UNIVERSIDADE FEDERAL
DE SANTA CATARINA

CurTo

CURRENT TOPICS
IN BIOCHEMISTRY

ABSTRACT BOOK

May 21th - 24th 2018 | Florianópolis, SC - Brazil

Current Topics in Biochemistry

ABSTRACT BOOK

Department of Biochemistry
Biological Sciences Centre
Federal University of Santa Catarina

Support: UFSC and FAPESC

MESSAGE FROM THE ORGANIZING COMMITTEE:

Welcome to Current Topics in Biochemistry (CurToBiochem), a symposium that aims to promote interdisciplinary scientific communication and discuss cutting-edge researches in Biochemistry, facilitating the networking among graduate students (advanced Master's Degree, PhD, MD, and Post-docs), principal investigators and professionals, bringing wide-ranging benefits to the society.

University Federal de Santa Catarina (UFSC) is moving towards internationalization, so that all written materials is in English language, including abstracts and posters. *However, oral/platform presentations and discussions can be presented in English, Spanish or Portuguese.*

CurToBiochem offers a variety of topics from biological, physic-chemical and health sciences, with a biochemical background, providing novel insights through collaborative efforts.

We are very happy for recieving you to in this exciting meeting with constructive discussions related to Biochemistry.

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SUMMARY

SCIENTIFIC PROGRAM

MAY 21, MONDAY		
8:00-9:00	**Accreditation**	
8:30-9:10	**Opening ceremony**	
Biochemistry of neuropsychiatric disorders		
9:10-10:00	Sâmia Regiane Lourenço Joca	New mechanisms for new antidepressants
10:00-10:30	Coffee break	
10:30-11:20	Ana Lúcia Severo Rodrigues	Glutamatergic modulators as fast-acting antidepressants
11:20-12:00	Manuella Pinto Kaster	Genetic variation in the NLRP3 gene and major depressive disorder
12:00-13:30	Lunch	
Biochemistry of neurodegenerative conditions neurotoxicity and neuroptotection		
13:30-14:20	Carla Inês Tasca	Purinergic and glutamatergic crosstalk as a protective mechanism against neurodegeneration
14:20-14:40	Andiara Espíndola de Freitas	Agmatine potentiates neuroprotective effects of subthreshold concentrations of ketamine via mTOR/S6 kinase signaling pathway
14:40-15:00	Jozimar Carlos Szczepanik	Altered glyoxalase system and dopamine levels were associated with poorer working memory performance after repeated treatment with methylglyoxal in mice
15:00-15:30	Coffee break	
15:30-16:20	Marcelo Farina	Neuroprotective effects of probucol and related-molecules
16:20-16:40	Ruth Liliám Quispe Gaspar	Diphenyl diselenide protects against the generation of superoxide anion and mitochondrial dysfunction in immortalized mouse hippocampal cell line (HT22) exposed to tBuOOH
16:40-17:00	Eduardo Benedetti Parisotto	Role of melatonin in neurodevelopment and redox imbalance in an animal model of Down syndrome
17:00-18:00	Stevens Kastrup Rehen	New insights about the biology of zika virus infection using human stem cells

MAY 22, TUESDAY		
Biochemistry of cancer		
8:30-9:20	Fábio Klamt	Bioenergetics in a cell model of melanoma's aggressiveness
9:20-10:00	Alfeu Zanotto Filho	NRF2 inhibits endoplasmic reticulum stress-mediated apoptosis by inducing GSH synthesis and protein thiol homeostasis in cancer cells exposed to alkylation
10:00-10:30	Coffee break	
10:30-11:20	Rozangela Curi Pedroza	DNA damage and triggering apoptosis: an old molecular target and promising novel molecules for cancer treatments
11:20-11:40	Naira Fernanda Zanchett Schneider	Cardiac glycoside glucoevatromonoside induces cancer type-specific cell death
11:40-12:00	Fabiana Ourique da Silva	Selenium-indole compounds monosubstituted interact with DNA and cause cytotoxicity in breast cancer cells
12:00-13:30	Lunch	
Biochemical aspects of metabolic disorders		
13:30-14:20	Fátima Regina Mena Barreto Silva	Potential anti-hyperglycemic and insulinomimetic effect of the nutraceutical theobromine
14:20-14:40	Marisa Jádna Silva Frederico	p-Methyl-phenyl-sulfonamideacts on insulin and incretin secretagogue and stimulates glucose uptake in insulin resistant rats
14:40-15:00	Débora da Luz Scheffer	Sepiapterin reductase inhibitors reduce rheumatic pain and increase urinary sepiapterin
15:00-15:30	Coffee break	
15:30-16:20	Alexandra Susana Latini	Modulation of brain tetrahydrobiopterin metabolism signals antioxidant pathways, anti-inflammation and enhanced cognition
16:20-17:00	Joana Margarida Navalho Gaspar	Downregulation of HIF complex in the hypothalamus exacerbates diet-induced obesity

MAY 23, WEDNESDAY		
Biomarkers of chemical exposure and toxicity		
8:30-9:20	José Maria Monserrat	New strategies for the toxicological evaluation of carbon nanomaterials
9:20-9:40	Tomás Bohn Pessatti	Alternative splicing for intracellular lipid binding protein in the oyster Crassostrea gigas
9:40-10:00	Flávia Lucena Zacchi	Molecular and biochemical biotransformation responses in oysters *Crassostrea brasiliana* (Lamarck, 1819) exposed to polycyclic aromatic hydrocarbons.
10:00-10:30	Coffee break	
10:30-11:20	Afonso Celso Dias Bainy	Identification of molecular biomarkers of exposure to contaminants in aquatic organisms
11:20-12:00	Carlos Henrique Lemos Soares	Metabolomic analysis as a tool in ecotoxicological studies
12:00-13:30	Lunch	
Biotechnology and structural biochemistry		
13:30-14:20	Hernán Francisco Terenzi	Protein phosphatases as model systems: from biomimetics to post-expression mutagenesis
14:20-15:10	Boris Juan Carlos Ugarte Stambuk	Biochemical and molecular biology challenges of first- and second-generation bioethanol production in Brazil
15:10-15:30	Ruth Fernandes Rocha	S-nitrosilation of protein tyrosine phosphatase YopH and its influence on protein structure and catalytic activity
15:30-15:50	Rodrigo Augusto da Silva	Epigenetic control of HOXA gene family cluster in osteoblastic differentiation
16:00-18:00	**Coffee break - POSTER SESSION**	

MAY 24, THURSDAY		
Oxidative/nitrosative stress, redox status and biological implications		
8:30-9:20	Sayuri Miyamoto	Lipidomics and oxi-lipidomics as powerful tools for mapping lipid alterations in diseases
9:20-10:10	Alcir Luiz Dafré	There is a link between the AMPK-independent modulation of thioredoxin interacting protein (Txnip) and inhibition of thioredoxin (Trx)
10:10-10:40	Coffee break	
10:40-11:30	Andreza Fabro de Bem	Metabolic derangements predisposing to neurodegenerative disease: Selenium compounds as a promising therapy
11:30-12:00	**Closing Ceremony – Poster Award – Future Meetings**	

ABSTRACTS

A. Biochemistry of cancer

A1

DNA DAMAGE AND CYTOTOXICITY OF NOVEL SELENO-DIHYDROPYRIMIDINONE AGAINST CANCER CELLS

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Introduction: The medicinal properties of dihydropyrimidinone derivatives and organic selenium compounds have been related as an important strategy in drug discovery as novel chemotherapeutic agents for any types of cancer including breast carcinoma. The effects of these compounds have been reported with a potential to binding to DNA, cell cycle arrest and apoptosis. **Objectives:** The aim for this study was to elucidate the antitumor activity of seleno-dihydropyrimidinone compound, named J2. **Material and Methods:** The cytotoxicity of the compound was verified by MTT assay after the treatment of cells for 72 h, in MCF-7 (breast cancer), HeLa (cervix cancer) and McCoy (normal) cells. The interaction between test compound and CT-DNA (thymus calf DNA) was done through UV-Vis spectrophotometry using a fixed concentration of CT-DNA (150 μM) and different compound concentration (50 μM to 350 μM). The clonogenic assay, comet assay and cell death (propidium iodide/acridine orange) analysis were performed in MCF-7 cells, after 72 h of treatment with non-toxic concentration of the compound (J2 = 7.85 μM). **Results and Discussion**: The MTT assay showed interesting cytotoxicity activity for MCF-7 (IC_{50} = 13.09 μM) and HeLa (IC_{50} = 22.38 μM) cells, but mainly against MCF-7 cell. The J2 compound caused contraction in conformation of CT-DNA (hyperchromism) and intercalation into DNA. In addition, the J2 compound caused DNA cleavage in MCF-7 cells and induction of apoptosis. **Conclusion:** The selenourea J2 seems to be a promising compound for the development of a new antitumor drug, since demonstrated higher cytotoxic and antiproliferative activities in a lower concentration. Also, it was able to induce DNA cleavage and cell death mainly by apoptosis.

Keywords: Cancer, selenoureas, cytotoxicity, DNA damage, apoptosis.

A2

SELENIUM-INDOLE COMPOUNDS MONOSUBSTITUTED INTERACT WITH DNA AND CAUSE CYTOTOXICITY IN BREAST CANCER CELLS

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Introduction: Previous studies have shown the antitumor activity of indolic compounds and selenium separately. Thus, studies of antitumor activity and possible mechanism of action of selenium-indole compounds have been gaining considerable interest in the medicinal chemistry. **Objective:** The aim of this work was to evaluate the antitumor activity of four selenium-indole compounds monosubstituted, comparing their activity with the selenium-indole nucleus. **Material and Methods:** The cytotoxicity of selenium-indole compounds (0.1 to 1000μM, 72 h) was evaluated in MCF-7 cells by assay tetrazolium salt (MTT), to obtaining the IC_{50}. The interaction and intercalation of these compounds (100 to 400 μM) with calf thymus DNA (CT-DNA 150μM) was evaluated by ultraviolet-visible spectrophotometry and by fluorescence measurement using the DNA intercalating propidium iodide (PI), respectively. Then, using the subtoxic concentration (33.66 μM) was evaluated the antiproliferative activity by the colony assay and proapoptotic activity was evaluated with propidium iodide (PI)/orange acridine (OA). **Discussion and Results:** The cytotoxicity evaluation has shown that the addition of R = $Phe^{(1)}$, Phe-OCH3, Phe-Cl and $Phe^{(2)}$ to the selenium-indole nucleus increased their cytotoxicity to MCF-7 cells ($IC_{50(N)}$ =1489 μM, $IC_{50Phe(1)}$ = 56.11 μM, $IC_{50Phe\text{-}OCH3}$ = 306.20 μM, $IC_{50Phe\text{-}Cl}$ = 694 μM and $IC_{50Phe(2)}$ = 115.80 μM). The $Phe^{(1)}$ was the most cytotoxic compound, thus it was chosen to continue the other assays. The interaction between $Phe^{(1)}$ with CT-DNA revealed that $Phe^{(1)}$ possess the hypochromic effect (absorbance reduction) and also decreased fluorescence of IP, suggesting that $Phe^{(1)}$ has the capable to bind to CT-DNA by intercalation. The $Phe^{(1)}$ showed a significant antiproliferative effect by inhibiting colonies almost in 50%. Moreover, $Phe^{(1)}$ has caused the cell death mainly by necrosis (26.19 ± 6.69%). The results suggest that $Phe^{(1)}$ can bind to DNA by intercalation, which may damage the DNA in tumor cell. Thereby

having a cytotoxic effect, triggering the cell death and consequently decreasing proliferation of tumor cells. **Conclusion:** The addition of Phe(1) to selenium indole nucleus increased their antitumor effect evidencing this compound as a promising antitumor molecule.

Keywords: Indole, selenium, cancer, cytotoxicity, DNA damage, antiproliferative.

A3

INTRANASAL PERILLYL ALCOHOL-BASED THERAPY EFFECTIVELY REDUCED CIRCULATING CELL-FREE DNA AND INCREASED SURVIVAL OF PATIENTS WITH BRAIN TUMOR

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Glioblastoma (GBM) and brain metastasis (BM) disseminate into brain parenchyma due to beneficial interactions between high proliferative, anabolic and invasive tumor cells and the microenvironment. Complete surgical tumor resection and image detectionare usually underscored considering the diffuse and ill-defined borders favoring tumor recurrence. Besides, brain biopsy does not reflect the complete molecular complexity and dynamicof tumor activity.Molecular markers are used to increase diagnostic accuracy, but with limited monitoring application. Circulating DNA (cfDNA) is detected in human serum as a result of apoptosis, necrosis or active release from proliferatingcells. Cancer patients present high concentration of small double stranded cfDNA with similar genetic and epigenetic alteration of the tumor tissue, thus representing a useful biomarker. Intranasal administration of perillyl alcohol (POH), a naturally occurring monoterpene has recognized efficacy for GBM temozolomide (TMZ) resistant patients, due to its cytotoxic, pro-apoptotic, anti-inflammatory and antiangiogenic properties. We evaluate the potential of cfDNA as molecular indicator of response to POH intranasal administration (ITN) therapy of patients with brain tumors. The cohort included 130 healthy subjects as control-paired group and patients at terminal stage with GBM(n=122) and BM (n=55) from stage IV adenocarcinoma. Serum cfDNA was isolated and quantified by fluorimetry. Compared to control (40.48± 0.52 ng/ml), patients with brain tumor before ITN-POH treatment had increased ($p<0.0001$) cfDNA levels, GBM (1,237.50 ± 314.29 ng/ml) and BM (1,237.55 ± 255.21 ng/ml). ITN-POH treatment caused a significant correlation (rho= -0.225; $p=0.024$) with survival>6 months (599 ± 221 ng/ml) and <6 months (1,626 ± 505 ng/ml), but a sharp and abrupt increase of cfDNA and tumor recurrence occurred after ITN-POH discontinuation. Patients under continuous ITN-POH treatment and with brain magnetic resonance image(MRI) compatible of stable disease had cfDNA levels similar to controls. cfDNA may be used as noninvasive prognostic and response molecular marker to ITN-POH therapy in brain tumors, representing a screening tool with accuracy for early detection of tumor progression.

Keywords: cell-freeDNA,circulating nucleic acids (CNA), perillyl alcohol, intranasal therapy, glioblastoma, brain metastasis.

A4

CYTOTOXIC ACTIVITY OF LECTINS FROM CANAVALIA BRASILIENSIS (ConBr) AND DIOCLEA REFLEXA (DrfL I) SEEDS ON C6 GLIOMA CELLS

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Introduction: Glioblastoma multiform (GBM) is the most aggressive type of glioma, with unfavorable prognosis and average survival of patients approximately 12 months. Taken in account a limited therapy available, new bioactive molecules have been investigated. Lectins are proteins of non-immune origin that bind glycans on cell surface displaying a diversity of biological activities. Since cancer cells may express modifications in the glycosilation pattern, the ability of plant lectins to induce selective tumoral cell death has been investigated. **Objectives:** the present study was designed to determine the cytotoxicity of the DrfL I and ConBr lectins against rat glioma cell lineage C6, characterizing a possible mechanism for this action. **Material and Methods:** The technique of affinity chromatography was used to purify the lectins. Cell viability was tested by the MTT method; the fluorescence microscopy assays for cell death and autophagy analysis were performed with the propidium iodide and acridine orange dyes, respectively; LC3 protein expression (autophagy marker) was analyzed by western blot; detection of cytostatic effects was performed by migration and clonogenic assays. **Results and Discussion:** The results showed that DrfL I and ConBr lectins (30 and 50 μg/mL) decreased cell viability (measured by MTT assay) and induced morphological changes (assessed by optic and fluorescence microscopy) on C6 cells. The lectins also induced inhibition of cell migration and clonogenic survival, especially at highest concentration of 50 μg/mL. Moreover, both ConBr and DrfL I were able to induce autophagy of C6 cells, as evaluated by acridin orange stain and western blot assay. **Conclusion:** Our study suggests an antitumor potential for DrfL I and ConBr, considering the induction of

autophagy and cytostatic effect. Moreover, future studies will be necessary in order to ascertain the DrfL I and ConBr mechanisms using in vitro and in vivo models of gliomas.

Keywords: Glioma, lectins, DrfL I, ConBr, autophagy

A5

PREDICTING MOLECULAR INTERACTIONS *IN SILICO* OF NOVEL SELENOESTER DIHYDROPYRIMIDINONE WITH dsDNA AND WITH PROTEINS HIGHLY EXPRESSED IN CANCER CELLS

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Introduction: Various studies demonstrated the importance of dihydropyrimidinones (DHPMs) and organoselenium compounds due their biological and pharmacological properties. Molecular docking improves rational drug design through *in silico* predictions done with macromolecule–ligand complex. Besides, DNA and proteins highly expressed in cancer cells can be target by new drugs developed to cancer treatment based on the inhibition of growth signals and triggering apoptosis. **Objectives:** This study aim to predict *in silico* molecular interactions between novel selenoester dihydropyrimidinone (ligand A) and macromolecules. **Material and Methods:** Ligand A 3D conformer with lowest energy level (Dreiding force field; Marvin Sketch ChemAxon) was converted to pdb format (Openbabel). Structures of dsDNA (PDB: 5OCZ), thioredoxin reductase (TrxR) (PDB: 1H6V) and Bcl-xL (PDB: 4TUH) were prepared (AutoDock MGLTools) and docked (AutoDock Vina), using default parameters. Predicted poses were visualized with LigPlot+ and PyMOL open source 1.8. **Results and Discussion:** Ligand A predicted pose 1 has the highest binding affinity energy (-7.8kcal/mol) and showed H-bond between the tetrahydropyrimidine moiety and thymine nucleotide (dt22; Chain B). Pose 2 and pose 3 were H-bonded with guanine (dg5, Chain A) (RMSD 3.12 and 2.89, respectively). Bcl-xL ring (C14-19) has hydrophobic interactions with DNA nucleotides positioned at chain A (dt 6; da 7; dc 8) and chain B (dt 18; da 19; da 21; dt 22; dc 23). Docking predicted that residue Thr^{373} of TrxR (chain A) was H-bonded with ligand A at pose 1 (binding affinity -8.3kcal/mol) and at pose 2 (RMSD 1.54). Ligand showed hydrophobic interactions with Lys^{67} (Pose 1; 47.73126) and Lys^{68} (Pose 2; 54.47784). Lys^{67} is the same residue where FAD is hydrogen bonded to TrxR. Ligand A (pose 1) also formed H-bonds with Bcl-xL chain A at residue Tyr^{195} (affinity -8.8kcal/mol) and at residue Asn^{136} (Pose 2; RMSD 1.771) as previously seen in Bcl-xL inhibitor. Hydrophobic interactions between ligand A and Bcl-xL (chain A) appeared at Tyr^{101}; Asp^{136}; Gly^{138} and at other residues at ring C14-19 (Leu^{130}; Arg^{139}; Phe^{97}; Phe^{105}; Ala^{142}). **Conclusions:** Based on predicted ligand A results, the interaction target-macromolecules through H-bond formation and hydrophobic interactions potentially suggests inhibitory interactions.

Supported by: CNPq, CAPES/PNPD

Keywords: dihydropyrimidinones, docking, dsDNA.

A6

EVALUATION OF ANTITUMOR POTENTIAL OF LECTIN FROM *Canavalia grandiflora* (ConGF) IN C6 GLIOMA CELLS

Mann, J.[1]*; Wolin, I.A.V.[1]; Nascimento, A.P.M.[1]; Heinrich, I.A.[1]; Welter, P.G.[1]; Cavada, B.S.[2]; Leal, R.B.[1] - [1]Departamento de Bioquímica, CCB-UFSC, Florianópolis, Brazil; [2]Departamento de Bioquímica e Biologia Molecular, BioMol-Lab-UFC, Fortaleza, Brazil. *E-mail: josianemann92@gmail.com

Glioblastoma (GBM) are the most aggressive tumors of the central nervous system. The prognosis of patients with GBM remains dismal, mainly because it is a cancer very resistant to therapies currently employed beyond their invasive nature. A characteristic that has been associated with malignant transformation and tumor progression is the presence of changes in the cellular glycosylation pattern. Lectins are carbohydrate recognition and binding proteins present in glycoconjugates being able to regulate numerous cellular processes in physiological and pathological events. ConA lectin with mannose/glucose-binding specificity, has shown to have antitumor activity, being able to trigger pro-apoptotic and autophagic signaling. ConGF is a mannose/glucose-binding lectin isolated from *Canavalia grandiflora* seeds that have mannose/glucose-binding activity like ConA. However, ConGF effects on the GBM are largely unknown. The present study aimed to investigate the antitumor effect of ConGF on C6 lines cell, as well as the related mechanism of cell death. Our results showed that ConGF (30-100μg/mL) reduces viability (measured by the MTT assay) and induced morphological changes (assessed by optic and fluorescence microscopy) on C6 cells. This lectin is also very promising in inhibiting proliferation rate of C6 cells, besides affecting the mitochondrial membrane potential. However, carbohydrate recognition domain blocker (CRD) assays demonstrated that the action of ConGF was dependent on CRD, since it had no effect after its preincubation with α-methyl-D-mannoside. Analysis of cell migration showed that treatment with ConGF at low concentrations was effective in promoting the inhibition of cell migration. We demonstrated that the treatment with ConGF promoted the induction of the autophagy process in the C6 lineage, evaluated by acridine orange stain. In addition, increased expression of the protein recruited during

autophagy (LC3AB-II) was observed in cells treated with ConGF. Therefore, we conclude that ConGF showed potent antitumor activity *in vitro*, and this effect may be related to the induction of the autophagy process. Further studies should be conducted to confirm these proposals and to evaluate the mechanism of action of this lectin.

Keywords: glioblastoma, lectin, ConGF, autophagy.

A7

KETOGENIC DIET WITH CONCOMITANT INTRANASAL PERILLYL ALCOHOL: STRATEGY THERAPY FOR DELAYING GROWTH OF RECURRENT GLIOBLASTOMA

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Background: Glioblastoma (GBM) is a highly aggressive primary brain cancer that is difficult to treat. The current standard of care consists of surgery, followed by radiation and chemotherapy with the alkylating agent temozolomide (TMZ). It has been hypothesized that ketogenic diet (KD) might represent a potential therapeutic strategy against high-grade gliomas. Perillyl alcohol (POH) is a non-toxic, naturally-occurring, hydroxylated monoterpene that exhibits cytotoxicity against temozolomide-resistant glioma cells, regardless of O6-methylguanine-methyltransferase promoter methylation status. This study aimed to evaluate the therapeutic efficacy of intranasal POH administered in combination with a ketogenic diet (KD) program for the treatment of patients with recurrent glioblastoma. **Methods**: Thirty-two patients, treated with intranasal POH (55 mg, four times daily), were divided into two groups - KD (n=17) or standard diet (n=15). The nutritional status and anthropometric parameters of patients were measured. Patients maintained the combination treatments for three months. Neurological examination and magnetic resonance imaging were used to monitor disease progression. In the KD patient, strict compliance with the KD was confirmed by measuring the levels of ketone bodies in the urine three times a week. **Results:** The effect of KD in combination with intranasal POH was significant in the reduction of tumor mass ($p = 0.035$; 33.8%) compared to the control group, as demonstrated by magnetic resonance. There was no significant difference in glucose levels at baseline and after 90 days between groups. However, statistical significance was observed between the levels of total cholesterol ($p = 0.003$), LDL-c ($p = 0.0002$) and triglycerides ($p = 0.004$) in treated group only. The KD combined with intranasal POH had a favorable response in reducing tumor growth. **Conclusion:** Altogether, the results suggest that KD associated with intranasal POH may represent a viable option as an adjunct therapy for recurrent GBM.

Keywords: glioblastoma, ketogenic diet, cancer.

A8

DIGITOXIGENIN BISDIGITOXOSIDE: A CYTOTOXIC NATURAL CARDENOLIDE

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Since lung cancer is the most aggressive and deadly type of cancer, research, development and innovation of new anticancer drugs are necessary. Digitoxigenin bisdigitoxoside (DGXB) is a cardenolide extracted from *Digitalis lanata*, and was investigated against two non-small cell lung cancer (NSCLC): A549 and H460. The objective of this study was to stablish DGXB impacts on NSCLC mechanism. All experiments described were performed after 24 h and/or 48 h of DGXB treatment. First, the DGXB inhibitory concentration to 50% of NSCLC (IC_{50}) was determined by sulforhodamine B assay (SFB). Propidium iodide (PI) staining was used to quantify cell cycle progression. The autophagy inhibitor chloroquine (CQ) was used to evaluate cytotoxicity reduction by SFB assay, and associated with acridine orange staining to observe acidic vesicular organelles (AVOs). Annexin V/PI was applied to investigate apoptosis and necrosis. Cell migration was performed by scratch assay, and the clonogenic survival was used to determine a long-term treatment impact on proliferation. The DGXB IC_{50} stablished for both NSCLC was 25nM. Then, to evaluate the mechanistic aspects, the concentrations used were $0.5 \times IC_{50}$ (scratch); 1, 2 and $4 \times IC_{50}$, for all experiments. Only A549 showed cell cycle interference, mainly arresting cells in subG0 phase. CQ co-treatment promoted a slight but not significate cytotoxic protection only in A549 cells. AVOs appeared in both cells after 48h. Apoptosis and necrosis were not induced in A549, and only $4 \times IC_{50}$ increased the number of H460 cells in early apoptosis. $1 \times IC_{50}$ inhibited A549 migration at 24 and 48h, but showed a slight impact on H460 cells. All concentrations reduced significantly A549 colony formation; 2 and $4 \times IC_{50}$ reduced H460 clonogenic survival. Taken together, autophagy, apoptosis and necrosis did not seem to interfere on DGXB cytotoxic mechanism of action. Clonogenic survival and migratory potential were reduced in both tumoral lung cells, especially in A549. DGXB mechanism of action in H460 remains unclear, but its effectiveness is robust against both tumoral lung cell lines. A549 is the most prevalent cell type in patients with lung cancer, and was the most sensitive NSCLC to DGXB

treatment.

Keywords: cardenolides, Digitoxigenin bisdigitoxoside, cytotoxicity, lung cancer, A549 and H460 cells.

A9

EVALUATION OF NOVEL SEMI-SYNTHETIC CARDENOLIDES WITH PROMISING *IN VITRO* ANTITUMOR ACTIVITY

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Cardenolides are cardiotonic glycosides found mainly in plants, and from the therapeutic point of view the most important are digoxin and digitoxin. Despite the wide use of cardenolides as positive inotropic agents, recently, they have been presenting new therapeutic possibilities. One of them is their potential anticancer action, since their cytotoxic and antitumor effects have already been reported. In this sense, we present here the results of a cytotoxic screening of sixteen new semi-synthetic Cardenolide Derivatives (CDs). The most active compounds were selected to have their effects evaluated on cell cycle and phosphatidylserine exposure (Annexin V-FITC/propidium iodide). For the cytotoxic screening, CDs were tested on PC3, A549, HCT-8 and LNCaP cells for 48h, and stained with sulforhodamine B. CDs **6c**, **7c**, **10**, **11**, **12**, **13**, **14** and **16** inhibited cell proliferation, wherein compounds **10** and **11** were the most cytotoxic. A549 cell line was the most sensitive, and CDs presented IC_{50} values ranging from 0.11 to 2.86 µM. The most cytotoxic compounds on A549 cells were tested on another NSCLC (H460 cells) and the most promising were: **7c**, **10**, **11**, **12** and **16**. This cell line was even more sensitive to the treatments and, therefore, selected for the subsequent assays. To explore the effects on cell cycle, H460 cells were treated with CDs **7c**, **10**, **11**, **12**, **13** and **16** at their IC_{50}. CDs **7c**, **12**, **13** and **16** showed no alterations in cell cycle up to 24h. On the other hand, CDs **10** and **11** induced a significant increase of cells in subG1, which is indicative of apoptosis, and a slight decrease of cells in G1 phase. To identify whether the increase of cells in subG1 was associated with cell death, Annexin V-FITC/PI was evaluated. Both compounds increased the number of early and late apoptotic cells. Compound **10** presented a time-dependent increase showing 10.7% at 24h and 51.8% at 48h. CDs **7c**, **12**, **13** and **16** showed a slight increase of Annexin V-positive cells although this effect was not statistically significant up to 48h. Thus, these data suggested that new CDs were able to stop cell proliferation by inducing cell death in H460 cells. Several experiments are in course to evaluate the biochemical events involved in cell death prompted by them.

Keywords: screening, new cardenolides, cytotoxic effects, apoptosis.

A10

CARDIAC GLYCOSIDE GLUCOEVATROMONOSIDE INDUCES CANCER TYPE-SPECIFIC CELL DEATH

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Lung cancer is the most common form of cancer worldwide with a poor 5-year survival rate (+/-25%), despite the recent implementation of targeted therapies, thus yet clearly needing new treatment avenues to be discovered. Cardiac glycosides (CGs) are natural compounds used traditionally to treat congestive heart diseases. Recent investigations repositioned CGs as potential anticancer agents as they demonstrated to trigger different cell death mechanisms. To discover novel cytotoxic CG scaffolds, we selected the cardenolide glucoevatromonoside (GEV) out of 46 CGs for its low nanomolar anti-lung cancer activity and a series of experiments were carried out. GEV presented reduced toxicity toward non-cancerous cell types (lung MRC-5 and Peripheral Blood Mononuclear Cells - PBMC) and high-affinity binding to the Na+/K+-ATPase α subunit, assessed by computational docking. Additionally, GEV reduced the proliferation and the viability of various cancer cell types. GEV-induced cell death was caspase-independent, as investigated by a multiparametric approach, and culminates in severe morphological alterations in A549 non-small cell lung cancer, monitored by transmission electron microscopy, live cell imaging and flow cytometry. This non-canonical cell death was not preceded or accompanied by exacerbation of autophagy. In the presence of GEV, markers of autophagic flux (e.g. LC3I-II conversion) were impacted, even in presence of bafilomycin A1. Cell death induction remained unaffected by calpain, cathepsin, parthanatos, or necroptosis inhibitors. Furthermore, we extended

our mechanistic studies to an example of hematological cancer by selecting an acute myeloid leukemia cells U937, which exhibit a similar susceptibility to GEV compared to A549 cells to be within a comparable concentration range for the induction of cell death modalities. Interestingly, GEV triggered caspase-dependent apoptosis in U937, witnessing cancer-type specific cell death induction. Differential cell cycle modulation by this CG led to a G2/M arrest, cyclin B1 and p53 downregulation in A549, but not in U937 cells. We further extended the anti-cancer potential of GEV to 3D cell culture using clonogenic and spheroid formation assays and validated our findings *in vivo* by zebrafish xenografts. Altogether, GEV shows an interesting anticancer profile with the ability to exert cytotoxic effects via induction of different cell death modalities.

Keywords: cardiac glycoside, glucoevatromonoside, apoptosis, non-canonical cell death, lung cancer**.**

A11

EVALUATION OF ANTITUMOR POTENTIAL OF LECTINS FROM *DIOCLEA VIOLACEA* (DVL) AND *CANAVALIA ENSIFORMIS* (CONA) IN U-87 GLIOMA CELLS

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Introduction: Lectins correspond to a highly diverse group of proteins, which allows them to selectively recognize and bind reversibly to free sugars or glycoconjugates. Their selective effects have been studied in antitumor therapy for several types of cancers. **Objective:** To evaluate the antitumor / cytotoxic capacity of lectins derived from legumes in human glioma tumor (U-87). **Material and Methods:** The technique of affinity chromatography was used to obtain the lectins and their purities were analyzed by SDS page. The cell viability was tested by the classical MTT method in 24, 48, 72 and 96h after lectin exposure; the fluorescence microscopy assays were performed with the acridine orange dyes and propidium iodide, the mitochondrial potential was verified through the JC-1 probe and LC3 protein expression was analyzed by western blot. The migration assay was performed to determine their ability in generating cytostatic effects. **Results and Discussion:** It was initially observed that lectins were able to generate a decrease in cells viability after 24h exposure in U-87 lines; in addition, there was a greater incorporation of propidium iodide in cells treated with DVL compared to those treated with ConA. In contrast, the cells treated with ConA demonstrated more sustained staining by the acridine orange dye, demonstrating formation acidic vesicles. The membrane potential was altered by the lectins at 6h after treatments, indicating mitochondrial damages. The migration assay demonstrated the cytostatic effect was sustained in 10 μg/ml for DVL and 30μg/ml for both lectins in 24 and 48h. Finally, the western blot assay demonstrated that lectin treatment is capable to enhance the cleavage of the LC3AB I protein, thereby characterizing increased autophagy. **Conclusions:** Our results demonstrated that leguminous lectins have the capacity to cause changes in the mitochondrial potential and trigger an increase autophagic event, which may be involved in the cell death process.

Acknowledgment: Capes and CNPQ for financial support.

Keywords: Glioma, Lectins, autophagy, cytotoxicity

B. Biochemical aspects of metabolic disorders

B1

OMEGA-3 HAS BENEFICIAL EFFECTS ON VISCERAL FAT IN DIET-INDUCED OBESITY MODELS

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Introduction: Excess fat in the abdominal area is associated with inflammation, metabolic dysfunction, and comorbidities. The increased prevalence of obesity and difficulties in its treatment show the necessity to explore different therapeutic approaches. Because of its anti-inflammatory properties, the use of omega-3 polyunsaturated fatty acids may be a strategy to treat obesity. However, many effects and mechanisms are still unclear. The effect of omega-3 on alterations related to the inflammatory process, such as oxidative stress and mitochondrial dysfunction,

were little explored. **Objective:** To evaluate the effects of omega-3 on oxidative stress and energy metabolism parameters in visceral fat of mice with high-fat diet-induced obesity. Dietary intake, body weight, and visceral fat were also evaluated. **Methods:** Male Swiss mice with 40 days old received either a control diet or a high-fat diet (obese group) for 6 weeks. After this period, the groups were divided into Control + Saline, Control + Omega-3, Obese + Saline, and Obese + Omega-3. For the experiment, 400 mg/kg/day of fish oil (or saline) was administered orally, for 4 weeks. Food intake and body weight were monitored throughout the experiment. In the 10th week, the animals were euthanized and the visceral adipose tissue (mesenteric fat) was removed, weighed and used in the analysis of Krebs cycle, mitochondrial respiratory chain and oxidative stress**. Results:** Animals that received high-fat diet had body weight (11%) and visceral fat (25%) significantly higher than the group that received standard diet. Omega-3 neither affected food intake nor body weight, but it reduced visceral fat weight. In visceral fat, omega-3 reduced the oxidative damage, as well as mitigated alterations in antioxidant defense and in the Krebs cycle, caused by the intake of a high-fat diet. The mitochondrial respiratory chain was neither altered by obesity nor by omega-3 in visceral fat. **Conclusion:** The study concluded that omega-3 had a beneficial effect on the visceral fat of obese animals, since it mitigates the alterations caused by the consumption of a high-fat diet.

Keywords: obesity, adipose tissue, omega-3, fish oil, oxidative stress, energy metabolism.

B2

MECHANISM OF ACTION OF CAMPHORYL-BENZENE SULFONAMIDE DERIVATIVE ON TARGET TISSUES OF INSULIN

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Aim: To study the mechanism of action of a camphoryl-benzene sulfonamide derivative (**CS**) on glucose uptake, GLUT4 content, intestinal disaccharidases activities and advanced glycation endproducts (AGEs) formation. **Methodology:** Fasted male *Wistar* rats (180 – 200g) were divided into groups of 5 animals for glucose tolerance test (GTT): I) Hyperglycemic control (4 g/kg of glucose body weight intraperitoneally (i.p.); II) Hyperglycemic + **CS** (10 mg/kg, i.p.) and III) Hyperglycemic + **CS** (10 mg/kg, i.p.) + diazoxide (30 mg/kg, i.p.). ^{14}C-Glucose uptake was measured in adipose tissue from these hyperglycemic animals. Moreover, glucose uptake was evaluated in muscle or adipose tissue, and isolated adipocytes with or without **CS** (1, 10 and 100 μM) or diazoxide100μM and/or glibenclamide (20 μM). GLUT4 citosolic and plasma membrane content was measured with or without diazoxide (100 μM). GLUT4/β-actin mRNA was determined. Intestinal disaccharidases activity was evaluated *ex vivo*. AGEs were measured in a bovine serum albumin/glucose system *in vitro* (Protocol CEUA/UFSC/PP00398/749). **Results**: The stimulatory effect of the **CS** on glucose uptake on adipose tissue was blocked by diazoxide *in vivo* (hyperglicemic experiment) and in adipose tissue. **CS** was able to stimulate glucose uptake in isolated adipocytes, adipose tissue and in soleus muscle. On the other hand, the effects of **CS** were not blocked by glibenclamide, an inhibitor of the K+-ATP channel. **CS** did not increase mRNA levels for GLUT4 in the adipose tissue. *In vivo*, this compound reduced intestinal disaccharidase activity, while, *in vitro*, **CS** reduced the formation of AGEs at 7, 14 and 28 days of incubation. **Conclusion:** The stimulatory effect of **CS** on glucose uptake requires the activation of the K+-ATP channel, translocation and fusion of GLUT4 vesicles to the plasma membrane on adipocytes for glucose homeostasis. In addition, the inhibition of disaccharidase activity contributes to the glucose homeostasis in a short-term as well as the remarkable reduction in AGE formation indicates that the **CS** may prevent of complications of late diabetes.

Financial support: CNPq; CAPES; PPG-BQA PROAP/UFSC.

Keywords: camphoryl-benzene sulfonamide derivative, glucose uptake, GLUT4, intestinal disaccharidases, AGES.

B4

RAPID RESPONSE OF 1,25(OH)$_2$ VITAMIN D3 ON CALCIUM INFLUX IN INTESTINE OF *DANIO rerio*

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Calcium is an important ion involved in the signal transduction, cell proliferation and apoptosis. The calcium provided by the diet is absorbed by intestine and 1,25-(OH)$_2$ vitamin D$_3$ (1,25-D$_3$), a hormone from the vitamin D endocrine system, modulates calcemia through a genomic and non-genomic mechanism in the intestine and bone. The regulation of ionic balance in fish occurs mainly by gills. However, there is scarce data about how does the physiological hyperosmolar environment happens in fish and which are the body consequence of calcium unbalance.

AIMS: To study the effect and the mechanism of action of 1,25-D_3 on calcium influx in the intestine from *Danio rerio*. **METHODS:** *Danio rerio* was euthanized by spinal transection (CEUA/UFSC nº P00968). The whole intestine was dissected, cut longitudinally and preincubated *in vitro* in Cortland's buffer at 28ºC, pH 7.4 and bubbled with O_2:CO_2 mixture (95%:5%; v/v). For time-course (5, 15, 30 and 60) or concentration-response curves (1 pM, 1 nM and µM), $^{45}Ca^{2+}$ was added with/without 1,25-D_3 for 30 minutes at the end of incubation, $LaCl_3$ was added to block calcium flux. The aliquots from homogenized intestines were used for total protein and radioactivity determination. **RESULTS:** The time-course of calcium influx in the intestine from fed fish was unchanged from 5 to 30 min of incubation but significant increase at 60 min when compared with 5, 15 and 30 min. The time-course of calcium influx in the intestine from fasted *Danio rerio* increased progressively from 5 to 15 min and showed a significant pic at 30 min compared to the initial time of 5 min. So, in fasted animals, calcium influx reached the balance between 30 and 60 min of incubation and then further experiments were carried out at 30 min of incubation. The concentration-response curve was assayed in the calcium influx and was observed that the 1,25-D_3 stimulated significantly calcium influx at 1 pM. **CONCLUSION:** 1,25-D_3 acts at plasma membrane and stimulates rapid responses by increasing $^{45}Ca^{2+}$ influx on intestine of *Danio rerio*. Studies are underway to characterize channels and/or ionic exchangers modulated by this hormone to assure calcium balance in the intestine.

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Keywords: Rapid response,1,25-D3, calcium, intestine, *Danio rerio*.

B5

SEPIAPTERIN REDUCTASE INHIBITORS REDUCE RHEUMATIC PAIN AND INCREASE URINARY SEPIAPTERIN

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Introduction: Sepiapterin reductase (SPR) is an enzyme involved in the biosynthesis of tetrahydrobiopterin (BH4). BH4 is an essential co-factor for the aromatic amino acid hydroxylases and nitric oxide synthases; however excessive BH4 levels has been involved in inflammatory and neuropathic pain. **Objective**: Investigate the effect of SPR inhibition on autoimmune inflammation-induced pain in Balb/c adult mice using the collagen antibody-induced autoimmune arthritis (CAIA) model of rheumatoid arthritis. **Materials and Methods**: On Day 0, control mice were injected with non-specific IgG and model mice received an injection (1.5mg/mouse) of a cocktail of 5 monoclonal antibodies recognizing the conserved epitopes on various species of type II collagen. On Day 3 all mice were injected i.p. with LPS (50 µg/mouse) to enhance arthritis induction. CAIA induced 2 phases; an early phase characterized by physical signs of inflammation of the joints as well hypersensitivity and a late phase characterized by exaggerated responses to peripheral stimuli but no inflammation. In both phases, the mice received a dose of SPR inhibitor (SPRi3). Also, a group of 10 healthy volunteers collected one sample of urine on day 1 (pre) and then on the following 3 days took a 500mg tablet of sulfasalazine (SSZ; every 6 h; total 2g/day) and collected the first voided urine each following morning. **Results and discussion**: SPRi3, significantly attenuated heat hyperalgesia in both phases, and mechanical allodynia in the late phase. Urinary sepiapterin levels in the mice showed high sensitivity and specificity as a pharmacological biomarker of SPR inhibition. SSZ, clinically used to treat rheumatoid arthritis, inhibited SPR and increased urinary sepiapterin levels in both mice and healthy human volunteers. **Conclusion**: We conclude that SPR inhibition reduces pain associated with autoimmune joint inflammation and that urinary sepiapterin levels may have utility as a non-invasive biomarker in the clinical use of SPR inhibitors.

Keywords: sepiapterin, pain, inflammation

B6

MECHANISM ACTION OF ASTRAGALIN IN RAT PANCREATIC ISLETS: POTENTIAL SECRETAGOGUE OF INSULIN EFFECT

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Diabetes mellitus is a complex and multifactorial pathology considered as a public health problem worldwide, is

characterized by hyperglycemia caused by defects in the action or secretion of insulin. Most of the treatments help to maintain the homeostatic levels of glucose by intervening in the intestinal absorption of glucose, the secretion of pancreatic insulin or uptake, utilization and storage of glucose in tissues, however, the adverse effects, as well as cultural and economic factors make it continue in research of new options for this pathology. Many plants are used in folk medicine due to hypoglycemic properties, but little has been studied of the compounds and the molecular mechanisms responsible for this activity. **Objective**: The aim of this study was to characterize the effect of astragalin, a flavonol, found as one of the major compounds of ethanolic fraction of an aqueous extract of *Passiflora ligularis*, in signaling pathways involved in glucose homeostasis, to evaluate the hypoglycemic or antihyperglycemic activity in a curve of glucose tolerance and signaling pathways involved in the influx of calcium into isolated pancreatic islets. **Methods:** Male Wistar rats (50-55 days) were used. For the glucose tolerance curve, blood was collected for the determination of glucose and serum insulin in 5 timepoints after an oral glucose load of 4g/kg. Pancreatic islets were isolated and incubated with $^{45}Ca^{2+}$ and astragalin in the presence or absence of different inhibitors or activators of intracellular signaling pathways and channels. **Results:** Astragalin showed an antihyperglycemic effect when compared to the control at all timepoints, also 50 μM of astragalin stimulated the influx of calcium in the pancreatic islets. This effect of astragalin in calcium influx involves the activation of potassium and calcium channels, as well as extracellular calcium and stocks. **Conclusion**: These results suggest that astragalin is one of the compounds responsible for the property of *Passiflora ligularis* to regulate glucose homeostasis. This property might contribute to the prevention of complications of diabetes in the near future.

Keywords: glycemic homeostasis; diabetes; astragalin; insulin secretion; calcium influx; pancreatic islets.

B7

BLOOD BRAIN BARRIER DYSFUNCTION IS ASSOCIATED TO BEHAVIORAL ALTERATIONS IN A DIET-INDUCED OBESITY MODEL

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Over the last few decades the prevalence of obesity has increased substantially, affecting more than 600 million adults worldwide. A hallmark of the increased obesity ocurrence is the overconsumption of energy dense foods, like high fat diets (HFD). Clinical and experimental evidence have shown that consumption of HFD triggers metabolic disorders characteristic of obesity, with gradual increase of body weight, glucose intolerance and insulin resistance. In addition, affected patients become more susceptible to the development of additional medical complications such as cardiovascular disease, type 2 diabetes mellitus, central nervous system (CNS) dysfunction, and early death. It is known that the blood brain barrier (BBB) integrity is essential for neuronal function and CNS homeostasis, since it prevents the passive solutes exchange between blood and brain. However, during systemic inflammatory processes (as observed in obesity), BBB dysfunction has been described. Here, our aim was to evaluate, in an animal model of diet-induced obesity, if the metabolic changes are associated to a disrupted BBB and CNS alterations that could lead to behavioral alterations. For this purpose, 40-day-old male Swiss mice were fed a HFD (60% calories from fat) for 7, 14 and 28 consecutive days. Student ttest was used to compare the difference between the control group (Lean) and diet-induced obesity (DIO) group. Metabolic parameters, including body weight, subcutaneous fat and lipid profile were evaluated. Cognition and emotionality assays, as well as assessment of the BBB function were performed after the experimental periods. The set of our results showed that even in a small period of diet exposure, 7 days, DIO leads to body mass and subcutaneous fat increase, besides spatial memory impairment and depressive-like behavior, a condition that persisted up to 28 days of obesity. These metabolic and behavioral changes were accompanied by the increase in BBB permeability at 7 days after diet induction. In addition, an increase in total cholesterol levels after 14 days of dietary intervention was observed. In conclusion, the behavioral changes observed in obese animals occur, associated to loss of function and homeostasis of the BBB, possibly contributing to the establishment of an inflammatory environment in the CNS.

Keywords: obesity, high-fat diet, cognition, depression, blood-brain barrier.

B8

REDUCED CONTENT OF CREATINE KINASE IN SKELETAL MUSCLE OF WISTAR RATS SUBMITTED TO CHRONIC HYPERGLYCEMIA

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Introduction: Diabetes mellitus (DM) is a metabolic condition of multiple etiologies characterized by persistent hyperglycemia resulting from lack of insulin and/or resistance of tissues to the hormone action. The metabolic

derangement can trigger numerous central and peripheral deleterious processes, including the deficiency of important enzymes involved in energy metabolism. **Objective**: To investigate the effect of chronic hyperglycemia in the induction of oxidative stress in male Wistar rats. **Materials and methods**: Chronic hyperglycemia was induced by the administration of a single dose of streptozotocin (STZ; 55 mg/kg, intraperitoneally) in adult Wistar rats, and oxidative stress markers, including non-protein thiol groups (NPSH) and tetrahydrobiopterin (BH4) levels (depicting antioxidant defenses), lipid peroxidation (TBA-RS; indicating lipid damage), and the content of creatine kinase, a key energy enzyme involved in energy metabolism, were investigated after 60 days of hyperglycemia. **Results**: It was observed that chronic hyperglycemia elicited oxidative stress, observed by a significant decrease in the plasma thiol groups levels, an increase in plasma lipid peroxidation and also a reduction of BH4 levels in cerebrospinal fluid. Moreover, a marked decreased was observed in the skeletal muscle creatine kinase content in hyperglycemic animals. However, no differences in the brain or heart were observed. **Conclusion**: These data suggest that persistent hyperglycemia results in central and peripheral oxidative stress that compromise the content of the skeletal muscle key energy enzyme creatine kinase.

Keywords: hyperglycemia, oxidative stress, creatine kinase

B9

METABOLIC AND BEHAVIORAL CHANGES INDUCED BY CHRONIC ADMINISTRATION OF FRUCTOSE IN MICE

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The consumption of fructose has increased by at least 25% in the past 30 years, due to the addition of sugars (e.g., table sugar and fructose rich corn syrup) to the diet,. Clinical and nonclinical evidence has shown that the high consumption of sugary beverages rich in fructose is directly related to the development of obesity and its consequences, e.g., metabolic syndrome. More recently, there has been increasing interest in the potential side effects of fructose on the central nervous system (CNS), with evidence that metabolic disorders may affect insulin signaling in the brain and contribute to the development of cognitive impairment and changes in synaptic plasticity. Thus, the aim of the present study was to characterize the behavioral (i.e., cognitive and emotional) and metabolic changes induced by chronic fructose administration in male and female Swiss mice. For this purpose, 20 male and 20 female mice, three months old, were used at the beginning of the experiments. The animals received, for 8 weeks, drinking water (filtered) or drinking water containing fructose (at a concentration of 30% w / v) ad libitum. All animals had free access to the standard rodent diet. After the 8-week period, the animals were submitted to a battery of behavioral tests in the following order: open field, T-maze, splash and tail suspension test. It was observed that the chronic consumption of fructose induced changes of the anxiogenic type (open field) and depressive-like type (tail suspension) in female mice. Furthermore, it induced cognitive impairment in male and female mice (T-maze). On the other hand, the chronic consumption of fructose induced a significant weight gain, hypercholesterolemia, hypertriacylglycerolemia and glucose intolerance in male and female mice. Collectively, the results emphasize that in addition to metabolic changes, chronic consumption of fructose may also trigger important behavioral, i.e., emotional and cognitive changes in rodents. There are several caveats when extrapolating the results of animal models for putative effects of fructose on human behavior, but the present study proposes an experimental model for the future study of the possible underlying neural mechanisms.

Keywords: Fructose, Metabolism, Behavior, Rodents.

B10

OMEGA-3 FATTY ACIDS ATTENUATE BRAIN ALTERATIONS IN HIGH-FAT DIET-INDUCED OBESITY MODEL

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Introduction: Obesity is defined as the accumulation abnormal or excessive fat that may impair health. Considering

the great impact of obesity on health and the gaps still existent in its pathophysiology, many researchers are searching to better understand the role of the central nervous system. Besides that, because of its anti-inflammatory properties, the use of omega-3 polyunsaturated fatty acids may be a strategy to treat obesity. **Objective:** This study evaluated the effects of omega-3 on inflammation, oxidative stress, and energy metabolism parameters in the brain of mice subjected to high-fat diet-induced obesity model. **Methods:** Body weight and visceral fat weight were evaluated as well. Male Swiss mice with 40 days old were divided into control (purified low-fat diet) and obese (purified high-fat diet). After 6 weeks, the groups were divided into Control + Saline, Control + Omega-3, Obese + Saline, and Obese + Omega-3. Omega-3 (400 mg/kg/day) or saline solution was administrated orally, during 4 weeks. When the experiment completed 10 weeks, the animals were euthanized and the brain and visceral fat were removed. The brain structures (hypothalamus, hippocampus, prefrontal cortex, and striatum) were isolated. **Results:** Treatment with omega-3 had no effect on body weight, but reduced the visceral fat. Obese animals presented TNF-α and IL-1β increased, and alteration in the IL-10 levels. The obese group presented increased in the oxidative damage to lipids and proteins. Obese animals had reduction of CAT activity and reduction of GSH levels, but not altered the activity of the SOD. The obese group had inhibition of the citrate synthase, isocitrate dehydrogenase and α-ketoglutarate dehydrogenase activity, and increased of the succinate dehydrogenase and malate dehydrogenase activity. Obese animals showed inhibition of complexes I, II and IV. Omega-3 treatment partially reversed the changes in the inflammatory and in the oxidative damage parameters, and attenuated the alterations in the antioxidant defense and in the energy metabolism. **Conclusion:** Omega-3 had a beneficial effect on the brain of obese animals, as it partially reversed the changes caused by the consumption of a high-fat diet and consequent obesity. Our results support studies that indicate omega-3 may contribute to obesity treatment.

Keywords: obesity, brain, omega-3, inflammation, oxidative stress, energy metabolism.

B11

p-METHYL-PHENYL-SULFONAMIDEACTS ON INSULIN AND INCRETIN SECRETAGOGUE AND STIMULATES GLUCOSE UPTAKE IN INSULIN RESISTANT RATS

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Aim: To highlight the insulin secretagogue mechanism of *p*-methyl-phenyl-sulfonamide (sulfonamide 3), and to evaluate the extrapancreatic actions of this compound on GLP-1 secretion, glucose uptake and activity of intestinal disaccharidases. **Methodology:** Fasted male *Wistar* rats (180–200 g) were divided into groups of 5 animals for glucose tolerance test (GTT): I) Hyperglycemic control (4 g/kg of glucose body weight intraperitoneally (i.p.) and II) Hyperglycemic plus sulfonamide 3 (10 mg / kg, i.p.). The glycaemia was measured before any treatment (zero time) and at 15, 30, 60 and 180 min after glucose overload. The serum GLP-1 was measured by ELISA. Isolated pancreatic islets were used to measure insulin and calcium influx. In another experiment the rats became insulin resistant by receiving daily injections of dexamethasone via the subcutaneous route for 5 consecutive days. For this, the rats were divided into four groups: I): vehicle (saline); II) dexamethasone (0.1 mg / kg), III) dexamethasone (0.1 mg / kg) plus sulfonamide 3 (10 mg / kg) and IV) sulfonamide 3 (10 mg / kg). Intestinal disaccharidases activity was evaluated *ex vivo* (Protocol CEUA/UFSC/PP00398/749). **Results**: Sulfonamide 3 increased insulin levels *in vitro* by a mechanism K^+ -ATP-dependent channels and by increasing GLP-1 *in vivo*. The stimulatory effect of sulfonamide 3 on calcium influx in isolated pancreatic islets depends on the activation of voltage-dependent calcium channels. In addition, Sulfonamide 3 increased glucose uptake in skeletal muscle and adipose tissue in insulin resistant animals. Still, this compound inhibited the activity of intestinal disaccharidases *in vitro*. **Conclusion:** Sulfonamide 3 is an insulin secretagogue agent and is able to ameliorate the insulin resistant status by increasing glucose uptake. Also, it is effective on the inhibition of glucose absorption. In a whole this compound is a potential new insulin secretague to be used for diabetes therapy, in the near future.

Financial support: CNPq; CAPES; PPG-BQA PROAP/UFSC.

Keywords: *p*-methyl-phenyl-sulfonamide, GLP-1 and insulin secretion, calcium influx, glucose uptake and insulin resistant rats.

B12

EFFECTS OF FRUCTOSE CONSUMPTION ON BIOCHEMICAL PARAMETERS IN MICE

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Introduction: Urbanization has led to ingestion of inadequate food, excessive consumption of sweetened beverages and high availability of fructose. In addition, the effects of high fructose intake are associated with biochemical changes and oxidative damage in the liver. In this sense, the biochemical evaluation in the hypothalamus and epididymal fat, after the consumption of fructose, can be better explored. **Objective:** To evaluate the effects of the consumption of different concentrations of fructose on biochemical parameters of mice. **Methods:** The experiment was based on water intake with different concentrations of fructose. Male Swiss mice were divided into four groups, with exclusive intake of water, water + 5% fructose, water + 10% fructose and water + 20% fructose. A standard chow was common to all groups. Food consumption and body weight were evaluated during the experiment. After 11 weeks the animals were euthanized for removal and analysis of the visceral adipose tissue and hypothalamus. **Results:** There were no differences in body weight gain, but there was an increase in the epididymal and visceral fat, in the fructose 10 and 20% groups. There was a tendency in the epididymal fat to increase lipid damage (malondialdehyde levels) in the fructose 10 and 20% groups, with alteration of the complexes I, II and IV of the mitochondrial respiratory chain. In the hypothalamus the 10 and 20% fructose groups presented oxidative damage to lipids and proteins (proteins carbonylation), but without alteration of the complexes of the mitochondrial respiratory chain. In the evaluation of food consumption, the 10 and 20% fructose groups showed a decrease in solid consumption and higher water intake. This contributed to the gradual increase of the total fructose consumption, in relation to the 5%, 10% and 20% groups. **Conclusion:** It was concluded that the groups that consumed the highest amounts of fructose (10 and 20%) had an increased deposition of visceral fat, mitochondrial changes in epididymal fat and increased oxidative damage in the hypothalamus.

Keywords: fructose, hypothalamus, fat, oxidative stress.

B13

EFFECT AND MECHANISM OF ACTION OF SULFONYLTHIOUREA DERIVATIVE ON GLYCAEMIA HOMEOSTASIS

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Introduction: Sulfonylureas are used worldwide as insulin secretagogues in the treatment of diabetes. In this sense, glibenclamide, a 2nd generation sulfonylurea, was chemically modified with the purpose to investigate its effect on glucose homeostasis. **Aim:** To assess the glycaemia, insulin and GLP-1 levels of a glibenclamide derivative sulfonylthiourea 7 (Sulp7) in the oral glucose tolerance test (oGTT). Next, the calcium influx and the mechanism of action of Sulp7 on static insulin secretion were investigated in pancreatic islets. Also, the role of Sulp7 on intestinal disaccharidases and incretion secretion was studied. **Methods:** Male *Wistar* rats (50 days old) were divided into three experimental groups: Group I, Hyperglycemic rats (4g/kg glucose, via oral (v.o)), Group II, Hyperglycemic rats plus Glibenclamide (10 mg/kg, v.o., 30 min before glucose overload), and Group III, Hyperglycemic rats plus Sulp7 (10 mg/kg, v.o., 30 min before glucose overload). Sitagliptin (10 mg/kg, v.o., 1 h before glucose overload) was administered to analyze GLP-1 levels. Blood samples were collected prior to glucose overload (time 0); and 15, 30, 60 and 180 min after, to quantify glucose, insulin and GLP-1 levels. At 180 min the animals were euthanized and gut samples were taken to analyze the activity of the disaccharidases. Pancreatic islets were isolated to study calcium influx and to quantify insulin. Data were expressed as mean ± S.E.M. ANOVA followed by the Bonferroni post-hoc test or unpaired Student's t-test was used (n=6), and the level of significance of 95% ($p < 0.05$), (CEUA/UFSC: PP00749). **Results:** Sulp7 reduced blood glucose and increased static insulin by a mechanism triggered by calcium. Furthermore, Sulp7 increased significantly serum GLP-1. Sulp7 decreased the activities of intestinal disaccharidases. **Conclusions:** Sup7 regulates glucose homeostasis by a mechanism involving insulin and incretin secretion and also points the intestine as an alternative target since reduced the activities of disaccharidases.

Financial Support: CAPES, CNPq agency and BQA/UFSC.

Keywords: insulin secretion, *Diabetes mellitus*, sulfonylthiourea

B14

PATTERN OF CALPAIN ACTIVITY AND CALCIUM DEPOSITS IN MDX SKELETAL MUSCLES AT DIFFERENT STAGES OF MUSCULAR DYSTROPHY

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Duchenne muscular dystrophy (DMD) is a fatal muscular disorder characterized by progressive muscle wasting due to a nonsense mutation in the dystrophin gene. Dystrophin is a cytoskeletal protein critical for maintaining sarcolemma integrity and activity of signaling complexes and channels. Calpains (CAPN) are a ubiquitous Ca^{2+}-

dependent cysteine proteases important for skeletal muscle remodeling. CAPN1 (μ-calpain) is half maximally activated by 3-50 μM Ca^{2+} whereas CAPN2 (m-calpain) requires high Ca^{2+} concentration (400-800 μM). CAPN-1 and -2 have similar molecular weight (80-kDa) and form a heterodimer with the small subunit (28-kDa), but in presence of Ca^{2+}, suffer autoproteolytic processing with reduction of molecular weight. This study aimed to draw a relation between Ca^{2+} accumulation and the activity of CAPNs in distinct skeletal muscles (gastrocnemius, triceps-brachii, soleus, and extensor longus digitorum - EDL) at different stages of muscular dystrophy at the peak of myonecrosis (4-weeks) and regeneration (12-weeks) in the murine model for muscular dystrophy (mdx mouse) comparing to nondystrophic murine (C57Bl10). To date, mdx mouse contains a point mutation that depletes the dystrophin protein. **Methods:** The gastrocnemius, triceps-brachii, soleus and EDL muscles of mdx mice and C57Bl10 at 4-weeks and 12-weeks were dissected for zymography and histology. Alizarin dye was used for detection of small quantities of Ca^{2+} deposits in paraffin-embedded blocks, and CAPN activity was determined by casein-zymography polyacrylamide gel. The muscles were macerated, centrifuged, the supernatant was recovered and the protein levels determined by Lowry method. In each lane was loaded 40μg of protein. **Results:** It was observed increased activity of all three isoforms of calpain but when compared to age-matched nondystrophic C57Bl10 control, mdx dystrophic muscles at both 4 and 12-weeks showed decreased μ-calpain activity. Conversely m-calpain was increased at both ages in mdx mice and the autolyzed calpain was exclusively detected in mdx with highest activity on mdx muscles at 12-weeks. Calcium deposits were consistently detected in all dystrophic muscles at both ages but with a slight increase at 12w. During mdx muscular regeneration an increased Ca^{2+} and CAPNs mobilization might be especially important for myofiber remodeling.

Keywords: mdx mouse, muscular dystrophy, calpain, calcium, alizarin

B15

URINARY BIOMARKERS FOLLOWING SULFASALAZINE TREATMENT IN HUMANS

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Introduction: Sepiapterin reductase (SPR) is an enzyme involved in the biosynthesis of tetrahydrobiopterin (BH4). BH4 is an essential co-factor for the aromatic amino acid hydroxylases and nitric oxide synthases; however excessive BH4 levels has been involved in inflammatory and neuropathic pain. Sulfasalazine (SSZ) a small molecule disease-modifying anti-rheumatic drug (DMARD) is commonly recommended as a first line treatment for rheumatoid patients. The mechanism of action of SSZ was not known until recently when it was discovered to be an SPR inhibitor. Therefore, inhibitors of SPR have been proposed to induce analgesia. **Objective**: To investigate urinary biomarkers involved in the BH4 metabolism in healthy subjects receiving SSZ. **Materials and Methods**: A group of 10 pain-free subjects (male n=4, female n=6, age range 31.9 ± 6.7) from Florianópolis, Brazil, were recruited (CEPSH 54297916.7.0000.0121). None of them were taking any medication for pain neither reported any symptoms of pain prior to or during the study. Each volunteer collected one sample of urine on day 1 (pre-treatment) and then on the following 3 days took a 500mg tablet, approximately every 6 h (total 2g/day) and collected the first voided urine each following morning. After the third day of SSZ treatment, volunteers did not to take any further doses of SSZ but continued to collect the first voided urine of the day for four more days. A total of 8 urine samples per volunteer were collected for analysis. BH4, neopterin and sepiapterin urinary levels were analyzed by high performance liquid chromatography. **Results and discussion**: SSZ administration increased sepiapterin levels in the urine, reaching statistical significance on the 3rd day of sampling following SSZ administration. Sepiapterin levels immediately returned to pre-treatment levels on cessation of SSZ. BH4 and neopterin levels were found not to change in response to SSZ treatment. **Conclusion**: Urinary sepiapterin following SSZ treatment is a sensitive and specific biomarker of BH4 pathway engagement. This measurement will allow to monitor the efficacy of analgesic therapies involving SPR inhibitors.

Keywords: sulfasalazine, sepiapterin, tetrahydrobiopterin, neopterin

B16

POTENTIAL ANTIHYPERGLYCEMIC EFFECT OF CHALCONE ANALOGUES

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Chalcones are a group of natural or synthetic compounds that can be found in their natural form widely in plants. They exhibit a wide biological activity, which varies according to the substituents of the molecule, being of great chemical and pharmacological interest. Chalcones are key intermediates in the biosynthesis pathway of flavonoids.

Some beneficial properties have been reported in the literature, such as: antifungal, antibacterial, antiviral, anti-inflammatory, anticancer, anti-platelet, anti-oxidant, among others. This research aimed to investigate the role of chalcones on glucose homeostasis. Eleven synthetic chalcones were investigated for their antihyperglycemic potential in male Wistar rats. For this purpuse, the glucose tolerance tests were performed in the presence or absence of 10 mg/kg chalcones, by oral gavage. Rats were fasted for 16h and chalcones were administered orally 30 min before glucose overloading (4 g/kg glucose, oral). The glycemia was checked at zero (before loading of glucose), 15, 30, 60 and 180 min in order to measure the glycemic profile. The results were expressed as percentage relative to the control. One-way analysis of variance (ANOVA) followed by the Bonferroni post-test or unpaired Student's t-test were used to determine the significant difference between grups. Differences were considered to be significant at p 0.05,(CEUA/UFSC: PP00749). The chalcone 160 induced serum glucose lowering in about 12% related to the control at 15 min. Furthermore, chalcones 102 and 134 decreased serum glucose by 18% and 12%, respectively compared to control at 30 min. In addition, chalcones 101 and 191 decreased the serum glucose by 35% and 12% at 180 min when compared to the respective control group. The other compounds did not present a significant decrease on glycemia. We conclude that the chalcone analogue that has the substituent trimethoxyphenil group at B ring exhibits a significant acute antihyperglycemic effect by improving glucose tolerance. Other trials are underway to characterize the mechanism of action of chalcone 101.

Financial support: CNPq; CAPES; PPG-BQA PROAP/UFSC.

Keywords: Chalcone; Hyperglycemia; Analogues, Diabetes *mellitus*.

B18

ACUTE EFFECT OF BIS(2-ETHYLHEXYL)PHTHALATE AND BISPHENOL A IN VITRO ON ENERGETIC SUPPORT FOR ZEBRAFISH TESTIS

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Introduction: The adequate energetic support, such as the lactate production by Sertoli cells through the action of lactate dehydrogenase (LDH) and gamma glutamyl transpeptidase (GGT) activity, that is considered a testicular biomarker of Sertoli cells activity, it is the main energy source to germ cells. However, the disturbance of this pathway by endocrine disruptors effect, such as phthalates and bisphenol A can negatively affect the testicular biochemistry, impacting on the animal reproductive physiology. **Objectives:** The objective of this study was to analyze the acute *in vitro* effect of bis(2-ethylhexyl)phthalate (BEHP) and bisphenol A (BPA) on the energetic support of Danio rerio (Zebrafish). **Methodology:** Danio rerio fish testis (CEUA PP00968) were used to analyze the acute effect (1 hour) of BEHP and BPA *in vitro* in a low concentration (1 μM) on lactate content, 14C-deoxy-glucose uptake, LDH activity and GGT activity. **Results:** The *in vitro* exposition to BEHP for 1 hour decreased the lactate content and the LDH activity, without altering glucose uptake, however, a concomitant reduction on LDH activity was observe. Furthermore, the acute treatment with BEHP did not change the GGT activity. In addition, the *in vitro* exposition to BPA for 1 hour was able to increase the lactate content, without altering glucose uptake and intracellular LDH activity. However, an increase on extracellular LDH activity was observed after BPA treatment. Furthermore, BPA acute treatment was also able to increase the activity of GGT. Conclusions: These results suggest that the reduction of the testicular energetic support can affect the spermatogenesis and reproductive physiology of the zebrafish.

Support: CNPq -PVE. 401410/2014-1; CAPES-PPG-Biochemistry.

Keywords: lactate, LDH, fish, testis, BEHP, BPA.

C. Biochemistry of neurodegenerative conditions, neurotoxicity and neuroprotection

C1

MECHANISMS MEDIATING PARAQUAT AND MANEB-INDUCED TOXICITY IN SH-SY5Y CELLS

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by loss of dopaminergic neurons in the substantia nigra and progressive decline in motor function. The etiology of PD seems to involve both genetic and environmental factors. Pesticides exposures have been implicated as risk factor for PD development. In this scenario, occupational exposure to the herbicide paraquat (PQ) and the fungicide maneb (MB) may be associated with the incidence of PD. Therefore, we investigated the cytotoxic effects of PQ and MB, isolated or in combination, in human neuroblastoma SH-SY5Y cells, with potential focus on oxidative stress-related parameters. Cells were exposed to PQ (1 - 10,000 µM) or MB (0.5 - 30 µM) for 24 hours. In a different experimental protocol, cells were exposed to 100 µM PQ and 10 µM MB, alone or in combination (PQ + MB) for 6, 12 and 24 hours. In parallel experiments, cells were pretreated with the well-established antioxidants trolox (50 µM) and N-acetylcysteine (NAC, 500 µM) 1 hour before pesticides exposures. The exposure to PQ and MB, alone induced a significant concentration-dependent decrease in the cell viability. Next, to investigate the effect of combined exposure to pesticides (PQ + MB), we exposed cells to 100 µM PQ plus 10 µM MB (the highest concentrations not affecting cell viability in single exposure). PQ + MB exposure for 24 hours significantly decreased the cell viability. MB, alone or in association with PQ, increased the reactive species (RS) production and induced a significant decrease in glutathione (GSH) levels. On the other hand, MB exposure for 12 hours increased GSH levels. In addition, PQ and/or PQ + MB exposures for 12 hours induced a significant increase in RS generation and a concomitant increase in the superoxide anion production. Notably, treatment with trolox and NAC protected SH-SY5Y cells from PQ + MB- induced toxicity. Taken together, these findings suggest that the combined exposure to PQ and MB at low concentrations induced more severe cytotoxic effects in SH-SY5Y cells than when cells are exposed to each pesticide alone. In addition, PQ + MB induces cytotoxicity through the induction of oxidative stress.

Keywords: Parkinson's disease, paraquat, maneb, oxidative stress, antioxidants.

C2

DEPRESSIVE-LIKE BEHAVIOR AND IMPAIRMENT OF NEUROTRANSMITTERS SYSTEMS INDUCED BY METHYLGLYOXAL, A ENDOGENOUS TOXIN

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Introduction: Methylglyoxal (MGO), a readily reactive dicarbonyl and a precursor of advanced glycation end products, is mainly formed from the non-enzymatic degradation of the glyocolytic intermediates, dihydroxyacetone phosphate and glyceraldehyde-3-phosphate. MGO is associated with several pathologies, including diabetes and neurodegenerative diseases. High levels of this compound can modify animal behavior, showing anti-depressive like and anxiolytic behaviors, although, there are controversies regarding existing data. **Objectives:** In the present study, we aimed to investigate the effects of MGO on depressive-like behavior, as well as to determine plasma concentration of MGO, and levels of serotonin, dopamine and epinephrine neurotransmitters. **Material and methods:** Mice received a single injection of MGO (10, 25 or 50 mg/kg) or daily injections for 7 days. Behavioral tests and euthanasia were conducted 4 hours after the single injection or 24 hours after the last injection in the 7-day protocol. **Results and discussion:** The acute treatment with MGO (25 and 50 mg/kg) induced a significant increase in MGO levels after 4 hours whilst this was not observed in the 7-days treatment. On the tail suspension test, in both protocols, animals presented a depressive-like behavior without decreasing the locomotor activity in the open-field test. This indicates that the higher immobility time is not related to decreased locomotion. Moreover, the hippocampus of the 7-days treatment these animals presented increased levels of serotonin and dopamine, on the concentration of 10 mg/kg and decreased levels of norepinephrine when animals were treated with 50 mg/kg of MGO. The prefrontal cortex has not shown any alteration on the serotonin, dopamine or epinephrine. **Conclusion:** Both MGO protocols induced a clear depression-like behavior, contrary to the literature data. The MGO-induced depressive-like behavior is potentially related to alterations in neurotransmitters found in the hippocampus. On this manner, increased levels of MGO could be one of the factors related with the development of neuropsychiatric diseases, like depression.

Keywords: methylglyoxal; depression, mood disorders, dopamine, serotonin, norepinephrine.

C3

EVALUATION OF THE NEUROPROTECTIVE EFFECT OF CONBR LECTIN IN A MODEL OF ORGANOTYPIC HIPPOCAMPAL CULTURES EXPOSED TO OXYGEN AND GLUCOSE DEPRIVATION

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Lectins are carbohydrate-binding proteins that can recognize glycoconjugates, regulating many cell functions in physiological and pathological events. ConBr is a lectin isolated from *Canavalia brasiliensis* seeds that displays high

affinity for mannose/glucose. Previous work by our group demonstrated that ConBr exhibits neuroprotective effect against seizures induced by quinolinic acid and glutamatergic excitotoxicity in hippocampal slices, through a mechanism involving NMDA inhibition and modulation of PI3K/Akt. Cerebral ischemia is one of the main causes of morbidity and mortality in the world, which makes this pathology target of many studies. Hence, it is fundamental to understand the mechanisms that trigger neuronal cell deathand search strategies for neuroprotection. Organotypic hippocampal cultures subjected to oxygen and glucose deprivation has been applied as *in vitro* model to study the neurochemical and cellular alterations in the cerebral ischemia, as well as to search compounds with neuroprotective activity. The aim of this study was to investigate the neuroprotective potential of ConBr on organotypic hippocampal cultures subjected to oxygen and glucose deprivation for 15min followed by 24 hours reperfusion (OGD/Rep). The organotypic hippocampal cultures incubated for 15 min with control medium or oxygen and glucose deprivation medium were treated for 24 h with ConBr (0,1 and 1μg/mL) diluted in the reperfusion medium. Cell death in the CA1 region of hippocampus was assessed by fluorescent image analysis of propidium iodide uptake. Our results demonstrate that treatment with ConBr (1μg/mL) significantly decreases OGD-induced cell death in CA1 region of the hippocampus. Through immunohistochemical analysis, it was detected changes in the morphology of the astrocytes, with scarce ramifications in the OGD/Rep group. Treatment with ConBr promoted a significant improvement in cell viability being able to maintain the density and morphology of the GFAP-positive astrocytes, similar to the control group. In addition, we observed a decrease of NeuN-positive cells in the OGD/Rep group and treatment with ConBr was able to improve neuronal survival. Although further studies will be necessary to evaluate the neuroprotective mechanisms induced by ConBr against ischemic damage, the results obtained in the present study suggest the potential of this lectin as a neuroprotective strategy.

Keywords: Lectin, ConBr, Oxygen and glucose deprivation, Organotypic hippocampal culture, Neuroprotection

C4

ALTERED GLYOXALASE SYSTEM AND DOPAMINE LEVELS WERE ASSOCIATED WITH POORER WORKING MEMORY PERFORMANCE AFTER REPEATED TREATMENT WITH METHYLGLYOXAL IN MICE

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Introduction: Methylglyoxal is a highly cytotoxic dicarbonyl molecule linked to several pathologies including diabetes, neurodegeneration and cognitive dysfunction. The major detoxification system of methylglyoxal is comprised of the enzymes glyoxalase 1 (GLO1) and glyoxalase 2 (GLO2). Dopamine in the prefrontal cortex is important to the stabilization of current goal representations in working memory. Drugs that increase dopamine availability, like methylphenidate and modafinil, can lead to cognitive enhancement. **Objectives:** To investigate the effects of methylglyoxal treatment on working memory performance and the monoamine, GLO1 and GLO2 levels in the brain. **Material and methods:** Three months old female Swiss mice (35-50 g) were treated by daily intraperitoneal injections with saline (control) or methylglyoxal (10, 25 or 50 mg/kg) (N=10-11) during 11 days. Evaluation of working memory was carried out by measuring the spontaneous alternation rate in a Y-maze. After treatment, plasma methylglyoxal and dopamine, noradrenaline and serotonin were measured by HPLC and protein levels of GLO1 and GLO2 were also evaluated by western blot in the prefrontal cortex and hippocampus. Statistical analyses were performed by ANOVA followed by Newman-Keuls *post hoc* test. **Results**: While the other groups did not show alterations, 50 mg/kg methylglyoxal-treated mice displayed lower alternation rate compared to control [$F(3.38)=4.36$; $p<0.05$], an evidence of lower memory retention. This result was accompanied with decreased dopamine levels in the prefrontal cortex [$F(3.19)= 3.15$; $p<0.05$]. GLO1 protein increased 52% in the hippocampus after treatment with 50 mg/kg methylglyoxal [$F(3.32)=11.166$; $p<0.05$]; GLO2 decreased 37% in the prefrontal cortex of mice treated with 10 mg/kg methylglyoxal [$F(3.34)=7.011$; $p<0.05$]. **Discussion**: Data from literature showed that deficits in attention/working memory processes were restored by dopamine in the prefrontal cortex of impaired animals. **Conclusion**: The results suggest that methylglyoxal treatment can alter glyoxalase system and impair working memory of mice by decreasing dopamine levels in the prefrontal cortex.

Keywords: methylglyoxal; glyoxalase system; dopamine; working memory.

C5

SYNTHESIS AND EVALUATION OF L-HYPAPHORINE AND D-HYPAPHORINE FOR ANTIACETYLCHOLINESTERASE ACTIVITY

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Alzheimer's disease (AD) is considered the most prevalent dementia worldwide in the elderly. AD is associated with

a deficiency of neurotransmitters, mainly acetylcholine (ACh). Despite the lack of cure, treatment with acetylcholinesterase inhibitors (AChEis) is the most used approach, since they prevent the hydrolysis of ACh and, therefore, increase cholinergic neurotransmission. Drugs approved by the Food and Drug Administration (FDA) for the treatment of AD have problems of toxicity and low bioavailability, so promising new compounds have been investigated. L-Hypaphorine, a natural indole alkaloid found in plants of the genus *Erythrina* (Leguminosae) has effects in the central nervous system, for example, as a sleep inducing agent in rats. Aqueous and ethanolic extracts of *Erythrina velutina* are able to inhibit AChE activity in the mouse cortex (*in vitro*). However, there are no reports in the literature about the inhibition of AChE by hypaphorine. In this context, since it has structural characteristics similar to ACh, this work proposed the synthesis, molecular docking and anticholinesterasic evaluation of L and D-Hypaphorine. In our study, L and D-Hypaphorine were obtained by reactions of L and D-tryptophan with methyl iodide in basic medium with good yields of 92% and 95%, respectively. Inhibition assays of AChE activity with L and D-Hypaphorine were performed in four brain regions: cortex, hippocampus, striatum and cerebellum. The results of AChE inhibitory activity showed a selective inhibition in different brain regions. L-Hypaphorine showed inhibition only in the cerebellum with an IC_{50} = 18.63 ± 0.14 μM and D-Hypaphorine showed inhibition only in the cerebellum and striatum with an IC_{50} = 19.12 ± 0.03 μM and 34.60 ± 0.34 μM, respectively. Through the studies of molecular docking, it was possible to observe different interactions of D-Hypaphorine with the amino acid residues present in the active site of AChE, when compared with the L-Hypaphorine. The selective inhibition presented by the enantiomers of hypaphorine is very important for the pharmacodynamic studies of these compounds, considering that each brain region performs different functions, such as reflexes and postural coordination (cerebellum), emotional function (striatum), learning and motor impulses (striatum).

Keywords: Alzheimer's disease, Acetylcholinesterase, Hypaphorine.

C6

BEHAVIOR, METABOLIC AND NEUROCHEMICAL EFFECTS OF ENVIRONMENTAL ENRICHMENT IN HIGH-FAT CHOLESTEROL ENRICHED FED MICE

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Introduction: There is increasing evidence that *hypercholesterolemia* during midlife may represent a predictor of dementia decades later. The effects of cognitive and physical stimulation on the brain and behavior are modeled in rodents using a paradigm called environmental enrichment (EE). EE has positive effects on brain function, including dendritic arborisation, synaptogenesis and improved memory function. **Objectives:** We sought to investigate the impact of EE on mice fed a *high-fat cholesterol enriched* diet (HFCED)(20 % fat and 1.5 % cholesterol). **Methods:** 3-month-old male Swiss mice were randomly divided into two experimental groups (n = 20) and fed daily during 8 weeks with different chows: (SD) standard diet and HFCED. After a period of four weeks, half of the animals in each group were exposed to a four-week period of concomitant exposure to EE. Afterward, mice were submitted to several behavioral tests including T-maze and object recognition. After the performance in the behavioral tests, mice were food deprived overnight and the glucose tolerance test was performed. Moreover, blood was collected from the heart to determine plasma lipid levels. The hippocampus was also dissected to determine the brain derived neurotrophic factor (BDNF) and IL-6 levels. **Results:** EE was able to prevent cognitive impairments induced by HFCED in the object recognition test (recognition index: 65.33 ± 2.26% vs. 57.85 ± 4.47%, respectively) and T maze task (% of time in the new arm: 50.55 ± 4.80% vs. 35.64 ± 3.79%). Furthermore, EE significantly mitigatedthe HFCED-induced glucose intolerance (AUC: 1094.66 ± 59.16 vs. 1290.12 ± 65.60). On the other hand, EE was not able to prevent the HFCED-induced hypercholesterolemia (mg/dl: 141.67 ± 8.05 vs. 127.43 ± 13.40). EE was also not able to prevent the significant decrease in BDNF (pg/mg protein: 124.96 ± 12.08 vs. 119.89 ± 13.83) and IL-6 (pg/mg protein: 5.54 ± 0.34 vs. 5.80 ± 0.39) levels observed in the hippocampus of mice exposed to HFCED, compared to control mice. **Conclusion**: EE is able to mitigate the cognitive impairments induced by HFCED in mice, although the underlying mechanisms did not involve the modulation of plasma cholesterol levels and/or BDNF levels in the hippocampus.

Keywords: Hypercholesterolemia; Environmental enrichment; Learning and Memory.

C7

IN VITRO EVALUATION OF A NOVEL PROBUCOL DERIVATIVE, RC513: PROTECTIVE ACTIVITY IN NEURONAL CELLS THROUGH GPX UPREGULATION

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Recent studies have shown that probucol (PB), a hipocholesterolemic agent with antioxidant and anti-inflammatory properties, presents neuroprotective properties. On the other hand, adverse effects have limited PB's clinical application. Thus, the search for PB derivatives with no or less adverse effects has been a topic of research. In this study, we present a novel organoselenium PB derivative (RC513) and investigate its potential protective activity in an *in vitro* experimental model of oxidative toxicity induced by tert-butyl hydroperoxide (tBuOOH) in HT22 neuronal cells, as well as exploit potential protective mechanisms. tBuOOH exposure caused a significant decrease in the cell viability, which was preceded by (i) increased oxidant species (OS) generation and (ii) decreased mitochondrial maximum oxygen consumption rate. RC513 pretreatment (48 h) significantly prevented the tBuOOH-induced decrease of cell viability, OS generation, and mitochondrial dysfunction. Of note, RC513 significantly increased glutathione peroxidase (GPx) activity and mRNA expression of GPx1, a key enzyme involved in peroxide detoxification. The use of mercaptosuccinic acid, an inhibitor of GPx, significantly decreased the protective activity of RC513 against tBuOOH-induced cytotoxicity in HT22 cells, highlighting the importance of GPx upregulation in the observed protection. In summary, the results showed a significant protective activity of a novel PB derivative against tBuOOH-induced oxidative stress and mitochondrial dysfunction, which was related to the upregulation of GPx. Our results point to RC513 as a promising neuroprotective molecule, even though studies concerning potential beneficial effects and safety aspects of RC513 under *in vivo* conditions are well warranted.

Keywords: Glutathione peroxidase; HT22 cells; Mitochondrial dysfunction; Probucol derivative; tBuOOH

C8

DIPHENYL DISELENIDE PROTECTS AGAINST THE GENERATION OF SUPEROXIDE ANION AND MITOCHONDRIAL DYSFUNCTION IN IMMORTALIZED MOUSE HIPPOCAMPAL CELL LINE (HT22) EXPOSED TO tBuOOH

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Oxidative stress and mitochondrial dysfunction are critical factors in neurodegeneration, especially in Alzheimer's and Parkinson's diseases. The hippocampus, which represents a brain region closely linked to memory and cognition, is commonly affected in several neurodegenerative diseases. New compounds that activate the antioxidant defenses have been evaluated as potential protective agents against oxidative stress and mitochondrial dysfunction. Diphenyl diselenide $(PhSe)_2$ is a simple diaryl diselenide, whose cytoprotective effects have been described in endothelial cells and macrophages. Recently, we also showed that $(PhSe)_2$ protects HT22 neuronal cells against oxidative stress (oxidant species) and tBuOOH-mediated cell death, involvement increase of GPx activity and level of GSH. Therefore, the aim of this study was to deepen into the understanding of the protection mechanism of $(PhSe)_2$, for which we pretreated the HT22 cells with $(PhSe)_2$ and evaluated i) the production of superoxide anion using MitoSOX at 4 h after tBuOOH exposure; ii) the oxygen consumption rate using high-resolution respirometry at 2-4 h after tBuOOH exposure, iii) temporal transcript levels of genes (*Gpx1*, *Cat*, *Gclc* and *HO-1*) at 3 h, 6 h, 12 h and 24 h after pretreatment, iv) temporal levels of GPx activity at 3 h, 24 h and 48 h after pretreatment, v) the effects of pretreatment time (3 h, 24 h and 48 h) with $(PhSe)_2$ on the viability of HT22 cells after 48 h of incubation and exposed to tBuOOH. Our results demonstrated that $(PhSe)_2$ decreased tBuOOH-induced mitochondrial superoxide anion production and mitochondrial dysfunction in HT22 cells by increasing GPx activity, transcript levels of the *Gpx1*, *Cat*, *Gclc*, and *HO-1* genes, as well as prevented tBuOOH-induced cell death in dependence of the time of pretreatment. In summary, this study demonstrated that the neuroprotective effects of $(PhSe)_2$ started in the first hours of pretreatment (GPx activity and gene expression) in HT22 cells, and expanded the knowledge about the mechanism of protection, which involves increase of antioxidant gene expression that indirectly indicates the activation of transcription factors such as FoxO or Nrf2. These results reinforced the idea that this compound is a promising molecule for further studies concerning neuroprotection in mitochondrial dysfunction-related conditions.

Keywords: Neurodegeneration, Diphenyl diselenide, Gpx activity, tBuOOH, mitochondrial dysfunction and HT22 cells.

D. Biochemistry of neuropsychiatric disorders

D1

AGMATINE POTENTIATES NEUROPROTECTIVE EFFECTS OF SUBTHRESHOLD CONCENTRATIONS OF KETAMINE VIA MTOR/S6 KINASE SIGNALING PATHWAY

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Dysregulation of the hypothalamic-pituitary-adrenal (HPA) axis is one of the most robust neurobiological findings in the pathophysiology of major depressive disorder (MDD) over the last 40 years. The persistent increase in glucocorticoids levels induces morphological and anatomical changes in the brain, especially in the hippocampus. Ketamine represents a major advance for the treatment of MDD, however the psychotomimetic effects of this compound limit its widespread use. Agmatine is a neuromodulator that has been shown to be a putative novel and well-tolerated antidepressant/augmenter drug. In this study, the exposure of HT22 hippocampal neuronal cell line to corticosterone (50 μM) induced a significant neuronal cell death. Interestingly, the incubation of HT22 cells with the fast-acting antidepressant drug ketamine (1 μM) prevented the corticosterone-induced toxicity. Similarly, agmatine caused a significant cytoprotection at the concentration of 0.1 μM against corticosterone (50 μM) cell damage. Notably, the incubation with a subthreshold concentration of ketamine (0.01 μM) in combination with a subthreshold concentration of agmatine (0.001 μM) prevented the neuronal damage elicited by corticosterone (50 μM). A 24 h co-incubation with subthreshold concentrations of ketamine (0.01 μM) and agmatine (0.001 μM) was able to cause a significant increase in the phosphorylation levels of Akt (Ser^{473}) and p70S6 kinase (Thr^{389}) as well as PSD95 immunocontent. Neither glycogen synthase kinase-3β (Ser^{9}) phosphorylation nor β catenin immunocontent were altered by a 24 h co-incubation period. Finally, the co-incubation of cells for 30 min did not produce any effect in the phosphorylation or immunocontent of any protein investigated. Taken together, our results support the notion that the combination of subthreshold concentrations of ketamine and agmatine has cytoprotective effects against corticosterone-induced cell death. This effect is accompanied by its ability to activate Akt and mTOR/S6 kinase signaling pathway, and increase the expression of synaptic proteins.

Keywords: agmatine; Akt; HT22; ketamine; neuroprotection; p70S6 kinase.

D2

GLYBURIDE TRETAMENT PREVENTS DEPRESSIVE-LIKE BEHAVIOR INDUCED BY CHRONIC UNPREDICTABLE STRESS IN MICE

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Neuroinflammatory processes activated in response to stressful challenges have recently been documented in major depressive disorder (MDD). The Nod-like receptor (NLRP3) is an intracellular multiprotein complex responsible for a number of innate immune processes. The assembly of the NLRP3 inflammasome leads to proteolysis and release of the pro-inflammatory cytokines interleukin-1β (IL-1β) and interleukin-18 (IL-18), which are known to be involved in the pathophysiology of MDD. A therapy that inhibits the NLRP3 is considered promising. In this way, the second-generation sulfonylurea hypoglycemic agent glyburide was previously demonstrated to inhibit NLRP3 activation and IL-1β release. The aim of this study was to investigate the potential effect of glyburide treatment in the behavior induced by chronic unpredictable stress (CUS). CUS, composed by uncontrollable daily stressors (tilted cage, food/water deprivation, foot shock, paired caging, continuous light, wet bedding), was applied in female Swiss mice (45-50days) for 21 days. Glyburide (5mg/kg, p.o., a NLRP3 inhibitor) or vehicle (1%DMSO, p.o.) was administered during all the CUS protocol. Twenty-four hours after the last stressor and treatment, animals were submitted to the open field test for locomotor activity evaluation, tail suspension test for depressive-like behavior evaluation and object location test with 180 minutes of interval to evaluate short-term memory. Data were analyzed by two-way ANOVA followed by *Duncan post hoc test* when appropriated. Results showed treatment with glyburide was able to prevent the depressive-like behavior induced by stress in the tail suspension test [$F(1,24)=0.05, p<0.05$]. Additionally, glyburide treatment prevented the impairment in short-term memory observed in stressed mice [$F(1,35)=6.43, p<0.015$]. No differences were observed in the locomotion in the open field test [$F(1,36)=0.42, p=0.522$]. Our results show that glyburide treatment successfully prevented the depressive-like behavior and the impairment in short-term memory caused by CUS. However, more studies need to be performed to evaluate the central and peripheral biochemical effects of glyburide treatment and to confirm its ability to inhibit NLRP3 complex.

Keywords: Major Depressive Disorder, Inflammation, NLRP3, Glyburide

D3

SYMPTOMS OF DEPRESSION, ANXIETY AND PERIPHERAL INFLAMMATION IN PATIENTS WITH OVERWEIGHT AND OBESITY

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Introduction: Symptoms of depression and anxiety are frequently observed in obese individuals. These conditions have a multifactorial and complex origin, however inflammation appears to play a significant role their etiology and progression. **Aim:** The present study evaluated the relationship between symptoms of depression, anxiety and peripheral inflammation in subjects with overweight/obesity. **Methods:** Our sample included 36 individuals, who attended a nutrition clinic in the city of Garopaba, SC. Symptoms of depression were evaluated using the Beck Depression Inventory (BDI), a 21-item self-reported instrument. Anxiety was evaluated with the State-Trait Anxiety Inventory (STAI), divided in a section related to how the person feels most of the time (trait) and a section related to how the person feels at the time of assessment (state). Detailed socio-demographic and clinical history was obtained (age, gender, physical activity and health habits, body composition, levels of C-reactive protein, CRP). **Results:** In our sample, 27 individuals (75%) were women, with an average age of 36.39±9.72 years, body mass index (BMI) of 26.57±kg/m^2 and 27.95±7.50% of body fat. In addition, 31 (86,1%) were non-smokers and 34 (94.5%) reported the habit of doing physical activity at least twice a week. Among these subjects, 14 (38.8%) were eutrophic (BMI<25) and 22 (61.2%) overweight/obese (BMI ≥25). When compared to eutrophic individuals, overweight/obese had higher levels of CRP (1.25 ± 1.85 vs 3.50 ± 5.26 p = 0.022), an indicative of peripheral inflammation. However, no differences were found in BDI scores between eutrophic and overweight/obese subjects (9.21 ± 6.47 vs 10.09 ± 5.17 p = 0.650). Additionally, no differences were found in the eutrophic and overweight/obese individuals in anxiety trait scores evaluated by the STAI (40.14 ± 6.78 vs 41.08 ± 8.15 p = 0.720) or in anxiety state scores (42.00 ± 8.33 vs 38.95 ± 8.37 p = 0.294). **Conclusion:** Our results suggest that despite the higher levels observed in overweight/obese individuals when compared to eutrophic, no behavioral differences in depressive or anxiety symptoms were observed.

Keywords: depression, inflammation, obesity, overweight.

D4

SEROTONERGIC SYSTEM IS REQUIRED FOR THE ANTIDEPRESSANT-LIKE EFFECT OF *Ilex paraguariensis* HYDROALCOHOLIC EXTRACT

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Ilex paraguariensis St. Hilaire (Aquifoliaceae) is a typical plant from South America, popularly known as "yerba mate" and extensively used for local community. Our group recently demonstrated neuroprotective and antidepressant-like effect of *Ilex paraguariensis* hydroalcoholic extract (*Ip*HE). Considering the importance of serotonergic system for the treatment of depression, as well as in the mechanism of antidepressants, the aim of this work is to investigate the participation of serotonergic system in the antidepressant-like effect of *Ip*HE. Male adult *Swiss* mice were (UnC – P031/12) were treated with *Ip*HE sub active dose (0.01 mg/kg, using gavage technique), alone or in combination with sub active doses of different antidepressants: fluoxetine (5.0 mg/kg, orally); paroxetine (0.1 mg/kg, orally) or sertraline (1.0 mg/kg, orally). After 1h of treatment, animals were subject to Tail Suspension Test (TST) and Open Field Test (OFT). In order to evaluate the participation of serotonergic receptors in the antidepressant-like effect of *Ip*HE, independent groups of animals were treated with centanserin (5.0 mg/kg, s.c., a 5-$HT_{2A/C}$R antagonist) or WAY100635 (0.1 mg/kg, s.c., a 5-HT_{1A}R antagonist) and 30 minutes later received the active dose of *Ip*HE (0.1 mg/kg, orally). One hour after the last administration, animals were teste in TST and OFT. In order to investigate the participation of 5-HT_3R, ondansetron was administrated (0.1 mg/kg, s.c.) to mice 30 minutes before the administration of *Ip*HE sub active dose (0.01 mg/kg, orally). After 1h, animals were tested in TST and OFT. Two-way analysis of variance followed by Tukey's HSD post-hoc test were performed. P<0.05 was considered to be significant. Results demonstrated a combined effect provided by *Ip*HE and classical antidepressants in TST. The participation of 5-$HT_{2A/C}$R and 5-HT_3R in the antidepressant-like effect of *Ip*HE were demonstrated. In the other hand, 5-HT_{1A}R seems to be not involved in the *Ip*HE antidepressant-like effect. In conclusion, the antidepressant-like effect of *Ip*HE depends on serotonergic system modulation.

Keywords: *Ip*HE, antidepressant-like effect, serotonergic system, serotonergic receptors

D5

LEVELS OF VITAMIN D, BIOCHEMICAL PARAMETERS AND SYMPTOMS OF DEPRESSION AND ANXIETY IN HEALTH INDIVIDUALS

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Introduction: Vitamin D is a steroid hormone obtained from the diet or endogenously produced after sun exposure. In the liver, it is hydroxylated to 25-hydroxyvitamin D and then, to the biologically active metabolite, 1,25-dihydroxyvitamin D3, in the kidney and other tissues. This metabolite can bind to nuclear receptors expressed in different tissues, including the brain, to mediate genomic and non-genomic responses. Growing evidence support the role of vitamin D in brain function and development, including neuronal growth, neurotransmission and neuroplasticity. **Aim:** This study investigated the relationship between peripheral levels of 25-hydroxyvitamin D, biochemical profile and symptoms of depression and anxiety in health individuals. **Methods:** Our sample included 36 individuals, which attended a nutrition clinic in Garopaba, SC. Symptoms of depression were assessed by the Beck Depression Inventory (BDI) and anxiety was evaluated with the State-Trait Anxiety Inventory (STAI). **Results:** Our sample included mostly women 27 (75%), 36.39±9.72 years old, non-smokers 31 (86,1%), body mass index (BMI) of 26.57±kg/m^2, and 27.95±7.50% body fat. No association was found between 25-hydroxyvitamin D3 levels and age, BMI, % body fat and changes in lipid profile. However, 25-hydroxyvitamin D3 levels were inversely associated with levels of glucose (p=0.04), insulin (p=0.05) and the homeostatic model assessment of insulin resistance (HOMA IR) index (p=0.048), using Pearson correlation. These associations remained significant even after adjustment for age, BMI, % body fat, lipid profile and mood symptoms, using multilinear regression. Additionally, when participants were divided into those with sufficient and insufficient 25-hydroxyvitamin D levels (<40 vs. ≥ 40 ng/mL), those with insufficient 25-hydroxyvitamin D had higher levels of glucose, insulin and HOMA IR index (p=0.019; p=0.011; p=0.008), using Student's t-test. However, STAI state (39.67±8.91 vs. 41.57±7.80; p=0.61), STAI trait (40.56±8.26 vs. 40.57±5.85; p=0.99) and BDI scores (10.04±5.05 vs. 7.86±7.98; p=0.375) were not associated with 25-hydroxyvitamin D levels and not different in individuals with 25-hydroxyvitamin D levels (<40 vs. ≥ 40 ng/mL, using Student's t-test. **Conclusion:** These results suggest that low levels of 25-hydroxyvitamin D are associated with dysfunction in glucose metabolism, but not with symptoms of depression and anxiety in healthy individuals.

Keywords: vitamin D, anxiety, depression, glucose profile.

D6

ANIMAL MODEL OF PARKINSON DISEASE POTENTIALIZES OXIDATIVE STRESS IN THE BRAIN OF RATS SUBJECTED TO CHRONIC MILD STRESS

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Introduction: Major depressive disorder (MDD) is one of the most prevalent form of mental illnesses affecting elderly people. Recent evidence suggests a relationship between MDD and neurodegenerative diseases, including Parkinson's disease (PD). PD patients has a predisposition to the development of MDD, and both neurobiological conditions are associated elevated oxidative stress. **Aim**: To investigate if rodents exposed to chronic stress and animal model of PD could show a higher damage in the brain areas involved with both MDD and PD. **Methods:** Wistar adult rats were subjected to chronic mild stress (CMS) by 40 days, and then it was induced PD using 6-hydroxydopamine into striatum. The experimental groups were: 1) Control+Sham; 2) CMS+Sham; 3) Control+PD; and 4) CMS+PD. Oxidative stress parameters were measured in the striatum, hippocampus and prefrontal cortex (PFC). **Results:** Lipid peroxidation was increased in the hippocampus and in the striatum in all groups ($p < 0.05$), compared to control. Carbonyl protein levels increased in the PFC and striatum in CMS+PD group ($p < 0.05$). Nitrite/Nitrate concentration were elevated in the PFC of CMS group, and in the striatum in all groups ($p < 0.05$). Myeloperoxidase activity was increased in the PFC of PD and CMS+PD groups, and in the striatum in the CMS+PD group ($p < 0.05$). The activities of antioxidant enzymes catalase and superoxide dismutase (SOD) were decreased in the PFC of CMS and CMS+PD groups ($p < 0.05$). Also, SOD was decreased in the hippocampus and striatum of PD and CMS+PD groups ($p < 0.05$). **Conclusion:** These findings suggest that CMS plus PD may induce a more pronounced oxidative stress in the brain, mainly in the striatum. These results may help to explain, at least in part, a common mechanism involved with the pathophysiology of PD and MDD.

Financial Support: UNESC, FAPESC e CNPq.

Keywords: oxidative stress, Parkinson's disease, major depressive disorder.

D7

SYSTEMIC P2X7 RECEPTOR BLOCKADE PREVENTS BEHAVIORAL AND NEUROCHEMICAL EFFECTS OF ACUTE RESTRAINT STRESS

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ATP has been implicated in the induction of acute and chronic inflammatory processes. The P2X7 receptors are activated by high concentrations of ATP, generally associated with stress or cellular damage. These receptors play a key role in the processing and release of the pro-inflammatory cytokine interleukin-1β (IL-1β) via activation of the inflammatory complex NLRP3 inflammasome. In this way, the present project aimed to evaluate the involvement of the P2X7 receptors in behavioral and neurochemical alterations induced by acute restraint stress in mice (CEUA protocol number: 9282200317). Animals were treated with the P2X7 receptor antagonist, Brilliant Blue G (BBG, 50 mg/kg, i.p., once a day for 7 days) or vehicle. On the seventh day, the acute restraint stress was applied for 7h, and forty minutes after the end of the stress, the animals were submitted to behavioral tests for the evaluation of locomotion, depressive-like and anxiety-like behaviors. IL-1β levels were measured in the hippocampus and prefrontal cortex (PFC), and the immunocontent of thioredoxin interacting protein (TXNIP) was evaluated in the hippocampus. Our results showed that acute restraint stress induced a depressive-like behavior, characterized by increased immobility in the forced swimming test (FST), with no changes in the locomotor activity in the open-field test. Additionally, acute restraint stress induced an anxiogenic-like effect characterized by the decrease in the amount of time spent in the open arms of the elevated plus-maze (EPM). Treatment with BBG prevented the depressive-like behavior induced by stress in FST but not the anxiogenic-like effect in the EPM. Concerning the neurochemical parameters, acute restraint stress decreased levels of IL-1β in the hippocampus and PFC, an effect that was partially prevented by BBG treatment. Moreover, acute restraint stress increased the immunocontent of TXNIP in the hippocampus, an effect that was further increased in the group treated with BBG and submitted to stress. Our results demonstrated that blockade of P2X7 receptors might represent a good strategy to prevent some of the behavioral alterations induced by acute stress, an effect that might be partially associated with the recovery of IL-1β levels in the hippocampus and PFC.

Keywords: Stress, ATP, P2X7, depression, inflammation.

D8

INVOLVEMENT OF ERK/GSK-3B SIGNALING PATHWAY AND HO-1 IN THE ANTIDEPRESSANT-LIKE EFFECT OF GUANOSINE IN THE TAIL SUSPENSION TEST

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Guanosine is an extracellular signaling molecule implicated in the modulation of glutamatergic transmission and neuroprotection. Our group has demonstrated that this endogenous nucleoside displays antidepressant-like properties. The present study investigated the involvement of ERK/GSK-3β signaling pathway and heme oxygenase-1 (HO-1) in the antidepressant-like effect of guanosine in the tail suspension test (TST) in mice. The experiments were performed after approval of the protocol by the Ethics Committee of the Institution (PP00795 Protocol). Female Swiss mice were treated with a sub-effective dose of guanosine (0.01 mg/kg, p.o.) or vehicle combined with a sub-effective dose of lithium chloride (a non-selective GSK-3β inhibitor, 10 mg/kg, p.o.), AR-A014418 (selective GSK-3β inhibitor, 0.01 μg/site, i.c.v.) or vehicle. In another set of experiments, mice were treated with an effective dose of guanosine (0.05 mg/kg, p.o.) or vehicle and 45 minutes after, they received U0126 (selective MEK1/2 inhibitor, 5 μg/site, i.c.v.), PD98059 (MEK1/2 inhibitor, 5 μg/site, i.c.v.), ZnPP (HO-1 inhibitor, 10 μg/site, i.c.v) or vehicle. Sixty min after guanosine administration the TST was carried out, followed by the open field test (OFT). Results showed that treatment with sub-effective doses of guanosine and lithium chloride or AR-A014418 produced a synergistic antidepressant-like effect in the TST. In addition, the antidepressant-like effect of guanosine was completely prevented by treatment of animals with U0126, PD98059 and ZnPP. The number of crossings in the OFT was not altered by any treatment. Altogether, these results provide evidence that the antidepressant-like effect of guanosine involves the activation of MEK1/2 and HO-1 and inhibition of GSK-3β.

Keywords: Depression, guanosine, ERK, GSK-3β, heme oxygenase-1.

D9

EFFECTS OF OMEGA-3, N-ACETYLCYSTEINE, AND FOLIC ACID ADMINISTRATIONS ON BEHAVIOR AND OXIDATIVE STRESS IN RATS SUBMITTED TO EARLY OR LATE LIFE STRESS

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According to studies, early or late stressful events can affect the brain and be involved with the development of major depressive disorder. We investigated the antidepressant and antioxidant effects of omega-3, folic acid and n-acetylcysteine (NAC) in rats subjected to early or late life stress. Early stress was induced by maternal deprivation (MD) and late stress by chronic mild stress (CMS). Young rats subjected to MD and adult rats subjected to CMS were administrated with omega-3 fatty acids (0.72 g/kg) or NAC (20 mg/kg) or folic acid (50 mg/kg) once by 20 days. After treatments, it was evaluated immobility time in the forced swimming test. Oxidative stress parameters were evaluated in the brain. Depressive-like behavior induced by CMS was prevented by NAC and folic acid, and depressive-like behavior induced by MD was prevented by NAC, folic acid and omega-3. Treatment with NAC, folic acid and omega-3 were able to exert antioxidant effects in the brain of rats subjected to CMS or MD. The treatments reduced protein carbonylation, lipid peroxidation, concentration of nitrite/nitrate, and the activity of myeloperoxidase (MPO) activity in the rat brain induced by CMS or MD. In addition, NAC, folic acid and omega-3 increased superoxide dismutase and catalase activities in the rat brain subjected to early or late life stress. In conclusion, NAC, omega-3 and folic acid based on it antioxidant properties could be interesting by it inhibition of behavioral and brain changes occurring from stressors life events.

Keywords: Omega-3; folic acid; antidepressant; animal model of depression; oxidative stress; major depressive disorde

E. Biomarkers of chemical exposure and toxicity

E1

EXACERBATED BH4 METABOLISM IN EXPERIMENTAL COLITIS PAIN

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Introduction: Pain is defined as an unpleasant sensory and emotional experience associated with actual or potential tissue damage. It is a common symptom in several disorders, particularly in inflammatory chronic diseases, including colitis. There is evidence showing that increased levels of tetrahydrobiopterin (BH4), a mandatory cofactor for enzymes with essential roles in the synthesis of the neurotransmitters and nitric oxide, intensify pain sensitivity, and that the reduction of BH4 intracellular concentrations confers analgesia. **Objective:** To investigate the participation of BH4 levels in pain hypersensitivity in an experimental model of colitis. **Methodology:** Colitis was induced by adding 2% dextran sodium sulfate (DSS) in drinking water, *ad libitum*, during 6 days, in adult Swiss male mice. In order to reduce BH4 levels, another group of animals received DSS plus an inhibitor of the BH4 synthesis, sulfasalazine (SSZ), during 7 days. Twenty-four h later, abdominal mechanical hypersensitivity to pain was determined by using the von Frey filaments, and afterwards mice were euthanized and colonic samples and urine were collected (PP00425/CEUA). The modulation of BH4 metabolism was assessed by measuring the levels of the metabolites neopterin and BH4, as well as, the content of the regulatory enzyme of the BH4 pathway, GTP-cyclohydrolase (GTPCH). **Results:** The intermediate score obtained in the clinical disease activity evidenced the induction of mild colitis in DSS-receiving mice. Abdominal mechanical hypersensitivity to pain was already observed at day 2 of DSS treatment and the administration of SZZ conferred analgesia. The content of GTPCH and

the levels of BH4 and neopterin were significantly increased in colonic samples from DSS-receiving mice, indicating the upregulation of the pathway during experimental colitis. In addition, neopterin was also significantly increased in the urine of DDS-receiving mice. However, BH4 levels were found to be reduced in the urine, probably due to the presence of inflammation/oxidative stress. **Conclusion:** To our knowledge, this is the first evidence showing the role of BH4 in colitis abdominal pain and that SZZ confers analgesia.

Keywords: pain, BH4, colitis, inflammation.

E2

EFFECTS OF MEDICINAL PLANT EXTRACTS ON CYP2D6 ENZYME-MEDIATED METABOLISM OF METOPROLOL

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The indiscriminate use of medicinal plants concomitantly with conventional drugs may result in herb-drug interactions that cause fluctuations in drug bioavailability, and consequent therapeutic failure and/or toxic effects. The cytochrome P450 superfamily of enzymes plays an important role in herb-drug interactions, and the CYP2D6 enzyme is one of the most significant, since it metabolizes 30% of the drugs on the market. The aim of the present study was to investigate the occurrence of *in vitro* interactions of medicinal plant extracts in metoprolol metabolism mediated by the CYP2D6 enzyme. Standardized extracts of ten medicinal plants (*Cynara scolymus; Matricaria recutita; Camellia sinensis; Cecropia glaziovii; Echinacea* sp*; Cimicifuga racemosa; Ilex paraguariensis; Ginkgo biloba; Bauhinia forficata* and *Glycine max)* were evaluated for their potential to modify metoprolol metabolism using a CYP2D6 recombinant enzyme. Among the tested extracts, *Camellia sinensis* (green tea) and *Cecropia glaziovii* (red embaúba) inhibited CYP2D6 activity *in vitro*, with similar inhibition profiles to that observed for quinidine, which was used as positive control. Both extracts showed concentration-dependent inhibition, with IC_{50} values of 384.3 and 396.0 µg/mL for *Camellia sinensis* and *Cecropia glaziovii*, respectively. The major phytoconstituents of these extracts were identified by UHPLC-ESI-MS/MS. To our knowledge, this is the first time the *in vitro* CYP2D6 inhibition detected for *Cecropia glaziovii* is described for this plant. In conclusion, the results demonstrate that *Camellia sinensis* and *Cecropia glaziovii* extracts inhibited CYP2D6 enzyme-mediated *in vitro* metabolism of metoprolol, an effect that could lead to clinically relevant interactions with substrates for this isoenzyme.

Keywords: Herb-drug interactions; CYP2D6; metoprolol metabolism; UHPLC-ESI-MS

E3

PAIN HYPERSENSITIVITY IN TWO STRAINS OF RODENTS IN COLITIS MODEL

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Pain is an unpleasant sensory and emotional experience associated with tissue damage. It is a symptom in inflammatory diseases like Colitis, which is characterized by loss of weight, diarrhea, hematochezia and pain. 50-70% of patients present pain and 20% of them still have pain even with negative lesions. These findings suggest that other factors might contribute to pain symptomatology. One of these factors could be genetic variations. This work aims to compare the pain hypersensitivity in two strains of rodents in colitis model: C57Bl/6 mouse (minimum genetic variation) and Swiss mouse (high variability). Colitis was induced by adding 2% dextran sodium sulfate (DSS) in autoclaved drinking water, *ad libitum*, during 6 days. Its severity was measured by the activity of the disease through observation of weight loss, stool consistency and hematochezia. The severity was calculated based on their combined scores (Index Score: IS; 0-12). The abdominal mechanical hypersensitivity was determined with a graded series of von Frey filaments that produced a bending force of 0.02 - 6 g. The stimuli were applied 10 times near the diaphragm in increasing order of force. The minimal force filament for which animals presented either a brisk abdominal withdrawal and/or abdominal licking in response to at least 5 of 10 stimulations was used to determine the mechanical response threshold. In C57Bl/6 mice the disease activity and nociception were evident after five days of treatment, eliciting mild colitis (IS ~ 4). In agreement with the intermediate IS, body weight was significantly reduced at days 6 and 7. A significant abdominal hypersensitivity to pain was observed at day 2 of treatment and reached a maximum response at days 5 and 7 (about 95 % of the nociceptive abdominal threshold). In Swiss mice, treatment also induced colitis. However, the disease activity was evident only after day 6 (mild colitis). The body weight was not compromised. There was increased abdominal hypersensitivity to pain starting and reaching the maximum effect at day 2 (98% of reduction in threshold). Altogether, the data show that even having genetic variability, the physiopathology of DSS-induced colitis can be investigated in both mouse strains.

Keywords: colitis, rodents, pain.

E4

SAFETY ASSESSMENT OF NANOPESTICIDES USING THE ROUNDWORM *Caenorhabditis elegans*

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The demand for food increases progressively, subordinated to the population growth. Agriculture provides such food sources. The use of agrochemicals, aiming high productivity, is a common practice for this purpose. Among them are pesticides, used in large quantities and, frequently, contributing significantly to environmental pollution, contamination of rivers, soils and food. The technological inefficiency of commercial pesticide formulations is a major cause for large volume of sales and frequent applications of these products. Nanotechnology offers, as an alternative to this scenario, nanopesticides with greater bioavailability and better use of the active principle, consequently decreasing the volume, frequency and environmental contamination. However, this field is new and still growing, especially areas that contemplate the interaction of nanopesticides with other organisms. This work, for the first time, investigated the toxicological implications in worms (*Caenorhabditis elegans*) exposed to three nanopesticides formulations: solid lipid nanoparticles loaded or not with atrazine and simazine, SLN; polymeric nanoparticles, NC_PCL loaded with atrazine; and chitosan/tripolyphosphate, CS/TPP, loaded or not with paraquat. Worms (L1 larval stage) were exposed chronically (48 hours) to nanoformulations on NGM (nematode growth media) plates with E. coli OP50. After this period, survival, reproduction, body size and uptake of the nanoparticles were analyzed or verified. All formulations, loaded or not with pesticides, increased lethality in a dose- dependent manner with similar LC50. Both loaded and unloaded NC_PCL were the most toxic formulations to developmental rate, significantly reducing worms length, even at low concentrations. In contrast, both CS/TPP nanoparticles were the least toxic, not affecting reproduction and body length at higher concentrations, probably due to the biocompatibility of chitosan. Notably, our results indicate that the observed effects were caused by the nanoparticles *per se*. These results suggest that the development of nanoparticles aiming agriculture applications needs more studies in order to optimize the composition and then reduce their toxicity to non-target organisms.

Keywords: Nanopesticides, C. *elegans*, atrazine, paraquat,

E5

PULP MILLEFFLUENT AFFECTS MAMMALIAM SPERMATOGENESIS

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The active and complete spermatogenesis is fundamental for male reproduction. It is known that pollutants can interfere in this important process, exerting effects on fertility. Pulp mill effluent (PME) is considered one of the major contributions of water pollution. Studies have shown the deleterious impacts of PME on fish reproduction but few data are available related to the mammalian reproduction disruption directly or indirectly by this contaminant. Thus, the aim of this study was to detect the effect of PME on the testis or Sertoli cells from immature and pubertal rats (*Rattus norvegicus*). For this, the acute effect, *in vitro*, during 1 hour, of the effluent at a low concentration (4%) have been studied in the testes from rats (10 and 30 days-old) by measuring: lactate content, glucose uptake, LDH enzyme activity and oxidative status (through the measurement of reactive oxygen species and evaluation of lipid peroxidation). The acute effect, *in vitro*, of the effluent at the same concentration and time of exposition also have been studied in the Sertoli cells from rats (10 and 30 days-old) by measurement: lactate content, LDH enzyme activity and cell secretion activity through the labeling of vesicles stained with quinacrine. The results have shown that the effluent decreased lactate content in the testis of 10-day-old rats and in the Sertoli cells of 30-day-old rats, decreased glucose uptake in the testis of both ages, decreased LDH activity in Sertoli cells from 30-day-old rats, increased lipid peroxidation and the content of reactive oxygen species in the testis of 30-day-old rats and caused a delay in secretion activity in Sertoli cells from rats of both the ages. These results indicate that the acute exposure to a low concentration of effluent changes the energetic metabolism, secretory activity and ROS generation in the testis. In a whole, these alterations produced by the effluent may disturb the spermatogenesis wave contributing to the infertility.

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Keywords: Spermatogenesis, Sertoli cell, testis, pulp mill effluent, energetic metabolism, oxidative stress.

E6

REGULATION OF CALCIUM INFLUX IN ZEBRAFISH GILLS: IONIC MODIFICATION AND ENDOCRINE DISRUPTORS EFFECT

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Introduction: The fish face the task of balancing the ionic gradients between the aquatic environment and their body fluids, they are in constant absorption of ions to avoid the ionic loss to aquatic environment. The gills are main responsibles for ionic regulation, including the regulation of calcium (Ca^{2+}), which is considered the most important intracellular ion. Nowadays, the environment is exposed to chemicals that are considered endocrine disruptors, producing estrogenic or anti-androgenic responses, among them are 2,2-bis(4-hydroxyphenyl)propane, known as bisphenol A (BPA) and dibutyl phthalate (DBP). **Objectives:** To study the influence of different concentrations of Ca^{2+} exposed in aquatic environment on Ca^{2+} influx and *in vitro* effect of BPA and DBP in *Danio rerio* (Zebrafish) gills. **Methodology:** *Danio rerio* fish were exposed in the aquatic environment for 12 hours with low Ca^{2+} (0,02 mM) and high Ca^{2+} concentrations (2 mM); animals from the control group were allocated in an aquarium containing water with no calcium. Subsequently, the gills were dissected to analyze radioactive Ca^{2+} ($^{45}Ca^{2+}$) influx at 30 and 60 minutes. Additionally, was analyze the acute effect *in vitro* (1 hour) of BPA (100 nM), 17β-estradiol/E2 (10 nM) and DBP at 1 pM, 1 nM and 1 μM on $^{45}Ca^{2+}$ influx in gills (CEUA PP00968). **Results:** After exposures to low and high Ca^{2+} levels, it was performed $^{45}Ca^{2+}$ influx (*in vitro*) in gills. High Ca^{2+} concentration (2 mM) exposure during 60 minutes led to a rise on $^{45}Ca^{2+}$ influx compared with the group exposed during 30 minutes, as well as when compared with the control group and with a group exposed to low Ca^{2+} concentration (0,02 mM) during 60 minutes. However, *in vitro* treatment at 60 minutes with BPA (100 nM), E2 (10 nM) and both associated, led to a rise on on $^{45}Ca^{2+}$ influx in relation to control group. Furthermore, only DBP treatment at 1 pM stimulated the $^{45}Ca^{2+}$ influx in gills when compared to the control group. **Conclusions:** Ca^{2+} influx in the gills is highly regulated since low and high Ca^{2+} concentrations in the aquatic environmental altered the *in vitro* Ca^{2+} influx, as well as the acute effect of endocrine disruptors at low concentrations, disturbing homeostasis ionic of fish.

Support: CNPq -PVE. 401410/2014-1; CAPES-PPG-Biochemistry.

Keywords: Ca^{2+}, Zebrafish, gills, BPA, DBP.

F. Biotechnology and structural biochemistry

F1

EVALUATION OF THE ANTICHOLINESTERASIC ACTIVITY OF A NOVEL MOLECULAR HYBRID BASED ON SEMICARBAZONE AND SEMICARBAZIDE COMPOUNDS

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The enzyme acetylcholinesterase (AChE) hydrolyzes the neurotransmitter acetylcholine (ACh) in the synaptic cleft and, thus, participates in the cholinergic neurotransmission. The knowledge on the cholinergic system has been used in the development of new treatments for neurological and psychiatric syndromes. Dysfunctions such as Alzheimer's disease (AD) exhibits massive synaptic loss and neuronal death in regions that are responsible by cognitive functions, including the cerebral cortex, hippocampus, and ventral striatum. Currently, the most commonly used treatment for AD is the use of AChE inhibitors (AChEi). However, adverse effects such as hepatotoxicity, bradycardia and arrhythmia, has led to the search for new compounds. Semicarbazones and semicarbazides are classes of compounds that present structural similarity to ACh, with studies already describing their anticholinesterasic and anticonvulsant activity, respectively. Para-substituted arylhydrazonecarbohydrazidamides are potential candidates for AChEi, since they are hybrid structures of the cited groups. The purpose of this research was to carry out molecular docking studies

to evaluate structure activity relationship of the compound RM78, which belongs to this chemical class, and in vitro assays to evaluate the anticholinesterasic activity in encephalic structures. The animals used in the experiments were adult Wistar rats. The activity of AChE was investigated in the homogenate of the brain regions, cerebral cortex, striatum, cerebellum and hippocampus. The synthesis of the salt was made by the addition of sodium hydroxide, and it was possible to evaluate its anticholinesterasic activity, with inhibition only in striatal samples at a concentration of 1000 μM. After enzymatic assays, molecular docking studies were performed and showed interactions between the compound and the catalytic site of the enzyme, which indicates that RM78 may be a potent AChEi due to the existence of interactions with important residues of the catalytic site, such as Tyr^{341} and Ser^{203}, the same observed in the interaction of huprine W.

Keywords: Cholinergic system. Acetylcholinesterase. Inhibitors. Hydrazonecarbohydrazidamides. Alzheimer's disease.

F2

INFLUENCE OF ACETIC AND FORMIC ACIDS ON *Spathaspora passalidarum* KINETIC GROWTH AND FERMENTATION PROFILE

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Introduction: Efficient xylose fermentation and high tolerance to lignocellulosic-derived inhibitory compounds are two key traits required by the microorganism to be used for second-generation ethanol production. In this way, several researches are bioprospecting microorganisms that could naturally have both traits. The newly isolated yeast *Spathaspora passalidarum* has attracted attention because this yeast has already the innate ability to convert with high yields xylose into ethanol. However, not much is known about this yeast tolerance to inhibitory compounds such as weak acids. **Objective:** In this work the influence of acetic and formic acid on the kinetic growth and fermentation profile of the *Sp. passalidarum* strain 2.1 was evaluated. **Material and Methods:** Kinetic growth assays were carried out in 96-well plates containing synthetic media with 20 $g.L^{-1}$ of glucose or xylose, added with increasing concentrations of acetic (0-4 $g.L^{-1}$) or formic (0-0.4 $g.L^{-1}$) acids. Batch fermentation was performed in hemicellulosic hydrolysates containing 15.2 $g.L^{-1}$ of xylose, 2.2 $g.L^{-1}$ of acetic acid and 0.44 $g.L^{-1}$ of formic acid. **Results and Discussion:** Independent from the carbon source used, during kinetic growth assays low concentrations of acetic acid (1.2 $g.L^{-1}$) or formic acid (0.18 $g.L^{-1}$) were enough to double the yeast *lag* phase and/or to decreased by half the yeast total cellular growth. Concentrations of both acids beyond those values completely inhibited the yeast cell growth. By contrast, no such severe inhibition was observed during batch fermentation of hemicellulosic hydrolysates, and the yeast showed a satisfactory xylose consumption rate (91.8%) and ethanol yield (0.35 $g.g^{-1}$). **Conclusion:** Although with a satisfactory fermentation performance, the *Sp. passalidarum* strain 2.1 has low tolerance to both acetic and formic acid in either glucose or xylose carbon sources. Therefore, this yeast would hardly be used directly as the fermentation microorganism for lignocellulosic second-generation ethanol production.

Keywords: inhibitors, hemicellulose hydrolysate, xylose, second-generation ethanol

F3

DEVELOPMENT OF GREEN COFFEE NANOEMULSION FOR COSMETIC PURPOSES

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Nanotechnology works in the development of nanometer scale materials (from 1 to 1000 nm), with application in the most different areas, such as biological, chemical, physical, and electronic areas. In the last years, coffee has been admitted for having antioxidant potential, helping in the fight against free radicals, being a promoter of human health. It is considered as a powerful source of phenolic compounds such as chlorogenic acid, which has antioxidants that act in the protection of the biological system to mitigate the damages caused by the oxidation reactions. In addition, the topical use of these compounds has been outstanding among skin professionals because of its biological properties of preventing photo aging, natural aging and as a sun protection agent. In this context, the objective of this work is to develop and characterize a nanoemulsion containing a combination of extract and oil of green coffee aiming the prevention of free radicals action for cosmetic purposes. Green coffee nanoemulsions were prepared by the spontaneous emulsification method. In order to optimize the preparation conditions, nanoemulsions were prepared with different concentrations of green coffee oil, aqueous extract green coffee, lipophilic surfactant lecithin, and hydrophilic surfactant poloxamer. Nanoemulsions were characterized in terms of mean particle size, polydispersity index (PdI), zeta potential, pH and stability. Subsequently, the antioxidant activity of nanoemulsions was investigated by DPPH and FRAP methods. Green coffee nanoemulsions showed monodisperse distribution of particles (PdI < 0.3), with a mean particle size between 218 and 320 nm. The zeta potential was around -41 and -49 mV. The pH was around 6.0-6.5, compatible to skin application. The stability study showed that the formulations remained stable after 30 days of storage at 4 °C (refrigerator) and room temperature. In relation to the analysis of the antioxidant potential,

the nanoemulsions showed higher activity when compared to the free green coffee extract and oil. Therefore, green coffee nanoemulsions show to have potential effect for cosmetic application aiming at the reduction of the damages caused by the free radicals, as well as help in the improvement of the quality of life.

Keywords: nanoemulsion, phenolic compounds, chlorogenic acid, green coffee, antioxidant activity, skin rejuvenation.

F4

NON-CLINICAL STUDY OF BIOTECHNOLOGICAL PRODUCTS FROM BACTERIAL CELLULOSE ON WOUND HEALING OF PRESSURE ULCERS

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Introduction: Pressure ulcers are an important health problem due to their impact on the morbidity and suffering of patients, and are increasing over the world owing to aging of the population. Moreover, pressure ulcers are defined as injuries in skin, which are compressed between the bony prominences of the patients and an external surface leading skin necrosis inducing pain and distress, leading to the impairment of the quality of life of patients. **Objectives:** Herein, we investigated the effects of biotechnological products from bacterial cellulose in an animal model of pressure ulcers. **Methods:** Non-invasive model of pressure injury was induced in male Swiss mice. Animals were exposed to four cycles of cutaneous ischemia–reperfusion (I/R) by trapping the dorsal skin between two magnetic plates for 12 hours, followed by plate removal. Four IR cycles were performed in each mouse to initiate decubitus ulcer formation. To investigate bacterial cellulose effect on wound healing of pressure ulcers, the mean area of skin ulcers, presence of exudate, redness and moisture were evaluated at 0, 3, 5, 7, 10, 12 and 15 days after skin injury. The results are expressed as percentage of original wound area. Bacterial cellulose hydrogel plus montmorillonite (BCH-M) was applied daily in right side, and left side was used with untreated control. Dersani hydrogel with alginate® was used as positive control drug. **Results:** Application of hydrogel incorporated with bacterial cellulose plus montmorillonite at the beginning of reperfusion markedly inhibited the formation of cutaneous pressure ulcers when compared to untreated contralateral injury. Wound areas in BCH-M-treated mice were significantly smaller than those in untreated contralateral control from 3 to 7 days after reperfusion. However, BCH-M did not alter inflammatory signs – exudate, redness and moisture – during pressure ulcers model. **Conclusion:** BCH-M application improves cutaneous wound healing of pressure ulcers in mice. Moreover, hydrogel showed a great potential for biotechnological innovation, particularly considering the low cost of production and easy therapeutic applicability. Thus, BCH-M application may be a good therapeutic alternative in the future for treatment of pressure ulcers and other skin injuries. **Financial Support:** CNPq; CAPES; FAPESC; INCT-INOVAMED; PGN-UFSC.

Keywords: pressure injury, bacterial cellulose membrane hydrogels.

F5

CLONING AND CHARACTERIZATION OF SUGAR TRANSPORTERS FROM *Spathaspora arborariae* IN RECOMBINANT *Saccharomyces cerevisiae*

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Introduction: *Saccharomyces cerevisiae* is the main microorganism employed for ethanol industry, but it is unable to use xylose as a carbon source. One bottleneck for the fermentation of xylose is the lack of efficient sugar transporters. A possible solution is cloning transporters from rotten wood yeasts, like *Spathaspora arborariae*, into *S. cerevisiae*. *S. arborariae*'s genome is sequenced and it displays ten genes that may translate to sugars transporters. Two were already characterized and do not exhibit activity over xylose. Cloned heterologous transporters have the tendendy to suffer endocytosis after being ubiquitinared, but removing some lysine residues from the transporter can avoid this. **Objectives:** Test the aptitude of two transporters from *S. arborariae* cloned into *S. cerevisiae* on growth and fermenting xylose and other sugars. Furthermore, we wanted to change the genes in a way that the transporters stayed longer in the membrane. **Material and Methods:** We used the DLG-K1 strain of *S. cerevisiae*, which lacks the major hexose transporters (*hxt*-null) and has high activity of the xylose reductase, xylitol dehydrogenase and xylulokinase enzymes allowing xylose utilization, and transform it with plasmids containing genes encoding transporters *SUT6* and *SaXUT1* from *S. arborariae*. These genes were also amplified removing the first 17 N-terminal residues of *SaXUT1*, removing 4 lysine residues that could be ubiquitinated (*T-SaXUT1*). The growth and fermentation performance of the new strains were tested. **Results and Discussion:** The transporter SUT6 is very similar to other sugars transceptors and allowed the consumption of glucose when expressed by the cell. The

transporter XUT1 allowed the cell to grow in both glucose and xylose, but the strain was not capable to ferment the last one. However when the gene is truncated, the strain with the transporter tXUT1 was capable to ferment xylose. **Conclusion:** Other genes not yet investigated could present transporters with more efficient activity over xylose. Still removing the ubiquitination sites may be a good way to increase the efficiency of xylose uptake in recombinant yeasts. These results reinforce the relevance of researches on sugar transporters for increase production in bioethanol.

Keywords: Biotechnology; Fermentation; Yeast; Xylose; Ethanol; Ubiquitination.

F6

EPIGENETIC CONTROL OF HOXA GENE FAMILY CLUSTER IN OSTEOBLASTIC DIFFERENTIATION

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Homeobox genes are transcriptional regulators that integrate a complex network of signals responsible for phenotypic changes during development by gene transcription activation or repression. They are controlled by epigenetic mechanisms that play a key role in coordinated gene expression control. HOX genes are able to determine important products such as the position and shape of bone throughout the vertebrate body by modulating important transcription factors, including RUNX2 and OSTERIX, which are essential for bone development. Recently, it has been shown that regenerative physiological processes are dependent on the action of HOX genes to coordinate fracture repair in adults. Here, we evaluated the involvement of epigenetic machinery in modulating the expression of the HOXA gene family of genes in a human primary osteoblastic cell differentiation model. The results obtained by qPCR showed clearly that there is a dynamic involvement of members of the HOXA gene family. To observe, the *HOXA1, 2, 3, 4, 5, 6* and *9* genes were upregulated, while *HOXA10* decreased and *HOXA7* did not present changes. Regard the epigenetic machinery, our results showed that there was a significant increase of the activation of genes encoding important enzymes capable of modulating the metabolism of the methyl moiety, such as DMNT3A and TET2. Additionally, we showed there is an epigenetic marker in promoter region of genes of *HOXA3, 6,* and *9* and establishing an important correlation between hypomethylation and gene activity. Furthermore, intergenic long non-coding RNAs (lncRNAs), HOTAIR and HOTTIP, were also evaluated. Our results show significant promoter region hypomethylation and gene expression increase (~ 150-fold changes) only for HOTTIP in osteogenic phenotype group. Taken together, our results showed for the first time the involvement of epigenetically modified HOXA genes in the family in osteoblasts and we believe that there may be a retro activation of HOXAs expression by lncHOTTIP and we suggest these new molecular parameters for the understanding of osteoblast renewal long of life.

Keywords: Bone, Osteoblast, HOXA methylation, HOXA genes, differentiation, development.

F7

INVESTIGATION OF THE POSSIBILITY OF EPIGENETIC INHERITANCE IN THE INDUCTION OF α-AMYLASES IN LARVAE OF *Zabrotes subfasciatus* (COLEOPTERA: CHRYSOMELIDAE: BRUCHINAE)

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Introduction: *Zabrotes subfasciatus* has great economic impact since it infests two of the main legume species cultivated in Brazil, *Phaseolus vulgaris* and *Vigna unguiculata*. Efficient defense mechanisms, which are not harmful to the environment and humans and that repel or intoxicate these insects, can be found in chemical substances of the plants. α-Amylase inhibitor 1 (αAI1) present in *P. vulgaris* seeds, has been shown to be extremely effective against Old World bruchids such as *Callosobruchus chinensis* and *Callosobruchus maculatus*, but ineffective against *Z. subfasciatus*. It tolerates the presence of αAI1 by having the ability to secrete two induced α-amylase isoforms, which are constitutively and irreversibly expressed and are insensitive to the inhibitor. The αAI1, besides representing an inducer for this enzymatic overexpression, also represents a biological stress for these insects, suggesting that mechanisms of epigenetic inheritance may be involved. **Objectives:** The adaptive mechanism investigated here is whether the induction of the α-amylase dimer can be transmitted from parents who have had a life cycle in *P. vulgaris* seeds, in the presence of αAI1, to generations in *V. unguiculata* where there is no α-amylase inhibitor. **Results and Discussion:** When analyzing the α-amylase patterns of larval *Z. subfasciatus*, which had a parent developed in *P. vulgaris* seeds, but now in *V. unguiculata* seeds, it was not observed the inheritance of the constitutive expression of the two α-amylase induced isoforms. We found that, when *Z. subfasciatus* starts growing in *V. unguiculata* seeds, the patterns of α-amylase activity characteristic of larvae from these grains are restored, regardless of whether the parental origin was. However, the presence of αAI1 in *P. vulgaris* seeds promotes a delay in development time, as well as a compromise in their offspring. **Conclusion:** Future studies with a greater number of

generational passages in seeds of *P. vulgaris* may give us more confirmations about the inheritance of the constitutive expression of the two isoforms of α-amylase and the impact in the insect fitness.

Keywords: *Zabrotes subfasciatus*, α-Amylase inhibitors; *Vigna unguiculata*, Epigenetic inheritance; *Phaseolus vulgaris*

G. Oxidative/nitrosative stress, redox status and biological implications

G1

EFFECTS OF CHRONIC FRUCTOSE CONSUMPTION IN THE PREFRONTAL CORTEX CELLULAR DEFENSE

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Introduction: Fructose (FRU) is a natural fruit sugar used on a large scale by the food industry as a cheap sweetener. Exacerbated FRU consumption is a risk factor for several pathologies such as obesity, diabetes and central nervous system disorders**. Objectives**: To investigate the effects of chronic FRU consumption on the activity of catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR), thioredoxin reductase (TrxR), and glyoxalase (GLO) enzymes in cerebral cortex, hippocampus, striate and liver. GPx, GR, TrxR1, GLO1, GLO2, and glutamate-cysteine ligase protein content were also evaluated in cerebral cortex and liver. **Material and methods:** Female mice at 9 months of age received water (CTL) or 10% fructose (FRU) in drinking water ad libitum for 12 weeks. Samples were collected for enzymatic and Western blot assays. **Results and discussion**: Among the tissues analyzed, the prefrontal cortex was more vulnerable to treatment, since CAT (CTL = 0.57 ± 0.10 vs FRU = 0.25 ± 0.03 umol/min/mg) and SOD (CTL = 177.8 ± 23.64 vs FRU = 82.23 ± 17.56 U/mg) activities decreased significantly, and GPX activity increased (CTL = 7.03 ± 0.27 vs FRU = 8.35 ± 0.36), which indicates an adaptive response to compensate for decrease CAT. In addition, minor alterations were observed for other analyzed enzyme activities and protein content. **Conclusions:** Our study suggests that chronic consumption of FRU decreases antioxidant defenses (SOD, CAT) mostly in cerebral cortex, which may be associated to higher vulnerability to oxidative stress. Cerebral cortex may be a target of FRU toxicity, which can be related to increased locomotor activity observed in these animals.

Support: CNPq

Keywords: Fructose, diabetes, antioxidante defenses, catalase, superoxide dismutase

G2

EFFECTS OF METHYLGLYOXAL TREATMENT ON ITS DETOXIFICATION SYSTEM IN MOUSE BRAIN

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Introduction: Methylglyoxal (MGO) is formed in the glycolytic pathway, and detoxified through the glyoxalase (Glo) system, comprised of Glo1 and Glo2. MGO can form adducts on proteins interfering with their functions. MGO is associated with neurodegenerative diseases, like Alzheimer's and Parkinson's diseases. **Objectives:** Investigate the effects of MGO treatment on the MGO detoxification system and parameters related to oxidative stress in mouse brain. **Material and methods:** Female mice were treated with single daily injections of MGO (10, 25, 50 mg/kg) for 7 days. Twenty-four hours after the last treatment, the prefrontal cortex and hippocampus were collected for evaluation of the levels of Glo1 and Glo2 by Western blot, and for the measurement of enzyme activities Glo1, glutathione reductase (GR), thioredoxin reductase (TrxR) and total glutathione levels (GSH-t). MGO and malondialdehyde adducts to proteins were assayed by dot blot. **Results and discussion:** We found no changes in the activity of the enzymes GR and TrxR or in the levels of GSH-t and MGO adducts to proteins. There was an increase in malondialdehyde adducts in hippocampus, but not in the cerebral cortex. The levels of Glo1 protein were increased in the cortex, but their enzymatic activity did not follow this increase. This indicates that in some way their activity was inhibited. Glo2 protein levels in the cerebral cortex decreased, showing that the cycle of MGO detoxification was impaired. Similar effects were found in the cell line HT22, whose Glo2 degradation was mediated by autophagy. **Conclusion:** Our data show that the cerebral cortex is more susceptible to MGO in comparison to the hippocampus and that the impairment in Glo system may be a molecular target of MGO, decreasing its own degradation. Further investigation will still be necessary to clarify possible mechanisms of action of MGO.

Keywords: methylglyoxal, glyoxalase, glutathione; thioredoxin reductase.

G3

METHYLGLYOXAL LEADS TO IMPAIRMENT OF BRAIN MITOCHONDRIAL FUNCTION

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Introduction: The cytotoxic dicarbonyl methylglyoxal (MGO) is physiologically generated by both enzymatic or nonenzymatic reactions, including glycolysis, a major site of MGO production. Several pathologies are highly associated to MGO. Literature consistently indicates that mitochondrial impairment may be the first step on the development of neurological disorders, such as Alzheimer´s and Parkinson´s diseases. **Objectives:** In the present work, we aimed to evaluate how MGO can affect mitochondrial function. **Material and methods**: Sinaptosomes were isolated from mouse cerebral cortices through differential centrifugation. Viability was analyzed using MTT and mitochondrial function was measured through oxygen consumption using a protocol of whole brain isolated mitochondria measured in the Oroboros™ apparatus, using substrates such as pyruvate, malate, succinate and ADP, as well as uncoupler FCCP and complexes inhibitors rotenone and antimycine. Parameters were later analyzed using O_2 flux. **Results and discussion:** Using different concentrations (0.3, 1, 3 and 10 mM) for 1 hour, we showed that viability was not affected up to 1 mM MGO, thus this concentration was used for further analyses. On the oxygen consumption analysis we verified that whole brain isolated mitochondria proton leak of complex I and II, oxidative phosphorylation (OXPHOS), maximum respiration, and OXPHOS associated to complex I and II were decreased by MGO. These effects might indicate an impairment on coupled functions, as OXPHOS, which may lead to a higher generation of reactive oxygen species (ROS). **Conclusion:** MGO at a non-toxic concentration clearly decreased brain mitochondrial respiratory parameters. Our data support the literature data indicating that MGO might be responsible for *in vivo* pathological mitochondrial alterations, which may lead to development of neurodegenerative disorders.

Support: CNPq and CAPES

Keywords: methylglyoxal; mitochondria; oxygen consumption, respiration, OXPHOS

G4

THE EFFECT OF VOLUNTARY RUNNING-WHEEL IN THE ANTIOXIDANT STATUS AND MITOCHONDRIAL FUNCTION IS DEPENDENT OF SOCIABILITY CONDITIONS

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The voluntary running-wheel is widely used as a physical activity model in rodents, but most studies analyze the beneficial effects of this intervention on protocols with isolated animals in the home cage. Social isolation is associated to several mental and physical health impairments. In line with this, isolated animals are more vulnerable to oxidative stress and mitochondria dysregulation. The aim of this study was to investigate the effect of free access to voluntary running-wheel by 21 days on the nonprotein thiols (NPSH) and malondialdehyde (MDA) levels, glutathione peroxidase (GPx) and glutathione reductase (GR) activity in the cerebral cortex and cerebellum, heart, skeletal muscle gastrocnemius, liver and blood of mice isolated or grouped (3 animals per cage). Furthermore, the activities of mitochondrial complexes I and II were also measured in the cerebral cortex and gastrocnemius in the same experimental groups. The results showed that social isolation increased MDA levels in the cerebral cortex and blood, and physical activity intervention in the voluntary running-wheel is not able to reverse such change. Interestingly, physical active protocol in mice grouped, but not in isolated mice, reduced MDA levels in the liver. In another way, physical activity protocol only in isolated mice reduced the MDA levels in the gastrocnemius. The results showed also that the physical activity increased the levels of NPSH in the cerebral cortex of mice that were grouped, as compared to the sedentary group. Furthermore, individualized animals, as compared to grouped animals, presented a lower activity of the GPx in the cerebellum and gastrocnemius, as well as the activity of GR in the cerebral cortex and liver. In contrast, the chronic deprivation of social stimuli in mice induced a greater activity of the enzyme GPx in the cerebral cortex and heart. Physical activity reduced GPx enzyme activity in the cerebral cortex of isolated animals and GR in the cerebral cortex and liver of isolated animals. Finally, individualized animals had a higher activity of mitochondrial complexes I and II in the cerebral cortex, but not in the gastrocnemius, and the exercise was not able to alter the mitochondrial complexes activity. In conclusion, the results showed the importance of social isolation for the effects of physical activity on the antioxidant status and mitochondrial functionality.

Keywords: Glutathione; Mitochondria; Oxidative stress; Physical activity; Voluntary running-wheel; Social

isolation.

G5

THE EFFECTS OF TERT-BUTYLHYDROQUINONE ON GLUTATHIONE REDUCTASE IN PACIFIC OYSTER

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Introduction: Three different antioxidant enzymes mainly mediate intracellular peroxide metabolism: catalase, glutathione peroxidase (GPx) and peroxiredoxin. GPx converts the reduced form of glutathione (GSH) to its disulfide form (GSSG) upon peroxide removal. GSSG is reduced back to GSH by glutathione reductase (GR) at expenses of NADPH. Thus, GR is a critical enzyme in maintaining high GSH/GSSG ratio, providing a continuous backup system for GPx. Our group has been repeatedly shown lower GR activity in bivalves exposed to electrophilic compounds. This indicates GR is highly relevant for peroxide removal and bivalve survival. **Objectives:** Here, we investigated the potential inhibitory effect of tBHQ on the GR activity, and possible biological relevance predicted, in Pacific oyster. **Material and methods:** Pacific oyster (*Crassostrea gigas*) were treat with 10 or 30 uM tert-butyhydroquinone (tBHQ) and gills were collected 24, 48 or 96h. Gill crude extract was obtained after centrifugation (20.000g, 20 min) for evaluation of the relative levels of GR protein by Western blot, and the enzymatic activity by a spectrophotometric method. *In vitro* studies were performed by incubating the crude extract or purified GR with 10, 30 or 100 uM tBHQ. **Results and discussion:** tBHQ (10 and 30 uM) treatment drastically increased GR activity at 96h; however, GR activity did not follow the same pattern, always being lower as compared to the protein increase. *In vitro* studies showed that GR, in the presence of NADPH, is almost fully inhibited by tBHQ after 4h of incubation, a pattern also observed in purified yeast GR. However, tBHQ was ineffective in inhibiting purified GR after 1h, indicating tBHQ derivatives are the active inhibitors. **Conclusion:** GR is an *in vivo* target of tBHQ, leading to decreased activity, as compared to protein levels, which may compromise oysters' capacity of degradate peroxide and survive.

Sponsorship: CNPq and CAPES.

Keywords: glutathione reductase, tert-butylhydroquinone, oyster, bivalves

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