).Quinones, a group of molecules, have shown several biological activities includingtrypanocidal(2).**Aim:**Evaluate the *in vitro* effect of three quinone derivatives (Q2, Q3 and Q4) combined with ascorbic acid (AA) on trypomastigote forms of *T. cruzi*Y strain. **Methods:**Quinones were associated with AA at a ratio of 1:100, at concentrations of 0.25 to 8 µM for quinones and 25 to 800 µM for AA.Trypomastigotes(1 x 10^7 parasites/mL) derived from tissue culture (LLC-MK2 cells previously infected) were incubated with different concentrations of quinones and AA, alone or in combination, in 96-well microplates for 24 h at 34 °C. After incubation the $IC_{50}$and Fractional Inhibitory Concentration Index (FICI) was calculated. Additionally, cytotoxicity on LLC-MK2 cells was determined.For this, LLC-MK2 cells (2.5 x 10^5 cells) were incubated for 96 h at 37°C and 5% CO_2 with the same concentrations as the combination assay. MTT (2 mg/ml) was used and absorbance measured at 570 nm in a microplate reader. **Results:**Quinones alone exhibited IC_{50} values of 2.08 µM (Q2), 6.62 µM (Q3) and 1.47 µM (Q4), while AA alone exhibited an IC_{50} of 230.29 µM. Quinones combined with AA showed reduction in IC_{50} values: 0.82 µM (Q2+AA), 0.95 µM (Q3+AA) and 0.65 µM (Q4+AA). Combined AA also exhibited a reduction in IC_{50}: 82.46 µM (Q2+AA), 95.32 µM (Q3+AA) and 65.26 µM (Q4+AA). The FICI values were 0.75 (Q2+AA), 0.56 (Q3+AA) and 0.73 (Q4+AA), indicating a synergistic effect. Regarding cytotoxicity in LLC-MK2 cells, only Q4 was cytotoxic at 8 µM isolated and combined with AA.**Conclusion:**In this study, Q2, Q3 and Q4 showed a promising trypanocidal activity together with AA in *T. cruzi* trypomastigotes, in addition it was not cytotoxic alone and combined in the IC_{50} concentrations.

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References:

(1) Rassi AJ, Rassi A, Rezende JM. American Trypanosomiasis (Chagas disease). Infect Dis Clin N Am. 2012; 26: 275-291.

(2) Zhang L, Zhang G, Xu S, Song Y. Recent advances of quinones as a privileged structure in drug Discovery. European Journal of Medical Chemistry. 2021; 223:113632.

DEVELOPMENT OF SOLID LIPID NANOPARTICLES CONTAINING CAFFEIC-PHTHALIMIDEDERIVATIVE AND EVALUATION OF THE PHOTOCHEMIOPROTECTIVE EFFECT AGAINST L929 FIBROBLASTS UVB-INDUCED

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Keywords: **Photoprotection; oxidative stress; caffeic-phthalimide derivate; solid lipid nanoparticles.**

Introduction: Ultraviolet B (UVB) radiation corresponds to 5% of the radiation that affects the Earth[1], being more genotoxic and mutagenic than UVA radiation, capable of penetrating the epidermis and the upper layer of the dermis. UVB causes direct DNA damage, and indirect damage through the generation of reactive oxygen species (ROS)[2], contributing to premature skin aging and even photocarcinogenesis. Solid lipid nanoparticles (SLNs) are used as carriers for the topical delivery of antioxidants to the skin, in addition they can act as UVR blockers[3]. Furthermore, the incorporation of photoprotective agents to the SLNs can lead to a synergistic protective effect. The phthalimide molecule Caffeic-phthalimide derivate (CP) was synthesized with Caffeic Acid, to increase its antioxidant potential. Vary its hydrophobic character and improve photochemoprotective effects, has also shown ability to inhibit the activity of NADPH oxidases[4]. **Aim:**Therefore, the present work aims to investigate the *in vitro*antioxidant properties and photoprotective activity of CP derivateand associateCP in SLNs.**Methods:**The antioxidant capacity of CP was studied using the 2,2-diphenyl-1-picrylhydrazyl method and the xanthine/luminol/xanthine oxidase system. The neutral red method was used to determine cell viability and cytoprotection against UVB radiation. The 2',7'-dichlorodecafluorescein diacetate marker was used to assess the production of ROS.The method of preparation of SLNs was hot with melting point of solid lipid (precirol ATO 5) at 65°C, with tween 80, mili Q water and CP (5mg). **Results:**CP showed antioxidant activity in both assays. The CP did not show cytotoxicity to L929 fibroblasts at concentrations of 1-100 µM. In the cytoprotection assay, concentrations of 1, 10 and 20 µM prevented 80, 88.91% of the unfeasibility caused by UVB radiation, respectively. Pretreatment with CP (1 and 10 µM) also significantly decreased the production of UVB-induced ROS (≅65% lower compared to the UVB group). When associated with SLNs andCP 2.5% it had a size of 335.33±13.3 nm, with a polydispersion index of 1.9 and zeta potential -21mV.**Conclusion:**The future use of CP associated with SLNs in cosmetic formulations designed to protect the skin against damage caused by UVB and photoaging is promising due to its antioxidant and photochemoprotective effects.

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References:

(1)HoelDG, Berwick M, De GruijlFR,HolickMF. The risks and benefits of sun exposure, Dermatoendocrinol.2016; 8.
(2) de Jager TL, Cockrell AE, Du Plessis SS. Ultraviolet Light Induced Generation of Reactive Oxygen Species, Adv Exp Med Biol. 2017; 996: 15–23.
(3)Chen-yu G, Chun-fen Y, qi-lu Y et al. Development of a quercetin-loaded nanostructured lipid carrier formulation for topical delivery. Int J Pharm. 2012. 1;430(1-2):292-8.
(4) dos Santos W,Yoguim HMI,Daré RG, da Silva-filho LC, Lautenschlager SOS, Ximenes VF. Development of a caffeic acid–phthalimide hybrid compound for NADPH oxidase inhibition, RSC adv.2021; 11:17880-178890.

EVALUATION OF IN VITRO ANTITUMOR ACTIVITY OF SYNTHETIC SUBSTANCE B32 DERIVED FROM BETA-CARBOLINE-ALPHA-AMINOPHOSPHONATE IN MCF-7 AND MDA-MB-231 BREAST ADENOCARCINOMA CELLS

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Keywords: Breast cancer, cytotoxic effect, beta-carboline-alpha-aminophosphonate

Introduction: Breast canceris the most common cancer in women worldwide. According to INCA, it was considered the leading cause of death from cancer in the female population in Brazil, with approximately 18.068 in 2019 [1]. It is a complex disease with great potential to invade adjacent organs and has high clinical, morphological and biological heterogeneity [2]. Cells of the MDA-MB-231 and MCF-7 subtype are representatives of two breast cancer cell lines with different characteristics used in research [3] and studying possible treatments for these lines is extremely relevant, since it is essential to find new therapeuticagents for breast cancer subtypes without targeted therapies. In this context, the substance B32 derived from the β-alkaloid-carboline-α-aminophosphonate, is described in the literature for having antitumor activity [4,5]. **Aim:**The aim of this work is to carry out a study of the *in vitro* antitumor activity of the substance((4-Fluorophenyl)-{2-[(1-phenyl-9H-b-carboline-3-carbonyl)-amino]ethylamino} dibutyl -methyl)phosphonate (B32) on MCF-7 and MDA-MB-231 cells, evaluating mechanisms of cytotoxicity, migration and oxidative stress. **Methods:**Cells were plated ($2.5x10^5$ cells/ml) and treated with substance B32 (2, 20, 50 and 100 µM) for 24 and 48 hours. Cell viability was determined by colorimetric MTT assay and the inhibitory concentration for 50% of cells (IC_{50}) was determined by linear regression. For subsequent tests, the IC_{50} of the substance was used. Morphology evaluations were obtained after 24 and 48 h (Olympus CKX41) and cell migration was performed by the wound healing assay. ROS production was verified by H2DCFDA labeling and TRME-labeled mitochondrial membrane potential, both were analyzed by VICTOR X3 fluorescence quantification, PerkinElmer. **Results:**The IC_{50} value of substance B32 was determined in 48 h for MCF-7 (32.33 µM) and MDA-MB-231 (38.60 µM) cells, and a change in morphology and decreased cell migration of both cells was verified. There was an increase in the production of ROS, which is generally associated with significant cell damage.There was a significant decrease in membrane potential in the two cell lines tested.**Conclusion:**The substance demonstrates cytotoxic potential against MCF-7 and MDA-MB-231 cells, in addition to altering cell morphology, decreasing cell migration, increasing ROS and decreasing membrane potential, which may be related to damage and increased cell permeability.

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References:

(1)José Alencar Gomes da Silva NationalCancerInstitute. Atlas ofmortality. Rio de Janeiro: INCA, 2021. Database. Available at: https://www.inca.gov.br/app/mortality Accessed on: 28th of 2021.
(2)(INCA), José Alencar Gomes da Silva National Cancer Institute; Ministry of Health. ABC do Câncer - Basic approaches to cancer control - 6th revised and updated edition Rio de Janeiro, RJ. 6th edition ed. Rio de Janeiro - RJ; 2020.
(3) Harbeck N, Gnant M, SeminarBreastcancer. The Lancet, v. 389, n. 10074, p. 1134–1150, 2017.
(4)Farouk L, Laroub A, Aboufatima, R, Benraheef A, Chait, A. Evaluation of the analgesic effect of alkaloid extract of Peganum harmala L.: Possible mechanisms involved. Journal of Ethnopharmacology, v. 115, p. 449–454, 2008.
(5)Li S, Cheng X, Wang C. A review on traditional uses, phytochemistry, pharmacology, pharmacokinetics and toxicology of the genus Peganum. Journal of Ethnopharmacology, v. 203, n. March, p. 127–162, 2017.

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DOXORUBICIN-LOADED IRON OXIDE NANOPARTICLES EFFECT IN BREAST CANCER CELLS

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Key-words: **oxidative stress, nanotechnology, MCF-7**

Introduction: Nanotechnology is an area of interdisciplinary and multidisciplinary research that has been used in different biomedical applications. Currently one of the cancer treatments choices is chemotherapy, but problems such as drug resistance, lack of selectivity and accelerated metabolism result in toxicity to healthy cells thus reducing the effectiveness of this treatment (1). Therefore, it is important to study the use and mechanism of nanocarriers that can be used on specific targets and consequently can reduce side effects caused by anti-tumor drugs in healthy organisms.In this respect magnetic nanoparticles are promising candidates due to their physicochemical and magnetic characteristics (2, 3). **Aim:**Therefore, this work aimed to evaluate the mechanism of action ofmagnetite (NPMag), silica-coated magnetite(NPMag+Si) nanoparticles and doxorubicin-functionalized nanoparticles (NPMag+Dox, NPMag+Si+Dox) on human breast adenocarcinoma cells (MCF-7).**Methods:**DLS and FTIR was used to verify the physico-chemical characteristics of the nanoparticles. Cells were exposed to concentrations of 5µg/mL and 10µg/mL of NPMag, NPMag+Si, NPMag+Dox and NPMag+Si+Doxand the equivalent concentrations of 0.3, 0.4, 0.6 and 0.8µg/mL of Doxorubicin (Dox) for 48 h. To search for the abilityof these nanoparticles to enhance oxidative stress in cancer cells it was evaluated the membrane integrity, reactive oxygen species (ROS) production, mitochondrial membrane potential, production of reduced thiols and cell migration. **Results:**The DLS shows that NPMag, NPMag+Si, NPMag+Dox and NPMag+Si+Dox have the hydrodynamic sizes ranges from 120nm to 240nm with a polydispersity index of 0.2. The FTIR shows the presence of Dox in the nanoparticles. The NPMag+Dox and NPMag+Si+Doxinduced an increase in cells with membrane integrity compromised but it was not higher than the free drug. Also,NPMag+Dox and NPMag+Si+Dox increased ROS formation and decreased mitochondrial membrane potential and the levels of reduced thiols compared with the free drug. In addition, NPMag+Dox and NPMag+Si+Doxinhibited cell migration compared with only Dox. **Conclusion:**The nanoparticles alone did not cause oxidative stress in cells but when conjugated with doxorubicin caused damage to the cancer cells. This indicates the potential of this formulation for drug delivery.

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References:

(1) Ulbrich, K et al. Targeted drug delivery with polymers and magnetic nanoparticles: covalent and noncovalent approaches, release control, and clinical studies. Chemical reviews. 2016; 9: 5338-5431.
(2) Agiotis, L et al. Magnetic manipulation of superparamagnetic nanoparticles in a microfluidic system for drug delivery applications. Journal of Magnetism and Magnetic Materials. 2016; 401: 956-964.
(3) El-Hammadi, Mm, Arias, Jl. Iron oxide-based multifunctional nanoparticulate systems for biomedical applications: a patent review (2008–present). Expert opinion on therapeutic patents.2015; 25: 691-709.

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ANTITUMOR ACTIVITY *in vitro* OF MENADIONE AGAINST CERVICAL CANCER

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Key-words: menadione, antitumor, cervical cancer.

Introduction: Cervical cancer is the fourth most common type of cancer among women worldwide, mainly due to human papillomavirus (HPV) infection with high-risk HPV16 and HPV18. It has a high mortality rate in patients aged 20 to 39 years. It is usually treated by surgery, chemotherapy and/or radiotherapy. Despite these therapeutic approaches already implemented in the clinic, the survival rate of these patients is still relatively low[1].Menadione, also known as vitamin K_3, has several functions in the body since it acts as a cofactor in glutamate residues, besides, it is already known that it has an antitumor effect, by causing the increase of reactive oxygen species (ROS)[2,3].**Aim:** So, the objective of this research was to evaluate the antitumor action of menadione against cervical cancer (Siha and Hela cells), as well as the production of reactive oxygen species and the reduced glutathione (GSH).**Methods:**Cytotoxicity was evaluated by MTT method, cell morphology, ROS by H_2DCFDA marker, and GSH by OPT (o-phthalaldehyde) reagent. **Results:**For the MTT assay, the $IC_{50}$and IC_{90} for menadione, respectively, was 2.09±0.05; 3.87±0.09 µg/mL for Siha cells, 2.95±0.04; 5.59±0.21 µg/mL for HeLa cells and 2.68±0.25; 5.27±0.58 µg/mL for HaCaT cells.Alterations of morphology were observed in cells treated with $IC_{50}$and IC_{90} of menadione, including irregularity of shape and cellular detachment, but cells pre-incubated with *N*-acetylcysteine (NAC), presented the preservation of morphology.When compared tothe negative control, there was a higher ROS production after the treatments for both cells and the pre-incubation with NAC reduced that production.For the quantification of reduced glutathione levels, there was a small decrease in treatments, which may indicate the oxidative stress process.**Conclusion:**Therefore, menadione treatment may be a good candidate for the treatment of cervical cancer, but further studies are needed.

Acknowledgments: CAPES; CNPq, FINEP, Fundação Araucária/PRONEX.

References:

(1) Xia, C. et al. Metformin inhibits cervical cancer cell proliferation by modulating PI3K/Akt-induced major histocompatibility complex class I-related chain A gene expression. Journal of Experimental and Clinical Cancer Research, v. 39, n. 1, p. 1–12, 2020.
(2) Acharya, B. R. et al. Vitamin K3 disrupts the microtubule networks by binding to tubulin: A novel mechanism of its antiproliferative activity. Biochemistry, v. 48, n. 29, p. 6963–6974, 2009.
(3) Ko, L. W.; Odawara, T.; Yen, S. H. C. Menadione-induced tau dephosphorylation in cultured human neuroblastoma cells. Brain Research, v. 760, n. 1–2, p. 118–128, 1997.

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SYRINGIC ACID PROTECTS L-929 FIBROBLASTS FROM UVB RADIATION AND CONTRIBUTES TO WOUND HEALING

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Keywords: **Antioxidant; ROS; UV protection; Syringic acid**

Introduction: The skin has many functions including vitamin D production, thermoregulation and protection againstchemical and physicalagents[1].Exposure to UVB radiationis associated withsuperficial burn on the skin, commonly seen after exposure without any protective agent[2].Among the main mechanisms that lead to damage from exposure to solar radiation is the generation of free radicals[3].To neutralize the deleterious effects of oxidizing molecules, the human body is equipped with some antioxidant components[4].Syringic acid (SA) is classified as a phenolic acid derived from hydroxybenzoic acid. SA has antioxidant activity and it becomes the target ofphotochemoprotection researches[5].**Aim:**The objective of this work was to evaluate the antioxidant activity, cell cytotoxicity and photochemoprotection of SA against UVB radiation in the L-929 fibroblast cell line. **Methods:**The first tests with SA were of antioxidant activity, in order to evaluate the DPPH and the $ABTS^{\cdot+}$free radical scavenging, evaluate the iron reducing potential (FRAP) and the antioxidant activity through the Xanthine/system XOD/Luminol. After that, SA cytotoxicity in L-929 fibroblasts was evaluated by MTT method. Cell viability tests were carried out together with UVB radiation, in order to evaluate the photochemoprotection of SA. The concentrations used were 100, 50, 25 and 10 µM, and the radiation dose was 500 mJ/cm^2. Reduced glutathione level (GSH) quantified using O-phthaldialdehyde fluorescence and Catalase activity (CAT) assessed by H_2O_2 consumption, were evaluated. A wound healing assay on L-929 fibroblasts was performed to assess whether SA would be able to influence cell migration.**Results:**The results showed excellent activity when comparing SA with a standard substance, quercetin (QT). Cell viability results showed that, in the concentration range from 9.81 to 630 µM, SA was not cytotoxic to cells and it could protect L-929 from UVB radiation. SA was able to significantly reduce the production of total ROS, immediately after exposure to radiation (0h), 1, 2 and 3h after UV irradiation. The levels of GSH and catalase were significantly higher with SA treatment when compared to cells irradiated with UVB and without treatment.Finally, it could be observed that SA contributed to cell migration.**Conclusion:** It is concluded that SA has photochemoprotective activity and contributes to wound healing, being an active potential in multifunctional photoprotective formulations.

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References:

(1) Talikowska M, Fu X, Lisak G. Biosensors and Bioelectronics Application of conducting polymers to wound care and skin tissue engineering : A review. Biosensors and Bioelectronic. 2019; 135:50–63.
(2) Masaki H. Role of antioxidants in the skin: Anti-aging effects. Journal of Dermatological Science. 2010; 58:85–90.
(3) Barbosa KBF, Costa NBM, Alfenas RDCGet al. Estresse oxidativo: Conceito, implicações e fatores modulatórios. Revista de Nutrição. 2010; 23:629–643.
(4) Birben E, Sahiner UM, Sackesen C. et al.Oxidative Stress and Antioxidant Defense. World Allergy Organization Journal. 2012; 44:9–19.
(5) Escher GB, Marques MB, Carmo MA et al. *Clitoria ternatea L.* petal bioactive compounds display antioxidant, antihemolytic and antihypertensive effects, inhibit α-amylase and α-glucosidase activities and reduce human LDL cholesterol and DNA induced oxidation. Food Research International. 2020; 128:1-14.

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INCLUSION OF ALGINATE MICROPARTICLES CONTAINING BERBERINE AND FLUCONAZOLE IN PHARMACEUTICAL FORM CREAM AND ARTIFICIAL SALIVA FOR THE TREATMENT OF VULVOVAGINAL AND ORAL CANDIDIASIS

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Keywords: **Berberine; Fluconazole; *Candida* spp.**

Introduction: *Candida* spp. is a commensal microorganism that can remain in the body without major damage. However, if there is any kind of imbalance in the host, they may manifest orally, intestinally or vaginally (1).Berberine (BBR), an alkaloid widely found in plant families including *Hydrastis canadensis* (goldenseal), *Berberis aquifolium* (Oregon grape), and *Berberis vulgaris* (barberry), is currently demonstrated to have antimicrobial activity against different kinds of organisms (2). Fluconazole is a well-tolerated antifungal triazole and a safe agent that exhibits good clinical activity against most *Candida*spp, but already shows fungal resistance due to its indiscriminate use (3)**.Aim:**Produce microparticles containing two substances that present synergism, performingthe physical, chemical and microbiological analysisaiming at the inclusion of these in a vaginal cream base and in an oral suspension (artificial saliva) for future testing of these two products in humans.**Methods:**The microparticles were produced by the spray drying technique to encapsulate Berberine and Fluconazole; morphological analysis, scanning electron microscopy, minimal inhibition assay (MIC), anti-biofilm effect in the formation phase, inclusion of microparticles in the vaginal cream base and saliva artificial, physical-chemical and microbiological tests of the final products, texture and component release test by RMNwere performed (4). **Results:**Analyzing the microparticles obtained through the spray dryer technique by scanning electron microscopy, it was possible to detect a structure consistent with the literature, demonstrating a circular morphology with some holes on its surface.The structure of the micro-organism treated with the microparticle and the drugs in their isolated form was also analyzed through scanning electron microscopy,and it was possible to observe the damage to the yeast surface as well as the reduction of its colony in the samples treated with microparticles when compared with the isolated forms of the drugs. The physical-chemical activities of the final products were as expected in the literature, the microbiological tests also showed an effect against the tested microorganism, texture was within the standard and the release tests of the microparticle components are still in progress.**Conclusion:**Thus far, it was concluded that the microparticle containing Berberine and Fluconazole presented synergistic activity against *Candida albicans* as well as in the final products - vaginal cream and artificial saliva observed in an agar diffusion test.

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References:

(1) Kashem, S. W., Igyártó, B. Z., Gerami-Nejad, M., Kumamoto, Y., Mohammed, J., Jarrett, E., Drummond, R. A., Zurawski, S. M., Zurawski, G., Berman, J. Candida albicans morphology and dendritic cell subsets determine T helper cell differentiation. Immunity.2015;42, 356- 366.
(2) Li Ping et al. Synergistic antifungal activity of berberine derivative B-7b and fluconazole. PLoS One.2015; v. 10, n. 5, p. e0126393.
(3) Yang, Z. et al. Fluconazole inhibits the cellular ergosterol synthesis to confer synergism to berberine against yeast cells. Journal of global antimicrobial resistance.2018; v. 13, p. 125-130.
(4) CLSI. Manual Clinical and Laboratory Standards Institute. Reference methods for broth dilution antifungal susceptibility tests for yeasts; approved standards, CLSI document M27-A3,Wayne, PA., 2008.