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# Revista de Ciências Médicas e Biológicas

## *Journal of Medical and Biological Sciences*

**V Simpósio Internacional de Neuroquímica e Fisiopatologia da Célula Glial**

***V International Symposium of Neurochemistry and Pathophysiology of the Glial Cell***

**X Simpósio de Atualização em Farmacologia da UFBA**

**X Symposium in Pharmacology of UFBA**

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# REVISTA DE CIÊNCIAS MÉDICAS E BIOLÓGICAS

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Pavilhão de Aulas Reitor Felipe Serpa (PAF-I) - 09 a 12 outubro de 2019

UFBA - Av. Adhemar de Barros, s/n - Campus Universitário de Ondina – Salvador

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**V Simpósio Internacional de Neuroquímica e Fisiopatologia da Célula Glial**
**X Simpósio de Atualização em Farmacologia da UFBA**



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**Endereço**: Instituto de Ciência da Saúde (ICS) — Universidade Federal da Bahia (UFBA). Av. Reitor Miguel Calmon, s/n.º, Vale do Canela. CEP: 40.110-100 Salvador Bahia Brasil. Fone: (0xx71) 3283-8959. Fax: (0xx71) 3283-8894. E-mail: cimedbio@ufba.br
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## SUMÁRIO


Volume 18 — Suplemento — Outubro 2019

**ABSTRACTS OF POSTER SECTION**

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# APRESENTAÇÃO

O 5º Simpósio Internacional de Neuroquímica e Fisiopatologia da Célula Glial aconteceu em conjunto com o 10º Simpósio de Atualização em Farmacologia da Universidade Federal da Bahia, de 09 a 12 de outubro de 2019 no Pavilhão Felipe Serpa (PAF I) no Campus Ondina, em Salvador-Bahia. Além de pesquisadores locais, o evento contou com a participação de renomados palestrantes do Brasil, das Américas e da Europa e Oceania, que apresentaram e discutiram avanços no entendimento de aspectos da neuroquímica, imunologia, fisiologia e fisiopatologia de doenças que afetam o sistema nervoso e sobre o entendimento de alvos moleculares para descoberta de drogas. Entre os palestrantes, um número significativo de professores da recém-criada Escola de Estudos Superiores em Neuroquímica (EAENq), cujo objetivo é a disseminação do conhecimento em Neuroquímica e o compartilhamento de conhecimento e tecnologia por meio de redes de cooperação, associado à formação de alunos de graduação e pós-graduação. Durante o simpósio, os participantes participaram de conferências, sessões científicas de jovens cientistas (estudantes de graduação e pós-graduação) e tiveram a oportunidade de discutir resultados e projetos em desenvolvimento durante a sessão de pôsteres. Com mais de 160 participantes entre estudantes de graduação e pós-graduação, pesquisadores e profissionais, o Simpósio trouxe a possibilidade de interação da comunidade acadêmica e científica local com renomados pesquisadores, permitindo o intercâmbio do conhecimento científico e tecnológico.

# PRESENTATION

The 5th International Symposium on Neurochemistry and Pathophysiology of the Glial Cell toke place in conjunction with the 10th Symposium on Updating in Pharmacology of the Federal University of Bahia from 09 to 12 October 2019 in the Felipe Serpa Pavilion (PAF I) at the Ondina Campus, in Salvador-Bahia. The event featured besides local researchers the participation of renowned speakers from Brazil, the Americas and researchers from Europe and Oceania, that presented and discussed advances in the understanding of aspects of neurochemistry, immunology, physiology and pathophysiology of diseases that affect the nervous system and about the understanding of molecular targets for drug discovery. Among the speakers a significant number of professors from the newly created School of Higher Studies in Neurochemistry (EAENq), whose objective is the dissemination of knowledge in Neurochemistry and sharing of knowledge and technology through networks of cooperation, associated with the formation of graduate and undergraduate students. During the symposium, participants attended conferences, scientific session of young scientists (graduate and post-graduate students), as well had the opportunity to discuss results and projects in development during the poster session. With more tham 160 participants between undergraduate students and graduate students, researchers and professionals, the Symposium brought the possibility of interaction of the local academic and scientific community with renowned researchers, allowing the exchange of scientific and technological knowledge.

*Silvia Lima Costa*
Chair
Professor, VMD, MPhil, PhD
PI-Laboratory of Neurochemistry and Cellular Biology
Department of Biochemistry, Institute of Health Science
Federal University of Bahia Universidade Federal da Bahia
Salvador, BA , 40.110-902, Brazil

# PROGRAM

***Quarta-feira, 09 de outubro***

**08:00 – 09:00** **Registro** [Registration]

**09:00 – 13:00** **Mini curso pré-simpósio - Escola de Altos Estudos em Neuroquímica (EAENq) –Bases do Conhecimento da Pesquisa em Neuroquímica.** [Bases of the knowledge of Research in Neurochemistry]. **Victor Diógenes Amaral da Silva** (LabNq-ICS/UFBA), **Balbino Lino dos Santos** (UNIVASF, LabNq-ICS/UFBA) **& Silvia Lima Costa** (LabNq-ICS/UFBA).

**14:00 – 15:00** **Mini curso pré-simpósio - Estratégias na formação de jovens cientistas: Publicando no Inicio da carreira.** [*Strategies in the formation of young scientists: publishing at the beginning of the care*er]. **Robyn Louise Tolhurst** (Red Fern Communication, Sydney, Austrália).

**15:30 – 15:40** **CERIMÔNIA DE ABERTURA [OPENING CEREMONY]. Silvia Lima Costa** (LabNq-ICS/UFBA)

**15:40 – 17:00** **Apresentação da Escola de Altos Estudos em Neuroquímica (EAENq). Estado Atual de Pesquisa e Formação de Recursos Humanos.** [Presentation of the School of High Studies in Neurochemistry (EAENq). Current State of Research and Training of Human Resources].
**Maria de Fátima Dias Costa.** Laboratório de Neuroquímica e Biologia celular, Instituto de Ciências da Saúde, Universidade Federal da Bahia, Salvador.
**Vivaldo Moura-Neto.** Laboratório de Biomedicina do Cérebro, Instituto Estadual do Cérebro Paulo Niemeyer, Rio de Janeiro.
**Henning Ulrich.** Laboratório de Neurociências, Instituto de Química, Universidade de São Paulo, São Paulo.
**Yanier Nuñez Figueredo.** Centro de Investigación y Desarrollo de Medicamentos (CIDEM), Havana, Cuba.
**Dora Brites.** Instituto de Investigação do Medicamento da Faculdade de Farmácia da Universidade de Lisboa, Portugal.
**Gilles Guillemin.** Neuroinflammation Group, Faculty of Medicine and Health Sciences, Macquarie University, Sydney, Austrália.
**Arthur Morgan Butt,** Cellular Neurophysiology Group, SPBS, University of Portsmouth, Reino Unido.´

**17:00 – 18:00** **CONFERÊNCIA – Imunointervenção como terapêutica em doenças neurodegenerativas.** [Immunointervention as therapeutics in neurodegenerative diseases]. **Dora Brites** (Universidade de Lisboa, Portugal).

***Quinta-feira, 10 de outubro***

**09:00 – 12:00** **CONFERÊNCIAS - Desafios e Experiências em Biotecnologia e Desenvolvimento de Fármacos.** [Challenges and experiences in Biotechnology and Drug Development].
**Medicamentos e Sociedade.** [Medications and Society]. **Eudes da Silva Velozo** (FAR/UFBA).
**Drogas multi-alvos: desafio no desenvolvimento de fármacos para doenças neurodegenerativas.** [Multi-target drugs: challenge in the development of drugs for neurodegenerative diseases]. **Yanier Nuñez Figueredo** (CIDEM, Univ. Havana- CU).
**Estratégias no registro de patentes aplicadas ao desenvolvimento de fármacos.** [Strategies in patent registration applied to drug development]. **Eduardo Muniz Santana Bastos** (PROFNIT-IQ/UFBA).
**BIOLINKER, a startup líder na síntese de proteínas recombinantes livres de células do**

**Brasil.** [BIOLINKER, the leading startup in the synthesis of recombinant free cell proteins in Brazil]**. Mona das Neves Oliveira & Sandi Ravbar** (BIOLINKER).

**13:00 – 14:00** **CONFERÊNCIA TÉCNICA - Citometria de Imagem 2020 - Da IHC à Citometria de Imagem Contextual.** [Image Cytometry 2020 – From IHC to Contextual Image Cytometry]. **Rupert Ecker** (TissueGnostics, Viena, Áustria).

**14:00 – 18:00** **CONFERÊNCIAS: Comunicação neurônio-glia na saúde e na doença cerebral.** [Neuron-Glia Communication in Health and Brain Disease].

**Mantendo o cérebro conectado: a importância da mielinização ao longo da vida.** [Keeping the brain wired: the importance of life-long myelination]. **Arthur Morgan Butt** (Neurophysiology Group, School of Pharmacy and Biomedical Sciences (SPBS) University of Portsmouth, Reino Unido).

**Promovendo a geração de oligodendrócitos via inibição direcionada de GSK3b na substância branca murina.** [Promoting oligodendrocyte generation via targeted inhibition of GSK3b in the murine white matter]. **Francesca Pieropan** (Cellular Neurophysiology Group –SPBS, University of Portsmouth, Reino Unido).

**Identificação de lise oxidase e PPAR gama como reguladores mestres da astrogliogênese .** [Identification of lysis oxidase and PPAR gamma as master regulators of astrogliogenesis]. **Andrea Domenico Rivera** (Cellular Neurophysiology Group –SPBS, University of Portsmouth, Reino Unido).

**Usando metabólitos da via da cinurenina como biomarcadores para o prognóstico e progressão de doenças.** [*Using kynurenine pathway metabolites as biomarkers for disease prognosis and progression*]. **Gilles Guillemin** (Faculty of Medicine and Health Sciences, Macquarie University, Sydney, Austrália).

***Sexta-feira, 11 de outubro***

**09:00 – 12:00** **CONFERÊNCIAS - Mecanismos de neuroinflamação *versus* neurodegeneração** [*Mechanisms of* Neuroinflammation versus Neurodegeneration].

**Interações entre o sistema nervoso entérico e a microbiota intestinal: impacto na fisiopatologia de doenças neurodegenerativas.** [Interactions between the enteric nervous system and the intestinal microbiota: impact on the pathophysiology of neurodegenerative diseases]. **Marcelo Biondaro** (UFRB).

**O sistema endocanabinoide e a diversidade da glia.** [The endocannabinoid signaling regulation and glial diversity]. **Clarissa de Sampaio Schitine (UFBA).**

**Um olhar neuroenergético sobre a Doença de Alzheimer: o papel do lactato.** [*A neuroenergetic look at Alzheimer's disease: the role of lactate*]. **Adriano Martimbianco de Assis** (CCPS/UCPEL).

**O papel da Inflamação em amiloidoses.** [The role of inflammation in amyloidosis]. **Debora Foguel** (IBqM/UFRJ).

**13:00 – 15:00** **SESSÃO DE POSTERS – [Poster Session]**

**15:00 – 18:00** **CONFERÊNCIAS - Diversidade e impacto patológico do microambiente glial em tumores cerebrais e desenvolvimento de fármacos.** [Diversity and pathological impact of the glial microenvironment on brain tumors and drug development].

**Mecanismos de interação astrócito/glioma em modelos 3D e novas estratégias terapêuticas através de biotecnologia marinha.** [Mechanisms of astrocyte/glioma interaction in 3D models and new therapeutic strategies in marine biotechnology]**. Giselle Pinto de Faria Lopes** (Instituto de Estudos do Mar Almirante Paulo Moreira, Instituto Nacional do Câncer, UFRJ).

**Cininas no sistema nervoso central: de biologia tumoral à neurorregeneração** [Kinins in the central nervous system: from tumor biology to neuroregeneration]. **Henning Ulrich** (IQ/USP).

**Receptores de bradicinina como alvo no controle da invasão, fusão celular e migração de glioblastoma e células mesenquimais tronco em co-cultura.** [Bradykinin receptors as a target in the control of invasion, cell fusion and migration of glioblastoma and mesenchymal stem cells in co-culture]. **Mona das Neves Oliveira** (IQ/USP).

***Sábado, 12 de outubro***

**09:00-11:00** **Apresentação oral de jovens cientistas** [Oral presentation of young scientists]. Mediadores: Silvia Lima Costa & Victor Diogenes Amaral da Silva (LabNq-ICS/UFBA)

**11:00 – 14:00** **CONFERÊNCIAS - Neurotoxicologia e Neuropsiquiatria** [*Neurotoxicology and Neuropsychiatry*].

**Papel da neuroinflamação em doenças neuropsiquiátricas/neurodegenerativas, ênfase na depressão e Doença de Parkinson.** [Role of neuroinflammation in neuropsychiatric/ neurodegenerative diseases, emphasis on depression and Parkinson's Disease]. **Yousef Tizabi** (Howard University – USA).

**Update do uso clínico de canabidiol para fins medicinais.** [Update of clinical use of cannabidiol for medicinal purposes]. **Antônio de Souza Andrade Filho** (FAMED/UFBA).

**15:00** **ENCERRAMENTO [CLOSURE]**

**Premiação de Pôsteres.** [Poster Awards].

# ABSTRACTS OF MINI-COURSES PRE-SYMPOSIUM

## BASES OF THE KNOWLEDGE OF RESEARCH IN NEUROCHEMISTRY

Victor Diógenes Amaral da Silva*[1], Balbino Lino dos Santos[1,2] & Silvia Lima Costa*[1]

**School of High Studies In Neurochemistry (EAENQ), [1]Laboratory of Neurochemistry of Cellular Biology (LabNq), Federal University of Bahia (UFBA), Salvador-BA, Brazil; [2] Federal University of Vale do São Francisco (UNIVASF), Petrolina-PE, Brazil.*

The knowledge of neural cell biology is fundamental to understand the pathogenesis of nervous system diseases. Multidisciplinary studies have contributed to the development of new study models, which make it possible to perform tests for the development of new therapies. The Laboratory of Neurochemistry and Cell Biology at Federal University of Bahia (UFBA) has adopted several study models to investigate the plasticity of these cells in response to inflammatory stimuli or pathological conditions and mechanisms of neurotoxicity/ neuroprotection triggered by natural or synthetics compounds. These and other current study models adopted by neuroscience will be addressed in this mini-course during the V International Symposium on Glial Cell Neurochemistry and Pathophysiology and X Symposium of Update in Pharmacology of UFBA. The short course is designed with a discussion of fundamental neurochemistry topics and covers interdisciplinary aspects in the development of new therapies for diseases affecting the central nervous system. The main topics are: i. cellular components of nervous tissue; ii. neurotransmitters and neuromodulators; iii. models and techniques applied in the study of the CNS. iv. *in vitro* and ex vivo models applied in the study of pathogenesis and discoveries of new therapies for CNS diseases; v. in vivo models applied in the study of the pathogenesis and discoveries of new therapies for diseases of the CNS. The methodology, applied as theoretical approaches are followed by discussion with the students. As a result, it is expected that students develop the ability to address related topics; recognize and value CNS cell strains and those that are targets of pharmacologically active compounds; have an updated on texts, themes and everyday situations linked to the functional and pathological relationships of the CNS; and acquire of basic knowledge about the most appropriate models to study the pathogenesis of CNS diseases and the development of new related therapies.

*Keywords: neuron, glial Cells, nervous system diseases, models, markers*

## STRATEGIES IN THE FORMATION OF YOUNG SCIENTISTS: PUBLISHING AT THE BEGINNING OF THE CAREER.

Robyn Louise Tolhurst

*Red Fern Communication, Sydney, NSW, Australia.*
*e-mail: info@redferncommunicaton.com.*

Navigating the publishing world and getting your work published can be challenging as an early career researcher. As the number of predatory journals continues to grow, many researchers are falling prey to these non-legitimate sources, which can hinder rather than help your reputation as a researcher. This presentation will take you on the journey of what to look for in a publisher, how to prepare a good cover letter, and importantly, how to identify and avoid the predators. You will gain an understanding of the key things editors look for in a scientific manuscript, the tried and tested techniques for preparing well-structured scientific papers, and how to present your work to increase your chances of success in getting your work published.

# ABSTRACTS OF CONFERENCES

## PRESENTATION OF THE SCHOOL OF HIGH STUDIES IN NEUROCHEMISTRY (EAENq). CURRENT STATE OF RESEARCH AND TRAINING OF HUMAN RESOURCES

Maria de Fátima Dias Costa*[1]

**School of High Studies In Neurochemistry (EAENq), [1]Laboratory of Neurochemistry of Cellular Biology (LabNq), Federal University of Bahia (UFBA), Salvador-BA, Brazil.*

The School for Advanced Studies in Neurochemistry (EAENq) is a project proposed by the Laboratory of Neurochemistry and Cellular Biology (LabNq-UFBA) to be sponsored by CAPES (Ministry of Education/ Brazil). The objective of EAENq is to offer to post graduate students a high-level intensive course taught by internationally renowned professors. The program of the first edition consists of (I) a theoretical approach on anatomical, physiological and metabolic aspects of the central nervous system and (II) mini courses on methods and tools for analyzing the brain and its circuits, both presented by local team during a week. On the second week conferences and round tables will be presented by foreign guests on the mainly research topics of theirs scientific cooperation with LabNq. Each theme will be followed by a presentation of review papers and discussion by the students. The project must collaborate to improving students profile and to develop the scientific and academic exchanges between Brazilian and foreign research groups

## IMMUNOINTERVENTON AS THERAPEUTICS IN NEURODEGENERATIVE DISEASES

Dora Brites

*Faculdade de Farmácia, Universidade de Lisboa, Portugal.*

Neurodegenerative diseases, such as Alzheimer's disease (AD) and amyotrophic lateral sclerosis (ALS), are not timely and efficiently treated using conventional therapies. This emphasizes the need for alternative strategies. In this respect, microRNA (miRNA) up- and down-regulation have been envisaged as potentially feasible alternative therapies, where extracellular vesicles (EVs), known to carry miRNAs, are indicated as potential biomarkers for AD and ALS diagnosis, as well as vehicle candidates for medicines. MiRNAs are increasingly recognized by their decisive role in AD and ALS pathologies and neuroinflammatory processes, being considered players in the onset and progression of such disorders. In this context, the key miRNA may be incorporated into EVs to increase the quantity and quality of the packed miRNA to be delivered to a specific neural cell and thus used for medical intervention. The pleiotropic nature of miRNAs makes them attractive candidates for the multifactorial AD and ALS diseases. MiRNA(miR)-155, miR-146a, miR-124 and miR-21 are among the most studied in neuroinflammation (inflamma-miRNAs). Although no miRNA drug candidates have entered in phase 3 trials yet (clinicaltrials.gov), Regulus has a new miRNA drug candidate targeting miR-10b to be tested for glioblastoma multiform and Miragen has an active phase 2 trial for miR-155 (MRG-106) for some T-cell lymphomas. Miragen is also developing a miR-155 antagonist (MRG-107) to target activated microglia in ALS. In this talk, the pathological consequences of inflamma-miRNA deregulation in neurons, astrocytes and microglia using mouse and human cell lines, primary rodent cells, or human astrocytes and neurons generated by reprogramming and direct conversion technologies, as AD and ALS in vitro models, will be addressed. Emphasis will be placed on how glial cell dysfunction interferes with nervous tissue homeostasis, cell-to-cell trafficking and exosome-mediated signaling. Impact of phenotypic

diversity and regional heterogeneity of microglia and astrocytes will be highlighted. In addition, effects by the use of miRNA mimics and inhibitors in recovering neuronal, astrocytic and microglia function, and their influence in soluble and EVs miRNA signature, will be presented. Taken together the possibility of specifically targeting miRNAs in neural cells and their EVs would enable precision transcriptomic medicine in ALS and AD therapeutic strategies.

Funded by SCML (ALS Research Grant ELA-2015-002) and FCT (PTDC/MED-NEU/31395/2017 and LISBOA-01-0145-FEDER-031395 to DB, and in part by UID/DTP/04138/2013 to iMed.ULisboa.

**Keywords:** Neuroinflammation & Glial Cell activation, microRNA modulation, secretome & extracellular vesicles

## KEEPING THE BRAIN WIRED: THE IMPORTANCE OF LIFE-LONG MYELINATION

Arthur Morgan Butt

*Institute for Biomedical and Biomolecular Sciences, University of Portsmouth, Portsmouth, United Kingdom.*

The massive computing power of the brain depends on myelinated fibres that are bundled together into the white matter (WM) — 'superhighways of information' that interconnect widely dispersed grey matter (GM). Myelin is produced by oligodendrocytes, which are derived from oligodendrocyte precursor cells (OPCs), a significant population of cells throughout WM and GM. OPCs form neuron-glial synapses and respond to neurotransmitters released by neurons via a range of receptors, which are proposed to regulate their differentiation into oligodendrocytes. However, WM is largely devoid of neuronal cell bodies and synapses. Nonetheless, neurotransmitter signalling is highly prominent and diverse in WM, with a predominance of glutamatergic and purinergic (ATP and adenosine) signalling. Experimental studies support a model of neurotransmitters being released from axons and astrocytes during action potential propagation to regulate myelination. Notably, there is life-long generation of oligodendrocytes from OPCs, which is required for replacement of myelin lost through natural 'wear and tear', for myelination of new neuronal circuits formed in response to new life experiences and regeneration of oligodendrocytes following pathological demyelination, such as occurs in multiple sclerosis (MS). However, myelination declines in the ageing brain, which may be important in the ultimate failure of remyelination in MS and the loss of WM in Alzheimer's diseases (AD). Significantly, we provide evidence that neurotransmitter signalling is dysregulated in ageing WM of the mouse optic nerve, comparable to that described in the GM of human ageing brain and AD. This leads us to propose a vicious cycle in the ageing brain, whereby disruption of neurotransmitter signalling results in impaired OPC regenerative potential and subsequent loss of oligodendrocytes and myelin, which is aggravated in diseases such as MS and AD. Supported by the BBSRC.

**Keywords:** ageing, oligodendrocytes and myelin

## PROMOTING OLIGODENDROCYTE GENERATION VIA TARGETED INHIBITION OF GSK3B IN THE MURINE WHITE MATTER

Francesca Pieropan

*Institute for Biomedical and Biomolecular Sciences, University of Portsmouth, Portsmouth, United Kingdom.*

Oligodendrocytes (OLs) are the myelin-forming cells of the central nervous system (CNS). Myelin insulates axons enabling rapid communication and its loss is the hallmark of demyelinating diseases such as multiple sclerosis (MS). Myelinating OLs are generated from precursors (OPCs) throughout life, under the control of multiple intrinsic and extrinsic factors. In the developing forebrain, OLs are generated from OPCs originating from neural stem cells (NSC) of the dorsal subventricular (SVZ). Previously, we looked at the dorsal subventricular zone (SVZ) one of the neurogenic niches in the developing forebrain and using a pharmacogenomic analysis we identified the GSK3β inhibitor AR-A014418 as an important regulator of oligodendrogenesis. Using the Sox10-eGFP mice to identify oligodendroglial lineage cells (OL and OPC), we have examined the effects of AR-A014418 in adult mouse CNS. Mice were killed humanely in accordance with the Home Office Animals (Scientific) Act 2012 (UK), and optic nerves (ONs) isolated with retina intact. ONs were maintained *ex vivo* in organotypic culture for 3 days *in vitro* (DIV) either in untreated control medium or medium containing 20 μM AR-A014418. Confocal microscopy analysis showed that AR-A014418 had a dramatical effect on increasing the number of Sox10+ cells in the adult nerve; Sox10 is expressed by both OLs and OPCs, but only the latter are proliferative, indicating that AR-A014418 promoted oligodendrogenesis in adult OPCs. This was further supported by preliminary data on the adult murine cortex of transgenic Cre/LoxP mice in which the number of YFP+ cells was nearly doubled following genetic ablation of Gsk3β specifically in OPCs. We are currently investigating the role of Gsk3β in driving OPCs proliferation and differentiation into myelinating OLs during development and following *ex vivo* demyelinating lesions. A.D. Rivera and A.M. Butt report to be shareholders in the company GliaGenesis. Supported by the BBSRC and Multiple Sclerosis Society of the UK.

**Keywords:** ageing, oligodendrogenesis, myelin, GSK3β

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## ASTROCYTES ARE DIRECT CELLULAR TARGETS OF LITHIUM TREATMENT: NOVEL ROLES FOR LYSYL OXIDASE (LOX) AND PEROXISOME-PROLIFERATOR ACTIVATED RECEPTOR-Γ (PPAR-Γ) AS ASTROGLIAL TARGETS OF LITHIUM

Andrea Domenico Rivera

*Institute for Biomedical and Biomolecular Sciences, University of Portsmouth, Portsmouth, United Kingdom.*

Astrocytes are multifunctional glial cells that play essential roles in supporting synaptic signalling and white matter-associated connectivity. There is increasing evidence that astrocyte dysfunction is involved in several brain disorders, including bipolar disorder, depression and schizophrenia. The mood stabilizer lithium is a frontline treatment for bipolar disorder, but the mechanisms of action remain unclear. Here, we demonstrate that astrocytes are direct targets of lithium and identify unique astroglial transcriptional networks that regulate specific molecular changes in astrocytes associated with BD and schizophrenia, together with Alzheimer's disease (AD). Using pharmacogenomic analyses, we identified novel roles for the extracellular matrix (ECM) regulatory enzyme Lysyl Oxidase (LOX) and Peroxisome Proliferator-Activated Receptor Gamma (PPAR-γ) as profound regulators of astrocyte morphogenesis. This study unravels new pathophysiological mechanisms in astrocytes that have potential as novel biomarkers and potential therapeutic targets for regulating astroglial responses in diverse neurological disorders.

**Keywords:** brain disorders, lithium , astrocytes, PPAR-γ

## USING KYNURENINE PATHWAY METABOLITES AS BIOMARKERS FOR DISEASE PROGNOSIS AND PROGRESSION.

Gilles J. Guillemin

*Neuroinflammation research group, Faculty of Medicine and Health Sciences, Macquarie University, NSW, 2109 Australia.*

The kynurenine pathway (KP) of tryptophan metabolism is one of the major regulatory mechanisms of the immune response. Activation of the KP is implicated in the pathogenesis of a wide range of neuroinflammatory diseases. Several pro-inflammatory mediators can activate indoleamine 2,3 dioxygenase (IDO-1) one of the first and regulatory enzymes of the KP. A prolonged activation of the KP leads to production and accumulation of several neuroactive metabolites including the potent excitotoxin quinolinic acid (QUIN). Every brain cell type appears to express differently the KP enzymes and producing different KP metabolites.
Over the last decade, together with our collaborators, we have shown that the KP is activated and QUIN level increases in most of the major neurodegenerative diseases (multiple sclerosis, Alzheimer's disease, amyotrophic lateral sclerosis, Parkinson's disease…), neuropsychiatric disorders (suicidality, schizophrenia, autism…) and cancers (glioblastomas, breast cancers…).
We demonstrated that some KP metabolites can be used as accurate biomarkers for the prognosis and the rate of progression of several diseases. Designing new, sensitive, accessible and low-cost tools is the critical next step to be able to use these markers in clinical, biological and hospital laboratories.
**Keywords:** Tryptophan metabolism, biomarkers, prognostic

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## THE ENDOCANNABINOID SIGNALING REGULATION AND GLIAL DIVERSITY

Clarissa Schitine

*Federal University of Rio de Janeiro*

Endocannabinoids are defined as endogenous compounds, produced in different tissues, that bind to cannabinoid receptors, CB1R and CB2R. It has been described that hemoglobinderived peptides, hemopressin (HP) modulate CB1R. The subventricular zone (SVZ) is a neurogenic niche that contributes to the development of the central nervous system (CNS) in embryonic stages and in adulthood. The endocannabinoid system has been implicated in the modulation of gliogenesis and neurogenesis in the SVZ. Activation of CB1R on self-renewal, proliferation and neuronal or oligodendrocyte differentiationwerestudied in the mouse neonatal SVZ stem/progenitorcell cultures (Xapelli et al., 2013). We have shown that CB1R expression isdetected in SVZ-derived immature cells (nestin-positive), neurons and astrocytes. Stimulation of the CB1R by (R)-(+)-Methanandamide (R-m-AEA) increased self- renewal of SVZ cells, as assessed by counting the number of secondaryneurospheres and the number of Sox2+/+ cell pairs. R-m-AEA also increased proliferation as assessed by BrdU incorporation assay. Stimulation of CB1R by R-m-AEA also promoted neuronal differentiation (without affecting glial differentiation), at 7 days. Intracellular calcium concentrations ([Ca2+]i) in single cells following KCl and histamine stimuli was performed and we observed an increase in neuronal-like cells. The proneurogenic effect was blocked when SVZ cells were co-incubated with R-m-AEA and the CB1R antagonist AM 251. On the other hand, in normal conditions, SVZ cells are poorly oligodendrogenic, and we show that Hp increased oligodendroglial differentiation in SVZ neural stem/progenitorcell cultures derived from neo natal mice (Xapelli et al., 2014). Chronic treatment with Hp in cultures of SVZ neurospheres promoted functional differentiation of progenitors into oligodendrocytes. And finally, data from our laboratoty have shown that Müller glia, the main retinal glia, underexcitotoxicity, modifies its expression of GAT-3, a GABA transporter (Schitine et al., 2015b) and that endocanabinoid signaling alters neuro-glia dinamycs in the

retina. Data on the heterogeneity of astrocytes in the CNS will be also discussed based on differences in progenitors cells and their roles in developmental processes and how astrocyte sub populations function on the onset of psychiatric and neurologicaldiseases (Schitine et al., 2015a).
**Keywords:** hemopressin, cannabinoid receptor, subventricular zone, Müller glia

## UM OLHAR NEUROENERGÉTICO SOBRE A DOENÇA DE ALZHEIMER: O PAPEL DO LACTATO

Adriano Martimbianco de Assis

*Catholic University of Pelotas (UCPEL), Pelotas — RS, Brazil.*

The decrease in life expectancy is related to the increased incidence of neurodegenerative diseases, such as Alzheimer's disease (AD). AD is an important neurodegenerative disease as it is the leading cause of dementia in the world and there is no treatment yet that can prevent its progression or cure the disease. Thus, further studies are needed to better understand this disease, and allow progress in seeking treatment. According to the β-amyloid cascade hypothesis, a neuron-centric view, AD is initially characterized by the accumulation of β-amyloid protein (Aβ) and hippocampal hypometabolism that progresses to neuronal death, which clinically translates into memory loss, cognitive decline and dementia. The cause of Aβ protein increase and deposition is still unknown, indicating the need for a better understanding of the disease ontology. However, a new hypothesis, called neuroenergetic hypothesis, suggests that the decrease in brain energy input caused by aging would lead to increase neuronal substrate competition, increasing reactive oxygen species (ROS) production and the formation of misfolded proteins such as Aβ, consequently leading to the death of many neurons. According to this hypothesis, this would be the initial and pre-symptomatic stage of AD. Lactate from astrocytes can be transferred to neurons for oxidative metabolism in a process known as the astrocyte-neuron lactate shuttle, and it plays a very important role in brain energy supply especially in pathological situations. The lactate shuttle system consists of monocarboxylate transporters (MCTs) located predominantly in astrocytes (MCT1 and 4) and neurons (MCT2). Recent studies in the literature demonstrate that Aβ peptide reduces MCT2 and cerebral lactate content in hippocampus and cerebral cortex in experimental models of AD. In agreement with other works, we demonstrated *in vitro* that by genetically silencing the neuronal lactate transporter (MCT2) together with $H_2O_2$, there was greater neuronal death and loss of cell viability. The results so far are showing that the neuroenergetic hypothesis has great potential to study the early alterations of Alzheimer's disease, however the continuation of this study and new experiments are necessary.
**Keywords:** Alzheimer's disease, Brain energy metabolism, Lactate.

## MECHANISMS OF ATROCYTE/GLIOMA INTERACTION IN 3D MODELS AND NEW THERAPEUTIC STRATEGIES IN MARINE BIOTECHNOLOGY

Giselle Pinto de Faria Lopes

*Instituto de Estudos do Mar Almirante Paulo Moreira e Instituto Nacional de Câncer (IEAPM/INCA), RJ, Brazil.*

For more than a decade, temozolomide has been used as a first-line chemotherapy in the treatment of glioblastoma (GBM), but the prognosis and survival of patients remains poor. During tumorigenesis, the Sonic hedgehog embryonic signaling pathway (SHH) is reactivated and is related to a worse prognosis in medulloblastoma. During tumorigenesis, increased expression of SHH pathway molecules has been demonstrated in reactive gliosis and activated microglia, cells that were transformed due to the neuroinflammatory microenvironment caused by GBM. In order to study new therapeutic strategies in vitro,

it is important to consider this signaling pathway, in a three-dimensional (3D) environment that mimics physiological aspects as the cellular heterogeneity of GBMs. As a new therapeutic strategy, this study will consider the pharmacological and environmental potential of the sea. The relevant biodiversity is a source of different natural marine products that were already used for the treatment of lymphoma and breast cancer. Preliminary results showed that the extract of an invertebrate invasive exotic ascidian species (Dideminum sp.) significantly decreased the viability of GBM cells without presenting cytotoxicity to healthy cells. Currently, bioactive compounds (indole alkaloids) have been isolated from this to evaluate the response of GBM spheroids with different molecular characteristics, considering the SHH pathway as a therapeutic target. We will also observe the influence of healthy and transformed astrocytes and microglia on this tumor response through cultivation with their conditioned media and 3D co-culture. GBM spheroids were evaluated by immunohistochemistry and spectrophotometry, considering their viability, cell death (caspase 3 and 9), proliferation (Ki-67), differentiation (GFAP, nestin, SHH, Gli1, Sox2), migration and resistance (TP53, survivin). The conclusion was that the secretome from non-transformed astrocytes prevents glioblastoma spheroids progression through Sonic hedgehog pathway inhibition and marine natural products presented an inedit anticancer potencial, being a new therapeutic strategy in vitro.
**Keywords:** glioblastoma, reactive gliosis, Dideminum, indole alkaloids

## KININS IN THE CENTRAL NERVOUS SYSTEM: FROM TUMOR BIOLOGY TO NEUROREGENERATION

Henning Ulrich

*Department of Biochemistry, Institute of Chemistry, University of São Paulo,São Paulo, Brazil.*

Bradykinin (BK) stimulating kinin-B2 receptors (B2BKR) participates in tumor biology as well as migration and differentiation processes. Kinin-B1 (B1BKR) and B2 receptors participate in glioblastoma migration and ectodermal-mesenchymal transdifferentiation in conditions of co-culture with mesenchymal stem cells (MSCs), contributing to tumor aggressiveness. Indirect cocultures, where the cells interact by paracrine mechanism, as well as direct interactions in co-cultures augmented B1BKR and B2BKR expression in U87 glioma cells, which was not observed in MSCs. U87 migration/invasion of 3D co-cultures with BM-MSCs were greatly enhanced by the kinin agonists and blocked by kinin antagonists. Enhanced migration and invasion correlated to significantly higher cell-cell interactions, such as more frequent heterotypic cell fusions and even cell cannibalism, as well as vesicle transfer between U87 and BM-MSC cells in direct co-cultures. Novel functions for BK in neurogenesis were obtained using neural progenitor cells (NPC) isolated from fetal rat as in vitro models for neural differentiation. Functional B2BKR and secretion of BK medium suggested the existence of an autocrine loop participating in neural differentiation. Chronic exposure of differentiating neurospheres to the B2BKR antagonist HOE-140 resulted in reduced migration and neurogenesis and enhanced gliogenesis. These results were confirmed in migration and differentiation assays with neurospheres isolated from B2BKR-knockout mice. Moreover, hippocampus of postnatal mice showed increased $Ki67^{+}$ cells were markedly increased in BK-treated mice and ERK inhibition affected this neurogenic response. Together, these results compose mechanisms of action for BK during neurogenesis. Neurogenic actions of bradykinin were further studied in a Parkinson's disease model induced by nigrostriatal injection of 6OH-dopamine. Degeneration of dopaminergic neurons and clinical symptoms, such as apomorphine-induced rotations, in a rat model of Parkinson's disease were mostly reversed following a single BK injection, providing novel strategies for brain repair. Furthermore, neuroprotective properties of BK were observed in an in vitro model of ischemic neuronal cell death. Following perfusion of hippocampal slices with NMDA for induction of cytotoxicity, BK

application reverted the loss of functional pyramidal neuron responses and apoptosis induction. Overall, stable B2 receptor agonists or protease inhibitors resulting in an increase of extracellular BK accumulation are promising tools for brain repair. Supported by grants from FAPESP and CNPq, Brazil.

**Keywords:** *Kinins, glioblastoma, neuroregeneration*

## STRATEGIES IN PATENT REGISTRATION APPLIED TO DRUG DEVELOPMENT

Eduardo Muniz Santana Bastos

*Programa de pós graduação em Propriedade Intelectual e transferência de Tecnologia para inovação (PROFNIT), Instituto de Química (IQ), Universidade federal da Bahia (UFBA).*

The constant discoveries of new drugs and medicinal plants derivatives and their applications in the most diverse areas of health are reflected in the economic importance and consequently in the patenting and licensing of technologies related to this subject. Even with resource constraints for R&D projects, Brazilian public universities play a significant role in the country's technological innovation process. Technological development by Universities is characterized as a challenge for the promotion of innovation. One of the barriers found by researchers to the development of new technologies is government contingency and partnerships with industry. This presentation will lead you to understand the main strategies used for technological mapping and patent registration allowing the evaluation of scientific and technological trajectory, able to significantly influence the scientific and technological research as a whole.
Keywords: Bioprospectings. cientific prospection. technological prospection.

## BRADYKININ RECEPTORS AS A TARGET IN THE CONTROL OF INVASION, CELL FUSION AND MIGRATION OF GLIOBLASTOMA AND MESENCHYMAL STEM CELLS IN CO-CULTURE.

Mona Oliveira[1,3], Micheli Pillat [1], Tamara Lah[2,3] e Henning Ulrich[1]

*[1]Department of Biochemistry, Institute of Chemistry, University of São Paulo, Av. Prof. Lineu Prestes 748, São Paulo — SP, Brazil; [2]Department of Genetic Toxicology and Cancer Biology, National Institute of Biology, Ljubljana, Slovenia; [3]Jožef Stefan International Postgraduate School, Ljubljana, Slovenia.*

Glioblastoma (GBM) is the most common primary tumour of the central nervous system and is highly aggressive with a median survival of about 15 months. Pronounced tumour intra- tumor and inter-tumour heterogeneity, as well as cancer (stem) cells' plasticity create obstacles to efficient treatment of glioblastoma patients. Tumour progression involves interactions between cancer cells and exogenous components, including various types of stromal cells recruited to the tumour. Among these are mesenchymal stem cells that contribute to the tumour microenvironment. The mutual interactions among mesenchymal stem cells various subpopulations of cancer cells is stil not sufficiently understood. In this study we aimed to compare the effects of bone-marrow-derived mesenchymal stem cells (MSCs) on two glioblastoma U87 and U373 cells in direct co-cultures, modestly modeling heterogeneity of glioblastoma. The interactions among these cells was addressed in the in-vitro two-dimensional (monolayer) and three-dimensional (spheroid) co-cultures. The U87 cells expresses higher levels of bradykinin receptor1 (B1R) than U373 cells and their migration/invasion was greatly enhanced by the agonist bradykinin (BK), but blocked by B1R antagonist (R715) and B2R antagonist (HOE) alone and when co-cultured with MSCs. BK receptors' activation correlated with significantly higher U87/MSC cell-cell interactions (heterotypic fusion, vesicle transfer and enosis. i.e. "cell cannibalism". Furthermore, the kinins triggered invasion, F-actin expression as well as epithelial-to-mesenchymal transition genes' expression in U87 cells upon U87/MSC co-cultures. After treatment with

both kinin receptors antagonists the invasion of U87 was significantly reduced. Altogether, these data support the on-going investigations of kinin receptors B2R and B1R as targets for adjuvant approach in glioblastoma therapy. Secondly, our results emphasize the need for further careful investigations of MSCs as potential candidates for cancer therapies, as they may adversely affect different cancer cell-subtypes, also depending on the levels of kinin/kinin receptors system.
**Keywords:** Bradykinin Receptors, Epithelial-to-Mesenchymal Transition (EMT); Glioma and Mesenchymal Stem Cells.

## BIOLINKER, THE LEADING STARTUP IN THE CELL-FREE SYNTHESIS OF RECOMBINANT PROTEINS IN BRAZIL.

Sandi Ravbar, Mona Oliveira, Ricardo Pereira, Phelipe Vitale e Mario Andrez

*Biolinker Biologia Sintética LTDA, Av. Professor Lineu Prestes 2242, sala 233, 2°andar-CIETEC/IPEN, Cidade Universitaria Butantan, São Paulo, Brazil.*

Headquartered in São Paulo, Biolinker is the leading Brazilian biotechnology company focused on cell-free protein synthesis (CFPS) and protein purification based on aptamers. We are developing a set of products to express and purify recombinant proteins 100 times faster compared to the protein production in living cells. You are then able to proceed faster with your research using that protein, including screening, structural analysis, high-throughput screening, or any other operation you choose. For exemple, CFPS has the capability to synthesize and evaluate thousands of potential therapeutic protein candidates in parallel. Thus, we accelerate pharmacology and safety assessments during the design and discovery phase of novel biologics and biosimilars. Our interdisciplinary and multinational team includes specialists in areas such as biochemistry, nanoscience and nanotechnologies, medical biochemistry, chemistry, veterinary medicine, chemical engineering, astrophysics/astrobiology and business administration. Biolinker was chosen as one of the 500 best deeptech startups in the world (HelloTomorrow 2019) among more than 4,500 applications from 119 countries.
**Keywords:** Cell-free protein expression system, deep-tech startups, science entrepreneurship.

## NEW PRECLINICAL EVIDENCES OF JM-20 AS NEUROPROTECTIVE COMPOUND FOR THE TREATMENT OF DIFFERENT NEUROLOGICAL DISORDERS.

Nuñez Y, Ramírez J, Pardo G, Wong M, Fonseca L, Ochoa E, Verdecia Y, Lima S, Amaral V, Delgado R, Merino N, Onofre D

Several neurological disorders, including Cerebral Ischemia, Parkinson Disease and Dementias have a very high incidence in the worldwide with negative impact in the society. At this time don´t exist effective drugs to treat these diseases. However an interesting strategy could be to design of multitarget molecules that could affect common pathological mechanisms. JM-20 is a novel multitarget-directed molecule with promising neuroprotective capacities. This molecule, is a strong antioxidant; 2) modulates the glutamatergic system; 3) preserves the mitochondrial functionality; 4) has anti-inflammatory and anti-apoptotic effects and 5) possesses a safe toxicological profile in rodents. Our group evaluated the effect of JM-20 on different cell systems (cell lines, cerebellar granules and organotypic cultures) under different cytotoxic damage. As results we demonstrated that JM-20 has a potent cytoprotective effect on several neurotoxic injury related with neurological disorders. Finally we evaluated the effect of JM-20 in animal models of cerebral ischemia (transient middle cerebral artery occlusion and permanent focal cortical ischemia induced by thermocoagulation), Parkinson's (icv administration of 6OHDA) and different types of dementia (vascular

dementia, ip administration of scopolamine, oral administration of Aluminum chloride and icv administration of beta-amyloid). As general results we demonstrated that: 1) in cerebral ischemia models, JM-20 improve the neurological behavior, decreases the infarct volume, has a very wide therapeutic windows and inhibits different neurotoxic mechanisms related to cerebral ischemia; 2) In dementia models, JM-20 improve the cognitive impairment induced by vascular dementia, scopolamine, aluminum and beta amyloid; and 3) In Parkinson models, JM-20 reduce the motor alterations in animals administrated with 6 OHDA. These results indicate that JM-20 is a multitarget molecule with promising neuroprotective properties for the treatment of different neurological disorders.

**Keywords:** JM-20, neuroprotection, neurodegenerative diseases

## ROLE OF NEUROINFLAMMATION IN NEUROPSYCHIATRIC/NEURODEGENERATIVE DISEASES, EMPHASIS ON DEPRESSION AND PARKINSON'S DISEASE

Yousef Tizabi, Ph.D.

*Howard University College of Medicine*

There is an urgent need to understand the mechanisms involved in age-related neurodegenerative diseases (NGDs), as invariably they are of progressive nature, and with the aging population on the rise, can have a devastating effect, not only on the afflicted individual, but also on the family and society in general. The challenge is further compounded by psychiatric co-morbid conditions, particularly the feeling of despair or depression in these individuals. Due to significant effort in this regard, it is now believed that inflammation may be a common culprit in manifestation of NGDs and mood disorders. Thus, over-activation of central microglia, considered to be the brain's defense mechanism, can lead to exaggerated release of cytokines and eventual destruction of the nerve cells and/or disruption of their communication. Hence, interruption of neuroplasticity and/or neuronal damage can lead to NDGs as well as behavioral consequences such as depression. In this presentation, following a brief description of the NGDs, particularly Parkinson's disease and mood disorders, the role of inflammation and the mechanism leading to these conditions is elaborated. Moreover, novel targets for pharmacological encounter of these diseases will also be discussed.

**Keywords:** Inflammation, Microglia, Cytokines, Neuroplasticity, Parkinson's Disease, Depression, Pharmacological Targets

## THE APPLICABILITY AND UPDATES OR CANNABIS USE FOR MEDICAL PURPOSES

Dos Nascimento, J. H. F.

**Introduction:** Cannabis (Cannabis Sativa) is a plant that has been used for many years as a source of fibers, food oil, regious moments, recreational habit as wll as for medical purposes. The firt recorded use or Cannabis as a medicinal compound appeared almost 5000 years ago in early chinese texts by the Emperor Chen Nung and the herb entered Brazil around the end of the 18$^{th}$ century, for use as a raw material for fiber protuction. However, It is also well-know for its curative compounds, incluing cannabinoids, terpenoids, flavonoids, and alkaloids. Among these, Δ9-tetrahydrocannabinol (THC) is a compound of the phenols family and it is the main component of the plant, being reponsible for its psychoative and hallucinogenic effects, and, because of this, it has been promoted widespread recreational use and misuse of the plant. Furthermore, the vannabinoids are derived from the C. sativa plant – considered to be a psychedelic (mild), hallucinogenic or depressant agnt – and the Cannabinoidiol (CBD) is one of the most exciting cannabinoids,

which are known today – natural compounds found in Cannabis. In neurophysiology, these agents act in Cannabinoid receptors (CB) and it triggers the biological effects. It is important to mention that the CBD consists of the main non-psychoactive component of C.sativa, presnt in up to 40% of the plant extract, and, in neurological pathways, is a competitive agent for receptors, with THC. Δ9-THC is the cannabinoid responsible form triggering psychotic effects in vulnerable individuals, which are related to increased presynaptic dopamine efflux in the medial lprefrontal cortex. On the otheer hand, CBD has become the target of several expenmental studies, revealing a broad spectrum of pharmacological properties such as analgesic and immunosuppressive action, action in the treatment of ischemia, diabetes nausea and cancer, effects on anxiety, sleects on anxiety, sleep and movement disorders, as well as in the treatment of symptoms resulting from eplipsy, schizophrenia, Parkinson's and Alzheimer´s disease. Besides, there are two receptors, CB1 and CB2, which are very similar, but the difference between them indicates that there are therapeutic substances that would act only on one or the other receptor and thus would activate or block the concentrations or active agents, the professional profile of the physician prescription and legal obstacles stimulate more robust research and discussions in order to improve, clarify and advance the applicability of the CBD for medical purposes, especially within neurology.

# ABSTRACTS OF POSTER SECTION

## INVESTIGATION OF THE ROLE OF GLIAL GLUCOCORTICOID RECEPTOR IN THE CONTEXT OF AGATHISFLAVONE SIGNALING IN AN *IN VITRO* MODEL OF NEUROINFLAMMATION

Almeida, Á. M. A. N.,[1,2]; Dos Santos, C. C. [1,2]; Da Silva, L. P.[1]; De Jesus, De Souza, G. C. [1]; Costa, V. M.[1,2]; A. E. D. V. [1,2]; Dos Santos, B. L. [1,2]; Da Silva, V. D. A. [1,2]; Costa, S. L. [1,2]; De Souza, S. B.[1]

[1]*Laboratory of Neurochemistry and Cellular Biology, BIochemistry and Biophysics Department, Institute of Health Sciences, Federal University of Bahia — UFBA. Salvador/Bahia. Brazil.*

[1]*Postgraduate Program in Immunology, Federal University of Bahia — UFBA. Salvador/Bahia. Brazil.*

**Introduction:** Oxidative stress and neuroinflammation are characteristic processes of neurodegenerative diseases and result from the release of proinflammatory molecules and the production of reactive oxygen species by glial cells. The co-occurrence of chronic dysregulation of the inflammatory and stress responses in neurodegenerative and psychiatric disorders suggests the participation of the glucocorticoid receptor (GR) in etiological mechanisms of neurodegeneration. Flavonoids are polyphenolic compounds extracted from plants, which have been shown to have neuroprotective, anti-inflammatory, antioxidant, anti-apoptotic and immunomodulatory actions in in vitro and in vivo studies. The biflavonoid agathisflavone is a dimer of the flavone apigenin, with neurogenic, neuroprotective and anti-inflammatory action demonstrated in *in vitro* models associated with estrogen and retinoid receptors. However, the mechanisms by which agathisflavone exerts its effects are still poorly understood. The aim of this study was to investigate whether the anti-inflammatory action of agathisflavone is mediated by GR. **Material and Methods:** An in vitro model of lipopolysaccharide-induced neuroinflammation (LPS; 1 µg / mL) was used in primary culture of cortical astrocytes and microglia obtained from neonatal rats (P0-P2). After 15 days in culture, the cells were treated with or without LPS, with agathisflavone (1 µM), and in the presence or not of mifepristone (1 µM), a GR antagonist. **Results:** Glial reactivity, an indicator of the inflammatory profile, was evaluated by immunocytochemistry for GFAP and for Iba-1, exclusive proteins of astrocytes and microglia, respectively. Astrocyte proliferation and relative GFAP expression increased in response to LPS treatment, but not in response to co-treatment with agathisflavone, neither in the presence or absence of mifepristone. Microglial proliferation increased in response to LPS treatment and was reduced to control levels when LPS was treated with agathisflavone flavonoid-associated LPS treatment, which was prevented in the presence of RU486. **Conclusion:** These results suggest that agathisflavone modulates microglial proliferation after inflammatory stimulation through GR signaling and contribute to elucidate the mechanisms by which agathisflavone exerts its anti-inflammatory activity.

**Keywords:** agathisflavone, microglia, glucocorticoid receptor.

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# *LIPPIA SIDOIDES* AS A SOURCE OF BIOACTIVE COMPOUNDS

Almeida, C. P. [1]; Cerqueira, M. D. [1]; Carvalho, J. R. M. [1]; Martins, D. [1]; Ribeiro, P. R. [1]

[1]*Metabolomics Research Group, Departamento de Química Orgânica, Instituto de Química, Universidade Federal da Bahia, Rua Barão de Jeremoabo s/n, 40170-115, Salvador, Brazil*

**Introduction:** *Lippia sidoides* popularly known as "*alecrim-pimenta*" is a native plant of northeastern Brazil, is used in popuplar medicine as antiseptic in the treatment of wounds and skin cuts, due to its strong action against microorganism and also antioxidant capacity. **Objective:** To evaluate the antimicrobial and antioxidant activity of *L. sidoides* leaf and stem extracts**. Material and Methods:** The extracts were obtained by maceration in hexane, ethyl acetate and ethanol. Antimicrobial activity was evaluated as minimum inhibitory concentration (MIC) using the successive 96-well microdilution assay against *Bacillus subtilis, B. cereus, Escherichia coli, Micrococcus luteus, Salmonella choleraesuis, Staphylococcus aureus* and *S. ephidermidis*. Nutrient broth was used as culture medium and chloramphenicol and gentamicin were used as positive control. The concentration of the extracts ranged from 7.80 to 1000 $\mu g.mL^{-1}$. MIC was determined by the appearance of turbidity in the wells. The extracts were considered active at concentrations less than or equal to 1000 $\mu g.mL^{-1}$. Antioxidant activity was determined by the 2,2 diphenyl-1-picrylhydraz radical scavenging assay and quantification was expressed in terms of effective concentration ($EC_{50}$). **Results:** *L. sidoides* ethyl acetate extracts showed high antimicrobial activity, with a MIC ranging from 125 to 1000 $\mu g.mL^{-1}$. The ethanol leaf extract showed activity against the bacteria: *B. subtilis* (1000 $\mu g.mL^{-1}$) and *E. coli* (1000 $\mu g.mL^{-1}$) . The ethanol stem extract showed activity against the bacteria: *S. aereus* (1000 $\mu g.mL^{-1}$) and *S. ephipermides* (1000 $\mu g.mL^{-1}$) . The ethyl acetate leaf extract showed the highest activity against all bacteria tested: *B. subtilis* (500 $\mu g.mL^{-1}$), *S. aereus* (250 $\mu g.mL^{-1}$), *B. cereus* (500 $\mu g.mL^{-1}$), *E. coli* (250 $\mu g.mL^{-1}$), *S. choleraesuis* (125 $\mu g.mL^{-1}$), *S. ephipermides* (250 $\mu g.mL^{-1}$) and *M. luteus* (500 $\mu g.mL^{-1}$). The ethyl acetate stem extract showed activity against the bacteria: *B. subtilis* (1000 $\mu g.mL^{-1}$), *S. aereus* (500 $\mu g.mL^{-1}$), *B.cereus* (500 $\mu g.mL^{-1}$), *S. ephipermides* (1000 $\mu g.mL^{-1}$) and *M. luteus* (250 $\mu g.mL^{-1}$). The leaf hexane extracts showed activity only against *Bacilus subtilis* (500 $\mu g.mL^{-1}$). The stem hexane extracts showed no activity. This shows that the leaf is the part of the plant where the species most produces compounds capable of inhibiting the growth of bacteria. The $IC_{50}$ for *L. sidoides* extracts varied from 25,24 to 240,06 $\mu g.mL^{-1}$. The ethanol leaf extract showed $IC_{50}$ of 240.06 $\mu g.mL^{-1}$, whereas the ethanol stem extract showed $IC_{50}$ of 53.13 $\mu g.mL^{-1}$. The ethyl acetate leaf extract showed $IC_{50}$ of 128.72 $\mu g.mL^{-1}$, whereas the ethyl acetate stem extract showed $IC_{50}$ of 25.24 $\mu g.mL^{-1}$. Hexane extracts were not active ($IC_{50}$ > 1000). therefore, stem extracts showed the highest antioxidant power. Ethyl acetate proved to be the best extracting solvent, given the best results for its extracts. **Conclusion:** The tests performed proved the high antioxidant and antimicrobial capacity of *L. sidoides,* proving its effectiveness in the use of the plant in traditional Brazilian medicine.

**Keywords:** bacteria, phytochemical, natural products

**Supported by**: UFBA, FAPESB, CNPq and CAPES.

# ANTIGLIOMA POTENTIAL OF NITROGENOUS HETEROCYCLIC COMPOUNDS DERIVED FROM SYNTHESIS AGAINST GLIOBLASTOMA

Amparo, J. A. O.[1]; Filho, R. F. S.[2]; Da Silva V. D. A.[1]; Cunha, S. do D.[2]; Costa, S. L.[1]

*[1] Laboratory of Neurochemistry and Cellular Biology, Institute of Health Sciences, Federal University of Bahia, Bahia, Brazil.*

*[2] Research Group in Chemical Synthesis and Molecular Bioactivity, Institute of Chemistry, Federal University of Bahia, Bahia, Brazil.*

**Introduction:** Glioblastoma multiform (GBM) is the most aggressive and common type of brain tumor that affects the central nervous system (CNS). Its localization, proliferation as well the induction of angiogenesis makes them highly resistant to multisystemic therapies, which justify the search for new treatments. Enaminone-derived molecules, obtained by organic synthesis using Green Chemistry-based principles, have already shown high biological potentials, such as anti-inflammatory and analgesic properties. These compounds may also act as inhibitors of the glioma cell growth, making them promising in the study of alternatives for the therapy for this tumor. **Objective:** The aim of this study was to perform a prospection in vitro of the effects of a library of thirty six new enaminone-derived molecules in terms of cytotoxicity and changes on morphology of chemoresistant GBM cells. **Material and methods:** Human GL-15 GBM cells were treated with the molecules at concentration of 100 µM or kept in control conditions (0.01% DMSO dilution vehicle). The cell viability and morphology were evaluated after 24 and 72 h of treatment by MTT [3-(4,5-dimethyltiazol-2-yl)-2,5-diphenyltetrazolium] assay and the phase-contrast microscopy analysis, respectively. Molecules (nitrogenous heterocyclic compounds) that followed criteria such as stronger effect on cell viability and induction of morphological alterations, as well as efficiency on synthesis and absence of precipitation in organic solvents, were selected for testing at different concentrations. Four molecules (RRF-D468, RRF-D542, RRF-D594 and RRF-D610), were selected and tested in GL-15 tumor cells and also normal glial cells at concentrations of 1, 10, 50 and 100 µM or kept in control conditions. Three independent experiments were performed, and eight replicate (wells) were used for each experimental condition. **Results:** The compounds RRF-D468, RRF-D542 and RRF-D610 induced concentration dependant reduction on viability of glioma cells since 24 h after treatments. However only the compound RRF-D610 presented cytotoxic and morphogenic activity against highly proliferating glioma cells, without toxic effect to normal glial cells. **Conclusion:** These data demonstrate the pharmacological potential of the synthetic enaminone RRF-D610 with selective *in vitro* cytotoxic effect against GBM cells, and it may be considered in further studies for the development of new drugs against this pathology. Support: FAPESB and CNPq.
**Keywords:** Glioma, nitrogenous heterocyclic compounds, cytotoxicity.

# ORAL ADMINISTRATION OF RUTIN PREVENTS GLIAL ACTIVATION AND DOPAMINERGIC DEGENERATION IN AN *IN VIVO* MODEL OF PARKINSON'S DISEASE INDUCED BY AMINOCHROME

Araújo, F. M.[1,2]; Frota, A. A.[1]; Jesus, L. B.[1]; Macedo, T. C.[2]; Sanchez-Rodrigo, C.[3]; Mendes, K. M. F[2]; Cuenca, L.[2]; Segura-Aguilar, J[3]; Costa, S. L[1]; Trinidad-Herrero, M [2].; Silva, V. D. A[1].

[1]*Laboratory of Neurochemistry and Cell Biology, Department of Biochemistry and Biophysics, Institute of Health Sciences, Federal University of Bahia, Salvador, Bahia, Brazil.*
[2]*Clinical & Experimental Neuroscience, Department of Human Anatomy & Psychobiology, Institute for Biomedical Research of Murcia (IMIB), School of Medicine, Campus Mare Nostrum, University of Murcia, Murcia Spain*
[3]*Department of Molecular & Clinical Pharmacology, Faculty of Medicine, ICBM, University of Chile, Santiago, Chile.*

**Introduction:** Parkinson's disease (PD) is a multifactorial neurodegenerative disorder that involves the deregulation of various cellular mechanisms that result in loss of dopaminergic neurons in the substantia nigra pars compacta, however the mechanisms responsible for the neurodegeneration remain unknown. Studies have been suggested aminochrome as an endogenous neurotoxin responsible for the dopaminergic neurons degeneration. On the other hand, flavonoids, such as rutin, have been described as neuroprotective in different study models of PD. **Objective**: The aim of this study was to determine glial reactivity induced by aminochrome in an *in vivo* PD model and to explore the protective action of flavonoid rutin in this model. **Material and Methods:** Experimental procedures were sanctioned by the local animal research ethics committee (protocol CEUA –ICS-UFBA- nº. 011/ 2017). Wistar rats (male, 250-270g) were divided. in 6 groups (**1**: saline control, **2**: rutin 10 mg/ kg, **3**: 6-hydroxydopamine 21µg/ µL; **4**: aminochrome 1,000µM; **5**: 6-hydroxydopamine (6-OHDA) + rutin and **6**: aminochrome + rutin). The animals were orally dosed with rutina 30 minutes before the stereotaxic injection of aminochrome or 6-OHDA in the striatum and daily orally treated with rutin until the 14th experimental day. Behavioral changes were assesed by open field test. After euthanasia, the brains were fixed in 4% PFA, slices were performed in microtome and immunohistochemical analyzes was performed for IBA-1, CD68, GFAP, S100ß and Tyrosine hydroxylase (TH) in the striatum and SNpc. **Results:** The 6-OHDA and aminochrome induced behavioral changes in wistar rats that was inhibited by rutin. We observed that the aminochrome and 6-OHDA were able to generate increase in the total number of microglia, as well as to increase the quantity of activated microglia evidenced by co-localized IBA-1$^+$/ CD68$^+$ cells. It was also observed that astrocyte activation marked by an increase of co-localized GFAP$^+$/ S100ß$^+$ expression. Dopaminergic neuronal (TH$^+$ cells) loss was also observed in the aminochrome or 6-OHDA-treated groups. On the other hand, rutin presented neuroprotective effect by inhibition effects of aminochrome or 6-OHDA in terms of microglial activation, astrogliosis and dopaminergic neuronal loss. **Conclusion**: rutin inhibits behavioral changes, glial activation and dopaminergic neurons degeneration induced by 6-OHDA and aminochrome. This is the first evidence of glial response in an *in vivo* model of PD induced by aminochrome. These results improve this new animal model that has been suggested as a more physiological model of PD and contribute to a development of a new therapeutic agent against neurodegeneration.

**Keywords:** Parkinson's disease, dopamine, neuroprotection flavonoid.
**Support**: FAPESB, CAPES and CNPQ.

## TRYPTOPHAN PATHWAY IN AN EXPERIMENTAL MODEL OF *Neospora caninum* INFECTION IN GLIAL CELLS

Argolo, D. S.[1]; Conceição, G. A. P. R.[1]; Branco, A.[1] Costa, S. L.[1]; Costa, M. De F. D.[1]

*[1]Postgraduate Program in immunology Federal University of Bahia — UFBA. Salvador/Bahia. Brazil. (Laboratório de Neuroquímica e Biologia Celular, Depto. Bioquímica e Biofísica);*
*[2]Postgrsduate program in biotechnology in state University of Feira de Santana UEFS Feira de Santana/Bahia.*

**Introduction**: *Neospora caninum* an Apicomplexa parasite, which has tropism by the central nervous system leading glial cells to astrogliosis and to release of TNF and IL-10. This infection activates indoleamine 2,3-dioxigenase (IDO), the first tryptophan (TP) pathway enzyme to produce kynurenine (KYN) and sequentially, quinolinic acid (QUIN) or kynurenic acid (KYNA), respectively by oxidation or transamination. This catabolism is induced by expression of pro inflammatory cytokines, chemokines and their cells receptors. Objective: The present study verified the TP in glial cells infected by tachyzoites of *N. caninum* and its relation with astrocyte reactivity and inflammatory response. **Material and Methods:** Primary cultures of rat cerebral cortex (P 0-2) were infected or not with
N. caninum (1:1 cell: parasite) during 24h; after, the culture medium was removed, filtered (Millipore 0.22-µm) and used to evaluate tryptophan catabolites by High Performance Liquid Cromatograph (HPLC). Astrocyte and microglia morphology and reactivity were investigated by immunocytochemistry (ICQ) for glial fibrillary acidic protein (GFAP) and for the ionized calcium binding adaptor molecule 1 (Iba-1), respectively. The expression of cytokines, chemokines and of TP enzymes were investigated by qPCR. **Results**: The infected cultures showed gliosis, with changes in astroglial and microglial morphology. Furthermore, in infected cells, the relative mRNA expressions for IL- 10, TNF, CCL5, CCL2 and kynurenine monoxidase (KMO) increased compared to uninfected control cultures. Infected cells also showed high concentration of quinolinic acid (QUIN), an agonist of NMDA receptor. **Conclusion**: This experimental model of N. caninum infection induced the activation of tryptophan catabolism by activated microglia with production of QUIN. The inflammatory condition associated with the KMO activation could increasing the expression of chemokine receptors in astrocytes which probably modulate the inflammatoryresponse to induce a consequent neuropreservation.
**Keywords:** Glia, Neospora caninum, quinolinic acid, neuroprotection, tryptophan pathway.
**Support:** CNPq/INNT, FAPESB.

## CASE IDENTIFICATION OF TEXT NECK SYNDROME IN A UNIVERSITY POPULATION

Barbosa, L. L. de S.[1]; De Oliveira, J. V. R.[1]; Carneiro, M. S.[1]; Santos, L. M.[1]; Almeida, G. M. S. D.[1]

[1]*Department: Biomorfologia, Laboratory: Anatomia, Institution: Instituto de Ciências da Saúde da Universidade Federal da Bahia — ICS/UFBA and State: Bahia and Country: Brazil.*

**Introduction:** The increase in cervical pain in the population motivated this study, with the description of text neck, a term most used today due to the increasing use of technologies such as smartphnes, are the loss of the natural curve of the cervical spine and the early wear of the joints. and nerve compressions. **Objectives:** To diagnose cervical pain in the university population by sampling. Perform intervention with health education actions, with the preventive intention of postural dysfunctions, as well as therapeutic interventions to alleviate and/ or treat mild cervicalgias detected. Refer moderate and severe cases to specialized treatments. Material and Methods: The study was carried out with cross-sectional observational methodological research to diagnose cervicalgias in the university population by sampling, using interview protocols. In a second phase, the study will be interventionist, with health education actions, preventive

actions of postural dysfunctions, **Results:** as partial results, in the first phase, people with neck pain were identified in the population. college student. It was found that 94.9% of respondents use the smartphone for more than two hours daily, 57.4% of the sample reported some type of neck pain. **Conclusions**: Thus, with preliminary data there is already inference about the diagnostic severity of neck problems prevalence in the studied population.

**Key words:** Text Neck, Pain, Smartphone.

## MEDICINAL PLANTS USED IN PERIPHERAL NEIGHBORHOODS OF SALVADOR AS POTENTIAL SOURCE OF BIOACTIVE COMPOUNDS

Barbosa, J. R.[1]; Santos, P. M[1]; Carvalho, J. R. M.[1]; Ribeiro, P. R.[1]

[1] *Metabolomics Research Group, Departamento de Química Orgânica, Instituto de Química, Universidade Federal da Bahia, Salvador, Brazil*

**Introduction:** Medicinal plants are used since the beginning of the civilization as medication for multiple diseases that afflict the humanity since the beginning. Especially in Brazil these herbs are used until these days by the poorest layers of society, which tends to keep the traditions and popular beliefs and maintain the use. However, with the evolution of pharmaceutical practices and industry the young generation start to leave aside the natural arts and focus only in consuming medicines. In this project we used the herbs *Matricaria chamomilla, Melissa officinalis, Miconia albicans* and *Peumus boldus* for the phenolic and antioxidants analyses, with the objective to verify if the information obtained through field researches of the popular believing would be proved true. Through field and online research we have found the supposedly benefits of the plants, which are: relaxing and anti-anxiety for chamomilla, stomach discomforts for lemongrass, liver injuries for boldo, spine and muscular injuries for *Miconia albicans.* **Objectives**: To evaluate the antioxidant activity and levels of phenolic compounds in *Matricaria chamomilla, Melissa officinalis, Miconia albicans* and *Peumus boldus* extracts. **Material and Methods:** Plants were collected in the courtyards and shops of the peripheral neighborhoods and the extracts were obtained by the maceration in ethanol. For the antioxidant analysis, the DPPH method was used, which consists in capturing the free radical DPPH (2,2-diphenyl-1-picrilhydraz) for antioxidants and the quantification of the total phenols, the Folin-Ciocalteau method was used. **Results:** Regarding the antioxidant activity, we concluded that the extract obtained from the Melissa officinalis flower presented IC50 7.68 µg.mL$^{-1}$ and the total phenol quantification was 330.81 mg.EAG.g$^{-1}$, followed by Peduncle of *Miconia albicans* that presented 9.78 µg.mL$^{-1}$ in its $IC_{50}$ and the quantification of total phenols presented 284.32 mg.EAG.g$^{-1}$. The extracts of the flower of *Matricaria chamomilla* presented $IC_{50}$ 87.12 µg.mL$^{-1}$ and phenolic compounds 63.78 mg.EAG.g$^{-1}$, while the stem of *Matricaria chamomilla* presented $IC_{50}$ 67.95 µg.mL-1 and phenolic compounds 99.85 mg.EAG.g$^{-1}$. **Conclusion:** Thus, we can correlate the highest antioxidant potential with the highest quantification of total phenols. Therefore, the analysis of antioxidant activity and total phenols showed differences in the activities of each plant that explain the population reports, demonstrating the potentiality of each medicinal plant.

**Keywords:** Antioxidant activities, medicinal plants, ethnobotanical study.

**Acknowledgment:** Financial supported was provided by UFBA via the PIBIC-Jr (Programa Institucional de Bolsas de Iniciação Cientifica Júnior) program.

## ISOLATION OF BIOACTIVE DITERPENOIDS FROM VELLOZIA PYRANTHA

Barboza, I. F. [1]; Ribeiro, L. A. F. [1]; Carvalho, J. R. M. [1]; Ribeiro. P. R. [1]

[1] *Metabolomics Research Group, Departamento de Química Orgânica, Instituto de Química, Universidade Federal da Bahia, Salvador, Brazil.*

**Introduction:** *Vellozia pyrantha* is a plant found in Chapada Diamantina National Park, famous for its flammable resin. It is traditionally used to light wood stoves and torches. It belongs to the Velloziacae family, but is popularly known as Candombá. We have demonstrated that *Vellozia pyrantha* extracts possess antimicrobial (Gram-positive and Gram-negative) and cytotoxic (MCF-7 human breast carcinoma, HCT116 human colon carcinoma, HepG2 human hepatocellular carcinoma, and HL-60 human leukemia) activities, which make this plant an excellent candidate to identify new bioactive compounds. **Objectives:** The present work aimed at isolating bioactive compounds from *Vellozia pyrantha*. **Material and Methods:** The extract was fractionated by liquid column chromatography using a gradient of hexane and ethyl acetate. Further steps included preparative thin layer chromatography to separate the substances, which had their structures elucidated by $^{1}H$ and $^{13}C$ nuclear magnetic resonance (NMR), as well as COSY, HMBC, HSQC. **Results:** From the first fractions of the chromatographic column, it was possible to identify four diterpenoids: cleisthantan-8,11,13-tetraene and cleisthantan-2,8,11,13-tetraene, cleisthantan-8,11,13-trien-7-one and cleisthantan-6,8,11,13-tetraene. Their structures were elucidated by the analysis of the $^{1}H$- and $^{13}$ C-NMR spectra and the two-dimensional COSY, HMBC, HSQC spectra. **Conclusions:** From the NMR spectra obtained from these substances is a skeleton known as Cleisthantan, standard for all. In addition, one of the substances known to date has been obtained with a very considerable mass, presenting as the majority compound of the extracts. Further analysis are necessary to establish the possible contribution of the isolated compounds to the activities observed in the extracts.

**Keywords:** biological activity, metabolomics, natural products.
**Acknowledgement:** UFBA, FAPESB and CNPQ.

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## BIOACTIVE COMPOUNDS FROM *PIPER ANISUM* EXTRACTS

Batista, D. L. J.[1]; Cerqueira, M. D.[1]; Ribeiro, P. R.1

[1] *Metabolomics Research Group, Departamento de Química Orgânica, Instituto de Química, Universidade Federal da Bahia, Rua Barão de Jeremoabo s/n, 40170-115, Salvador, Brazil*

**Introduction:** *Piper anisum* (Spreng.) Angely, popularly known as jaborandi, belongs to the Piperaceae family. This species is widely used by traditional indigenous communities of Central and South America in the form of decoction and infusion to treat stomach pain, rheumatoid arthritis, ulcers, diarrhea, infections, pain anesthesia for tooth extraction and a wide range of gastrointestinal disorders. In addition, the essential oils of Piper species are used as antimicrobial and antiprotozoal agents, and demonstrate acetylcholinesterase inhibitory activity, antinociceptive, anti-inflammatory and cytotoxic. Large-scale metabolomics provide important tools for the qualitative and quantitative study of metabolites in a given biological system. Metabolomics data analysis can be performed by several techniques, including nuclear magnetic resonance (NMR), mass spectrometer-coupled high performance liquid chromatography (HPLC-MS) and mass spectrometer-coupled gas chromatography (GC-MS). **Objectives**: The objective of this study was to perform the metabolomic and pharmacological activities evaluation of ethanolic extracts obtained from *P. anisum* roots, stem and leaves. **Material and Methods**: The extracts were prepared by maceration in ethanol for 72 hours. Antioxidant activity was evaluated by the free radical sequestration method 2,2-diphenyl-1-picrylhydraza (DPPH), antimicrobial activity was evaluated as minimum inhibitory

concentration (MIC) against *Bacillus subtilis, B. cereus, Staphylococcus aureus, Escherichia coli, Micrococcus luteus, Pseudomonas aeruginosa, Salmonella typhimurium, Candida albicans* and *C. glabrata*, the cytotoxicity was evaluated against tumor cell lines MCF-7 (human breast carcinoma), HCT116 (colon carcinoma HepG2 (human hepatocellular carcinoma), HL-60 (human leukemia) and MRC-5 (human lung fibroblast). In addition, the quantification of total phenols was performed by the Folin-Ciocalteau method and metabolic profile of extracts were determined by NMR, UPLC-MS and GC-MS. **Results:** *P. anisum* root extract presented the best antioxidant activity ($IC_{50}$ of 35.54 ± 0.70 μg.mL $^{-1}$), followed by the stem ($IC_{50}$ of 180.38 ± 6.85 μg.mL$^{-1}$) and leaf ($IC_{50}$ of 271.18 ± 6.37 μg.mL $^{-1}$). The root extract presented the highest total phenolic content (49.86 ± 2.33 GAE / g dry weight)$^{-1}$, followed by the stem (31.48 ± 1.51 GAE / g dry weight) and leaf (27.53 ± 1.29 GAE / g dry weight) $^{-1}$. There was a positive correlation with the root extracts that showed a better antioxidant activity and higher total phenol content. *P. anisum* extracts presented profile highly selective antimicrobial; root extract showed activity against *M. luteus* and *C. albicans* (MIC of 500 μg.mL$^{-1}$) and leaf extract against *B.subtilis* (500 μg.mL$^{-1}$ MIC) and *S. aureus* (MIC of 62.5 μg.mL$^{-1}$). In addition, root extract presented the highest inhibition percentages against MCF-7 cell lines (59.5%), HCT116 (49.2%), HepG2 (61.0%) and MRC-5 (38.6%). Leaf extract was the least active against MCF-7 (31.9%) and HepG2 cell lines (5.2%), while there was no difference in percentages of cellular inhibition between extracts of leaves and stem in the HCT116 and HCT116 cell lines. The NMR spectrum of *P. anisum* root extract shows a higher number of signals attributed to aromatic compounds (6-8.5 ppm) compared to *P. anisum* leaf and stem extracts. This may indicate that root extract has a higher amount of simple phenolics than leaf and stem extracts, which may contribute to the higher antioxidant capacity and higher total phenolic content of root extract. Forty-eight metabolites were detected by GC-MS and 28 metabolites were detected by HPLC-MS which included alkaloids (naproanilide, obliquin, pilosine), phenolic compounds ((-) epicatechin, hydroquinone, p-cresol), carbohydrates (fructose, leukrose and sucrose) and carbohydrate derivatives, fatty acids (palmitic acid, linoleic acid and stearic acid), hydrocarbons (1-methyl-1,3-cyclohexadiene and (Z) -1,5-tridecadienol), organic acids (glycolic acid, benzoic acid and mucicide) and terpenes (anopterin and pubescenol). In GC-MS higher levels of phenolic compounds were identified in the root extracts, which explains the higher antioxidant capacity and content of total phenolics. In addition, root and stem extracts were found to have higher levels of alkaloids that may be occasional to the higher cytotoxic activity of these extracts. In general, stem and root extracts had the highest carbohydrate and carbohydrate derivatives, with the exception of treose and trehalose. In addition, root and stem extracts had higher levels of unsaturated fatty acids, which may contribute to the higher antioxidant capacity of *P. anisum*. Many acids have pharmacological properties, such as antimicrobial and cytotoxic activities. Thus the fatty acids identified in *P. anisum* extracts are good candidates for exploration as antioxidant, antimicrobial and cytotoxic metabolites. **Conclusion**: This study allowed us to identify potential antioxidant, antimicrobial and cytotoxic metabolites present in *P. anisum* extracts. Therefore, this data provided important avenues for the discovery of new active metabolites and possibly for the development of new drugs.

**Keywords:** drug, medicinal plants, natural products.
Acknowledgment: Financial support was provided by UFBA, FAPESB, CNPq, and CAPES.

# ACUTE EFFECTS OF DIFFERENT KETAMINE ISOMERSON PLASMA BDNF LEVELS IN RATS SUBMITTED TO MATERNAL DEPRIVATION MODEL

Beanes, G.[1]; Carneiro, B.[2]; Queiroz, G.[2]; Marques, G.[2]; Dos Santos Y. V. M.[2]; De Jesus, G. C.[2], Costa, R.dos S.[3]; Santana, R. C.[3]; Quarantini, L. C.[4]

[1]*Medicine and HealthPostgraduate Program, Federal University of Bahia, Salvador, Brazil*
[2] *Federal University of Bahia,Salvador, Brazil*
[3]*Department of Bioregulation, Federal University of Bahia, Salvador, Brazil*
[4]*Department of Neuroscience and Mental Health, Federal University of Bahia, Salvador, Brazil*

**Introduction:** Major depressive disorder (MDD) is a chronic, recurrent and disabling mental disorder with a highprevalence worldwide. Conventional treatment for MDD produces low response rates and is associated to tolerability issuesand treatment resistancein about thirty percent of patients. Maternal separation is a validated animal model that promotes neurological and behavioral changes such as the presented in depressedindividuals.This is a widely used animal modelto study new antidepressant drugs, such as ketamine.A subanesthetic dose of ketamine has shown promising results asa new therapeutic optionfor MDD. However, most studies have investigated its racemic form (R,S)ketamine. It is still not clearwhether ketamineenantiomers, R(-)ketamine and S(+)ketamine, would be equivalent to the standardized racemic one. Moreover, there is a lack of data about which presentation of ketamine is the most effective antidepressant. Peripheral BDNF changes after ketamine administration have been proposed as a biomarker for brain BDNF changes. However, published data are conflicting and come from studies in paired animal groups. **Objective:** To investigate the acute antidepressant effects of ketamine enantiomersand their racemic formon plasma BDNF levels in ratssubjected tomaternal deprivation model. **Material and Methods:** An experimental preclinical study was performed with 52 male *Wistar* rats. They wererandomized into seven experimental groups: non-deprivedwith placebo and other six deprived split into control and intervention groups.The enantiomers and doses used were(R,S)ketamine (10mg/kg), S(+)Ketamine (10mg/kg), and R(–ketamine(5,10 or 20mg/kg). The blood was collected by cardiac puncture. The BDNF plasma levels were measured with an ELISA kit (Sigma), according to the manufacturer's instructions. The differences between the experimental groups were examined with the Mann-Whitney U-Test for independent samples. **Results:** Maternal deprivation did not influence plasma BDNF levels. However, the mean BDNF value was visually higher in control rats (123.57 ±36.57) when compared to private rats (107.05 ± 73.99), both treated with placebo (saline). Ketamine treatment did not cause statistically significant changes in plasma BDNF in private rats compared to placebo treatment. However it can be visually noted than the mean BDNF in ketamine-treated private rats (140.54± 47.15) is higher than the BDNF mean in placebo-treated private rats (107.05± 73.99). **Conclusion:** Present results suggest thatKetamine increases BDNF levels of rats subjected to maternal deprivation. Although no statistical difference was found between enantiomers, R Ketamine appears to be more effective than S(+)Ketamine and R,S-Ketamine in doses studied.

**Key words:** BDNF levels, ketamine, maternal deprivation

## CYTOTOXICITY OF CHEMICAL CONSTITUENTS FROM *KIELMEYERA* SPECIES IN HUMAN U251 GLIOMA CELLS.

Boness, H. V. M. [1], Oliveira, M. S. [1], Ramos, F. S. [1]; Boffo, El-Bachá, E. F. [1]; R.[1]; Cruz, F. G.[1]

*[1] Instituto de Química, Universidade Federal da Bahia*
*[1] Instituto de Ciências da Saúde, Universidade Federal da Bahia*

**Introduction:** The genus *Kielmeyera* (Calophyllaceae) aggregates 47 native species to Brazil that are found in regions of Cerrado, Restinga and Campo Rupestre. Studies of these species have revealed the occurrence mainly of xanthones, 4-phenyl and 4-alkylcoumarins. Glioblastomas are tumors of the central nervous system classified by the WHO as a type of astrocytoma (grade IV). Although these tumors are relatively rare, they usually have showed a high mortality. **Objectives:** From this perspective, we evaluate the cytotoxic activity of chemical constituents from *Kielmeyera* species against human U251 glioma cell line. **Material and Methods :** The specimens were collected in the Metropolitan Park of Abaeté, in Salvador-BA. We isolated the 4-alkylcoumarins **1** and **2** from hexane extracts of roots of the *K. argentea* and *K. reticulata*, respectively. We have purified both compounds by preparative HPLC and their structures were proposed from analysis of NMR data. Human U251 glioma cell lines were cultured in 96-well plates containing DMEM, supplemented with 10% fetal bovine serum (FBS) and antibiotics (100 IU/ml penicillin and 100 µg/ml streptomycin), and incubated at 37°C under humid atmosphere containing 5% $CO_2$. The compounds **1** and **2** were dissolved in DMSO and added to the medium. The cytotoxicity evaluation was performed by the MTT method and statistical analysis by GraphPadPrism software v. 5.0. **Results:** The cell viability was detected by measuring the optical density at the absorbance of 595 nm, after data normalization, the lowest cytotoxic concentration for the compound **1** corresponded to 2.7 µM [median (25th-75th percentile), variance: 2.7 — 8.1 µM, n = 3] while for the compound **2** corresponded to 1.6 µM [median (25th-75th percentile), variance: 1.6 — 16 µM, n = 3]. **Conclusions**: Similarly to other 4-alkylcoumarins, both compounds have shown cytotoxicity against human U251 cell line. Next step, we pretend to evaluate the $EC_{50}$ as well as the toxicity against rat glioma (C6) cells and rat astrocytes.

**Keywords:** glioblastoma, 4-alkylcoumarins, Kielmeyera.
**Financial support**: FINEP, CAPES, CNPq, FAPESB.

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## BRAIN ENDOTHELIAL CELLS CONDITIONED-MEDIUM MODULATE ASTROCYTES TO PROTECT AGAINST CATECHOL-INDUCED OXIDATIVE DAMAGE

Borges, J. M. P.[1;2]; Clarêncio, J.[3]; Silva V. D. A.[4]; Costa, S. L.[4]; Costa, F. D[4]; El-Bachá, R. S.[4]

*[1]Departament of Sciences end Technology, UESB, BA;*
*[2]Laboratory of Neurochemistry and Cell Biology (LabNq), ICS- UFBA-BA, Brazil.*
*[3]Departament Research — Fiocruz — Ba; [4] Laboratory of Neurochemistry and Cell Biology, Department of Biochemistry and Biophysics, Institute of Health Sciences, Federal University of Bahia UFBA-BA, Brazil.*

**Introduction:** The selectivity to blood-brain barrier, which consist astrocytes and endothelial cells, restricts the entry of harmful substances into the brain, mainly by biotransformation molecular in metabolic barrier. **Objective:** To evaluate the interaction of endothelial cells and astrocytes in catechol-induced damages. **Material and Methods:** Cerebral endothelial cells (CEC) obtained from Wistar rats were cultured in DMEM-F12 under collagen-coated plates to obtain conditioned medium for 24 h (MCCEC) and for treated witch 30 — 6,000 µM catechol. Glial cells of newborn Wistar rats were cultured in supplemented DMEM-F12, incubated at 37 ºC, 5% $CO_2$. Astrocytes were treated with 10 — 2,000 µM catechol in the absence or in the presence of 50% (v/v) or 100% MCCEC for 72 h. For analysis, quinone production (405 nm) and catechol cytotoxicity (by MTT test, 595 nm) by spectrophotometer, immunocytochemistry,

comet assay, quantification of UGT1A6 by western blot and of GSTpi and gp-P by flow cytometry were carried out. Human glioblastoma GL-15 cells treated with 600 µM catechol in the presence of 50% (v/v) and 100% MCCEC for 72 h for analysis of GSH depletion by fluorescence microscopy. **Results:** Catechol (in higher concentrations), did not alter the activity of mitochondrial dehydrogenases in CEC. Catechol is toxic to astrocytes: Median $EC_{50}$**:** 88 µM (range: 28-151 µM, n=8). However, the presence of 50% or 100% MCCEC induced a resistance of astrocytes to catechol: $EC_{50}$ 514 µM (range: 184-766 µM, n = 8) and 389 µM (181 — 827 µM, n = 9) respectively. Furthermore, MCCEC significantly induced the expression of UGT1A6, GSTpi and gp-P in astrocytes. MCCEC also protected astrocytes treated with 40 µM catechol against DNA lessons and diminished GSH depletion in GL-15 cells. **Conclusion**: CEC is resistant to oxidative damage caused by catechol, conferring protection to astrocytes by quinones-induced damage, modeling detoxifying mechanisms of xenobiotics an interacting to form a common metabolic cooperation.
**Keywords**: catechol, brain endothelial cells, astrocytes; oxidative damage.
Supported by CNPq.

## AMYLOID FIBRILS RECRUIT NEUTROPHILS AND INDUCE THEIR EXTRACELLULAR TRAPS *IN VIVO*

Cardim-Pires T. R. [1]; Guimarães, F. V.[2]; Saraiva, E. M.[3]; Paula-Neto, H. A.[4]; Silva, P. M. R.[2]; Foguel, D.[1]

*[1]Laboratório de Agregação de Proteínas e Amiloidoses, Instituto de Bioquímica Médica Leopoldo de Meis, UFRJ, RJ, Brazil*
*[2]Laboratório de Inflamação, Instituto Oswaldo Cruz, Fiocruz, RJ, Brazil*
*[3]Laboratório de Imunobiologia das Leishmanioses, IMPG, UFRJ, RJ, Brazil*
*[4]Laboratório de Alvos Moleculares, Faculdade de Farmácia, UFRJ, RJ, Brazil*

**Introduction:** Neutrophil Extracellular Traps (NET) are chromatin derived structures decorated with neutrophil enzymes such elastase and myeloperoxidase. Our group has shown that amyloid fibrils (AF) are able to induce NET*in vitro*. NET formation trigged by amyloid fibrils in human isolated neutrophils is dependent onthe formation of reactive oxygen species (ROS) by NOX2, an isoform of NADPH oxidase present in phagocytes.**Objectives:**The aim of the present work is to determine whether TTR or α-synuclein -composed amyloid fibrils induce NET formation *in vitro* and *in vivo* in mice lacking NOX2 and to dissect the role of NOX2 in this process. **Material and methods:** Amyloid fibrils were prepared by incubatingTTR-A25T or α-synucleinin PBS for 15 days. Six-weeks-old C57BL/6 wildtype (WT) and knockout (KO) for NOX2 mice were used for*in vitro*and *in vivo* experiments. For *in vitro* experiments neutrophils were purified from mouse bone marrow in Percoll gradient. For *in vivo* experiments 50µg of amyloid fibrils were injected into peritoneal cavity or lungs. Four hours after injection, peritoneal cavity and lungs were washed with 3mL of PBS-EDTA and cellular fraction were staining with Hematoxilin/Eosin for cell counting. Soluble fraction of peritoneal or lung wash was used to measure NET formation. **Results:** Our data show that amyloid fibrils were able to induce NET in murine neutrophils only in WT animal. Both WT and KO mice were able to recruit neutrophils to the same extent after fibril injection suggesting that NOX2 is not necessary for neutrophil recruitment. Interestingly, extracellular DNA, a marker of NET formation, wasonly found in the peritoneal wash of WT mice injected with AF, suggesting that NOX2 is important to trigger NET formation *in vivo*as well. In addition, small toxic oligomers were found only in wildtype mice pointing that NET may has a role in amyloidosis prognosis. **Conclusion:** Our data show that amyloid fibrils are able to induce NET in a NADPH oxidase dependent manner and to recruit neutrophil *in vivo*.
Keywords: Amyloid Fibrils, Neutrophil Extracelular Traps, NADPH oxidase
Financial support: FAPERJ, CNPq and CAPES

## UPLC-MSBASED METABOLITE PROFILING OF *LEPIDIUM MEYENII* EXTRACTS

Carvalho, F.[1]; Ribeiro, P.[1]

[1.] *Metabolomics Research Group, Departamento de Química Orgânica, Instituto de Química, Universidade Federal da Bahia, Rua Barão de Jeremoabo s/n, 40170-115, Salvador, Brazil.*

**Introduction:** *Lepidium meyenii* is a plant that has several medicinal properties, however, the correlation between the metabolites and the biological activities tested in the plant is limited. **Objective:** To characterize the metabolomic profile as well as to evaluate the antioxidant and antimicrobial activities of extracts obtained from the dehydrated plant and its commercial products and to correlate the metabolomic profile with biological activities. **Materials and Methods:** The extracts were obtained from the root of the plant and its commercial products by maceration in different organic solvents such as hexane, ethyl acetate and ethanol. The metabolomic profile was evaluated by Mass Performance Coupled Ultra Chromatography (UPLC-MS) and the metabolite identification was performed by searching m/z in the Metlin database. The antioxidant activity was determined by the free radical sequestration method 2,2-diphenyl-1-picrylhydrazyl (DPPH) and total phenols was quantified by the Folin-Ciocalteu method. Antimicrobial activity was evaluated against Gram-positive and Gram-negative bacteria, as well as non-filamentous fungi by broth microdilution method. **Results:** The $IC_{50}$ of *L. meyeniivaried* from 64.97 µg.mL$^{-1}$ for ethyl acetate extract of the dehydrated plant to 935.61 µg.mL$^{-1}$ for hexane extract obtained from commercial products. Total phenol varied from 6.83 mg. GAE.g$^{-1}$ for ethanol extract 2 obtained from commercial product 3 at 49.83 mg.GAE.g$^{-1}$ for ethyl acetate extract obtained from commercial product 2. Ethanol extracts 1 and ethyl acetate were the most active and presented higher antioxidant potential, and also showed antimicrobial activity against *M. luteus* and *B. cereus*. Possible metabolites correlated with these activities are meyeniihydantoin A, mamamide B and N-benzyl-9-oxo-12Z-octadecenamide because they were found in high concentrations exclusively in the most active extracts. **Conclusion:** *L. meyenii* presents potential metabolites for a possible development of new drugs due to the antioxidant and antimicrobial activities presented.
Keywords: Metabolomics, Natural Products, Peruvian Maca

## ALZHEIMER DISEASE: MOLECULAR APPROACHES AND EPIGENETIC UPDATE

Conceição, L. N. S.[1]; Junior, M. S. O.[2]; Almeida, P. R. M.[1,3]

[1.] *Faculdade Nobre (FAN), Feira de Santana, Bahia, Brazil.*
[2.] *Neurology Department, University Clinic, Heinrich Heine University, Duesseldorf, Germany.*
[3.] *Universidade Estadual de Feira de Santana (UEFS), Biology of Department, Bahia, Brazil.*

**Introduction**: Alzheimer's disease (AD) is an irreversible neurodegenerative disease that presents as a pathophysiological feature a decrease in motor skills followed by episodic memory loss and death. AD affects especially elderly individuals and there is currently no developed treatment that can reverse neurodegeneration. This represents one of the greatest medical challenges of this century, given the increased life expectancy of the population. *Objectives*: Understand, at the genomic and molecular level, the different epigenetic events associated with known physio-metabolic factors that trigger AD. **Material and Methods**: To elucidate these molecular processes, a bibliographic survey was carried out in SciELO, MEDLINE, SCIENCE DIRECT, LILACS and PUBMED databases. A timeframe was established from 2005 to 2019, in which 50 articles were selected to support this study. We excluded papers that did not describe the factors of AD, and also did not bring the associated epigenetic mechanisms. **Results**: Genomic approaches have shown significant changes in the brain and blood of AD patients. DNA methylation is a common occurrence in age-related diseases, including neurodegenerative diseases such as AD. Studies have shown that the methylation

status of promoter regions of various genes involved with AD is associated with the pathophysiological disorders of this disease. Highlighting the records of cyclooxygenase 2 (PTGS2) and nuclear transcription factor kappa B (NF-kB) hypomethylation. In addition, the hypermethylation of the genes involved in the expression of brain-derived neurotrophic factors (BDNF), the cAMP response element transcription factor, and the synaptofin synaptic protein (SYP) — all associated with sporadic AD inflammatory processes. Allied to these molecular events also occur microRNA hypermethylation leading to changes in the expression of beta-amyloid and tau protein genes, which promote the formation of amyloid plaques and tau protein hyperphosphorylation, respectively. Also noteworthy is the hypomethylation of the PSEN1 gene (expressed as the presenilin-1 protein involved in the gamma secretase complex), associated with familial AD. Moreover, the e4 allele of the APOE gene (the most prevalent isoform among AD patients) has a hypermethylated CpG island. *Conclusions:* Epigenetic events are strongly associated with AD. These discoveries are of fundamental importance in the evolution of neuroscience studies, especially in the elucidation of these molecular and biochemical processes that will enable more precise interventions against this disease.
**Keywords:** Alzheimer disease, neurosciences, epigenomics.

## *IN VITRO* CYTOTOXIC ACTIVITY OF *OCOTEA ACIPHYLLA* FRACTION ON RAT C6 GLIOMA CELLS

Conceição, R. S. [1]; Amparo, J. A. O.[2]; Reis, I. M. A.[1]; Ferreira, R. S.[2]; Da Silva, V. D. A.[2]; Costa, S. L.[2]; Branco, A. [1]; Botura, M. B.[1]

[1]*Department of Health, Estadual de Feira de Santana, Brazil*
[2]*Department of Biochemistry and Biophysic, Institute of Health Sciences, Universidade Federal da Bahia, Brazil.*

**Introduction**: Glioma is a broad term describing neuroepithelial tumors originating from the glial or supporting cells of the CNS. The glioblastoma (GBM) is the most representative and aggressive type of glioma. People with this type of tumor present short lifetime due the tumor resistance and recurrence problems. Therefore, it is justified the research efforts with antitumor approach to discover new anticancer compounds. **Objectives**: The aim of this study was evaluating the antiproliferative activity of fraction obtained from the hexane extract of *Ocotea aciphylla* (Lauraceae) against rat C6 glioma cells. **Material and methods**: The hexane extract of *O. aciphylla* leaves was fractionated by column chromatography to furnish 51 fractions. The fraction 38 (Fr 38) was selected for biological assays due to the purity and quantity of mass. The rat C6 glioma and astrocytes cells were exposed to the Fr 38 (25 to 100 µg/mL) for 24 hours, and the cell viability was evaluated by the MTT [3-(4,5-dimethyltiazol-2-yl)-2,5-diphenyltetrazolium] assay and the phase-contrast microscopy analysis. The data were analyzed by ANOVA, followed by Tukey test ($p < 0.05$). **Results**: Fraction 38 induced significative decreases in rat C6 glioma cell viability, in a concentration-dependent manner after 24 hours exposition. This fraction promoted a reduction of 3, 12 and 62% on mitochondrial dehydrogenase activity at the 50, 75 and 100µg/mL concentration, respectively. The fraction did not produce toxicity in astrocyte cells in concentrations evaluated (75 and 100 µg/mL). **Conclusions**: The evaluated fraction isolated from *O. aciphylla* showed potential antiproliferative activity against rat (C6) glioma. Further experiments are needed to define the death cell mechanism, and to investigate the *in vivo* anticancer activity.
**Keywords:** Ocotea aciphylla; cytotoxicity; glioma.
**Acknowledgments:** Fundação de Amparo à Pesquisa do Estado da Bahia (FAPESB); Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

## CYTOTOXIC EVALUATION OF THE MAMMEA A/BB TO GLIOMA CELLS

Costa, I.[1]; Cruz, F.[1], Boness, H.[1]; Marques, E.[1]; Borges, J. M.[1]; Lima, A. da S.[1], El-Bachá, R.[1]

[1.]*Department of Biochemistry and Biophysics, Laboratory of Neurochemistry and Cell Biology,Institute of Health Sciences, Universidade Federal da Bahia (UFBA), Salvador-BA, Brazil.*

**Introduction:** Glioblastoma is the most aggressive form of gliomas and the available therapeutic resources are limited and involve surgical resection, radiotherapy and chemotherapy. The search for more effective and less aggressive pharmacological alternatives places mammea A/BB under analysis. This plant metabolite is found in the bark of roots of the botanical family Calophyllaceae, of the genus *Kielmeyera*. **Objectives:**The objective of this study was to evaluate the cytotoxic potential of mammea A/BB in human glioblastoma U251 and C6 rat glioma cells. **Material and Methods:**The pharmacological action of mammea A/BB was tested in both strains by cell viability assays by MTT, migration test, cariopnosis quantification and p-glycoprotein expression.The effect of the drug on normal murine astrocytic cells and a comparison of cell viability between the test drug and the first-line chemotherapy, temozolomide, were also analyzed. **Results:** The experiments showed that the U251 and C6 cell lines had a concentration dependent sensitivity to mammae A/BB and resistance to temozolomide in the concentration range of 2 µM to 200 µM in all the analyzed samples. The concentrations of 27 ± 2.31 µM and 56.85 ± 14.17 µM of mammea A/BB were able to inhibit the cell population in 50% in the lines U251 and C6, respectively, as well as the migratory potential of these lines. The test drug shows no cytotoxic action on murine astrocyte cells. Pinocytic nuclei were found in greater quantity in the treatment with mammea A/BB when compared to the control and DMSO conditions. Mammea A/BB showed no significant difference in the induction of P-glycoprotein expression. **CONCLUSIONS:** The results indicate a potential pharmacological action in the induction of cytotoxicity of the drug in tumor cell types and non-cytotoxic in the normal cells evaluated.

**Keywords:** astrocytoma; glioblastoma; phenylcoumarin.

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## THE FLAVONOIDS RUTIN REGULATES THE INFLAMMATORY RESPONSE OF MICROGLIA: IMPACT ON THE ANTIGLIOMA ACTIVITY

Costa, S.[1]; Da Silva, A. B. [1], Oliveira, M.[1]; Coelho, P.L.[1]; Amparo, J.[1]; Da Silva, K. C.[1]; Soares, J.[1], Souza, C.[1], Silva, V. D.[1], Moura-Neto, V.[2], Costa, M. D. F.[1]

[1.]*Federal University of Bahia — Institute of Health Sciences, Laboratory of Neurochemistry and Cell Biology- Department of Biochemistry and Biophysics, Salvador, Brazil.*

[2.]*Instituto Estadual do Cérebro Paulo Niemeyer, Laboratório de Biomedicina do Cérebro, Rio de Janeiro, Brazil.*

**Introduction**: Glioblastoma is the most common high grade brain tumor with bad prognosis. Classically, microglia is the immune effector cell in the central nervous system; however microglia contributes more to glioma progression than to its elimination. Previous *in vitro* studies have demonstrated the antiglioma potential of rutin, flavonoid present in the Brazilian plant *Dimorphandra mollis* Bent., in addition to the its immunomodulatory effect on astrocytes and microglia. **Objectives**: In this study we investigated the antitumor and immunomodulatory properties of rutin on the viability of glioma cells alone and/or under interaction with microglia. **Results:** In cultures of C6 rat glioma cells and U251 or TG1 human glioblastoma cells rutin inhibited proliferation and modulated the mRNA expression for IL-6 and IL-10 negatively and the mRNA for TNF expression positively, characterizing change in the regulatory profile. C6 cells treated with rutin induced chemotaxia of miroglia. Rutin treatment of microglia and C6 cells in co-cultures, or during indirect interaction, via conditioned media from treated glioma cells, reduced viability and proliferation of glioma

cells, and directed cells to a inflammatory profile with increased mRNA expression for proinflammatory cytokines IL-1-beta, IL-6, IL-18 and decreased expression for NOS2 and PTGS2, arginase and TGF-beta, as well as IGF growth factor. Treatment of U251 cells reduced tumorigenesis and also directed cells to an inflammatory profile *in vivo*. **Conclusions:** Together, these results demonstrate that the flavonoid rutin present antitumor effect related to the alteration in the microglial profile, and may be considered for molecular and preclinical studies.

**Keywords:** Rutin, glioma, micróglia.
**Support:** FAPESB; CAPES; CNPq; INCT/CNPq- Neurociência Translacional.

## ANTI-INFLAMMATORY EFFECT OF *PASSIFLORA CINCINNATA* SEED OIL IN THE ARTHRITIS MODEL IN KNEE OF MICE

Cruz, A. P. D. de A.[1]; Gomes, R. O.[1]; Pereira, N de P.[1]; Soares, D. de M.[1]

[1] *Pharmacy College, Laboratory of Research in Medicines and Cosmetics, Federal University of Bahia, Salvador, Brazil. Pharmacology Laboratory of Inflammation and Fever, Federal University of Bahia, Salvador, Brazil.*

**Introduction:** Chronic inflammatory process affecting mainly as joints and clinical manifestations common in autoimmune diseases, such as rheumatoid arthritis. As personalized therapies are used in anti-inflammatory and anti-rheumatic that control symptoms, minimize pain, inflammation and reduce automatic response. However, it carries with it various effects that cause discomfort and interfere with the patient's well-being. Therefore, search for alternative treatments derived from natural products has gained prominence. The *Passiflora cincinnata* seeds fixed oil, a semi-arid wild species, is one of the biodiversity resources attributed to phytoconstituents that confer anti-inflammatory and antioxidant activity. **Objectives:** To evaluate the potential anti-inflammatory activity of *P. cincinnata* seed oil, along with its incorporation in cold self-emulsifying systems in the mouse knee arthritis model. **Material and Methods:** An intra-articular zymosan (30 µL) solution was injected as cartilage aggressor, thus simulating the clinical manifestation of rheumatoid arthritis, causing acute inflammation, evaluated within 6 hours, considering as parameters for evaluation, the knee edema measurement, along with the leukocyte count, each group consisted of n = 5 mice. Initially three fixed oil concentrations were chosen to determine which would be the best response and after obtaining this result it was evaluated whether the activity remained when incorporated into cold prepared emulsions. **Results**: it was found a potential anti-inflammatory activity of *P. cincinnata* seed oil, with the best performance being the concentration of 10% relative to the others, confirming this activity when applied as a pharmaceutical emulsion. **Conclusion:** It can be suggested that the cold self-emulsified system containing the fixed oil of *P. cincinnata* seed has good prospects to be one of the alternative sources for the treatment of inflammatory responses generated by rheumatoid arthritis.

**Keywords:** Arthritis; Anti-inflammatories; Passionflower.
**This work has no funding source, so there is no conflict of interests.**

# THE FLAVONOIDS RUTIN AND QUERCETIN REGULATE THE INFLAMMATORY RESPONSE OF MICROGLIA: IMPACT ON THE ANTIGLIOMA ACTIVITY

Da Silva, A. B.[1]; Coelho, P. L. C.[1]; Oliveira, M. Das N.[1]; Amparo, J. A. O.[1]; Da Silva, K. C.[1]; Soares, J. R. P.[1]; Souza, C. Dos S.[1]; Pitanga, B. P. S.[1]; De Faria G. P.[1]; Da Silva, V. D. A.[1]; Costa, M. De F. D.[1,4], Junier, M. P.[2]; Chneiweiss, H.[2]; Moura-Neto, V,[3,4]; Costa, S. L.[1,4]

[1]*Laboratory of Neurochemistry and Cell Biology, Department of Biochemistry and Biophysics, Institute of Health Sciences, Federal University of Bahia, Salvador, Brazil;* [2]*INSERM, UMR-S 1130, Neuroscience Paris Seine (IBPS), Campus Pierre et Marie Curie, Paris, France;* [3]*State Institute of the Brain Paulo Niemeyer, Rio de Janeiro, Brazil;* [4]*INCT/CNPq-Neurociência Translacional (INNT).*

**Introduction**: Glioblastoma is the most common high grade brain tumor with bad prognosis. Classically, microglia is the immune effector cell in the central nervous system; however microglia contributes more to glioma progression than to its elimination. Previous *in vitro* studies have demonstrated the antiglioma potential of rutin, flavonoid present in the Brazilian plant *Dimorphandra mollis* Bent., in addition to the its immunomodulatory effect on astrocytes and microglia. **Objectives**: In this study we investigated the antitumor and immunomodulatory properties of rutin and its aglycone quercetin on the viability of glioma cells alone and/or under interaction with microglia. **Material and methods:** In cultures U251 or TG1 human glioblastoma cells flavonoids inhibited proliferation and modulated the mRNA expression for IL-6 and IL-10 negatively and the mRNA for TNF expression positively, characterizing change in the regulatory profile. Treatment of C6 rat glioma cells induced inhibition of proliferation and migration, and also induced chemotaxia of rat microglia that was associated to the upregulation of TNF and downregulation of IL-10 at protein and mRNA expression levels, as well as regulation of mRNA expression for chemokines CCL2, CCL5 and CX3CL1. Treatment of microglia and C6 cells in co-cultures, or during indirect interaction, via conditioned media from treated glioma cells, reduced viability and proliferation of glioma cells, and directed cells to a inflammatory profile with increased mRNA expression for proinflammatory cytokines IL-1-beta, IL-6, IL-18 and decreased expression for NOS2 and PTGS2, arginase and TGF-beta, as well as IGF growth factor. Moreover, treatment of U251 cells with flavonoids reduced tumorigenesis in the brain of xenotransplated rats and also directed microglia and astrocytes in the microenvironment of tumor cell implantation as well as in the brain parenchyma to a not favorable molecular inflammatory profile to the glioma growth. **Conclusion:** Together, these results demonstrate that the flavonoid rutin and quercetin present antitumor effect related to the alteration in the glial inflammatory profile, and may be considered for molecular and preclinical studies as adjuvant molecules for treatment of gliomas.

**Keyword:** Rutin, glioma, microglia

**Support:** FAPESB; CAPES; CNPq; INCT/CNPq- Neurociência Translacional.

# PROGNOSTIC IMPACT OF PERINEURAL AND INTRANEURAL M2 MACROPHAGES INFILTRATION IN ORAL SQUAMOUS CELL CARCINOMA

Da Silva-Souza, C.[1,2]; Pena, L. da C.[1,2]; Calumby, R. F. A. T.[1,2]; Alves, A. B. P. S. [2]; Mercuri, R. L. V. [2], Da Silva, D. S. C.[2]; De Araújo, A. C. V. S. C.[2], Silva, L. G.[3]; Araújo, I.[4]; Meyer, R.[5], Costa, S.L.[6]; Dos Santos, J. N.[7]; Vilas-Bôas D. S.[1,2,8].

*[1]Postgraduate Program in Immunology. Federal University of Bahia — UFBA. Salvador/Bahia. Brazil. [2]Laboratory of Immunopathology and Molecular Biology. Institute of Health Sciences. Federal University of Bahia — UFBA. Salvador/Bahia. Brazil. [3]Department of Head and Neck Surgery. Aristides Maltez Hospital (Liga Baiana Contra o Câncer). Salvador/Bahia. Brazil. [4]Department of Pathology and Forensic Medicine. University Hospital Professor Edgard Santos. Federal University of Bahia — UFBA. Salvador/Bahia. Brazil. [5]Laboratory of Immunology and Molecular Biology. Institute of Health Sciences. Federal University of Bahia — UFBA. Salvador/Bahia. Brazil. [c]Laboratory of Neurochemistry and Cell Biology. Institute of Health Sciences. Federal University of Bahia — UFBA. Salvador/Bahia. Brazil. [7]Laboratory of Oral Surgical Pathology. School of Dentistry. Federal University of Bahia — UFBA. Salvador/Bahia. Brazil. [8]Department of Biomorphology. Institute of Health Sciences. Federal University of Bahia — UFBA. Salvador/Bahia. Brazil.*

**Introduction:** Oral squamous cell carcinoma (OSCC) is a major tumor of the head and neck region, representing the most common oral malignancy and sixth leading cancer by incidence worldwide. OSCC presents high grades of macrophages infiltration and these inflammatory components may favor tumor development and metastasis if alternatively activated (aka M2). In OSCC, perineural invasion by tumor cells in early disease has a particularly high impact on survival. In turn, tumor-associated macrophages (TAM) are observed into and around peripheral nerves in OSCC. However, little is known about the role of these cells in the parameter of perineural invasion. **Objectives:** The aim of this study is to characterize in OSCC the distribution and diversity of M2 macrophages in both perineural and intraneural regions in relation to clinicopathological characteristics of patients. **Material and Methods:** This study was approved by Ethics Committees of Aristides Maltez Hospital (HAM)– Salvador, Bahia, Brazil (protocol number 10/2010). A total of 60 OSCC patients who were treated between 2008 and 2012 at Department of Head and Neck Surgery of HAM were enrolled and evaluated retrospectively. The clinicopathological data were collected from medical records. Using immunohistochemistry, CD163+, CD204+ and CD206+ M2-like macrophages were examined in 60 paraffin-embedded OSCC samples by an experient morphologist. The TAMs distribution was described in two localizations: into the nerve and in circumferential infiltration around the nerve. Chi-square and Fisher's exact tests, were used to associate TAMs distribution in relation to clinicopathological parameters. Overall survival (OS), cancer- specific survival (CSS), disease-free survival (DFS) and progression-free Survival (PFS) in relation to investigated markers were analyzed using the Kaplan-Meier method. **Results and Conclusions:** TAMs semi-quantification as well as correlations with clinicopathological variables and overall survival were not yet concluded. The partial analysis indicate that perineural and intraneural regions in OSCC are infiltrated only by the CD206+ M2 macrophages. The results of this study may contribute to clarify if macrophages are involved in perineural invasion of OSCC and to describe the involved TAMs subpopulation.

**Keywords:** oral cancer, tumor-associated macrophages, perineural invasion.
**Financial Support:** CNPq, FAPESB, PIBIC-UFBA and Programa Permanecer (MEC/SESu).

## BETA GLUCORONIDASE: A DAMAGE MARKER IN ASTROCYTES INFECTION

Del'arco, A. E.[1,2]; Argolo, D. S.[1]; Pina, G. A.[1]; Costa, M. de F. D.[1]; Costa, S. L.[1]; Pinheiro, A. M.[2]

[1] *Neurochemistry Laboratory, Federal Univertity of Bahia, Bahia, Brazil;*
[2] *Biochemistry and immunology Laboratory, Federal University of Recôncavo da Bahia Bahia, Brazil.*

**Introduction:** The enzyme beta-glicuronidase is expressed in a variety of tissues and cataliseshydrolisis of residues of the beta-glucuronic acid in the non-reducing end of glycosaminoglycans (chondroitin sulfate, heparan sulfate, dermatan and keratin sulfate and hyaluronic acid). The increase of the lisossomal activity of this enzyme point towards the presence of several diseases, such as, tissue inflammation, acquired immunodeficiency syndrome, tuberculosis and cancer. Some studies point to an increased release of beta glucoronidase from activated microglia during the process of neuroinflammation caused by viruses or in demyelinating diseases such as multiple sclerosis. Glial cells when infected with *Neosporacaninum*, an obligate intracellular coccidian parasite, respond to this stimulus by changing their morphology and releasing potentially harmful inflammatory mediators for this microenvironment. **Objectives**: The aim of this study was to investigate the release of beta glucoronidase in *N. caninum* infection in astrocytes. **Material and methods**: Astrocytes were obtained from the cortex of newborn rats (<48 h) and cultures was maintained in DMEM (Dulbecco's modified Eagle's medium) supplemented with 10% (v/v) fetal bovine serum, 24 mM glucose, 2 mM glutamine and 100 UI/ml penicillin/streptomycin. After 10 days, microglial cells were removed and astrocytes were plated in 24 wells plate. The cells were infected with *N. caninum* (NC-Bahia strain) in a ratio of 1:1, 1:2 and 1:4 (astrocytes/parasite) and astrocytes not infected were used as control of infection. After 72 hours of infection, the enzyme assay was performed with the culture medium and the cytotoxicity was determined by using the MTT. **Results**: MTT results shown that infected groups had a decrease of mitochondrial metabolism of 24.64% (1:1); 17.38% (1:2); 41.6% (1:4). The difference was statistically significant between the astrocyte groups: control and 1:1, 1:2 and 1:4. The production of beta glucoronidase (µg of NO/µg of protein) was $1.047x10^{-3}$ in the control plate (astrocytes only), while infected groups show an increase in enzyme levels: $1.336x10^{-3}$ (1:1), $1.268x10^{-3}$ (1:2), and $1.488x10^{-3}$ in 1:4 ratio. The difference was statistically significant between the astrocytes groups and 1:4. **Conclusion:** The enzyme beta glucoronidase was released in infected astrocytes and it can be used as an indicator for cellular damage.

**Keywords:** MTT, betaglucoronidase, glia, astrocytes.
**Financial support:** INCT, FAPESB, CAPES, CNPQ

## CELL SIGNALING PATHWAYS ASSOCIATED WITH ZIKV INFECTION IN ASTROCYTES: A SYSTEMATIC REVIEW

Dos Santos, A. L.[1]; Ferrúa, C. P.[1]; Nedel, F.[1]; Pinheiro, R. T.[1]; Souza, D. O.[2]; De Assis, A. M.[1]

[1] *PPG Saúde e Comportamento, Universidade Católica de Pelotas, RS, Brasil.*
[2] *PPG Ciências Biológicas: Bioquímica, Universidade Federal do Rio Grande do Sul, RS, Brasil.*

**Introduction:** The ZIKV infection has been tightly associated with fetal brain malformation called microcephaly. These macroscopic pathological changes in the ZIKV-infected brain are observed in the middle gestation period. The physiology and/or morphology of ZIKV-infected neural cells in the fetus was altered sometime after infection, such as activated microglial cells, reactive astrocytes, and granular assemblies in neural structures. Astrocytes are the most abundant glial cells located near the capillaries and are key components of the blood brain barrier. Therefore, astrocytes may be the first targets affected by ZIKV after entering the CNS. In addition, ZIKV glial infection can cause neuroinflammation by releasing proinflammatory molecules through the blood brain barrier. Thus, in an attempt to better understand fetal CNS malformation, it is relevant

to investigate which cellular signaling pathways are affected by ZIKV infection in astrocytes. **Objectives:** Systematically review the literature to find what are the cellular signaling pathways associated with ZIKV infection in CNS astrocytes. **Material and Methods:** This systematic review realized an electronic search in PubMed, Scopus, Scielo and ISI Web of Knowledge databases, using the relevant MeSH and entry terms: (zika virus OR zika virus infection) AND (neuroglia OR astrocytes OR Ependymoglial Cells OR Microglia OR Oligodendroglia OR Glial cells). All references were managed in the EndNote X7 software (Thomson Reuters, New York, NY, US). Initially, duplicate references were excluded. Then, the studies were screened based on the inclusion and exclusion criteria, first by tittle and abstract, second by full text. Data were extracted and tabulated independently by two reviewers (ALS and CPF) to be submitted to a descriptive analysis. Lists were compared and in case of disagreement, a consensus was reached by discussion. When a consensus was not achieved, a third reviewer decided if the article should be included (AMA). This systematic review followed the PRISMA statements, with some adjustments. **Results:** The initial search yielded 283 articles; after removing duplicate titles, the title and abstract were screening. After reading the full text, 20 articles satisfied the inclusion criteria. After analysis of the selected papers, 19 astrocytic signaling pathways related to ZIKV infection were observed. The most prevalent was the Interferon pathway, which appeared in 31% of the studies. Among the evaluated strains there was a predominance of strains originating from Africa, Asia and Puerto Rico. **Conclusions:** These data are of scientific importance because they unify information about ZIKV infection in astrocytes and may direct new experimental studies on this subject.
**Keywords:** ZIKV; astrocytes; cell signaling pathway.
**Acknowledgments:** CNPq/CAPES/ZIKA Project 440763/2016-9

## INVESTIGATION OF THE EFFECT OF AGATHISFLAVONE FLAVONOID ON NRLP3-INFLAMOSOME MODULATION, MICRO-RNAS AND PROINFLAMMATORY CYTOKINES ASSOCIATED WITH GLIAL RESPONSE TO NEUROINFLAMATION

Dos santos, B. L.[1,2,3]; Costa, M. De F. D. [1]; Da Silva, V. D. A.[1]; Costa, S. L. [1]

[1]Instituto de Ciências da Saúde — UFBA Laboratório de Neuroquímica e Biologia Celular, Salvador/BA, Brazil.
[2]Postgraduate Program in Immunology. Federal University of Bahia — UFBA. Salvador/Bahia, Brazil.
*[3]College of Nursing, Federal University of San Francisco Valley — UNIVASF, Petrolina/PE, Brazil.*

**Introduction:** Alzheimer's disease (AD), the most common neurodegenerative disorder in the world, is characterized by the accumulation of β-amyloid protein (Aβ) in the brain parenchyma, formation of neurofibrillary tangles, glial activation and consequent production of inflammatory mediators such as NO and pro-inflammatory cytokines such as interleukin 1β (IL-1β), interleukin 6 (IL-6) and tumor necrosis factor-α (TNF-α), which eventually contribute to neuronal toxicity. Flavonoids, polyphenolic compounds present in a wide variety of plants, have potent anti-inflammatory and antioxidant properties. **Objectives:** Here, the anti-inflammatory potential of the flavonoid agathisflavone (FAB), which is derived from the Brazilian plant *Poincianella pyramidalis*, will be evaluated, using the lipopolysaccharide (LPS), IL-1β and Aβ o*ligomers as* in vitro models of neuroinflammation. **Material and Methods:** cultures of microglia and glial cells have been obtained from the cortex of newborn Wistar rats. After 21 days the cell cultures are exposed for 24h to LPS (1μg / mL) or IL-1β (10ng / mL) or for 4h to Aβ oligomers (500 nM) and then treated with agathisflavone (1μM or 10μM) for an additional 24h. After inflammatory stimulation and treatment, immunocytochemical analysis will be performed to evaluate the microglial activation profile and the expression of inflammatory markers. The immunomodulatory effect of agathisflavone on the expression of proinflammatory cytokines (IL1β, TNF-α, INF, IL-2 and IL-6) will be analyzed by ELISA and RT-qPCR. In addition, the immunomodulatory effect on the expression of the NLRP3 inflamosome complex and miR-155 and miR-146a microRNAs

associated with regulation of the inflammatory process will be also evaluated by RT-qPCR. **Results and Conclusions:** At the moment, microglia culture was performed and inflammatory stimulus was made with LPS. Then the cultures were treated with agathisflavone. Cultures were grown on different culture plates for immunocytochemical labeling and RNA extraction. Culture supernatants were frozen for further analysis by ELISA. In preliminary analysis by phase contrast microscopy, we observed that the treatment of microglia

## NEUROIMMUNOMODULATORY AND NEUROPROTECTIVE EFFECTS OF FLAVONOID APIGENIN IN *IN VITRO* MODELS OF NEUROINFLAMMATION ASSOCIATED WITH ALZHEIMER'S DISEASE

Dourado, N. S.[1]; Souza, C. S.[1]; Almeida, M. M. A.[1]; Santos, B. L.[1]; Silva, V. D. A.[1]; Assis, A. M.[2]; Silva, J. S.[3]; Souza, D. O.[3]; Costa, S. L.[1]

[1]*Laboratory of Neurochemistry and Cell Biology, Institute of Health Sciences — UFBA Salvador — BA, Brazil.*
[2]*Catholic University of Pelotas (UCPEL), Pelotas — RS, 96015-560, Brazil.*
[3]*Department of Biochemistry, Institute of Basic Health Sciences, Federal University of Rio Grande do Sul (UFRGS), Porto Alegre — RS, 90035-190, Brazil.*

**Introduction:** Alzheimer's disease (AD) is characterized by accumulation of the β amyloid protein (Aβ) and increase of inflammatory response mediated by microglia and astrocytes, which once activated, release pro-inflammatory cytokines such as IL1β, resulting in neurodegeneration. Apigenin (4,5,7 — trihydroxyflavone) is a flavonoid found in abundance in many fruits and vegetables, such as orange, chamomile, celery, parsley, and onion, in red wine and especially *Passiflora* (Passionflower). **Objectives:** This study evaluated the neuroprotective and neuroimmunomodulatory potential of flavonoid apigenin in *in vitro* models of neuroinflammation associated with AD. **Material and Methods:** Co-cultures of neurons and glial cells were obtained from the cortex of newborn and embryonary Wistar rats. After 21 days in cultures cells were exposed to LPS (1μg/mL) or IL-1β (10 ng/mL) for 24 h, or to Aβ o*ligomers* (500 nM) for 4 h, and then treated with apigenin (1 μM) for more 24 h. **Results:** It was observed by Fluoro Jade B and caspase 3 immunostaining that apigenin was not neurotoxic and has a neuroprotector effect against inflammatory damages. The immunocytochemistry analysis revealed that apigenin reduced microglial activation after inflammatory stimulus with LPS, IL-1β or Aβ o*ligomers,* characterized by decrease in expression of M1 inflammatory marker CD68, inhibition of proliferation (BrdU+ cells), and by modulation of microglia morphology (Iba-1+ cells). Moreover, apigenin treatment also preserved neurons and astrocytes integrity, determined by immunocytochemistry of β-tubulin III and GFAP proteins, respectively. The immunomodulatory effect of apigenin, also evaluated by RT-qPCR, demonstrated that inflammatory stimuli with IL-1β or Aβ o*ligomers* induced increase in expression of mRNA for IL-1β and CCL5, and decrease in the expression of mRNA for IL-10. However, the pre-treatment of cultures with apigenin before both inflammatory stimuli induced decrease in the expression of mRNA for IL-1β and CCL5 and increase the expression of mRNA for IL-10. **Conclusions:** Together these data demonstrate that apigenin presents neuroprotective and neuroimmunomodulatory effects in vitro, which may be considered for pre-clinical studies as adjuvant for the treatment of neurodegenerative diseases.

**Keywords:** Alzheimer's disease, apigenin, neuroprotection
**Support:** CAPES and FAPESB.

## RUTIN MODULATES GLIAL CELL RESPONSE IN AN *IN VITRO* MODEL OF NEUROINFLAMMATION

Farias, A. A.[1]; Santana-Bastos, E. M.[1,2]; Pinheiro, P. C; Costa, S. L. [1]; Silva, V. D. A[1].; Costa, M. F. D.[1]

*[1] Laboratory of Neurochemistry and Cell Biology, Department of Biochemistry and Biophysics, Institute of Health Sciences, Federal University of Bahia, Salvador – Ba, Brazil.*
*[2] Institute of Chemistry, Post-Graduate Program in Intellectual Property and Technology Transfer for Innovation PROFNIT/UFBA.*

**Introduction:** Flavonoids are secondary metabolites found in various plant structures and are of great pharmacological importance because they are important biological functions. Thus, clinicians and pharmacologists are interested in investigating the role of these compounds in neurodegenerative disorders, and the interest has been reflected in the worldwide literature that provides various citations with the descriptor "Rutin" in various areas of research. Among of them, it is demonstrated that rutin (quercetin-3-orutinoside) presents neuroprotective action in the classic *in vitro* model of the two most frequent neurological diseases, Alzheimer's disease and Parkinson's disease. **Objective:** Thus, we aimed to evaluate the protective effects of rutin in the mixed culture of glial cells with damage induced by aminochrome, a dopamine derived molecule. **Material and Methods:** Neonatal Wistar rats (P0-2) were used according to the recommendations of the UFBA Ethics Committee (Protocol No. 0272012). Their cortical hemispheres were aseptically isolated, the meninges removed, and cells dissociated and cultured for 15 days. The glial culture was pretreated with the rutin (1 µM) for 18 h, followed by treatment with aminochrome (10 µM) and rutin (1 µM) for 6 h. The number of microglial cells was evaluated by immunocytochemistry for ionizing calcium binding adapter molecule (Iba-1). **Results:** In the aminochrome treated cultures there is a predominance of microglia with amoeboid or spherical shape, and in some fields it is possible to observe cell clusters. However, in cultures pre-treated with rutin the microglia presented fewer prolongations and increase the number of cells with fusiform and polygonal morphology. **Conclusion:** These results show that rutin induces morphological alteration with fusiform and polygonal cellular form that suggests a protective profile against toxicity caused by aminochrome. Thus, such data corroborate the findings of the scientific literature and supplementary cellular and molecular analysis will be performed to characterize inflammatory profile modulate by flavonoid rutin under this cytotoxic damage associated with PD.
Key words: Rutin, Neuroinflammation, Neuroprotection, Mixed Culture of Glial Cells.
Supported by FAPESB, CAPES. INCT- Translational Neuroscience

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## BIOACTIVITY OF *ABAREMA COCHLIACARPOS* EXTRACTS

Farias, C. S.[1]; Carvalho, J. R. M.[1]; Ribeiro, P. R.[1]

[1]*Metabolomics Research Group, Departamento de Química Orgânica, Instituto de Química, Universidade Federal da Bahia, Rua Barão de Jeremoabo s/n, 40170-115, Salvador, Brazil.*

**Introduction:** *Abarema cochliacarpos* is a species native to Brazil, belonging to the family Leguminosae — Mimosoidae. It is popularly known as Barbatimão and it is used in alternative medicine to treat several diseases such as candidiasis, inflammations, cavities and gastritis. In relation to its chemical composition, it contains catechins, saponins, tannins and proanthocyanidins. **Objective:** To assess in vitro antioxidant activity and the total phenolics content of *Abarema cochliacarpos* extracts. **Material and Methods:** Leaf, stem and bark were collected and ground to a fine powder. Extracts were obtained by maceration in hexane and ethyl acetate. Antioxidant activity was determined by the 2,2 diphenyl-1-picryl-hydrazyl radical scavenging assay and quantification was expressed as $IC_{50}$. Total phenolics were quantified by Folin-Ciocalteau method and expressed as $mgGAE.g^{-1}$ (gallic acid equivalents). Four crude extracts were fractionated by column chromatography with different proportions of ethyl acetate, hexane and ethanol, yielding 5 distinct fractions of each of the initial extracts. Antioxidant activity and total phenolics were also determined for the fractionated extracts. **Results:** The $IC_{50}$ for *A. cochliacarpos* crude extracts varied from 4.69 to over 1000

μg.mL$^{-1}$ for the stem, from 3.89 to over 1000 μg.mL$^{-1}$ for the bark and from 17.77 to 85.84 μg.mL$^{-1}$ for the leaf. Total phenolics for *A. cochliacarpos* crude extracts varied from 36.38 to 359.05 mgGAE.g$^{-1}$ for the stem, from 43.79 to 355.90 mgGAE.g$^{-1}$ for the bark and from 71.97 to 117.37 mgGAE.g$^{-1}$ for the leaf. The $IC_{50}$ for *A. cochliacarpos* fractionated extracts varied from 2.16 to 155.14 μg.mL$^{-1}$ for the stem extracted with ethyl acetate, 4.31 to 600.56 μg.mL$^{-1}$ for the bark extracted with ethyl acetate, 6.56 to 114.73 μg.mL$^{-1}$ for the leaf extracted with ethyl acetate and 78.20 to over 1,000 μg.mL$^{-1}$ for the leaf extracted with hexane. Total phenolics for *A. cochliacarpos* fractionated extracts varied from 176.13 to 571.84 mgGAE.g$^{-1}$ for the stem extracted with ethyl acetate, 88.35 to 405.71 mgGAE.g$^{-1}$ for the bark extracted with ethyl acetate, 112.38 to 909.34 mgGAE.g$^{-1}$ for the leaf extracted with ethyl acetate and 72.22 to 228.47 mgGAE.g-1 for the leaf extracted with hexane. There is a high correlation between antioxidant activity and total phenolics content, thus it can be inferred that the phenolic compounds are most likely responsible for the antioxidant activity of *A. cochliacarpos* leaf, stem and bark extracts. For the fractionated extracts, in general, the better results was obtained with extracts with ethyl acetate. **Conclusion:** *A. cochliacarpos* ethyl acetate extracts of the stem showed greater antioxidant activity and higher levels of total phenolics, which consist of promising samples for further pharmacological investments. The metabolite profiling analysis showed differences in the metabolome that could explain the antioxidant activity of the extract.

**Keywords:** metabolomics, natural products, phytochemical
**Supported by**: UFBA, FAPESB, CNPq and CAPES.

## DISSECTING THE MECHANISM OF TOXICITY TRIGGERED BY AGGREGATES OF TRANSTHYRETIN

Ferreira, P.[1]; Conceição, J. P.; Oliveira, L. [1;] Foguel, D. F.[1]

[1]*Centro de Ciências da Saúde, Instituto de Bioquímica Médica Leopoldo de Meis, Universidade Federal do Rio de Janeiro- Rio de Janeiro, Brazil*

**Introduction:** The Transthyretin (TTR) related amyloidosis are neurodegenerative disorders characterized by the extracellular deposition of TTR fibrils in several tissues causing the Famylial Amiloidotic Polineuropathy (FAP), Famylial Amiloidotic Cardiomyiopathy (FAC) and Oculoleptomeningeal Amyloidosis (OA). The aggregates are deposited in particularly in the peripheral nervous system, kidney, retin and cardiovascular system in the case of FAC. Nerve biopsies in patients with different stages of disease progression shows deposition and presence of amyloid fibrils. Early in FAP, TTR is already deposited in an aggregated nonfibrillar form, negative for Congo Red birefringence. This suggested that pre-amyloidogenic forms of TTR exist in the nerve of FAP patients, in a stage before fibril formation. The cellular effects of TTR deposition on neuronal function in FAP remain however to be elucidated. **Objectives:** The objective of this work is to dissect the transthyretin toxicity elucidating if the amyloid fibers or intermediate aggregates are toxic in different cell types and describe the mechanism by which these species exert its toxic effects. **Material and Methods:** We used Live/Dead, MTT and LDH assay for viability measures. Caspase and annexin assay were used to characterize the mechanisms of death. Immunocytochemistry were done to confocal images. We used lineages of N2A, HEK, HepG2 and primary culture of chick retinal neurons and cardiomyocytes. **Results:** Our data show that only oligomeric species and not fibrils of TTR are toxic to neural and cardiac cell lineage and primary culture of retinal neuron. However, none of the toxic species formed in the TTR aggregation process was toxic to HEPG2 strain, indicating that these *in vitro* produced oligomers have tissue specificity, just as inventive occurs *in vivo*. These data may explain why TTR also does not aggregate in liver *in vivo*. We observed caspase release and presence of annexin in neural cells treated with oligomers of TTR. We evaluate if these oligomers can internalize in these cells and our data show that the oligomers of TTR are able to internalize in retinal neurons but not in HEPG2. We also analyze the contribution of RAGE receptor for internalization and toxicity of oligomers since this receptor is involved in the toxic mechanism of other

amyloidosis, and our data show that after blocking cells with anti-RAGE and after brand trypsinization the oligomers are not able to internalize. However, cell death produced by oligomers was not reduced after these treatments. **Conclusions:** TTR aggregates are specifically toxic for neural lineages and the oligomers are the most toxic species. These oligomers seem to trigger an apoptotic mechanism to exert its toxic effect and maybe its internalization are not related to its toxicity since they are not able to internalize after trypsinization, but toxicity is not reverse. The absence of toxicity of oligomers in human liver lineage may be due to another mechanism.

**Keywords:** Transthyretin, aggregation, amyloidosis.
**Acknowledgment:** Faperj, CAPES, CNPQ e IMBEB

## DICHLOROMETHANE EXTRACT FROM *Amburana cearensis* SEEDS PROMOTES ASTROCYTIC REACTIVITY AND PROTECTS NEURAL CELLS IN *IN VITRO* MODELS OF ISCHEMIC STROKE

Ferreira, R. S.[1,2]; Silva, J. H. C.[1]; Hoppe, J. B.[4]; Carneiro, M. M. A. A.[1, 2]; Pieropan, F.[3]; Pereira, E. P. L.[1]; Rivera, A.[2]; Ferreira, B. C. L.1; Andrade, G. B.[1]; Ribeiro, P. R.[5]; Fernandez, L. G.[5]; Salbego, C.G.[4]; Moreira, J.C.F.[4]; Costa, S.L. [1]; Butt, A.M.[2]; Silva, V.D.A.[1, 2]

[1]*Laboratory of Neurochemistry and Cell Biology, Institute of Life Sciences, UFBA, Salvador, Brazil;* [2]*Institute of Biomedical and Biomolecular Sciences, University of Portsmouth, England;* [3]*Department of Biochemistry, Institute of Basic Health Sciences, UFRGS, Porto Alegre, Brazil;* [4]*Department of Organic Chemistry, Institute of Chemistry, UFBA, Salvador, Brazil;* [5]*Biochemistry, Biotechnology and Bioproducts Laboratory, Institute of Life Sciences, UFBA, Salvador, Brazil*

**Introduction:** Glutamate-mediated excitotoxicity is a pathophysiological event present in several neurodegenerative diseases and stroke. This event also occurs in the hippocampus, a region rich in glutamatergic neurons. Under these conditions, there is a large increase in neuronal death. Astrocytes are the major cells responsible for the detoxification of glutamate excess. Studies have shown promising effects related to neuroprotection with derivatives of *Amburana cearensis* in *in vitro* models. **Objective:** To evaluate the neuronal and astrocytic response by treatment with Dichloromethane Extract of *Amburana cearensis* (EDAC) in models of glutamate excitotoxicity or deprivation of oxygen and glucose in the hippocampus. **Material and Methods:** Hippocampal slices from neonatal (P10-12) transgenic mice in which the fluorescent reporter enhanced green fluorescence protein (EGFP) is driven by GFAP in astrocytes or SOX-10 in oligodendrocytes were submitted to EDAC (1 or 10 µg/ mL)pretreatment for 1 h and then to normal conditions(OGN) or oxygen and glucose deprivation (OGD) for 1 h in the presence or absence of EDAC. Organotypic hippocampal slice cultures (OHSC) from wild type Wistar rats (P10-P12) were treated with glutamate and/ or EDAC. The *cornu ammonis 1* regions of the slices were analyzed by immunohistochemistry (IHC) for NeuN. The expression of GFAP, SOX-10 and NeuN was assessed by confocal microscopy. Cell viability was analyzed by propidium iodide uptake. **Results:** Our results showed that the OGD condition tends to reduce GFEC expression in *Stratum oriens, pyramidale and radiatumin* CA1 region and the presence of EDAC increases GFEC expression. In oligodendrocytes, it was observed that there was a reduction of SOX-10 expression under OGD conditions that was inhibited by EDAC treatment. In OHSC, we observed that glutamate induced an increased uptake of propidium iodide which was inhibited by treatment with EDAC. Glutamate also reduced NeuN expression in OHSC that was inhibited by treatments with EDAC and glutamate. **Conclusion:** EDAC has a potential pharmacological effect for stroke. Further studies with these two models are being carried out to better understand the ex vivo/ in vitro mechanism of EDAC action in neural cells.

**Keywords:** *Amburana cearensis; Hippocampal slices; astrocytes; oligodendrocytes; neuroprotection.*

**Acknowledgments:** *FAPESB, CAPES, CNPq and INNT.*

# REGULATION OF ASTROCYTIC METABOLISM, ANTIOXIDANT AND MITOPROTECTIVE ACTION ARE MECHANISM OF RUTIN PROTECTION AGAINST GLUTAMATERGIC EXCITOTOXICITY

Ferreira, R.S.[1*]; Teles-Souza, J.[1*]; Pereira, E.P.L.[1]; Araújo, F.M. [1]; Silva, J.H.C.[1]; Costa, A.C.S[1]; Sanches, F.S[1]; Souza, B.V.[1]; Costa, M.F.D. [1]; Souza, C.S.[1]; Nonose, Y.[3]; Nunez-Figueredo, Y. [2]; Assis, A.M.[3]; Moreira, J.C.F.[3]; Costa, S.L.[1]; Silva, V.D.A.[1, 2]

*These authors equally contributed to this work.

*[1]Laboratory of Neurochemistry and Cell Biology, Institute of Health Sciences, UFBA, Salvador, Brazil.*
*[2]Centro de Investigación y Desarrollo de Medicamentos, Habana, Cuba.*
*[3]Department of Biochemistry, Institute of Health Basic Sciences UFRGS, Porto Alegre, Brazil.*

**Introduction:** Rutin is a flavonoid with anti-inflammatory, antigliomatic and neuroprotector effects on CNS. Previous studies have been demonstrated that rutin is able to modulate astroglial and microglial response. Glutamate-mediated excitotoxicity is involved in the pathogenesis of many neurodegenerative diseases. To prevent over-stimulation, glutamate is removed from the synaptic cleft by astrocytes trough Excitatory Amino Acid Transporters (EAAT) and converted to L-glutamine through the action of glutamine synthetase (GS). **Objective:** the aim of this study was to investigate the mechanism of neuroprotective effect of rutin against glutamate excitotoxicity. **Material and methods:** studies with neuroprotective effects were performed in organotypic brain cultures (P8) (OBC) or PC12 cell cultures exposed to glutamate (10 – 60 mM) for 24 hours, concomitantly treating with rutin (0.5 – 1 µM), and cell viability was analyzed by propidium iodide (PI) test. PC12 cells were also treated with OBC conditioned medium derived from the different groups of the OBC treatments. The evaluation of GS expression levels was analyzed by Western blotting and GLAST and GLT1 expression by RTq-PCR. Glutamate uptake was analyzed in cerebral cortex slices (300 µm) from 90 days old Wistar rats. The slices were treated with rutin (5 µM) and/or glutamate (15 and 30 mM) diluted in HBSS at 37 °C, with addition of 0.33 µCi of L-[3H]-glutamate. Mitoprotection tests (membrane potential dissipation, ROS production and calcium influx) with rutin (10 µM) in isolated mitochondria from brain of adult Wistar rats exposed to rotenone (5 µM). **Results:** In OBC glutamate induced cell death demonstrated by increased relative fluorescence of PI and rutin (0.5 and 1 µM) was able to reduce relative fluorescence of PI. Rutin also protected PC12 cells against 10 mM glutamate exposure, but not against 60 mM glutamate exposure, however the OBC conditioned medium from 60 mM glutamate plus rutin treatment induced a fewer PC12 death cells, when compared with direct treatment. Furthermore in OBC, rutin induced increase in the GS and GLAST expression and increase of glutamate uptake in cerebral slices. Moreover, rutin reduced ROS production, membrane potential dissipation and calcium influx in isolated mitochondria. **Conclusion:** rutin present neuroprotective effect against glutamatergic excitotoxicity. The results presented here suggest that this effect involves antioxidative action, mitochondrial protection and regulation of glutamate metabolism by astrocytes.

*Keywords: glutamate uptake, mitoprotection, rutin.*

**Acknowledgements:** *FAPESB, CAPES e CNPq.*

## PHYSALIS ANGULATA MODULATES THE CELL RESPONSE IN NEOSPORA CANINUM GLIAL CELL INFECTION

Freitas, L. dos S. [1, 2]; Novais, É. S.[1]; Frota, A. F. [1]; Argolo, D.[1]; Conceição, R. S. [2]; Ifa, D.[2]; Branco, A.[3]; Costa, S. L.[1]; Costa, M. de F.[1]; Pinheiro, A. M.[1, 2]

*[1.] Laboratory of Neurochemistry and Cell Biology, Institute of Health Science, Federal University of Bahia –FFBA, Av. Miguel Calmon s / n, Canela Valley, Zip Code 41100-10, Salvador, Bahia, Brazil.*
*[2.] Veterinary Biochemistry and Immunology Laboratory — Center for Environmental and Biological Agrarian Sciences — UFRB, Rua Rui Barbosa, 710, CEP 44380-000, Cruz das Almas, Bahia, Brazil.*
*[3.] Departamento de Saúde, Universidade Estadual de Feira de Santana, Feira de Santana, BA, Brazil.*

**Introduction**: *Neospora caninum*, on obligate intracellular parasite, is capable to infect cells on central nervous system, causing proinflammatory molecules and neurological damage to the host. **Objectives:** This study evaluated the protective effect of ethanolic extract of *Physalis angulata* (EEPA) on glial cells infected with *N. caninum*. The EEPA was characterized by sequential mass spectrometry (ESI-MS/MS). **Material and Methods:** Glial cells were obtained from cortex of neonatal Wistar rats, treated for 24 h with EEPA and subsequently infected with *N. caninum* for 72 h. The cytotoxicity of the extract was evaluated by the 3-(-dimethylthiazolyl-2)-2,5-diphenyltetrazolium (MTT) assay. Astrocyte and microglial morphology were assessed by immunocytochemistry of the markers glial fibrillar acid protein (GFAP) and ionized calcium binding adapter protein 1 (IBA-1), respectively, while cell proliferation was through the 5'-bromo-2 marker. '-deoxyuridine (BrdU). Inflammation was assessed by necrosis factor (TNF) and nitric oxide (NO) levels. **Results**: EEPA showed no cytotoxicity at the concentrations tested. Infection led to morphological changes in astrocytes, but treatment with EEPA preserved polygonal morphology. EEPA modulated proliferation and microglial morphological profile in infected cultures. Increased levels of these mediators were observed in infected cultures; however, extract treatment was able to reduce NO levels. In addition, there was a 37% parasitic reduction in EEPA-treated cells (5µg/mL). **Conclusion**: The results obtained in this study demonstrated the protective potential of EEPA in *N. caninum* infected glial cells.
**Keywords:** Infection, cell glial, ethanolic extract.

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## HISTOPATHOLOGICAL ALTERATIONS IN INTESTINAL MUCOSA IN EXPERIMENTAL MODEL OF PARKINSON'S DISEASE IN RATS

Jesus, L. B.[1]; Frota, A. F.[2]; Araújo, F. M.[1]; Passos, F.[1]; Rossi, R. C.[3]; Aragão, C. B.[3]; Costa, M. De F. D.[1]; Silva, V. D. A.[1]; Gois, M. B.[1,3]; Baccan, G. C.[1]; Costa, S. L.[1]

*[1] Immunology Postgraduate Program, ICS- UFBA, BA, Brazil; [2] Postgraduate Program in Biochemistry, UFC, CE-Brazil; [3] Health Sciences Center, UFRB, BA, Brazil.*

**Introduction**: Parkinson's disease (PD) is the second most important neurodegenerative disorder after Alzheimer's disease and the most prevalent motor disorder. In addition to motor problems, the quality of life of patients with Parkinson's disease is severely impaired by a variety of non-motor symptoms, including gastrointestinal dysfunction. The enteric nervous system (ENS) is responsible for controlling intestinal motility. The involvement of ENS has been shown to be important in the pathophysiology of PD. **Objective:** To evaluate the effects of pathogenicityin a PD model induced by intracranial injection of 6-hydroxydopamine (6-OHDA) in the enteric nervous system on the gastrointestinal transit, immunohistochemical and histological parameters in the jejunum of rats. MATERIAL AND **Methods:** Twelve male Wistar rats weighing 250 — 300g were anesthetized and submitted to unilateral nigrostriatal injection of 6-OHDA (21 µg) or Saline (0.9%). Rats were also submitted to behavioral tests (Open-field test, Cylinder test and Rotation test induced by apomorphine [3 mg/kg, i.p.]). Gastrointestinal transit time and total fecal matter was evaluated, before

euthanasia. After the trial period, the rats were anaesthetized with ketamine and xylazine intraperitoneally, the jejunum was collected, washed with 0.1 M phosphate buffered saline pH 7.2 (PBS), carefully filled with paraformaldehyde fixative solution, and stored in PBS with 0.08% of azide and subject performing of the immunohistochemical techniques where the jejunum was microdissected to obtain the tunica muscularis. The enteric nervous system evaluation was performed by analyses of neurons enteric in the myenteric plexus. The immunohistochemical markers used were HuC/D (1:800, Molecular Probes) and β-S100 (1:800, Molecular Probes), respectively. Histopathological analysis (semi-quantitative and quantitative) was performed on hematoxylin/eosin (HE) stained slides. The production of extracellular matrix (collagen) components was determined by spectrophotometer at 540 nm using Sirius Red dye solution. **Results:** The 6-OHDA group showed decreased locomotor activity in the Open-field test (30.0 ± 5.1) compared to the control group (54.2 ± 2.9), also showing reduced performance on the cylinder test. In the rotational test, the 6-OHDA group increased the number of rotations in comparison with the control group ($p<0.05$). The rats in the 6-OHDA group exhibited a significant decrease in fecal water content (22%), reduced fecal yield and delay in gastrointestinal transit time of approximately 2h when compared to the control group. Immunofluorescence demonstrated that there was an increase over 25% in HuC/D and glia protein expression in the jejunum myenteric plexus of the rats of the 6-OHDA lesion group when compared to the control. Semiquantitative histopathological analysis revealed the discreet presence of diffuse inflammatory infiltrate with mononuclear predominance in ileo and slight increase the paneth cell in the je
junum. Collagen dosing results showed that the jejunal concentration increased slightly while the other segments (duodenum, jejunum and ileum) demonstrated collagen reduction thus exhibiting abnormal healing characteristics. **Conclusion:** The results showed that the rats submitted to the 6OHDA lesion, in addition to presenting motor deficit, presented altered intestinal transit, with reduction of gastrointestinal motility, reduction of pellets and fecal moisture. These data suggest that this model is adequate to evaluate the participation of ENS in the pathophysiology of PD.

**Keywords:** Parkinson's Disease, Enteric Nervous System, Enteric Glia
**Support**: FAPESB, CNPq and CAPES.

## *SPIRULINA PLATENSIS* LEB-18 BIOMASS INDUCES CENTRALLY MEDIATED ANTINOCICEPTION VIA THE OPIOID SYSTEM

Lauria, P. S. S. [1]Santos, D. S.[1]; Evangelista, A. F.[2]; Azeredo, F. J.[3]; Costa, J. A. V.[4]; Soares, M. B. P. [2]; Druzian, J. I.[5]; Villarreal, C. F. [1,2]

[1]*Laboratório de Farmacologia e Terapêutica Experimental, Faculdade de Farmácia, Universidade Federal da Bahia. Bahia, Brazil.*
[2]*Laboratório de Engenharia Tecidual e Imunofarmacologia, Instituto Gonçalo Moniz, Fiocruz. Bahia, Brazil.*
[3]*Laboratório de Farmacocinética, Faculdade de Farmácia, Universidade Federal da Bahia. Bahia, Brazil.*
[4]*Laboratório de Engenharia Bioquímica, Escola de Química e Alimentos, Universidade Federal do Rio Grande. Rio Grande do Sul, Brazil.* [5]*Laboratório de Pescados e Cromatografia Aplicada, Faculdade de Farmácia, Universidade Federal da Bahia. Bahia, Brazil.*

**Introduction:** The clinical management of pain is a world health issue. The analgesic drugs currently available induce several undesired side effects and are inefficient for some painful conditions. Natural products are rich sources of bioactive compounds that have been used for several decades to the development of new therapeutic drugs. *Spirulina platensis* is a cyanobacteria species whose biomass promotes remarkable anti-inflammatory activity in both preclinical and clinical trials. **Objectives:** This work investigates the antinociceptive properties of *Spirulina platensis* LEB-18 biomass (SP-LEB18) and their mechanisms. **Material and methods:** The antinociceptive effect of SP-LEB18 was initially investigated in the CFA-induced inflammatory pain model in mice. Using this model, paw edema formation and nociceptive thresholds were evaluated. The effect of SP-LEB18 on cytokines levels (IL-10, TNF-α, and IL-1β) was accessed by ELISA. The tail flick test was performed by immersion of the tip of the tail in hot water (47°C) until tail withdrawal was

recorded. **Results:** SP-LEB18 (100 — 400 mg/kg) exhibited consistent antinociceptive activity, reducing CFA-induced mechanical allodynia. Paw edema formation was also inhibited by SP-LEB18 (50 — 400 mg/kg), confirming its well-established anti-inflammatory action. SP-LEB18 (200 mg/kg) promoted an increase in IL-10 levels as well as the reduction of the levels of the pro-inflammatory cytokines TNF-α and IL-1β. Apart from its anti-inflammatory action mediated by IL-10 production, SP-LEB18 (100 — 400 mg/kg) exhibited a centrally mediated antinociceptive activity, as suggested by the increase of latency time in the tail flick test. When the same set of experiments was conducted with IL-10 knockout (IL-10 KO) mice, no pharmacologic effect was observed in the CFA model, but the intrinsic antinociceptive action of SP-LEB18 was still detected in the tail flick test. This action was abolished when mice were pretreated with naloxone, demonstrating the activation of the opioid system. **Conclusions:** This is the first demonstration of a pure antinociceptive action of *Spirulina* biomass, which uncovers new potential therapeutic applications of this product in the treatment of painful conditions.

**Keywords:** analgesic, IL-10, opioid.

## *IN VITRO ANTICOLINESTERASIC ACTIVITY OF ETHYL ACETATE FRACTIONS OF Ocotea daphnifolia BIOACTIVE COMPOUNDS FROM HANCORNIA SPECIOSA EXTRACTS*

Luz, R. L. S. de A.[1]; Albuquerque, M. M. S.[2]; Almeida, R. B. M.[1]; Branco, A.[2]; Botura, M. B.[1]

[1] Health Department, Toxicology Laboratory, Stadual University of Feira de Santana, Bahia, Brazil.
[2] *Health Department, Phytochemistry Laboratory, Stadual University of Feira de Santana, Bahia, Brazil.*

**Introduction**: Plant metabolites represent an important source for the discovery of new molecules with ability to inhibit acetylcholinesterase (AChE), the key enzyme in the pathology of Alzheimer's disease. Previous studies have identified *in vitro* anticholinesterase effect of *Ocotea daphnifolia* ethyl acetate extract. **Objectives**: The objective of this work was to evaluate the *in vitro* inhibitory effect on acetylcholinesterase activity of ethyl acetate extract fractions of *O. daphnifolia* and to perform the phytochemical caracterization of the active fraction. **Material and Methods**: The ethyl acetate extract was fractionated using column chromatography, and 14 fractions were obtained. After Thin Layer Chromatography analyses, fractions were combined by chromatographic profile resulting in 10 fractions. The anticholinesterase activity of fractions (1 mg /mL) was evaluated by spectrophotometry in microplate assays. The most active fraction was characterized using High Performance Liquid Chromatography (HPLC) in a semi-preparative column and Nuclear Magnetic Resonance (NMR). The data were analyzed by ANOVA, followed by Tukey test ($p < 0.05$). **Results**: Fraction 9 had the greatest anticholinesterase effect, with inhibition percentages of 82%. The fraction 6 showed a moderate inhibitory effect (47%), while the fractions 4, 5, 7 and 8 presented low anti-AChE activity (17% to 30%). The positive control, eserine, achieved 94% inhibition of AChE. Phytochemical analysis of fraction 9 suggested the presence of a aliphatic glucoside. **Conclusions**: The fraction 9 of *O. daphinifolia* ethyl acetate extract presented the highest inhibitory effect *in vitro* against acetylcholinesterase activity.

**Keywords:** *acetylcholinesterase; Alzheimer; Ocotea daphnifolia.*

**Financial Support:** *State University of Feira de Santana.*

## BIOACTIVE COMPOUNDS FROM *HANCORNIA SPECIOSA* EXTRACTS

Moura, D.[1]; Batista, D.L.J.[1]; Carvalho, J. R. M.[1]; Cerqueira, M. D.[1]; Martins, D.; Ribeiro, P. R.[1]

[1]*Metabolomics Research Group, Departamento de Química Orgânica, Instituto de Química, Universidade Federal da Bahia, Rua Barão de Jeremoabo s/n, 40170-115, Salvador, Brazil*

**Introduction:** Plant extracts are important allies for the development of new drugs from natural sources. *Hancornia speciosa,* as known as mangabeira, is a very popular species in Brazil, which is widely used in traditional medicine for treating several diseases. **Objectives**: To quantify antioxidant activity and total phenolics of *Hancornia speciosa* extracts and fractions and to determine the bioactivy compounds responsible for such activitys. **Material and Methods**: *H. speciosa* extracts were obtained by maceration in ethanol. Then, a mixture of ethanol and water (80:20) was used for partitioning with hexane and ethyl acetate. The ethyl acetate fraction was subjected to column chromatography (100 × 16 cm i.d. column, Merck silica gel 70–230 mesh), eluting with a gradient hexane and ethyl acetate to produce seven fractions (HS1-HS6) and ethanol (HS7). Antioxidant activity was determined by the 2,2-diphenyl-1-picryl-hydrazyl radical scavenging assay and expressed as $IC_{50}$. Total phenolics were quantified by Folin-Ciocalteau method. The metabolite profile of the extracts and fractions was analized by LC-MS and data were compilated using the MetaboAnalyst software and a Heat-Map and a Principal Component Analysis (PCA) was used to analyze the data. **Results:** The $IC_{50}$ for *H. speciosa* stem extract was 6.09 µg.mL$^{-1}$. Solvent extraction and partitioning in ethyl acetate slightly dimished the antioxidant activity ($IC_{50}$ 7.27 µg.mL$^{-1}$), whereas partitioning in hexane diminished dramatically the antioxidant activity ($IC_{50}$ 1527.39 µg.mL$^{-1}$). Total phenolics of *H. speciosa* stem extracts was 381.96 mg.EAG.g$^{-1}$. Similarly, solvent extraction and partitioning in ethyl acetate enhanced total phenolics (414.87 mg.EAG.g$^{-1}$), whereas partitioning in hexane dimished total phenolics (83.92 mg.EAG.g$^{-1}$). The $IC_{50}$ varied from 5.50 µg.mL$^{-1}$ for HS7 and 366.00 µg.mL$^{-1}$ for HS4, whereas total phenolics varied from 502.66 mg.EAG.g$^{-1}$ for HS7 and 39.06 mg.EAG.g$^{-1}$ for HS4. **Conclusion**: *H. speciosa* stem extracts showed antioxidant activity, which could be enhanced by using partitioning in ethyl acetate and further chromatographic fractionation. The metabolite profiling analysis showed 24 different compounds including Panaxydol linoleate, Notoginsenoside R10, that are known in the literature for the antioxidant properties they have stated.

**Keywork:** metabolomics, natural products, phythochemical analysis

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## MESENCHYMAL STEM CELLS PRETREATED WITH PHYTOESTROGEN AGATHISFLAVONE IMPROVE ACUTE SPINAL CORD INJURY IN WISTAR RATS

**Nascimento, R. P.**[1,2]; Moreira, E. L. T.[3]; David, J. M.[4]; David, J. P.[5]; Paredes, B.[6] Silva, V. D. [1] ; Costa, S. L.[1] .

[1] *Laboratory of Neurochemistry of Cellular Biology, Federal University of Bahia, Brazil;* [2]*Post-graduate Program in Biotechnology, State University of de Feira de Santana, UEFS, BA, Brazil;* [3] *Department of Anatomy, Pathology and Veterinary Clinics, Hospital of Veterinary Medicine, Federal University of Bahia (UFBA), Salvador, 40.110-170, Brazil;* [4]*Departament of General and Inorganic Chemistry, Institute of Chemistry, Federal University of Bahia, Brazil;* [5]*Departament of Medication, Faculty of Pharmacy, Federal University of Bahia, Brazil; Hospital São Rafael, Center for Biotechnology and Cell Therapy, Hospital São Rafael, Salvador-BA, Brazil.*

**Introduction**: Mesenchymal stem cells (MSCs) are characterized by their ability to self-renew and differentiate into various cell types, contributing to the functional reconstitution of certain tissues. The flavonoid agathisflavone (FAB) is a phytoestrogen capable of inducing neurogenesis in embryonic stem cells, in pluripotent stem cells, and in culture of cortical neuron and glial cells, also presenting anti-inflammatory properties. **Objectives:** The present study investigated the neuroprotective and anti-inflammatory effects

of treatment with FAB or with implant of rat MSCs (rMSCs) pre-treated with FAB (rMSCs+FAB) in a model of acute spinal cord injury. **Materials and Methods:** rMSCs were obtained from the femur bone marrow from adult Wistar rats and culture in supplemented DMEM. Male Wistar rats underwent acute spinal cord injury with an F-2 fogarty catheter. The animals were divided into 5 groups: Sham; with spinal cord injury (SCI); with SCI and treated with methylprednisolone (MP); with SCI and treated with FAB (10 mg/ kg IP daily) for 6 days; with SCI and a single application (via caudal vein) of $1x10^6$ control rMSCs (rMSCs); with SCI and with a single application (via caudal vein) of $1x10^6$ rMSCs pretreated with FAB (1 µM, each 2 days, for 21 days in vitro). After 6 days the BBB scale was used to evaluate the motor functions of the animals, the SCI area was analyzed after H&E staining, and RtT-qPCR was performed to measure neurotrophins such as GDNF, NGF and arginase. Giemsa staining was used to analyze myenteric plexus neurons. **Results:** Treatment of animals with flavonoid agathisflavone alone was able to protect injured spinal cord tissue, increased expression of neurotrophins that are related to nerve growth, and arginase expression, a enzyme directly related to the M2 anti-inflammatory profile of macrophages. In addition, the administration of 21-day FAB-treated rMSCs showed neuroprotective and anti-inflammatory properties, protecting the injured spinal cord tissue, increasing NGF and BDNF expression and improving the motor test of the group with the highest BBB score. Moreover, in this condition the integrity of the myenteric plexus neurons was mantained, reflecting a lower rate of weight loss in the animals of this group. **Conclusion:** Treatment with agathisflavone or with MSCs pretreated with this flavonoid modulate the inflammatory profile of the spinal cord injury to an anti-inflammatory profile and favor the spinal cord integrity and may be considered in further studies to characterize modulation of glial response and a possible neuroprotective effect.
**Keywords:** mesenchymal stem cells, agathisflavone, acute spinal cord injury
**Support:** CAPES and CNPq.

## IN SILICO AND IN VIVO APPROACHES ON UNDERSTANDING THE MECHANISM OF ACTION OF A NATURAL CHROMONE

Opretzka, L. C. F.[1]; Freitas, H. F.[2]; Espírito-Santo, R. F.[4]; Nascimento, O. A.[1]; Alves, I. M.[3]; Abreu, L. S.[3]; Velozo, E. S.[3]; Castilho, M. S.[2]; Villarreal, C. F.[1, 4]

[1]*Laboratório de Farmacologia e Terapêutica Experimental, Faculdade de Farmácia, Universidade Federal da Bahia, Brasil.*
[2]Laboratório de Bioinformática e Modelagem Molecular, Faculdade de Farmácia, Universidade Federal da Bahia, Brasil. [3]Laboratório de Pesquisa em Matéria Médica, Faculdade de Farmácia, Universidade Federal da Bahia, Brasil. [4]*Laboratório de Engenharia Tecidual e Imunofarmacologia, Centro de Pesquisas Gonçalo Moniz, Bahia, Brazil*

**Introduction:** The proper pharmacological control of pain is a continuous challenge for patients and health care providers. The ongoing treatment is primarily based on the use of opioid analgesics and non-steroidal anti-inflammatory drugs (NSAIDs). Even though those pharmacological classes are among the most widely used medications, they are still ineffective or unsafe for some patients, especially to those who suffer from chronic pain. Yet, there is a lack of development of new substances that target novel pathophysiological mechanisms, in order to supply the treatment of patients who respond inadequately. Natural products have long been an important source of bioactive molecules for drug discovery. Substances containing the chromone scaffold have shown a variety of biological activities, most importantly anti-inflammatory activity. We have previously presented the antinociceptive effect of the natural chromone 5-(methoxy) cneorumchomone (CR5) isolated from *Dictyoloma vandellianum* (Adr. Juss). CR5 promoted a dose-dependent and long-lasting antinociceptive effect in both tail flick and cold plate tests without affecting the motor function of mice, indicating that this effect may be centrally mediated. **Objectives:** This work aims to address some of the mechanisms by which CR5 causes its effect and the possible participation on

one or several pathways related with antinociception. **Material and Methods:** *In vivo* experiments were performed on groups of 6 male Swiss Webster mice weighting 22-28 g. An *in silico* screening was performed to determine possible pharmacological targets of CR5. The Tanimoto topological analysis strategy was used to investigate the fingerprint (2D) similarity between CR5 and antagonists of different receptors involved with the descending inhibitory system. The Surflex-sim program was used to perform the similarity analysis by 3D alignment. Antagonism assays were conducted employing the tail flick and the cold plate tests using the dose of maximum effect (50 mg/kg). The tail flick test was previously described by Dos Santos et al. (2012), and the cold plate test by Ta et al. (2013). The substances used as reference for both 2D and 3D similarity analysis and for antagonism assays were: naloxone (1 mg/kg, i.p., non-selective opioid antagonist), yohimbine (2 mg/kg i.p., α2 adrenoceptor antagonist), L-arginine (600 mg/kg, i.p., nitric oxide synthase substrate), methysergide maleate (5 mg/kg, i.p., serotoninergic receptors antagonist), atropine (10 mg/kg, i.p., non-selective cholinergic receptor antagonist), glibenclamide (2 mg/kg i.p., ATP-sensitive K+ channel blocker), bicuculline (1 mg/kg, i.p., $GABA_A$ receptor antagonists), or phaclofen (2 mg/kg, i.p., $GABA_B$ receptor antagonist). **Results:** First, we evaluated if the antinociception caused by CR5 was reversed by naloxe, a non-selective antagonist of opioid receptors, on tail flick and cold plate tests. The pretreatment (15 min, i.p.) with naloxe did not reverse the maximum antinociceptive effect (50mg/kg) caused by CR5, indicating that this chromone does not exerts its properties through activation of opioid receptors. Next, we used an *in silico* approach to predict the most probable target of CR5 within the descending inhibitory system by comparing its structure with other antagonists of these related receptors. The Tanimoto topological analysis strategy showed that bicuculline (18.4% similarity) and Methysergide (13.6% similarity) are the most similar antagonist to CR5, thus, the possible targets for activity investigation are $GABA_A$ and serotonergic receptors, respectively. The other compounds showed less than 8% similarity. In order to establish another comparison parameter, we used the 3D alignment as a similarity measure with the Surflex-sim program. In this analysis, CR5 scored higher with atropine (7.28) and bicuculline (7.03). Methysergide scored 6.71 and the CR5 itself scored 9.8. The combined 2D and 3D result suggests that the most likely target is the $GABA_A$ receptor. To prove our hypothesis, we performed antagonism assays on tail flick and cold plate tests. Pretreatment (15 or 30m, i.p.) with the different antagonists showed the CR5's effect was reversed by $GABA_A$ e $GABA_B$ antagonists (bicuculline and faclofen, respectively, $p<0,05$), but not by the modulation of adrenergic, serotonergic, cholinergic and nitric oxide pathways and of $K^+_{ATP}$ channels, confirming our hypothesis. These results encouraged us to use the Tanimoto similarity and Surflex-sim scores of CR5 against 53 ligands related to orthosteric and alosteric binding sites on $GABA_A$ receptor. The benzodiazepine, GABA, and neurosteroid sites were evaluated in order to investigate with which site on $GABA_A$ CR5 would probably interact. The result indicates that among the 5 most similar compounds 80% bind to the benzodiazepine site and 60% of the 5 highest scoring compounds in Surflex-sim are benzodiazepine binding site ligands. Thus, these two combined data suggest that CR5 could modulate the benzodiazepine's binding site. **Conclusions:** We conclude that CR5 probably exerts its antinociceptive activity via $GABA_A$ receptors, most likely by activating benzodiazepine allosteric receptors. Molecular docking assays on benzodiazepine site for investigating the mode of interaction of CR5 are being refined.

**Keywords:** pain, GABA, chromone.

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# *LEISHMANIA INFANTUM* CHANGES THE AREA OF COLONIC ENTERIC GANGLIA DURING THE CHRONIC INFECTION OF HAMSTERS

Passos, F. C.[1]; Sousa, A. D.[1]; Marinho, A. I. L.[1]; De Jesus, L. B.[1]; Góes, M. B.[2]; Baccan, G. C. [1]

[1]*Departamento de Bioquímica e Biofísica, Instituto de Ciências da Saúde, Universidade Federal da Bahia, Salvador, Brazil.*
[1]*Departamento de Saúde, Universidade Federal do Recôncavo Baiano, Santo Antônio de Jesus, Brazil.*

**Introduction:** Visceral leishmaniasis (VL) is a neglected disease that affect 12 million of people around the word, mainly in non developed countries. This disease is caused by parasites of the genus *Leishmania*, which are transmitted by the bite of vector insect from Phebotominie family. The parasites multiplicate in several organs, including spleen, liver and in gastrointestinal tract (IT). Notwithstanding the parasite cycle and the pathophysiology mechanisms have been well studied, the alterations in the IT caused by the presence of the parasites were not been fully characterized. The Enteric Nervous System (ENS) includes a number of neural circuits which control the motor functions, local blood flow, mucosal transport and secretions, and modulate the immune and endocrine functions. **Objectives:** The aim of this study was to evaluate the profile area (µm2) of myenteric and submucosal ganglia in the colon of hamsters during the experimental VL. **Material and Methods:** Male golden hamsters (*Mesocricetus auratus)* with age between 6 to 10 weeks were intradermal inoculated in the ear, with *L. infantum* ($1 \times 10^5$, 20 µl). After 4 and 8 months different groups of hamsters were euthanized and the colon was processed for histological analysis. An 1-cm ring from the colon, of each hamster per group, was fixed in buffered paraformaldehyde (10%) for 24 h. The segments were dehydrated in ascending series of ethyl alcohol, diaphanized in xylol and embedded in paraffin to obtain semi-serial cross-sections of 5-µm, which were stained with Hematoxylin and Eosin (HE). Images of all microscopic fields with myenteric and submucosal plexus ganglia were obtained using an optical microscope (Olympus® BX43F) and 40X objective lens with a high-resolution camera (Olympus® SC30) coupled to a Microcomputer. Morphometry (profiles) were performed with the aid of Image Pro Plus 3.0.1 image analysis Software. For analysis, the GraphPad Prism program was used. Data are expressed as mean ± SE. **Results:** Infected hamsters exhibit an increase in profiles of the area (µm2) of the myenteric ganglia when compared with non-infected controls, in both times: 4 (807.90 ± 42.59 and 545.20 ± 43.96, $P<0.001$) and 8 months (789.30 ± 33.43 and 542.40 ± 34.45, $P<0.001$), respectively. Similar data were obtained from the analysis of the submucosal ganglia, since infected hamsters also shown an increase in profiles of the area ($\mu m^2$) after 4 months (301.02 ± 12.41 and 152.40 ± 6.23, $P<0.001$) and 8 months (381.30 ± 16.03 and 228.10 ± 10.31, $P<0.001$) when compared with non-infected, respectively. **Conclusions:** Taken together, our results suggest that *L. infantum* infection caused changes in components of the ENS of the colon, including hypertrophy in the myenteric and submucosal ganglia.More studies are necessary to evaluated the mechanisms implicated in these changes and the role of these alterations during the infection.

**Keywords:** Leishmania Infantum. Colon. Enteric Ganglia.
**Support:** FAPESB.

## METABOLOMIC ANALYSIS OF PLASMA OF INDIVIDUALS WITH OSTEONECROSIS SECONDARY TO SICKLE CELL DISEASE

Pereira, T. C. S.[1]; Daltro, P. B.[2]; Carosio, M. G. A.[3]; Ferreira, A. G.[3]; Fortuna, V.[2]; Ribeiro, P. R.[1]

[1]*Metabolomics Research Group, Departamento de Química Orgânica, Instituto de Química, Universidade Federal da Bahia, Rua Barão de Jeremoabo s/n, 40170-115, Salvador, Brazil;*
[2]*Laboratório de Imunologia e Biologia Molecular, Dep. de Ciências da Biointeração, ICS-UFBA, Brazil;*
[3]*Laboratório de Ressonância Magnética Nuclear, Departamento de Química, Universidade Federal de São Carlos, São Carlos, SP, Brasil.*

**Introduction:** Sickle cell disease is the most common hemoglobinopathy in Brazil and is a worldwide public health problem, with major impact on morbidity and mortality of the affected population. This disease is multisystemic and its predominant joint clinical manifestation is osteonecrosis, which has serious complications, often progressing to terminal disease in patients with sickle cell disease. Because it is considered a complex disease, a broad biological view, especially of the metabolic pathways, is important for its better understanding. For this, the "omic" sciences have been widely used, especially the metabolomics, which aims to identify and quantify metabolites through analytical technologies. **Objectives:** In this context, this work had as main focus the identification of potential biomarkers for the early diagnosis of osteonecrosis secondary to sickle cell disease using nuclear magnetic resonance in a metabolomics approach. **Material and Methods:** Nuclear magnetic resonance spectra were acquired from blood plasma samples from healthy individuals and sickle cell disease patients with and without osteonecrosis. Spectra acquisition was performed after the optimization of the blood plasma sample preparation protocol. Metabolite identification was performed using Chenomx NMR Suite 8.4 software and data available in scientific articles. Quantification was performed using NMRProcflow software. After identification/ quantification of metabolites, statistical analyzes were performed by using MetaboAnalyst 3.0 software. **Results:** Preliminary results identified differences between the metabolic profile of the study groups of this work. Citrate, alanine, sn-glycerol-3-phosphocholine, glucose and histidine have all shown to be promising candidates for future investigations of osteonecrosis secondary to sickle cell disease and promising 2-hydroxyisovalerate candidates for future investigations of sickle cell biomarker. **Conclusions:** The present study may provide support for the validation of biomarkers for osteonecrosis secondary to sickle cell disease.
**Keywords:** Sickle cell disease, osteonecrosis, metabolomics, nuclear magnetic resonance.
Financial support was provided by **UFBA**, **FAPESB**, **CNPq**, and **CAPES**.

## ANALYZE PROSPECTIVE OF EUGENOL IN CROTON ZEHNTNERI (EUPHORBIACEAE) AGAINST ALZHEIMER'S DISEASE

Quintanilha, A. L.[1]; Cerqueira, D. S.[1]; Lima, L. B. De S.[1], Veríssimo, E. D.[1]; Filho, J. S. S.[1], Santana-Bastos, E. M.[1]

[1]*Maurício de Nassau University Center (UNINASSAU), Campus Pituba, Salvador, Bahia, Brazil.*

**Introduction:** Eugenol is a phenylpropanoid formally derived from guaiacol, presenting on the Central Nervous System (CNS). Evidence suggests that this molecule may be used as a drug for the treatment of Alzheimer's disease (AD) (ADAMS; SWEATT, 2002). Eugenol, like other antidepressants, increases expression of the Brain Derived Neurotrophic Factor (BDNF) gene and inhibits Monoamine Oxidase A (MAO-A) in an in vitro model (CHYAN et al., 1999). Croton zehntneri (Cz), Euphorbiaceae plants present in the rich flora of the State of Bahia, little explored, presents eugenol, one of the main active compounds. **Objectives:** Through scientific prospecting, investigating the pharmacological activity of eugenol in Alzheimer disease and its main experimental models in vitro and in vivo. **Material and Methods:** It is an exploratory methodology (PRODANOV; FREITAS, 2013). The open access online platforms: LILACS-bvs, PubMed and Science Direct were

used to search for articles through the descriptors Croton sp., Eugenol and Alzheimer. The data obtained were organized and analyzed as graphs by countries, year of publication of articles and journals. **Results:** They were evidenced after publications in 1992 to 2018. Brazil among other countries presented research on the genus Croton, eugenol compound, and its action in the CNS. Eugenol in an in vitro experimental model suppressed COX-2 gene expression in lipopolysaccharide-stimulated macrophages (LPS) and in vivo attenuated rat depression and inflammation by generating neuroprotective activity by inhibiting $Ca^{++}$ influx by PC-12 cells. (BERGMAN; DEUSCHL, 2002). **Conclusions:** Pharmacological activities observed in *C. zehntneri* eugenol experiments range from anti-inflammatory, anticonvulsant and antidepressant. Thus, further studies are necessary to optimize the process of obtaining and isolation, and elucidation of mechanisms of action of eugenol CNS.

**Keywords:** Croton zehntneri. Eugenol. Alzheimer.
**Funding source:** Institute Ser Educacional

## EVALUATION OF CYTOTOXIC POTENTIAL OF SUBSTANCES F001 AND E002 EXTRACTED FROM THE *CAESALPINIA GENUS* TO C6 AND U251 GLIOMA CELLS *IN VITRO*

Ramos, F. S.[1]; **Marques, E. J.[1]**; Boness, H. V.[1]; **El-Bachá, R. Dos S.[1]; Velozo, E. S.[2]**

[1]*Department of Biochemistry and Biophysics; Laboratory of Neurochemistry and Cell Biology, Salvador Bahia Brazil.*
[2]*Department of Medicines; Research Laboratory in Medical Matters, Federal University of Bahia; Salvador Bahia Brazil.*

**Introduction:** Glial cells are among the main ones of the human brain. Brain stem cells may change and multiply, resulting in glioblastoma (GBM). The *Caesalpinia genus* has metabolites derived from polyphenols, steroids, diterpenes and triterpenes that exhibit analgesic, antibacterial, anti-inflammatory, antioxidant, antiproliferative, and immunomodulatory activities. **Objectives**: This study aims to identify the potential of compounds F001 and E002 extracted from *Caesalpinia genus* to decrease the viability of murine (C6) and human (U251) glioma cells *in vitro*. **Material and methods:** The applied methodologies were: MTT test, determination of concentration that kills 50%, trypan blue cell viability test (TB), Cell Migration Inhibition test (BMI). As controls, C6 and U251 cells were used in DMEM and DMEM with DMSO. **Results:** F001 had no significant activity by using the MTT assay at concentrations between 1.2 - 200 µM, in both glioma strains. However, in TB assay, cells presented blebbing at concentrations above 40 µM. Furthermore, it inhibited cell migration above this same concentration. The substance E002 presented a mean $EC_{50}$ of 38 µM ± 0,008 (n = 9). The TB test confirmed the reduction of C6 and U251 viability, as well as the CMI test showed no cell migration at the same concentrations. **Conclusions** The results allow to conclude that F001 of the *Caesalpinia genus* inhibited cell migration, meanwhile the E002 was cytotoxic to the murine and human glioma strains.

**Keyword:** *Caesalpinia genus*; inhibited cell migration; glioma.
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# ANTIMICROBIAL ACTIVITY AND METABOLOMICS EVALUATION OF *Vellozia pyrantha* RESIN EXTRACTS

Ribeiro, L. A. F.[1,2]; Neta, L. C. de S.[2]; De Jesus P. R. R.[1]

*[1]Metabolomics Research Group, Instituto de Química, Universidade Federal da Bahia, Rua Barão de Jeremoabo s/n, 40170-115, Salvador, Brazil [2]Departamento de Ciências Exatas e da Terra I, Universidade do Estado da Bahia, Salvador, Brazil.*

**Introduction:** *Vellozia pyrantha* is a typical shrub of brazilian Cerrado. Its resin is commonly used as torches because of its flammability, being considered influencer of the natural cycle of nutrients in its Bioma since its flowers starts to appear after wildfires providing a big floresciment of the plant. This plant has medicinal use in its oil as anti-inflammatory and against insect bites. The study of biological activities of *V. pyrantha* is necessary because of the lack of reports in literature. **Objectives:** To assess the antimicrobial activities of resin extracts of *Vellozia pyrantha* and identify possible responsible metabolites using LC/MS. **Material and Methods:** The resin (VV-EB) was collected in Chapada Diamantina, on Vale do Capão and subjected to column chromatography, resulting in 8 other fractions (VV-Fr1-8). The 9 samples were used to assess MIC by broth microdilution. The antimicrobial activity was assessed against *Bacillus subtilis*, *B. cereus*, *Staphylococcus aureus*, *S. epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonela choleraesuis*, *Candida albicans* and *C. glabrata*. Nutrient broth and yeast/malt extract were used as culture media and chloramphenicol, gentamicin and loprox were used as positive control. The concentrations ranged from 3.9 to 500 $\mu g.mL^{-1}$. The MIC was determined through the emergence of turbidity in the wells. Extracts were considered active at concentrations below or equal to 500 $\mu g.mL^{-1}$. After that, the active samples were transferred to solid culture media and inoculated to MBC essays. Concomitant, the samples were subjected to LC/MS for metabolomics analysis. **Results:** VV-EB, VV-Fr7 and VV-Fr-8 showed activity against *S. aureus* with MIC of 62.5, 3.125 and 12.5 $\mu g.mL^{-1}$, respectively, having VV-Fr7 and VV-Fr8 as bactericides with MBC of 6.25 and 25 $\mu g.mL^{-1}$. VV-Fr2 and VV-Fr4 to 6 showed activity against *S. epidermidis* with MIC of 100 and 25 (Fr4-6) $\mu g.mL^{-1}$. VV-Fr1 to 6 showed activity against *B. subtilis* with MIC of 500, 100, 100, 250, 500 and 500 $\mu g.mL^{-1}$, respectively and with VV-Fr3 as bactericide with MBC of 100 $\mu g.mL^{-1}$. VV-Fr7 and VV-Fr8 were active against *B. cereus* with MIC of 250 and 500 (Fr4-6) $\mu g.mL^{-1}$. All samples were active against *S. choleraesius* with MIC between 100 and 50 $\mu g.mL^{-1}$ for fractions and 500 $\mu g.mL^{-1}$ for the VV-EB. Against *E. coli*, only VV-Fr6 was active (100 $\mu g.mL^{-1}$). Furthermore, no activity was observed for the other tested microorganism. The metabolomics analysis resulted in the identification of 14 metabolites until now, 2 pentacyclic triterpenes (with ursane and lupine skeleton) and 12 diterpenes (cleistantha-8,11,13-trien-7-one; (-)-ent-3β-hydroxylabd-8(17)-en-15-oic acid; cleistantha-1,8,11,13-tetraen-3,7-dione; candidenodiol; candidalactone; 20-carboxaldehyde-cleistanthan-8,11,13-trien-7-one; epoxivellozin; epoxicorcovadin; veadeirol; 8(9),15-isopimaradien-1,3,7,11-tetraone; and two not identified but with cleistanthane and isopimaradiene skeleton. Two of the diterpenes were used in bioassays envolving DNA repair deficient mutants of *Saccharomyces cerevisiae* while another was used in phytotoxic and larvicide bioassays. These metabolites probably are responsible for the antimicrobial activity. **Conclusions:** The extracts of *Vellozia pyrantha* showed good activity against most of the available microorganisms being microbicide in some cases but no selectivity was observed. More metabolites are being searching in the LC/MS results for justify others biological activities already obtained, like antioxidant and cytotoxic.

**Keywords:** metabolomics, phytochemistry and biological activity

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# SWEET TASTE RECEPTORS IN PARKINSON'S DISEASE AND INSULIN RESISTANCE: A GENETIC CORRELATION

Rubio, É. P.[1]; Santos, G.[2]

[1] *Department of Science and Technology, State University of Southwest of Bahia, Brazil*
[2]*Department of Biologic Science, State University of Southwest of Bahia, Brazil*

**Introduction:** Parkinson's disease (PD) is a neurodegenerative disorder whose main symptom is alteration in motor conditions, such as rest tremor, muscle stiffness and difficulty in walking. However, Parkinson's complexity goes far beyond these changes. The first development of Parkinson´s disease is usually between the ages of 55 and 65 year old, and the higher prevalence of PD is in males and by age, from 0.04% in people aged 40 to 49 years to 1.9% in people aged 80 years and older. PD is characterized mainly by dopaminergic neurons loss in the substantia nigra pars compacta and the Lewy bodies. The etiology of PD is still unknown, however, the search has been bringing strong associations between genetic and environmental factors. Studies indicate a relationship between the pathogenesis of insulin resistance and PD. Increased insulin resistance in the brain is closely related to age and more prominent in PD, as well as lower IGF-1 signaling in these patients. In addition, the death of dopaminergic neurons has been identified after effects of insulin resistance. Another situation about Parkinson's patients concerns the change in taste function sensitivity, but the results are often inconclusive, because the assessment of taste changes in PD's patients is hampered by the dementia symptom attributed to this pathology in the evaluated cases. Besides, it is well known that conventional drugs used in the treatment of PD can also trigger changes in taste, so there is bias against those studies. In contrast, changes in taste receptors are generally related to genetic polymorphisms. This makes them an excellent tool for assessing molecular changes from physiological parameters. **Objectives:** The objective of this study was to verify genetic correlations of the sweet taste receptors, insulin resistance and PD. **Material and Methods:** The identification of the main genes related to sweet taste receptors, insulin resistance and PD was performed by using Allen Brain Atlas´sdata, from which 49 genes were selected from their pathology significance level by using set of Gene Set Enrichment Analysis algorithms using X2 Kinase, these genes were clustered according to centrality measurements. The visualization was made through String. **Results:** The results obtained reveal the association of the various genes in question. The presence of sweet taste receptors (TAS1R) in regions such as the brain is already known. Functional abnormality would lead to impaired efficacy in circulating glucose signaling, compromising ATP production and, consequently, non-release of insulin. This correlation is observed by the genetic relationship between the sweet taste receptors and the KCNJ11 and ABCC8 genes. These genes, in turn, are responsible for providing the instructions for making proteins that form ATP-sensitive potassium channels (K-ATP). These channels respond according to the concentration of glucose in the blood, closing with its increase and releasing insulin. Thus, mutations in these genes characterize changes in insulin response. Joint expression of TAS1R sweet taste genes and DBH genes was also observed. DBH gene is involved in the production of the enzyme dopamine β-hydroxylase, which converts dopamine to norepinephrine, and relates to PD by limiting the conversion rate of dopamine. The next steps will be to evaluate the sequencing those two hubs genes in Parkinsonian patient through SNPs. **Conclusions:** The correlations of the sweet taste genes, insulin resistance and PD are evident. This study brings data that reinforce the association of changes in taste perception with PD, it doesn´t limit the development of this characteristic to the use of drugs or the symptom of dementia caused by pathology. In addition, this study showed that mutations in sweet taste genes may be directly related to insulin resistance also present PD.

**Keywords:** *Parkinson's disease (PD), insulin resistance, sweet taste receptors.*

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# ETHNOBOTANICAL EVALUATION AND QUANTIFICATION OF TOTAL PHENOLS OF MEDICINAL PLANTS USED IN PERIPHERAL NEIGHBORHOODS OF SALVADOR

Santos, A. J. O. S.[1,2]; Batista, D. L. J.[1]; Costa, C. O. D. S.[2]; Ribeiro, P. R.[1]; Carvalho, J. R. M.[1]

[1]*Metabolomics Research Group, Departamento de Química Orgânica, Instituto de Química, Universidade Federal da Bahia, Salvador, Brazil.* [2]*Colégio da Polícia Militar, João Florêncio Gomes, Ribeira, Salvador, Bahia.*

**Introduction:** Plants still these days continue to be used for the treatment, cure and prevention of diseases. It is one of the oldest forms of medicinal practice by the least developed population, and is the main source of therapeutic resources. Currently there is a growth in the use of herbal medicines by the Brazilian population, due to the advance in the scientific area, which allowed the development of safe and effective herbal medicines. Cymbopogon citratus (Familia Gramineas), Morus nigra (Familia Moraceae), Eugenia uniflora (Myrtaceae family) popularly known as Capim Santo, Amora and Pitanga are widely used in the Northeast region and in the peripheral neighborhoods of Salvador for the treatment of digestive problems disorders, immunity enhancement and blood pressure control, respectively. From a field survey, it was found that the peripheral neighborhoods of Itapuã and São Joaquim have a growing sale of the mentioned medicinal plants, besides these, the broom, open path, boldo and cinnamon are also popular in the region. These plants can be used in various ways, but their use is more prevalent in the form of teas by infusion, leaf baths, oils or as a spice. In the present work, we selected the species *Cymbopogoncitratus*, *Morusnigra* and *Eugeniauniflora* were due to their traditional use in peripheral neighborhoods of Salvador. *C.citratus* is used for Helps relieve depression, stress andbody tension, relieves muscle spasms, reducing all symptoms related to abdominal pain, headaches andjoint pain. *M. nigra* is used to help treat diabetes, toothache, bleeding, mouth inflammation, kidney stones and intestinal problems. *E. unifloras* used for depression, uncontrolled blood pressure, agitation and deep sadness.**Objectives**: Perform ethnobotanical and biological evaluations of medicinal plants marketed in peripheral neighborhoods of Salvador. **Material and Methods**: Initially there was a survey between fairs free of peripheral neighborhoods of Salvador on the most commercialized medicinal plants amongst them *C*. *citratus*, *M*. *nigra*, *E*. *uniflora*. Leaf and stem samples were collected from in the garden of the student's aunt, who cultivates medicinal plants. The extracts were prepared by maceration in ethanol for 72 hours.Quantification of total phenols was performed by the Folin-Ciocalteau method. **Results**: In data surveys in the neighborhoods of Itapuã and São Joaquim, we identified the greatest use of medicinal plants known as Pitanga (*Eugenia uniflora*), Amora (*Morusnigra*) and Capim Santo (*Cymbopogoncitratus*), plants used in the research. *M.Nigra* leaf extract presented the best total phenolic content (97.69 ± 3.81 GAE / g dry weight)−1, followed by the stem (49.16 ± 0.27 GAE / g dry weight). *E.Uniflora* stem extract (92.52 ± 1.78 GAE / g dry weight) followed by the leaf (90.37 ± 2.48 GAE / g dry weight) and *C.Citratus* (34.69 ± 2.33 GAE / g dry weight). Phenolic compounds are defined as substances that have a ring with one or more hydroxyl substituents, including groups thereof functional. These include flavonoids, phenolic acids, tannins and tocopherols as the most common phenolic antioxidants from a natural source. In addition these compounds have important pharmacological activities as antioxidants, antimutagenic and antiviral. **Conclusion:** The study allowed the ethnobotanical and biological survey of medicinal plants most commercialized in peripheral neighborhoods of Salvador.Later studies will be carried out to reach the isolation and identification of the main phenolic constituents of the extracts and to perform metabolomic evaluation.

**Keywords:** *medicinal plants, ethnobotanicalstudy, laboratorypractices, natural medicines.*

**Acknowledgment:** *Financial supported was provided by UFBA via the PIBIC-Jr (ProgramaInstitucional de Bolsas de IniciaçãoCientificaJúnior) program.*

# EFFECTS OF AGATHISFLAVONE AGAINST ALPHA-SYNUCLEIN AGGREGATION AND NEUROINFLAMMATION

Santos, C. C.[1]; Cardim-Pires, T. R.[2]; David, J. M.[3]; David, J.P.[4]; Costa, M. F. D.; Palhano, F. L.[2], Foguel, D.[2]; Costa, S. L.[1]; Silva, V. D. A.[1]

[1] *Laboratório de Neuroquímica e Biologia Celular (LabNq), Departamento de Bioquímica e Biofísica, Instituto de Ciências da Saúde (ICS), Universidade Federal da Bahia (UFBA), Bahia, Brazil;*
[2] *Instituto de Bioquímica Médica Leopoldo De Meis (CCS), Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil;*
[3] *Departamento de Química orgânica; Instituto de Química, Universidade Federal da Bahia (UFBA), Bahia, Brazil;*
[4] *Departamento de medicamento, Faculdade de Farmácia, Universidade Federal da Bahia (UFBA), Bahia, Brazil*

**Introduction:** Parkinson's disease (PD) is one of the most prevalent neurodegenerative diseases. There is a hypothesis that alpha-synuclein (alpha-syn) aggregation generates the formation of neurotoxic protofibrils leading to neuroinflammation and neuronal loss associated to the pathogenesis of PD. On the other hand, agathisflavone presents pharmacological potential for PD because of its reported antioxidant, anti-inflammatory, neurogenic and neuroprotective effects. **Objective:** The aim of this work was to evaluate the neuroprotective potential of agathisflavone in alpha-synuclein-based models of Parkinson's disease. **Material and Methods:** Agathisflavone was extract from *Poincianella pyramidalis*. Primary Microglial culture from Wistar rats (P0 -2) were exposed to alpha-syn fibril and treated with agathisflavone. The microglial morphology was analyzed by phase-contrast microscopy and immunohistochemistry for Iba-1. Moreover, alpha-syn was incubated with agathisflavone at 37 °C. The inhibition of alpha-syn aggregation was assessed by quantification of species formed using the reading of the D.O.; as well by the red dye of the congo and Electron Microscopy. **Results:** We observed that agathisflavone was able to inhibit microglial activation, marked by a reduction of the number of amoeboid cells and cytoplasm vacuolation induced by alpha-syn fibrils. Moreover, agathisflavone decreased the formation of alpha-syn aggregates. **Conclusion:** These results demonstrate the neuroprotective ability of agathisflavone for PD and suggest the inhibition of neuroinflammation and alpha-syn aggregation as mechanisms involved in its protective effect.

**Keywords:** *Parkinson's disease, flavonoid, neuroprotection*

**Support:** *FAPESB, CAPES, CNPq.*

# BIOACTIVE COMPOUNDS FROM *Ricinus communis* EXTRACTS

Santos, P. M[1]; Carvalho, J. R. M. [1]; Ribeiro, P. R.[1]

[1]*Metabolomics Research Group, Departamento de Química Orgânica, Instituto de Química, Universidade Federal da Bahia, Salvador, Brazil*

**Introduction:** *Ricinus communis* is known for its healing, anti-inflammatory and antimicrobial properties. In its composition we have the presence of alkaloids, flavonoids, coumarins, tocopherols and terpenoids. Various biological activities are reported for the different extracts. Thus, we conducted a large-scale study of the metabolic profile that correlated the chemical composition of the extracts and their biological activities. **Objectives**: To evaluate the chemical composition, antioxidant, antimicrobial and cytotoxic capacity of fractions obtained from ethanolic extracts of leaves, stem and roots of *R. communis*. **Material and Methods**: The extracts of leaves, stem and roots of *R. communis* were obtained through the maceration process, performed in organic solvents (hexane, ethyl acetate and ethanol). The identification and characterization of the extracts were performed by Nuclear Magnetic Resonance and chromatographic methods (GC-MS and CL-MS). Antioxidant activity was performed by the DPPH free radical capture method (2,2-diphenyl-1-picril-hydrazine). Antimicrobial activity was performed by microdilution in nutrient broth, used to evaluate the biological potential of the species. The toxicity of the extracts was analyzed in *Artemia salina*. **Results**: The antioxidant activity $IC_{50}$ of the extracts ranged from 31.73 to 571.74 $\mu g.mL^{-1}$, and the ethanolic extracts of the leaves (34.68 ± 0.24 $\mu g.mL^{-1}$), stem (31.73 ± 2.59 $\mu g.mL^{-1}$) and roots (34.55 ± 0.34 $\mu g.mL^{-1}$) showed better $IC_{50}$. Hexane leaf extracts showed $IC_{50}$ of 571.74 ± 0.24 $\mu g.mL^{-1}$, and stem and root extracts were not active ($IC_{50}$>1000 $\mu g.mL^{-1}$). The total phenol content of the leaf ethanolic extract was 72.99 ± 1.49 $mg.EAG.g^{-1}$, the stem 72.03 ± 1.92 $mg.EAG.g^{-1}$and the roots 135.06 ± 1.69 $mg.EAG.g^{-1}$. The $IC_{50}$ of the fractions ranged from 9.46 ± 0.04 to 157.6 ± 3.86 $\mu g.mL^{-1}$ and quantification of total phenols of the fractions, the results obtained for *R. communis* ranged from 22.23 ± 1.56 to 272.34 ± 18.21 $mg.EAG.g^{-1}$. The total phenol content showed a good correlation with the antioxidant activity in the extracts obtained with ethanol, which would probably explain its higher antioxidant activity (lower $IC_{50}$ values). The crude ethanolic extract of leaves showed antibacterial activity against *Pseudomonas aeruginosa* (500 $\mu g.mL^{-1}$) and *Salmonella choleraesius* (500 $\mu g.mL^{-1}$) as well as antifungal activity against *Candida* albicans (500 $\mu g.mL^{-1}$). The ethanolic extract of the roots showed antibacterial activity against *Pseudomonas aeruginosa* (500 $\mu g.mL^{-1}$) as well as antifungal activity against *Candida albicans* while the ethanolic extract of the stem showed antibacterial activity against *Pseudomonas. aeruginosa* (500 $\mu g.mL^{-1}$). Some fractions potentiated the antimicrobial activity, highlighting the gradient fraction (60 Hexane and 40 acetate) that showed bacteriostatic and bactericidal action in the concentration of (100 $\mu g.mL^{-1}$) against *Bacillus cereus*. In its composition, we have the presence of Ricinine alkaloid, identified by CL-MS, coumarins and terpenoids. The CG-MS technique identified phenolic compounds such as gallic acid, ferrulic acid and catechin. Various biological activities are reported for the different extracts. **Conclusion**: Thus, we conducted a large-scale metabolic profile study that correlated the chemical composition of extracts and fractions to correlate with biological activities.

**Keywords:** *extracts, pharmacology, bioactive molecules.*

**Acknowledgment:** *Financial supported was provided by UFBA via the PERMANECER.*

# COMPARISON OF THE EFFECT OF CELL THERAPY ON BONE MARROW MESENCHYMAL CELLS AND CELL FREE THERAPY IN EXPERIMENTAL MODEL OF TRIGEMINAL NEURALGIA

Silva, G. S. A.[1]; Santos, D. S.[1]; Evangelista, A. F.[2]; Santos, G. C.[2]; Dos Santos R. R.[2]; Soares, M. B. P.[2]; Villarreal, C. F. [1,2]

[1] *Faculty of Pharmacy, Federal University of Bahia, Salvador, Bahia, Brazil.*
[2] *Gonçalo Moniz Institute, Oswaldo Cruz Foundation, Salvador, Bahia, Brazil.*

**Introduction.** Trigeminal neuralgia (TN) is the most prevalent form of craniofacial neuropathic pain, with a debilitating and disabling profile. Considering the negative impact on the quality of life of individuals and the ineffectiveness of current treatments available, the development of new therapeutic approaches for this syndrome is of great relevance. Experimental evidence indicates that bone marrow mesenchymal stem cells (BMSC) have great therapeutic potential in neuropathies by exerting neuro-regenerative and analgesic action. Based on its paracrine mechanism of action, a new way of exploring the therapeutic potential without the need for cell transplantation was evidenced. This approach, called free-cell therapy, uses stem cells as the source of therapeutic molecules, which can be released freely into the culture medium (secretoma — SC) or inside on extracellular vesicles (EVs). **Objectives.** Compare the effect of BMSC transplantation, its secretoma (SC) and extracellular vesicles (EVs) in an experimental model of TN. **Material and Methods**. Mesenchymal cells were obtained from bone marrow of C57Bl / 6 mice from IGM — FIOCRUZ-BA (CEUA: L-IGM — 022/2015), characterized by flow cytometry and in vitro cell differentiation assays. The SC was obtained from the BMSC culture supernatant and the fraction of EVs obtained by ultracentrifugation and characterized by its protein content by mass spectrometry, quantity and size by particle tracking analysis (Nanosight). Animals of the same lineage underwent partial infraorbital nerve ligation surgery to induce the TN model and 5 days after surgery received intravenous administration of BMSC (1x106), SC (100µL) or EVs (100µL). Mechanical and thermal nociceptive thresholds were evaluated through the filaments von Frey and Hargreaves, respectively throughout the experimental period (30 days). **Results**. The cells presented characteristic markers of BMSC and when stimulated were able to differentiate into chondrogenic, adipogenic and osteogenic strains. The characterization of the EVs revealed vesicles with an average size of 205 nm, but the proteomic analysis of the protein content of the EVs was inconclusive. Soon after model induction, animals presented mechanical allodynia ($p < 0.001$) and thermal hyperalgesia ($p < 0.001$). Treatment of neuropathic animals with a single intravenous administration of BMSC ($p < 0.001$), SC ($p < 0.001$), and EVs ($p < 0.001$) was able to reverse mecanoalodynia and installed thermal hyperalgesia. **Conclusions**. Our results demonstrate that, similarly, a single administration of BMSC, its secretoma or EVs produced lasting antinociceptive effect, proving the paracrine effect of BMSC on TN and highlighting cell free therapy as a good therapeutic option for TN treatment.

**Keywords:** *trigeminal neuralgia, mesenchymal stem cells, extracellular vesicles, secretoma.*

# EFFECT OF GABAPENTIN TREATMENT IN AN EXPERIMENTAL MODEL OF SENSORY DIABETIC NEUROPATHY

Silva G. S. de A. [2]; Evangelista, A. F. [1]; Silva, D. N. [1]; Dos Santos, R. R. [1]; Soares, M. B. P.[1], Villarreal, C. F. [1,2]

[1] *Laboratory of Tissue Engineering and Immunopharmacology (LETI), Gonçalo Moniz Research Institute (IGM/FIOCRUZ), Salvador — BA;* [2] *Laboratory of Pharmacology and Experimental Therapeutics (LAFTE), Department of Medicine, Federal University of Bahia, Salvador — BA.*

**Introduction:** Diabetes is a disease that often causes damage in the nervous system. Sensory diabetic neuropathy (SDN) is a common complication of diabetes caused by metabolic changes that promote neuroinflammation and peripheral nerve degeneration, resulting in antagonistic sensory changes, such as loss of sensation and neuropathic pain, interfering with patients' quality of life. For this painful condition, the pharmacological treatment is based on the use of antidepressants and anticonvulsants, among which gabapentin (GBP) is one of the most widely used drugs because it has higher acceptability and lower price compared to other therapeutic options. However, its mechanism of action in SDN is still poorly understood. **Objective:** Evaluate the effect of GBP treatment on an experimental model of SDN. **Material and Methods:** C57Bl/6 mice (CEUA: L-IGM-025/2011) were diabetes induced by streptozotocin (STZ) (80 mg/ kg; ip for 3 consecutive days) and four weeks after model induction, the animals were treated with oral administrations of GBP (70 mg/kg; every 12 hours for 6 days). Mechanical and thermal nociceptive thresholds were evaluated by von Frey filaments and hargreaves test, respectively. At the end of the experimental period (12 weeks), sciatic nerve segments were collected and submitted to morphological and morphometric analysis by optical and transmission electron microscopy. In addition, the L4-L5 segment of the spinal cord was collected for cytokine (IL-1β, TNF-α, TGF-β e IL-10) dosage by ELISA. **Results:** Behavioral data showed that treatment with GBP was able to reverse mechanical allodynia and thermal hypoalgesia only during the treatment ($p < 0.001$). Morphometric and morphological analyzes of the sciatic nerve showed that treatment with GBP reduced myelin sheath thickness and mitochondrial atypia in myelinated fibers ($p < 0.05$). However, the treatment was not able to reverse changes in fiber diameter, number of myelinated fibers, area, density and mitochondrial atypia in unmyelinated fibers ($p < 0.05$). ELISA data demonstrated that GBP administration was able to decrease IL-1β and TNF-α levels in a sustained manner until the end of the experimental period and to raise TGF-β levels one week after initiation of treatment. **Conclusion.** These results indicate that despite promoting improvement in sensitivity changes only during the treatment period, GBP was able to permanently reduce proinflammatory cytokine levels in the spinal cord, indicating a possible modulating action of neuroinflammation present in sensory diabetic neuropathy.

**Keywords:** *Diabetic neuropathy, neuroinflammation, gabapentin.*

**Acknowledgment:** *CAPES and CNPQ.*

# EVALUATION OF THE IMMUNOMODULATORY PROPERTIES OF MONNIERISIDE A FROM *Evolvulus linarioides*

Silva, D. F. de A. [1,2]; Santo, R. F. do E. [2]; Pereira, L. C. O.[3]; Abreu, L. S.[3]; Tavares, J. F.[3]; Da Silva, M. S.[3]; Villarreal, C. F.[1,2].

[1] *Pharmacology and Experimental Therapeutics Laboratory, Faculty of Pharmacy, Federal University of Bahia, Salvador, Bahia, Brazil.*

[2] *Tissue Engineering and Immunopharmacology Laboratory, Gonçalo Moniz Institute, Oswaldo Cruz Foundation, Salvador, Brazil.*

[3] *Multi-user Characterization and Analysis Laboratory, Postgraduate Program in Natural and Synthetic Bioactive Products, Federal University of Paraíba.*

**Introduction:** Traditional use of medicinal plants often provides clues about the pharmacological potential of compounds derived from the secondary metabolism of distinct species from the plant kingdom. The genus *Evolvulus* is known in the ethnobotanical literature for containing species used for treating inflammation and disorders of the central nervous system. *Evovulvus linarioides (*Meins) stands out as an endemic species of tropical zones. It is a rich source of bioactive compounds, including monnieriside A. This molecule belongs to the flavonoids class and it has in its chemical structure a benzo-gamma-pyrone nucleus, a feature often associated with immunomodulatory effects. To date, few studies have investigated the pharmacological properties of monnieriside A, with only one study showing inhibition of nitric oxide (NO) production in the murine macrophage cell line. **Objective:** This study aimed to evaluate the immunomodulatory properties of monnieriside A in experimental models of pain and inflammation. **Material and Methods**: Monnieriside A was isolated from the fresh aerial parts of *E. linarioides* (Convolvulaceae). Samples were collected at Matureia, Paraíba, Brazil in June 2009 and were deposited at the herbarium Lauro Pires Xavier, Federal University of Paraíba, Brazil. Initially, the cytotoxicity of monnieriside A was determined in J774 macrophage lineage. Immunomodulatory activity of monnieriside A was next evaluated in activation-induced macrophage cytokine and NO production. Cytokines (IL-1β) concentrations in supernatants from macrophage cultures were determined by ELISA. NO production was estimated by measuring nitrite content in supernatants using the Griess method. Spontaneous nociceptive behavior was assessed by intraperitoneal (i.p.) injection of different doses of mononieriside A (0.01–100 mg/kg) and saline 200 uL (vehicle) 30 minutes before the intraplantar (i.pl.) 2.5% formalin were used as treatment and positive control, respectively. The nociceptive response was determined by counting the time the animal spent licking, biting, shaking or protecting the inoculated paw during a 30 minutes observation period. **Results:** Addition of monnieriside A (6.25 — 100 µM) to macrophage cultures stimulated with lipopolysaccaride (LPS) and interferon-γ (INF-γ) caused a concentration-dependent inhibition in NO production, as indicated by the nitrite concentrations in supernatants ($p < 0.05$). The effects of monnieriside A on NO production by activated macrophages were not due to toxicity, as J774 macrophage cultured in the presence of monnieriside A at 100 µM showed no cytotoxicity. The effects of monnieriside A on cytokine production by activated macrophages was next evaluated. Addition of monnieriside A (6.25 — 100 µM) to macrophage cultures stimulated with LPS and INF-γ significantly ($p < 0.05$) decreased the IL-1β production. This effect was concentration-dependent. Spontaneous nociception was the initial parameter used to characterize the antinociceptive properties of monnieriside A when quantifying pain-like behaviors. Treatment with monnieriside A i.p. 30 minutes before the test ($p \leq 0.05$) at doses of 0.1 to 100 mg/kg ($p \leq 0.05$) caused a reduction in the second phase of spontaneous nociceptive response in a dose-dependent fashion in mice. **Conclusion:** These results indicate that monnieriside A in non-cytotoxic concentrations, inhibited the production of inflammatory mediators by stimulated macrophages, and reduced the second phase of the formalin test in mice, thus exhibiting potential anti-inflammatory action.

**Keywords:** *Anti-inflammatory Pharmacology, Immunomodulation, Natural Products.*

**Acknowledgment:** *FAPESB*

# COMPARISON OF THE EFFECT OF CELL THERAPY ON BONE MARROW MESENCHYMAL CELLS AND CELL FREE THERAPY IN EXPERIMENTAL MODEL OF TRIGEMINAL NEURALGIA

Silva, G. S. A.[1]; **Santos D. S.** [1]; Evangelista, A. F.[2]; Santos, G. C.[2]; Dos Santos; R. R.[2]; Soares, M. B. P.[2]; Villarreal, C. F.[1,2]

[1] *Faculty of Pharmacy, Federal University of Bahia, Salvador, Bahia, Brazil.*

[2] *Gonçalo Moniz Institute, Oswaldo Cruz Foundation, Salvador, Bahia, Brazil.*

**Introduction:** Trigeminal neuralgia (TN) is the most prevalent form of craniofacial neuropathic pain, with a debilitating and disabling profile. Considering the negative impact on the quality of life of individuals and the ineffectiveness of current treatments available, the development of new therapeutic approaches for this syndrome is of great relevance. Experimental evidence indicates that bone marrow mesenchymal stem cells (BMSC) have great therapeutic potential in neuropathies by exerting neuro-regenerative and analgesic action. Based on its paracrine mechanism of action, a new way of exploring the therapeutic potential without the need for cell transplantation was evidenced. This approach, called free-cell therapy, uses stem cells as the source of therapeutic molecules, which can be released freely into the culture medium (secretoma — SC) or inside on extracellular vesicles (EVs). **Objectives:** Compare the effect of BMSC transplantation, its secretoma (SC) and extracellular vesicles (EVs) in an experimental model of TN. **Material and Methods:** Mesenchymal cells were obtained from bone marrow of C57Bl / 6 mice from IGM — FIOCRUZ-BA (CEUA: L-IGM — 022/2015), characterized by flow cytometry and in vitro cell differentiation assays. The SC was obtained from the BMSC culture supernatant and the fraction of EVs obtained by ultracentrifugation and characterized by its protein content by mass spectrometry, quantity and size by particle tracking analysis (Nanosight). Animals of the same lineage underwent partial infraorbital nerve ligation surgery to induce the TN model and 5 days after surgery received intravenous administration of BMSC (1x106), SC (100µL) or EVs (100µL). Mechanical and thermal nociceptive thresholds were evaluated through the filaments von Frey and Hargreaves, respectively throughout the experimental period (30 days). **Results:** The cells presented characteristic markers of BMSC and when stimulated were able to differentiate into chondrogenic, adipogenic and osteogenic strains. The characterization of the EVs revealed vesicles with an average size of 205 nm, but the proteomic analysis of the protein content of the EVs was inconclusive. Soon after model induction, animals presented mechanical allodynia ($p < 0.001$) and thermal hyperalgesia ($p < 0.001$). Treatment of neuropathic animals with a single intravenous administration of BMSC ($p < 0.001$), SC ($p < 0.001$), and EVs ($p < 0.001$) was able to reverse mecanoalodynia and installed thermal hyperalgesia. **Conclusions**. Our results demonstrate that, similarly, a single administration of BMSC, its secretoma or EVs produced lasting antinociceptive effect, proving the paracrine effect of BMSC on TN and highlighting cell free therapy as a good therapeutic option for TN treatment.

**Keywords:** *trigeminal neuralgia, mesenchymal stem cells, extracellular vesicles, secretoma.*

## SYNTHESIS OF QUERCETIN STRUCTURAL ANALOGUES FOR NEURAL CELL TESTS

Silva, S. V. S. [1]; Barboza, O. M.[2]; Da Silva V. D. A.[3]; De Santana L. L. B.[2]; Júnior, A. de F. S.[1,2]

[1] *State University of Bahia, Department of Life Sciences, Stricto sensu Postgraduate Program in Pharmaceutical Sciences (PPGFARMA), Salvador/Bahia/Brazil.*
[2] *State University of Bahia, Department of Life Sciences, Pharmacy Collegiate, Salvador/Bahia/Brazil.*
[3] *Federal University of Bahia, Laboratory of Neurochemistry and Cell Biology, Biochemistry and Biophysics, Salvador/Bahia/Brazil.*

**Introduction:** Quercetin is a flavonoid with antioxidat action and arise intererst like potencial neuroprotetive agent to use in neurodegenerative disease. Low water and the extensive metabolism associated with low biodisponibility reduce the biofarmacological use of the molecule. **Objectives:** This work was proposed to perform structural modifications in the quercetin molecule aiming to increase the actions of these analogues biological targets, improving permeability and bioavailability activities. **Material and Methods:** Two structual analogs were obtained after quercetin molecular modification from more acessible and reproducible synthetic routes (tetra and penta acetylation), applied the green chemistry principles, with minimization of substances use. These analogs were characterized by melt point and Fourier Transform-Infra Red spectroscopy (FTIR) and compared with literature data. **Results:** For reactions of obtaining quercetin analoges acetyl anidride was used as acylating agent and pyridine as a catalyst. The yields obtainded where 50 and 60 per cent for the tetra and penta acetylated respectively. The syntesized structural analogs where caracterized featuring melting points in the range of 168-173 and 178-186°C for acetylated tetra and penta quercetin derivatives respectively, compatible with literature data. Additionally, FTIR analysis showed absense of absorptions bands in 3,400 and appearance of bands around 1600 — 1650 demonstrating the replacement of hidroxyl groups by acetyl groups. The spectra is comparatible with those described in literature. Stands out the hydroxyl groups protection may promote improved cellular absorption of these analogues by the acetyl groups insertion favoring various biological tests, especially in neural cells. **Conclusions:** The conditions and reactions synthesis media of quercetin analogues showed efficient, obtaining two compounds duly characterized, according to the data described in the literature. This study will allow for the first time the application of structurally modified quercetin to neural cells for potential neuroprotetive effect.

**Keywords:** *Quercetin, structural modifications, neuroprotection.*

**Funding source:** *State University of Bahia (UNEB) and Federal University of Bahia (UFBA).*

## NICOTINE PROTECTS NEURAL CELLS AGAINST AMINOCHROME CYTOTOCICITY

Soares, É. N.[1]; Dos Santos, C. C.[1]; Macedo, T. C.[1]; Silva, I. T. F.[1]; Costa, M. de F. D.[1]; EL-Bachá, R. dos S.[1]; Segura-Aguilar, J.[2]; TsabI, Y. [3]; Costa, S. L.[1]; Da Silva, V. D. A.[1]

[1.] *Laboratory of Neurochemistry and Cell Biology, Department of Biochemistry and Biophysics, Institute of Health Sciences, Federal University of Bahia, Salvador, Bahia, Brazil;*
[2.] *Department of Molecular & Clinical Pharmacology, Faculty of Medicine, ICBM, University of Chile, Santiago, Chile;*
[3.] *Department of Pharmacology, Howard University College of Medicine, 520 W Street NW, Washington, DC 20059, USA.*

**Introduction:** Parkinson's disease (PD) is a neurodegenerative disorder that affects brain tissue, especially midbrain dopaminergic neurons. Because of the low levels of dopamine, PD is clinically characterized by motor symptoms such as dyskinesia, muscle stiffness, posture instability and tremors at rest, which appear after years of degenerative processes and are preceded by non-motor symptoms such as olfactory and mood disorders. Studies have been suggested aminochrome as an endogenous neurotoxin responsible for the dopaminergic neurons degeneration in PD. It is a natural molecule in dopaminergic neurons derived from dopamine oxidation that has been used as inductor of PD study model. On the other hand, studies

have been demonstrated that nicotine protects neuronal cells against aminochrome-toxicity. **Objetictive:** The aim of this study was to evaluate the effect of nicotine on glial cell response in models of Parkinson's disease induced by aminochrome. **Material and Methods**: Mesencephalic primary cultures of neurons and glial cells were obtained from wistar rat embryos (15 — 16 days) CEUA Protocol 127A/2017 and cultured for 7 days. Primary cultures were treated with aminochrome (25 µM) and/ or nicotine (1 µM) for a period of 48 h, then the cultures were analyzed by propidium iodide (IP) test, Fluoro-Jade B and Rosenfeld's staining. **Results:** It was observed that aminochrome induced a increase of IP red and Fluoro- Jade B green fluorescence and disorder of neural cell network in the in the primary culture that was inhibited by nicotine. We also observed that nicotine induced cellular Vacuolation. **Conclusion:** We conclude that nicotine is able to protect neural cells against aminochrome cytotocicity. More studies most be provide to clarify the mechanism of nicotine action and the involvement of the cytoplasmic vacuolation in the nicotine neuroprotective effect.

**Keywords***: Parkinson' disease, nicotine, neuroprotection*

**Support:** *FAPESB, CAPES and CNPQ.*

## CYTOTOXICITY OF SYNTHETIC FLAVONOID DERIVATIVE COMPOUNDS TOWARDS GLIOBLASTOMA CELLS

Soares, J. R. P.[1]; Franca, E. Silva, V. D. A.[1]; Victor, M. M.[2], Silvia L. Costa[1]

*[1]Laboratory of Neurochemistry and Cellular Biology, Institute of Health Sciences, Federal University of Bahia, Bahia, Brazil.*
*[2]Research Group in Chemical Synthesis and Molecular Bioactivity, Institute of Chemistry, Federal University of Bahia, Bahia, Brazil.*

**Introduction:** Human glioblastoma multiform (GBM) is the most aggressive malignant tumor of the central nervous system with bad prognosis. Natural occurring flavonoids with different degrees in hydroxylation and methylation have demonstrated antiglioma activities, which puts them as prototypes for the development of new synthesis-derived molecules that have better interaction with cellular targets and may effectively control the growth of malignant brain tumors. **Objective:** The present study aimed to conduct a prospecting on the effect of flavonoids in terms of cytotoxicity and changes on morphology of chemoresistant GBM cells. **Material and methods:** Human GL-15 cells were cultured in DMEM medium supplemented with FBS and antibiotics and the cytotoxic effect of the compounds apigenin and 7,4'-O-diprenylapigenin, naringenin, (S) -naringenin, 7-O- prenylnaringenin, 7,4'-O-diprenylnaringenin, (S) -7,4'-O-diprenylnaringenin, at concentrations from 1 to 100 µg/ mL or kept in control conditions (0.01% DMSO dilution vehicle). The cell viability and morphology were evaluated after 24 hours treatment by MTT [3-(4,5-dimethyltiazol-2-yl)-2,5-diphenyltetrazolium] assay and the phase-contrast microscopy analysis, respectively. **Results and Conclusion:** Among the compounds tested, the results showed that 7-O-prenylnaringenin flavonoid induced a concentration-dependent toxic effect to cells with approximately 50% inhibition of cell viability at 100 µg/ mL, an effect accompanied by changes in morphology and reduction in cellularity in the cultures, suggesting antitumorigenic potential.

*Key-words: Glioma, synthetic flavonoid derivative, cytotoxicity.*

## BEHAVIORAL SENSITIZATION CHANGES INDUCED BY INTERACTION OF PSYCHOACTIVE DRUGS

Soares, L. M.[1]; Ferreira, S. E. M. M.[1,2]; Leão, R. M.[1,2]

*[1] Neuroscience Laboratory, Department of Bioregulation, Institute of Health Sciences, Federal University of Bahia, Salvador (BA), Brazil. [2] Pharmacy Post-Graduate Studies Program, Faculty of Pharmacy, Federal University of Bahia, Salvador (BA), Brazil.*

**Introduction:** Drug addiction is a public health problem in Brazil and worldwide. Alcohol and tobacco are the most consumed. Evidence suggests that combined use of these drugs increases the risk of developing drug dependence. Neuroadaptations caused by repeated drug exposure are related to the development of addiction. This can be investigated by assessing behavioral sensitization which in animal models is characterized by gradual increase in locomotor activity following repeated administrations of a drug. *Objective*: The objective of this work was to investigate the interaction between ethanol and nicotine in the development and expression of locomotor sensitization in mice. **Materials and Methods:** For this analysis an open field was used and the male Swiss mice were divided into 3 groups: saline (SAL 0.1 ml / kg; ip), nicotine (NIC 4 mg / kg; sc) and ethanol + nicotine (EtOH 2.2 g / kg ip + NIC 4 mg / kg; sc) called MIX. Development was performed with daily administration of SAL, NIC or concomitant administration of EtOH + NIC for 21 days and locomotor activity soon after each administration was recorded for 30 minutes on days 5, 10, 15 and 21. Expression test consisted of the same procedure of administration and recording of locomotor activity performed on the thirtieth day (nine days after the last day of development). **Results:** Our results showed that the MIX group increased when compared to the control (SAL) and NIC groups on day 21 (last day of development) and expression test. As well as the SAL group presented greater locomotion than the NIC group on day 5. A predominantly depressing effect of isolated nicotine administration was evidenced, as well as the apparent neutralization of this effect by the co-administration of ethanol and the predominance of stimulating effect by it. These changes in locomotor activity indicate the occurrence of central nervous system adaptations. Future experiments using the immunohistochemistry technique will analyze differences in the activation of CNS areas, such as Nucleus accumbens, Habenula and Amygdala. And molecular changes in activated neural populations will be analyzed by Western Blotting. **Conclusions:** our work indicates that nicotine exposure associated with ethanol potentiates the inducing effects of nicotine dependence.

**Keywords:** *Dependence, Psychoactive Drugs, Locomotor Sensitization.*

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# EFFECT OF BRAIN ENDOTHELIAL CELLS EXPOSURE TO THE PYRROLIZIDINE ALKALOID MONOCROTALINE AND IMPACT ON GLIAL RESPONSE AND NEUROTOXICITY

**Santos, L. O.**[1]; Nascimento, R.P.[1]; Santos, C.C., Borges, J.M.P.[1], Pinheiro, P.[1]; Velozo, E.S.[3]; Batatinha, M.J.M.[4]; El-Bachá, R.S., Silva, V. D. [1] ; Costa, S. L.[1] .

[1] Laboratory of Neurochemistry of Cellular Biology (LabNq), Institute of Science of Health, Federal University of Bahia, Brazil;
[2] Laboratory of Research in Medical Matters (LAPEMM), Faculty of Pharmacy, Federal University of Bahia, Salvador, Brazil;
[3]Laboratory of Toxicology (Latox), School of Veterinary Medicine and Animal Science, Federal University of Bahia, Salvador, Brazil.

**Introduction**:*Crotalaria retusa* (Leguminosae) is a plant found in Northeastern Brazil, used in folk medicine but have reported to induce toxicity in animals and humans, which is attributed to the pyrrolizidine alkaloid monocrotaline (MCT), characterized mainly by hepatotoxicity and pneumotoxicity, but also compromising the central nervous system (CNS). In vitro studies demonstrated that MCT and its pyrrole derivative, dehydromonocrotaline (DHMC), interact with macromolecules in astrocytes and neurons. DHMC presents higher toxicity indicating that metabolism of MCT is a critical step of alkaloid toxicity. Recently the involvement of astrocytic CYP 1A1 isoform in the metabolism and toxicity of MCT was demonstrated. Moreover, behavioral changes associated with cerebrovascular lesions and gliosis were observed after experimental acute intoxication with MCT. Astrocytes together with brain edothelial cells (BECs) form the blood-brain barrier (BBB), and are the major drug metabolizing cells in the CNS, conferring selectivity. Therefore, it is important to investigate the involvement of these cells in MCT metabolism and neurotoxicity. **Objectives:** The present study aimed to evaluate the cytotoxic effects of MCT on BECs and effect on neuron and astrocyte. **Materials and Methods:** For this, BECs were obtained from the brain of adult Wistar rats, cultured in DMEM medium supplemented with 10% SFB and antibiotics. BECs were treated with MCT at concentrations of 1, 10, 100 and 500 µM. After 24 h and 72 h the effect of MCT on cell viability was analyzed by measuring dehydrogenase activity by the MTT test. Cell morphology and growth was analyzed by phase contrast microscopy and by immunocytochemistry for cytoskeleton protein β-actin. Co-cultures of neurons and glial cells were obtained from the cortex of newborn and embryonic Wistar rats and cultured in DMEM medium supplemented with 10% SFB and antibiotics; after 21 days co-cultures were exposed to the conditioned medium derived from BECs in control conditions (CBCM) or to the conditioned medium derived from BECs treated for 24 h with MCT (500 µM, MBCM) in view to investigate the effect of MCT metabolism on viability of CNS cells. Cell morphology and growth was analyzed by phase contrast microscopy, by Rosenfeld's staining and by immunocytochemistry for cytoskeleton protein GFAP, specific of astrocytes, and β-tubulin III, specific of neurons. **Results:** It was observed by MTT test that MCT is not toxic to BEC at concentrations adopted and, on the other hand, induced a concentration-dependent increase in cell dehydrogenase activity and cell vacuolation, especially after 72 h treatments, suggesting resistance to damage and drug metabolism. Moreover, exposure of glia/neurons co-cultures to the conditioned media derived from BECs treated for 24 h with MCT (500 µM) induced change on the morphology and vacuolation of the cells, with apparent reduction on neurites and over expression of GFAP. **Conclusion:** These results suggest that BEC metabolize MCT to compounds that may induce cytotoxicity and astrogliosis. Further studies of the BEC's secretome and molecular pattern of glial response will be performed to well understand the role of BECs associated with glial on neurotoxicity of this pyrrolizidine alkaloid and its metabolites.

**Keywords:** monocrotaline, brain edothelial cell, emetabolism, astrogliosis, neurotoxicity.

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Instituto de Ciências da Saúde
Av. Reitor Miguel Calmon s/nº
40.110-100 Salvador, BA – Brasil

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