UNIVERSIDADE FEDERAL DE PELOTAS

CENTRO DE CIÊNCIAS QUÍMICAS, FARMACÊUTICAS E DE ALIMENTOS

PROGRAMA DE PÓS-GRADUAÇÃO EM BIOQUÍMICA E BIOPROSPECÇÃO

DOUTORADO EM BIOQUÍMICA E BIOPROSPECÇÃO

Tese

Efeito neuroprotetor do ácido gálico em modelos experimentais de mania e neuroinflamação

Vânia Machado Recart

Pelotas, 2021

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Tese de doutorado apresentada ao Programa de Pós-Graduação em Bioquímica e Bioprospecção da Universidade Federal de Pelotas, como requisito parcial à obtenção do título de Doutora em Ciências (Bioquímica e Bioprospecção).

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Pelotas, 2021

Universidade Federal de Pelotas / Sistema de Bibliotecas
Catalogação na Publicação

R294e Recart, Vânia Machado

Efeito neuroprotetor do ácido gálico em modelos experimentais de mania e neuroinflamação / Vânia Machado Recart ; Roselia Maria Spanevello, orientadora ; Giovana Duzzo Gamaro, coorientadora. — Pelotas, 2021.

150 f.

Tese (Doutorado) — Programa de Pós-Graduação Bioquímica e Bioprospecção, Centro de Ciências Químicas Farmacêuticas e de Alimentos, Universidade Federal de Pelotas, 2021.

1. Transtorno afetivo bipolar. 2. Inflamação. 3. Estresse oxidativo. 4. Ácido gálico. 5. Cetamina. I. Spanevello, Roselia Maria, orient. II. Gamaro, Giovana Duzzo, coorient. III. Título.

CDD : 574.192

Elaborada por Gabriela Machado Lopes CRB: 10/1842

Vânia Machado Recart

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Tese apresentada, como requisito parcial, para obtenção do grau de Doutora em Bioquímica e Bioprospecção, Programa de Pós-Graduação em Bioquímica e Bioprospecção, Centro de Ciências Químicas, Farmacêuticas e de Alimentos, Universidade Federal de Pelotas.

Data da Defesa: 26 de fevereiro de 2021

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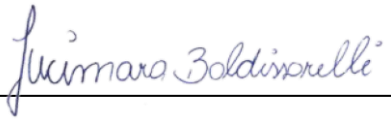

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Dedico este trabalho a minha família, principalmente ao meu filho Vítor Recart Mendes, minha fonte de luz e inspiração.

Agradecimentos

Agradeço primeiramente a Deus por guiar os meus passos, me dar forças para suportar os momentos mais difíceis, paciência e sabedoria para compreender que tudo na vida tem o seu momento certo de acontecer.

Aos meus pais, Sidnei e Nelimar, que me concederam o direito à vida, deixo um agradecimento especial por todo o apoio, incentivo e amor durante toda a minha vida. Amo vocês!

A minha querida irmã Eliane, que mesmo morando muito distante, sempre se fez muito presente em minha vida, com incansáveis manifestações de apoio, incentivo e carinho. Te amo mana! Agradeço também ao meu cunhado Thiago, pelo apoio e incentivo de sempre!

Ao meu querido irmão Wagner, por suas palavras de incentivo, otimismo e orgulho que sempre me fizeram muito bem. Te amo mano! As minhas amadas sobrinhas, Eduarda, Elisa e Laura pela alegria de tê-las em minha vida!

Agradeço profundamente ao meu esposo Miguel, por estar o tempo todo ao meu lado, me incentivando e apoiando. Nos momentos mais difíceis, que não foram poucos, principalmente neste último ano, ele sempre acreditou que venceria esta difícil, porém gratificante etapa. Estes quatro anos de doutorado nos revelou a verdade sobre nosso relacionamento: somos uma família! Sou grata por cada abraço, sorriso e gesto de amor. Te amo!

Ao meu amado filho Vítor que ainda não tem idade para compreender o que é uma tese, agradeço pela espontaneidade, alegria e amor incondicional que sempre me estimularam nos momentos mais difíceis. Nada disso teria sentido se você não fizesse parte da minha vida. A sua existência é o reflexo mais perfeito da existência de Deus! Te amo!

A Beti, pelo apoio, fé, otimismo e amizade.

A minha orientadora Roselia Spanevello, pela competência, profissionalismo, amizade e por acreditar que eu merecia esta oportunidade de viver esta experiência tão sonhada e desejada por mim, mas principalmente, porque seu apoio e supervisão não subtraiu a liberdade necessária para que eu ganhasse autonomia no pensar e no agir. Obrigada por tudo!

A minha co-orientadora Giovana Gamaro, pelo apoio, profissionalismo e amizade.

Aos demais professores do Programa de Pós-Graduação em Bioquímica e Bioprospecção, pela amizade e ensinamentos. Agradeço, principalmente, aos professores Cristiane Bortollato, César Brüning e Francieli Stefanello pela disponibilidade e atenção com que sempre me acolheram em todos os momentos.

Aos colegas e amigos dos laboratórios NEUROCAN e Biomarcadores, Mayara, Fernanda, Luiza, Natália Bona, Nathália Pedra, Juliane, Júlia, Karina, Fernando, Anita, Sara, Sabrina, Kelen, Alana e Bernardo. Obrigada pelos inúmeros conselhos e ajudas nos experimentos. As risadas e os cafés que vocês compartilharam comigo nessa etapa tão desafiadora da minha vida, com certeza fizeram toda a diferença. Minha eterna gratidão. Essa tese também é de vocês!

A Mayara, pela disponibilidade de ajudar e ensinar, nos protocolos, nos cálculos, na estatística, enfim, pela sua calma, ao me apoiar toda vez que o artigo era recusado, com sua frase: “Calma, é assim mesmo.”

A Fernanda, pelas ajudas, apoio, torcida, amizade e companheirismo. Nossas conversas e desabafos eram uma terapia.

A Juliane, pela amizade, apoio e disponibilidade de ajudar sempre.

Aos meus amigos e colegas de trabalho Cristiane, Maicon e José, pela amizade, ajudas, compreensão e companheirismo. Vocês são demais!

Ao biotério central e a Anelize Campello pelas ajudas prestadas.

Aos professores da banca, por aceitarem o convite para avaliação deste trabalho.

À Universidade Federal de Pelotas por grande parte da minha formação acadêmica.

"Apesar dos nossos defeitos, precisamos enxergar que somos pérolas únicas no teatro da vida e entender que não existem pessoas de sucesso e pessoas fracassadas. O que existem são pessoas que lutam pelos seus sonhos ou desistem deles."

Augusto Cury

Resumo

RECART, Vânia Machado. **Efeito neuroprotetor do ácido gálico em modelos experimentais de mania e neuroinflamação**. 2021. 150f. Tese (Doutorado) - Programa de Pós-Graduação em Bioquímica e Bioprospecção. Universidade Federal de Pelotas, Pelotas, 2021.

O Transtorno afetivo bipolar (TAB) é uma doença psiquiátrica crônica que apresenta episódios de depressão e mania. Alterações neuroquímicas, inflamatórias e de estresse oxidativo já foram relatadas em pacientes com esse distúrbio psiquiátrico bem como em modelos experimentais. O ácido gálico é um polifenol encontrado em vários produtos naturais e tem importantes propriedades farmacológicas tais como ações antioxidantes, anti-inflamatórias e neuroprotetoras. O objetivo desta tese foi fazer uma revisão da literatura sobre os avanços do potencial terapêutico dos compostos naturais e derivados frente ao TAB, bem como avaliar o efeito neuroprotetor do ácido gálico em dois modelos experimentais que mimetizam mecanismos patológicos associados ao TAB. Para o protocolo de mania induzido por cetamina foram utilizados ratos machos adultos, os quais foram divididos em sete grupos: I (salina); II (ácido gálico 50 mg/kg), III (ácido gálico 100 mg/kg), IV (cetamina), V (cetamina + ácido gálico 50 mg/Kg) VI (cetamina + ácido gálico 100 mg/Kg) e VII (cetamina + lítio 45 mg/Kg). Durante 14 dias, os animais receberam solução salina, ácido gálico ou lítio via oral. Entre os dias 8 e 14 os animais receberam também cetamina (25 mg/kg) ou salina via intraperitoneal. Para o protocolo de neuroinflamação induzido por lipopolissacarídeo (LPS) foram utilizados camundongos machos os quais foram divididos em quatro grupos: I (salina); II (LPS), III (LPS + ácido gálico 50 mg/kg), IV (LPS + ácido gálico 100 mg/Kg). Os animais receberam durante 14 dias água ou ácido gálico via oral. Entre o 8º e o 14º dia os animais receberam também salina ou LPS (250 µg/kg, i.p.). Em ambos os protocolos, no final do tratamento foram realizados testes comportamentais para avaliar locomoção e memória e após os animais foram submetidos a eutanásia e o córtex cerebral, hipocampo e estriado foram coletados para as análises bioquímicas. Primeiramente o manuscrito de revisão demonstrou que tanto estudos pré-clínicos e clínicos confirmaram o potencial dos compostos como ômega-3, inositol, N-acetilcisteína, extratos de amora, mirtilo e *Cecropia pachystachya*, quercetina, ácido alfa-lipóico e carvona na prevenção e no manejo das fases do TAB. Além disso, os resultados desta tese demonstraram também que o ácido gálico foi capaz de prevenir a hiperlocomoção, o dano oxidativo e alterações na atividade da enzima acetilcolinesterase induzida pela cetamina nas estruturas cerebrais de ratos. O LPS induziu prejuízo na memória dos camundongos e causou dano oxidativo em córtex, hipocampo e estriado, o qual pode ser evidenciado pelo aumento de espécies reativas de oxigênio, peroxidação lipídica e nitritos, bem como diminuição das defesas antioxidantes. O tratamento com ácido gálico mostrou-se efetivo em reverter os *déficits* de memória e proteger as regiões cerebrais do estresse oxidativo induzido pelo LPS. Assim, os achados do presente trabalho demonstram que o ácido gálico, um produto natural, pode ser considerado uma terapia promissora para o TAB.

Palavras-chave: transtorno afetivo bipolar, inflamação, estresse oxidativo, ácido gálico, cetamina, lipopolissacarídeo

Abstract

RECART, Vânia Machado. **Neuroprotective effect of gallic acid in experimental models of mania and neuroinflammation**. 2021. 150f. Thesis (Doctorate) - Programa de Pós-Graduação em Bioquímica e Bioprospecção. Universidade Federal de Pelotas, Pelotas, 2020.

Bipolar affective disorder (BD) is a chronic psychiatric illness that presents episodes of depression and mania. Neurochemical, inflammatory and oxidative stress changes have been reported in patients with this psychiatric disorder as well as in experimental models. Gallic acid is a polyphenol found in several natural products and has important pharmacological properties such as antioxidant, anti-inflammatory and neuroprotective actions. The aim of this thesis was to review the literature on the advances in the therapeutic potential of natural compounds and derivatives against TAB, as well as to evaluate the neuroprotective effect of gallic acid in two experimental models that mimic pathological mechanisms associated with TAB. For the ketamine-induced mania protocol, adult male rats were used, which were divided into seven groups: I (saline); II (gallic acid 50 mg / kg), III (gallic acid 100 mg / kg), IV (ketamine), V (ketamine + gallic acid 50 mg / kg) VI (ketamine + gallic acid 100 mg / kg) and VII (ketamine + lithium 45 mg / kg). For 14 days, the animals received saline, gallic acid or lithium orally. Between days 8 and 14 the animals also received ketamine (25 mg / kg, i.p.) or saline. For the lipopolysaccharide-induced neuroinflammation protocol (LPS), male mice were used, which were divided into four groups: I (saline); II (LPS), III (LPS + gallic acid 50 mg / kg), IV (LPS + gallic acid 100 mg / kg). The animals received oral water or gallic acid for 14 days. Between the 8th and the 14th day the animals also received saline or LPS (250 µg / kg) via intraperitoneal. In both protocols, behavioral tests were carried out at the end of the treatment to assess locomotion and memory and after the animals were euthanized and the cerebral cortex, hippocampus and striatum were collected for biochemical analyzes. First, the review manuscript demonstrated that both preclinical and clinical studies confirmed the potential of compounds such as omega-3, inositol, N-acetylcysteine, blackberry extracts, blueberry and Cecropia pachystachya, quercetin, alpha-lipoic acid and carbon in prevention in managing the phases of BD. In addition, the results of this thesis also demonstrated that gallic acid was able to prevent hyperlocomotion, oxidative damage and changes in ketamine-induced acetylcholinesterase activity in the brain structures of rats. LPS induced impairment in the memory of mice and caused oxidative damage in the cortex, hippocampus and striatum, which can be evidenced by the increase in reactive oxygen species, lipid peroxidation and nitrites, as well as decreased antioxidant defenses. Treatment with gallic acid has been shown to be effective in reversing memory deficits and protecting brain regions from oxidative stress induced by LPS. Thus, the findings of the present study demonstrate that gallic acid, a natural product, can be considered a promising therapy for BD.

Keywords: bipolar affective disorder, inflammation, oxidative stress, gallic acid, ketamine, lipopolysaccharide

Lista de Figuras

Lista de Abreviaturas e Siglas

ACh – Acetilcolina

AChE – Acetilcolinesterase

ALA – Ácido alfa-lipóico

BDNF – Fator Neurotrófico Derivado do Cérebro

CAT- Catalase

ChAT – Colina Acetiltransferase

CID-10 – Classificação Internacional de Doenças

COX-2 – Cicloxigenase 2

DNA – Ácido Desoxirribonucleico

DHA – Ácido Docosahexaenóico

DSM-V – Manual Diagnóstico e Estatístico de Desordens Mentais – 5ª Edição

EPA – Ácido Eicosapentanóico

EROs – Espécies Reativas de Oxigênio

FEWP – Free and Easy Wanderer Plus

GFAP – Proteína Ácida Fibrilar Glial

GPx – Glutationa Peroxidase

GR - Glutationa Redutase

GSH – Glutationa Reduzida

GSK3 - Glicogênio Sintase Quinase 3

GSSG – Glutationa Oxidada

GST – Glutationa S-transferase

HClO – Ácido Hipocloroso

H_2O_2 – Peróxido de Hidrogênio

Iba-1 – Molécula Adaptadora Ligante de Cálcio Ionizado 1

ICV - Intracerebroventricular

IL-1 – Interleucina 1

IL-4 – Interleucina 4

IL-6 – Interleucina 6

IL-10 – Interleucina 10

IL-1β – Interleucina -1-β

JNK – c-Jun N-terminal cinase

LPS – Lipopolissacarídeo

MAO-A – Monoamina oxidase A

NAC – N-acetilcisteína

NF-kB – Fator Nuclear Kappa B

NMDA – N-Metil D-Aspartato

PCR - Proteína C- reativa

PKC – Proteína Quinase C

PUFAs – Ácidos Graxos Poliinsaturados

sIL-2R - Receptor de IL-2 solúvel

SNC – Sistema Nervoso Central

SOD – Superóxido Dismutase

STZ - Estreptozocina

TAB – Transtorno Afetivo Bipolar

TBARS – Substâncias Reativas ao Ácido Tiobarbitúrico

THB – Transtorno de Humor Bipolar

TLR4 – Receptor Toll-like 4

TNF-α – Fator de Necrose Tumoral Alfa

TrKB - Trompomiosina Quinase

WSE – Withania somnifera

Sumário

1. INTRODUÇÃO

Os transtornos de humor podem ser considerados como uma alteração no padrão psicológico de um indivíduo e, potencialmente, refletida em seu comportamento (AMERICAN PSYCHIATRIC ASSOCIATION, 2013). O diagnóstico de pacientes com estes transtornos é realizado através das classificações de referência no serviço de atendimento à saúde pública que são o Manual Diagnóstico e Estatístico de Desordens Mentais (DSM V) e a classificação Internacional de Doenças (CID-10) (SILVA et al., 2012). Estes transtornos, como é o caso do transtorno afetivo bipolar (TAB), caracterizam-se por morte prematura combinada a anos vividos com incapacidade.

O TAB é uma doença psiquiátrica crônica e severa (PRICE e MARZANI-NISSEN, 2012), caracterizada por episódios alternados de depressão e mania ou hipomania (PRICE e MARZANI-NISSEN, 2012; SAUNDERS e GEDDES, 2016). Essa doença afeta de 1% a 4% da população do mundo, independentemente da nacionalidade, origem étnica ou condição socioeconômica (MERIKANGAS et al., 2011). O TAB é uma das principais causas de incapacidade entre os jovens, levando ao comprometimento cognitivo e funcional e mortalidade elevada, particularmente a morte por suicídio (GRANDE et al., 2016).

Embora o TAB seja amplamente estudado, sua fisiopatologia ainda não está totalmente elucidada (BUDNI et al., 2013, KAPCZINSKI e QUEVEDO, 2016). Entretanto, dados da literatura têm apontado que esse transtorno envolve mecanismos associados a alterações nos níveis de neurotransmissores (LAHERA et al., 2013, HASHIMOTO et al., 2007), neurotrofinas, processo inflamatório (MCNAMARA & LOTRICH, 2012) e estresse oxidativo (ANDREAZZA et al., 2008, BERK et al., 2011, DEBOM et al., 2016). Nos últimos anos, várias pesquisas abordam a relação do estresse oxidativo e do processo inflamatório com o TAB, em função disto, estes temas têm adquirido relevância clínica, sendo que estudos têm levantado à hipótese de que ambos seriam partes importantes da fisiopatologia e neuroprogressão do distúrbio.

O estresse oxidativo é representado pelo desequílibrio persistente entre processos pró-oxidantes e o potencial antioxidante do organismo (SIWEK et al., 2013). O resultado deste desequílibrio está associado a lesões celulares como a peroxidação de lipídeos, oxidação de proteínas, danos ao ácido desoxirribonucleico (DNA), inativação enzimática e ativação excessiva de citocinas pró-inflamatórias,

como, fator de necrose tumoral alfa (TNF- α) e interleucinas (IL) (SILVA-FERRARI, 2011). Estudos têm demonstrado um aumento nos níveis de marcadores inflamatórios tanto no sistema nervoso central (SNC) como em tecidos periféricos (BARBOSA et al. 2014; NASSAR e AZAB, 2014), tais como, as citocinas pró-inflamatórias, principalmente a interleucina 6 (IL-6), que apresenta taxas elevadas em pacientes durante os episódios maníacos e depressivos, quando comparados aos pacientes eutímicos (BERK et al., 2011; RODA et al., 2015).

Em função disso os modelos experimentais que utilizam animais para o estudo de transtornos psiquiátricos são de grande valia para novas hipóteses sobre a fisiopatologia, diagnóstico e tratamento dessas doenças. O tratamento desses transtornos, geralmente, é feito com fármacos, porém esses muitas vezes apresentam efeitos adversos. Em conjunto com a grande diferenciação da sintomática de acordo com cada paciente, e da dificuldade em se estabelecer uma dosagem específica sem efeitos indesejáveis, a pesquisa por tratamentos alternativos torna-se necessária (LAFER e NERY, 2011).

Neste contexto, diversos compostos têm sido estudados, entre eles, o ácido gálico (3,4,5- trihidróxibenzóico) e seus derivados que apresentam um grande número de aplicações em várias áreas da ciência (CHOUBEY et al., 2015). Na natureza, estes compostos estão distribuídos em plantas e frutas e em algumas bebidas processadas, tais como, vinhos e chás verde (MOHAPATRA et al., 2006). Dados da literatura têm demonstrado que o ácido gálico possui importantes propriedades biológicas, dentre as quais pode-se citar ações antivirais, antibacterianas, antitumorais, antioxidantes, anti-inflamatórias e neuroprotetoras (VERMA et al 2013).

Visto que já existem na literatura estudos demonstrando o efeito do ácido gálico no comportamento tipo-depressivo (CHHILLAR e DHINGRA, 2012; CAN et al., 2017) e ainda não existem modelos experimentais que mimetizam os sintomas de ambos os polos do humor, mania e depressão, em um mesmo animal, neste estudo procurou-se dar ênfase ao polo maníaco, que é o mais característico do transtorno.

Sendo assim, um dos objetivos deste trabalho foi avaliar o efeito preventivo do ácido gálico em parâmetros comportamentais e neuroquímicos em animais submetidos a um modelo de mania induzido por cetamina. Além disso, considerando que a neuroinflamação é uma condição envolvida na patogênese de diversas doenças psiquiátricas incluindo o TAB, este estudo também avaliou o efeito

neuroprotetor do ácido gálico em um modelo de neuroinflamação induzido por lipopolissacarídeo (LPS).

2 Objetivos

2.1 Objetivo geral

Realizar uma revisão da literatura sobre os avanços do potencial terapêutico dos compostos naturais e derivados frente ao TAB, bem como, avaliar os efeitos do ácido gálico em parâmetros comportamentais e neuroquímicos em animais submetidos a modelos experimentais de mania e neuroinflamação em roedores.

2.2 Objetivos específicos

a) Escrever um artigo de revisão sobre compostos naturais e derivados frente ao TAB, levando em consideração estudos pré-clínicos e clínicos.

b) Avaliar em ratos machos *Wistar* tratados com ácido gálico e submetidos a um modelo de mania induzido por cetamina os seguintes parâmetros:

- Atividade locomotora através do teste do campo aberto;

- Parâmetros de estresse oxidativo como: níveis de espécies reativas de oxigênio (EROs); níveis de substâncias reativas ao ácido tiobarbitúrico (TBARS) e nitrito, conteúdo tiólico total e atividade das enzimas antioxidantes superóxido dismutase (SOD), catalase (CAT) e glutationa peroxidase (GPx) em córtex cerebral, hipocampo e estriado;

- Atividade da enzima acetilcolinesterase (AChE) em córtex cerebral, hipocampo e estriado.

c) Avaliar em camundongos *Swiss* tratados com ácido gálico e submetidos a um modelo de neuroinflamação induzido por LPS os seguintes parâmetros:

- Atividade locomotora através do teste do campo aberto;

- Memória através do teste de reconhecimento de objetos;

- Parâmetros de estresse oxidativo como: níveis de EROs, TBARS e nitrito, conteúdo tiólico total e atividade das enzimas antioxidantes SOD, CAT e glutationa S-transferase (GST) em córtex cerebral, hipocampo e estriado.

3. Revisão da literatura

3.1 Transtorno Afetivo Bipolar (TAB)

Os transtornos de humor são problemas relevantes de saúde mental no Brasil e no mundo. Incluem transtornos unipolares (transtornos depressivos) e transtornos bipolares (episódios maníacos, hipomaníacos, depressivos) (AMERICAN PSYCHIATRIC ASSOCIATION, 2013). O TAB, também conhecido como transtorno de humor bipolar (THB) e originalmente chamado de “Insanidade Maníaco-Depressiva” é um distúrbio crônico caracterizado pela alternância de episódios depressivos e maníacos ou hipomaníacos. A mudança cíclica entre estes polos opostos de humor distingue este distúrbio das demais desordens psiquiátricas de humor, como a depressão maior (ENKHUIZEN et al., 2015).

O TAB é uma condição psiquiátrica relativamente frequente, que afeta entre 1% a 4% da população mundial (MERIKANGAS et al., 2011; SAUNDEERS e GEDDES, 2016). Além disso, esta patologia representa uma das principais causas da redução do tempo de vida e saúde na população entre 15 e 44 anos de idade (YOUNG e DULCIS, 2015).

Os episódios maníacos são definidos pela presença de sintomas eufóricos, como autoestima exagerada ou sensação de grandiosidade e poder, insônia, loquacidade aumentada, pensamentos exagerados e aumento da distração (AMERICAN PSYCHIATRIC ASSOCIATION, 2013). Episódios hipomaníacos são aqueles onde o paciente apresenta estes sintomas maníacos de maneira não tão acentuada, sem causar maior prejuízo no funcionamento social ou profissional, porém associados a uma mudança clara no funcionamento que não é característica do indivíduo quando assintomático (ENKHUIZEN et al., 2015; AMERICAN PSYCHIATRIC ASSOCIATION, 2013). O diagnóstico tanto da mania ou hipomania requer pelo menos três alterações pelo período mínimo de uma semana (AMERICAN PSYCHIATRIC ASSOCIATION, 2013).

Já o outro polo do TAB, a depressão, é caracterizado por sintomas como recorrência de pensamentos negativos, fadiga, sentimento de inutilidade e culpa, anedonia, além de distúrbios de apetite e sono. O conjunto de cinco ou mais desses

critérios devem estar presentes por pelo menos duas semanas, para compor o diagnóstico. O episódio depressivo causa sofrimento clinicamente significativo ou prejuízo no funcionamento social, profissional e em outras áreas da vida do indivíduo (ENKHUIZEN et al., 2015; AMERICAN PSYCHIATRIC ASSOCIATION, 2013). O período entre os estados extremos é denominado estado eutímico e o paciente apresenta comportamento relativamente normal (YOUNG e DULCIS, 2015).

Episódios de humor, às vezes, incluem ambos os sintomas de episódio maníacos e depressivos, denominado estado misto. O Manual Diagnóstico e Estatístico de Desordens Mentais 5° edição (DSM V) e a classificação Internacional de Doenças (CID-10), caracteriza isso como episódios simultâneos de síndromes depressiva e maníaca, denominada mania mista, mania disfórica ou depressão durante a mania. Apresentam sintomas depressivos com pelo menos dois dos seguintes sintomas: agitação motora, agitação psíquica, pensamentos acelerados (AMERICAN PSYCHIATRIC ASSOCIATION, 2013). Quando sintomas acentuados de ambos os pólos interagem, ou seja, a impulsividade potencializada da mania, juntamente com o sofrimento profundo da depressão, geram comportamentos incontroláveis, como tentativa de suicídios, agressões físicas e verbais, uso abusivo de álcool e drogas, sintomas obsessivos compulsivos, dentre outros (MORENO e MORENO, 2005).

Além dos próprios sintomas presentes no TAB, o indivíduo portador desta patologia ainda tem que conviver com outras comorbidades associadas a este. Muitos pacientes com TAB terão pelo menos um dos transtornos mentais comórbidos e muitos terão dois ou mais, sendo o transtorno de ansiedade o mais frequente (70%), enquanto transtornos relacionados ao abuso de substâncias, como álcool e drogas são relatados em mais de 40% dos pacientes (SAUNDERS e GEDDES, 2016). O transtorno obsessivo-compulsivo está presente em cerca de 20%, enquanto o transtorno de déficit de atenção e hiperatividade atinge 9% dos mesmos (MERIKANGAS et al., 2007). Cabe ressaltar também que a comorbidade pode afetar o funcionamento social, a qualidade de vida e agravar o prognóstico do caso, aumentando assim o risco de suicídio.

O diagnóstico de um paciente com TAB é realizado através de exame clínico, baseado na presença ou ausência da sintomatologia que está descrita no DSM-V. Existem três tipos de TAB: Transtorno de Humor Bipolar Tipo I, caracterizado por episódios decorrentes de mania e depressão, tendo pelo menos um episódio

maníaco ao longo da vida; o Transtorno de Humor Bipolar do Tipo II que é definido pela alternância entre episódios de depressão e hipomania, e o Transtorno Ciclotímico, que se trata da forma mais branda da doença, onde envolve mudanças de humor, com episódios hipomaníacos e depressivos que ocorrem frequentemente e constantemente (AMERICAN PSYCHIATRIC ASSOCIATION, 2013).

A identificação do TAB, muitas vezes, pode ocorrer tardiamente, sendo que o diagnóstico incorreto pode ter origem devido à vasta prevalência de comorbidades envolvidas e o paciente ser diagnosticado com os mais variados problemas, como dependência de drogas, obesidade, distúrbio de caráter, síndrome do pânico, por exemplo. Entretanto, o diagnóstico equivocado mais comum é a depressão maior, visto que o pólo depressivo costuma-se apresentar primeiramente, além do portador não possuir uma percepção clara do estado maníaco (PEREIRA et al., 2010).

De acordo com dados epidemiológicos, o risco de se desenvolver o TAB é maior em adultos jovens, numa faixa etária entre 15-44 anos (YOUNG e DULCIS, 2015). Estudos populacionais não encontraram diferenças significativas entre os sexos no que se refere ao TAB I, enquanto o TAB II é mais comum no sexo feminino (BURT e RASGON, 2004). Dados da literatura, os quais se referem a outras características sócio demográficas, demonstraram que não houve diferenças significativas ao correlacionar a doença com o estado civil, a raça e a renda familiar (MERIKANGAS et al., 2011).

O tratamento usual para o TAB é realizado através de fármacos estabilizadores do humor, e este é dividido em três fases: aguda, continuação e manutenção. Os objetivos do tratamento durante a fase aguda são: tratar a mania sem causar depressão e vice-versa, a fase de continuação busca estabilizar os benefícios, reduzir os efeitos colaterais, tratar até a remissão e finalmente, os objetivos do tratamento medicamentoso na fase de manutenção são: prevenir a mania e/ou depressão e maximizar a recuperação funcional (SOUZA, 2005). O lítio é o medicamento utilizado como primeira linha (NOLEN, 2015; ORUCH et al., 2014) e este apresenta efeitos tanto em episódios maníacos quanto em episódios depressivos, além de atuar na prevenção da recorrência de novos episódios agudos (DINIZ et al., 2013). O mecanismo específico de ação do lítio no tratamento da mania é desconhecido, porém estudos relatam múltiplos mecanismos de ação (PASQUALI et al., 2010). Foi demonstrado que o lítio previne o ácido desoxirribonucleico (DNA), a formação de radicais livres e a peroxidação lipídica em

diversos modelos (KHAIROVA et al., 2012). Outro mecanismo de ação do lítio é seu efeito inibitório sobre a enzima proteína quinase C (PKC), o que pode estar relacionado com a sua ação antimaníaca. Este também é capaz de aumentar a expressão da principal proteína neuroprotetora Bcl-2 (CHUANG et al., 2002). Além de inibir a cascata de transdução de sinal da glicogênio sintase quinase 3 (GSK3) (JING et al., 2013).

Outros tratamentos incluem os anticonvulsivantes, ácido valproico, carbamazepina e iamatrigina (CHIU et al., 2013). Entretanto, cabe ressaltar que, muitos pacientes podem apresentar uma resposta insatisfatória aos fármacos atualmente disponíveis. A demora em obter as primeiras respostas à terapia farmacológica, associada aos efeitos colaterais como, por exemplo, xerostomia, polidipsia, poliúria e ganho de peso também contribuem negativamente para a baixa aderência ao tratamento por parte dos pacientes (HASHIMOTO et al., 2007; KASPER, 2014).

Apesar de ser um transtorno amplamente estudado e com grande quantidade de dados existentes na literatura, a fisiopatologia do TAB ainda não está totalmente compreendida. Entretanto, dados da literatura têm apontado que a patogênese deste transtorno envolve mecanismos associados a atividades de neurotransmissores, neurotrofinas, inflamação e estresse oxidativo, bem como a uma série de alterações associadas à resiliência celular e aspectos genéticos (Figura 1) (SIGITOVA et al., 2016).

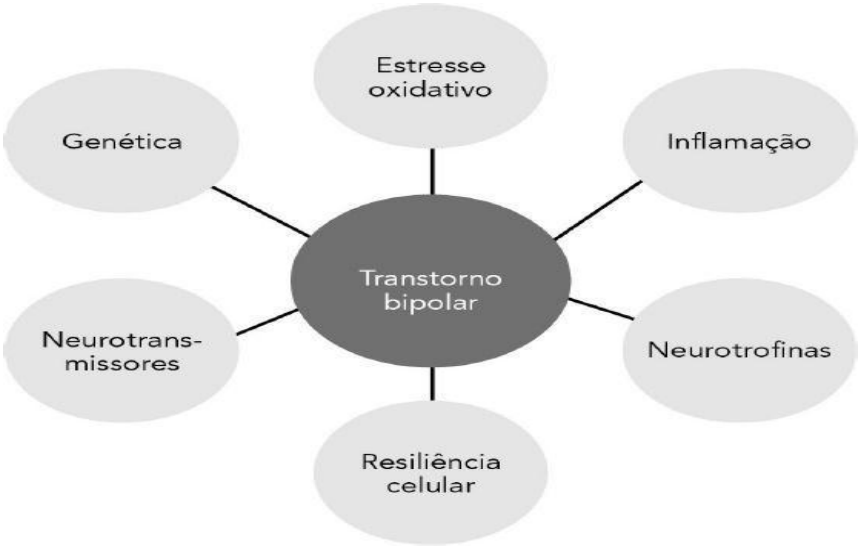

Figura 1: Fatores associados à fisiopatologia do Transtorno Afetivo Bipolar (TAB) (KAPCZINSKI e QUEVEDO, 2016)

3.2 Etiologia

A etiologia do TAB é objeto de exaustivas investigações clínicas e neurológicas. Sabe-se que fatores genéticos e ambientais contribuem para o desenvolvimento do transtorno. O histórico familiar é um dos fatores de riscos mais fortes e mais consistentes para o TAB, ou seja, estudos em gêmeos idênticos, demostram que os distúrbios afetivos são hereditários e, que o risco é dez vezes maior entre parentes adultos de indivíduos com o transtorno e a magnitude do risco aumenta com o grau de parentesco (MICHELON e VALLADA, 2004; BARNETT e SMOLLER, 2009).

Fatores psicossociais também podem estar envolvidos, como eventos de vida traumáticos, ou seja, perda de pessoas importantes, fim de relacionamentos, acidentes, agressões e outros abusos físicos e psicológicos. Além da utilização de drogas e/ou álcool, que pode desencadear este transtorno (ALDINGER e SCHULZE, 2017).

Alguns estudos têm sugerido que a dopamina tem papel fundamental no TAB, pois foi observado um aumento deste neurotransmissor em pacientes durante episódios maníacos (KAPCZINSKI e QUEVEDO, 2016). Níveis aumentados de glutamato também têm sido encontrados em córtex pré-frontal de pacientes bipolares, (MICHAEL et al., 2003; DAGER et al., 2004) sugerindo que alterações na neurotransmissão glutamatérgica podem estar associadas à patogênese do TAB. Além disso, estudos prévios demonstram níveis aumentados de noradrenalina em indivíduos maníacos; e níveis reduzidos de serotonina em pacientes que apresentavam agressividade e tentativas de suicídio (KAPCZINSKI e QUEVEDO, 2016).

Alterações neuroquímicas relacionadas aos fatores neurotróficos também têm sido descritos na literatura em relação ao TAB (SCOLA e ANDREAZZA, 2015; KAPCZINSKI et al., 2008). As neurotrofinas compreendem uma classe de proteínas altamente abundantes no sistema nervoso central (SNC), com funções fundamentais na sobrevivência, no crescimento e na plasticidade das células neuronais (SONG et al., 2017). O fator neurotrófico derivado do cérebro (BDNF) é a neurotrofina mais abundante no cérebro de mamíferos adultos, sendo essencial durante o processo de formação do SNC até a fase adulta, durante o qual desempenha um amplo espectro

funcional como regulação de conexões sinápticas, estrutura sináptica, liberação de neurotransmissores e plasticidade sináptica (SONG et al., 2017).

O BDNF exerce seus efeitos ligando-se ao receptor de alta afinidade trompomiosina quinase B (TrKB) e, consequentemente, as suas cascatas de sinalização promovem uma série de efeitos para a plasticidade sináptica e função cognitiva (SASI et al., 2017; SONG et al., 2017). Assim, o BDNF e sua cascata de sinalização parecem mediar processos dependentes de estímulos externos, como aprendizado e memória. De modo geral, a diminuição nos níveis circulantes desta neurotrofina é prejudicial (TWISS et al., 2006). Os níveis séricos de BDNF foram descritos como reduzidos em pacientes em episódios agudos de mania e depressão, sendo estes, associados a prejuízos cognitivos e também a progressão do transtorno (KAPCZINSKI et al., 2008; FERNANDES et al., 2011; BERK et al., 2011; GRANDE et al, 2012). Outros estudos confirmaram esta observação em pacientes sem medicação (MACHADO-VIEIRA et al., 2007; DE OLIVEIRA et al., 2009). Embora nem todos os estudos sustentem essa hipótese (AMEELE et al., 2017). O lítio, assim como outros agentes com propriedades antidepressivas é capaz de elevar a expressão de BDNF hipocampal e promover neuroproteção (DE SOUSA et al., 2011; MACHADO-VIEIRA et al., 2009). Além de todos os fatores citados anteriormente, muitos estudos têm focado no papel da neuroinflamação, da disfunção colinérgica e do estresse oxidativo na fisiopatologia do TAB.

3.2.1 Neuroinflamação

A inflamação do SNC é denominada de neuroinflamação e, este mecanismo tornou-se uma ferramenta importante para compreender o papel dos processos inflamatórios na fisiopatologia das doenças neurológicas (GARDNER & BOLES, 2011; FÜLÖP et al., 2016).

A inflamação é geralmente benéfica ao organismo, agindo para remover os agentes lesivos, promover a sobrevivência do tecido e restaurar suas funções normais, entretanto uma inflamação extensiva ou não regulada é altamente prejudicial ao organismo (ABBAS e JANEWAY, 2000), promovendo o comprometimento de funções cognitivas (declínio de memória) e considerada como um processo crucial para a progressão de diversas doenças/transtornos, entre elas, o TAB (ROSENBLAT et al., 2014).

O envolvimento da neuroinflamação em determinadas doenças está relacionado com alterações patológicas, que incluem a ativação de células gliais, como astrócitos e microglia (CHING et al., 2007), pelo aumento da concentração de diferentes citocinas e quimiocinas (O"CALLAGHAN & SRIRAM, 2005) e, em situações mais graves, ocorre o aumento da permeabilidade da barreira hematoencefálica (BANKS, 2006).

As células gliais contribuem de várias maneiras para a sustentação da homeostase tecidual e para neuroinflamação. Estas podem ser divididas principalmente, em três classes diferentes, como por exemplo, oligodentrócitos responsáveis pela formação e manutenção das bainhas de mielina dos axônios (PFEIFFER et al., 1993), os astrócitos, que são as células gliais mais abundantes do SNC (GUILLAMÓN-VIVANCOS et al., 2015) e desempenham funções essenciais para homeostase, como, formação da barreira hematoencefálica, controle do metabolismo neuronal e regulação dos neurotransmissores (OBERHEIM et al., 2012); e a microglia, que são as menores de todas as células gliais, e são responsáveis por monitorar o ambiente externo, respondendo aos sinais de desequilíbrio na homeostase (KREUTZBERG, 1996). Porém, quando ocorre a ativação crônica das células gliais, podem causar o comprometimento de órgãos e sistema, levando a descompensação, disfunção orgânica e morte neuronal, devido à liberação de compostos danosos como espécies reativas de oxigênio e nitrogênio, dentre outras (STREIT, 2004).

Vários estudos têm demonstrado que a neuroinflamação contribui para etiologia e o agravamento de determinadas doenças neurológicas e que mediadores inflamatórios são considerados essenciais para o monitoramento, diagnóstico, progressão e resposta à terapia destas doenças (ALBRECHT et al., 2016). Entre alguns biomarcadores presentes no sangue, podemos citar as citocinas e quimiocinas (ALBRECHT et al., 2016; KOTHUR et al., 2016).

As citocinas inflamatórias são uma família de polipeptídeos ou glicoproteínas hidrossolúveis produzidos a partir de diferentes células, entre elas as células do sistema imunológico, e são mediadores sistêmicos cruciais para uma resposta adequada do organismo frente a infecções e processos inflamatórios, através da ativação de receptores específicos (KUNZ et al., 2011; ALTAMURA et al., 2014). Existem citocinas pró-inflamatórias, tais como, fator de necrose tumoral alfa (TNF-α), interleucina 1 (IL-1) e interleucina 6 (IL-6), que atuam promovendo o processo

inflamatório e estimulam a resposta inflamatória, e as citocinas anti-inflamatórias, como por exemplo, a interleucina 4 (IL-4) e interleucina 10 (IL-10), que reduzem os danos deste processo, impedindo uma resposta inflamatória exacerbada (KIM et al., 2007).

A produção anormal de algumas citocinas, como TNF- α, interleucina -1- beta (IL-1β), receptor de IL-2 solúvel (sIL-2R), IL-6 e quimiocinas tem sido implicada na patogênese de várias doenças inflamatórias e auto-imunes (EVEREKLIOGLU et al., 2002). Citocinas específicas podem estar diretamente implicadas na fisiopatologia do TAB e pode, portanto, apresentar novos alvos para o tratamento deste transtorno (ROSENBLAT e MCINTYRE, 2017). Estudos mostraram consistentemente níveis elevadosde citocinas pró-inflamatórias empacientes com TAB, sugerindo inflamação crônica de baixo grau. Níveis séricos de moléculas pró-inflamatórias, incluindo IL-4, TNF- α, sIL-2R, IL-1β, IL-6 e proteína C- reativa (PCR) são elevados em pacientes com TAB comparados com controles saudáveis (MODABBERNIA et al., 2013; BARBOSA et al., 2014; BRIETZKE et al., 2009). Outra observação importante tem sido a variabilidade nos perfis de citocinas, dependendo do estado de humor, ou seja, perfis de citocinas diferentes durante períodos de depressão, mania, hipomania e eutímia (MODABBERNIA et al., 2013; MUNKHOLM et al., 2013).

Os dados mais consistentemente descritos na literatura demonstram o aumento dos níveis da IL-6 em soro de pacientes principalmente durante episódios maníacos, inclusive sendo sugerido por alguns autores como um possível marcador para estes estados (KAPCZINSKI et al., 2011; RODA et al., 2015; MUNEER, 2016). Já quanto aos níveis de IL-10, alguns estudos trazem uma diminuição desta citocina anti-inflamatória somente em pacientes em estágio mais avançado do transtorno, não sendo observadas alterações em pacientes recém-diagnosticados (RODA et al., 2015). Além disso, podem ser encontrados níveis elevados de outros mediadores pró-inflamatórios, como IL- 1β e TNFα (BERK et al., 2011; RODA et al., 2015). O aumento nos níveis destes mediadores, principalmente a IL-6, contribui também para o aumento nos níveis de PCR, portanto sendo possível observar um estado pró- inflamatório característico na fase maníaca do TAB (BAUER et al., 2014).

3.2.2 Sistema Colinérgico

O sistema colinérgico desempenha um papel essencial nos processos de aprendizado e memória (WINKLER et al., 1995). Este inclui a sinalização mediada

pela acetilcolina (ACh) e abrange todo o processo de síntese, armazenamento, transporte e degradação dessa molécula. A ACh é um neurotransmissor clássico cuja produção é oriunda do SNC e periférico (BRUNEAU e AKAABOUNE, 2006). No SNC, a ACh age como neuromodulador envolvido em processos comportamentais, de cognição, aprendizado, memória e atenção. No sistema periférico desempenha um papel de neurotransmissor excitatório promovendo ativação dos músculos (PICCIOTTO et al., 2012).

A síntese de ACh ocorre nos terminais nervosos a partir de dois precursores, a colina e a acetilcoenzima A. A colina é acetilada por uma enzima citosólica, a colina acetiltransferase (ChAT) que transfere o grupo acetil da molécula de acetilcoenzima A (RANG et al., 2008). Uma vez sintetizada, a ACh é armazenada em vesículas sinápticas e liberada por exocitose em resposta à entrada de Ca^{2+} na terminação nervosa, após esta interage com os seus receptores muscarínicos e nicotínicos presentes nas membranas das células (PICCIOTTO et al., 2012; BECKMANN e LIPS, 2013). Por fim, a ação da ACh cessa quando esta é hidrolisada em acetato e colina pela enzima AChE, presente na fenda sináptica (TAYLOR e BROWN, 1999). Uma parte da colina resultante é recaptada pelo terminal axonal colinérgico e reutilizada para síntese de novas moléculas de ACh, pois considerando que os neurônios não produzem colina suficiente e que maior parte desta provem da dieta, sua recaptação para o interior neuronal se torna essencial (PRADO et al., 2002; RIBEIRO et al., 2006).

A AChE é uma glicoproteína, sendo a principal enzima moduladora dos níveis de ACh no meio extracelular, possui uma alta eficiência catalítica, com capacidade de hidrolisar até $6X10^5$ moléculas de ACh por molécula de enzima por minuto (SILMAN e SUSSMAN, 2005). Esta enzima possui um sítio ativo onde existem três resíduos principais de aminoácidos, os quais estão envolvidos diretamente no processo de hidrólise da ACh, a serina número 200 (Ser-200), a histidina número 440 (His-440) e o ácido glutâmico número 327 (Glu-327) (POHANKA, 2014). A AChE está presente majoritariamente no SNC, porém é encontrada também em eritrócitos, linfócitos e plaquetas. Estudos têm demonstrado alterações na atividade da AChE e, estas estão associadas à diversas patologias, inclusive com o TAB (SIGITOVA et al., 2016; SPOHR et al., 2019). Tanto a ChAT quanto a AChE são proteínas marcadoras específicas da atividade fisiológica dos neurônios colinérgicos e, ambas

desempenham um papel importante na homeostase neuronal de ACh (FONNUM, 1975).

De fato, evidências sugerem que as disfunções do sistema colinérgico se associam a quadros de transtornos de humor (MINEUR e PICCIOTTO, 2010; PHILIP et al., 2010). CUMMINGS (2000) demonstrou que a redução dos níveis de ACh estão relacionados com a manifestação de doenças psiquiátricas, principalmente psicoses. Além disso, estudos prévios do nosso grupo de pesquisa demonstraram um aumento na atividade da AChE em córtex cerebral, hipocampo e estriado de ratos submetidos a um modelo experimental de mania induzida por cetamina (SPOHR et al., 2019; CHAVES et al., 2020), sendo que, estas alterações foram prevenidas pelo tratamento com lítio.

Dados da literatura tem sugerido que a diminuição na atividade colinérgica está associada com a mania, enquanto o aumento da atividade de ACh está associado com a depressão, ou seja, o aumento na atividade da AChE pode levar à diminuição dos níveis do neurotransmissor ACh, contribuindo para a ocorrência de episódios maníacos (VAN ENKHUIZEN et al., 2015, SIGITOVA et al., 2016). Assim, o uso de inibidores da AChE tem sido associado com a redução de sintomas neuropsiquiátricos (DIGBY et al., 2012; OBERMAYER et al., 2017). Uma vez inibida, esta pode gerar um acúmulo de ACh na fenda sináptica, tal mecanismo melhora a transmissão colinérgica.

3.2.3 Estresse Oxidativo

Vários estudos também demonstram a existência de disfunção mitocondrial e estresse oxidativo em indivíduos com TAB (CATALDO et al., 2010; BENES et al., 2006). As mitocôndrias regulam a produção de energia, através da cadeia transportadora de elétrons. Durante este processo, EROs são produzidas, o que pode levar ao estresse oxidativo e danos celulares, particularmente, na ausência de defesas antioxidantes (TOBE, 2013; NG et al., 2008).

As EROs podem ser divididas em dois grupos: os radicais livres e os compostos não radicalares. Denomina-se radical livre toda molécula que possui um elétron ímpar em sua órbita externa, gravitando em sentido oposto aos outros elétrons (elétrons desemparelhados) (KOHEN e NYSKA, 2002; CAROCHO e FERREIRA, 2013), como por exemplo, o ânion superóxido ($O_2^{\bullet}$), radical hidroxila ($OH^{\bullet}$) e oxigênio singleto. Entre alguns derivados não radicalares de oxigênio, o

peróxido de hidrogênio (H_2O_2) e ácido hipocloroso (HClO), não possuem elétrons livres, sendo, portanto, menos instáveis que os radicais livres, mas também podem reagir com moléculas do meio (HALLIWELL & GUTTERIDGE, 2007; RAJENDRAN et. al., 2014).

A produção destas espécies ocorre no organismo geralmente de forma benéfica, auxiliando em funções fisiológicas normais, ou seja, podendo proteger células contra infecções ou até mesmo regular a concentração do cálcio intracelular, envolvido em processos de fosforilação de proteínas (SALIM, 2014). Entretanto, quando formadas em excesso ou em detrimento da velocidade de remoção das mesmas, estas podem oxidar diversas biomoléculas, como os lipídios, as proteínas e o DNA (HALLIWELL & GUTTERIDGE, 2007; VENTURINI et al., 2011; HOLMSTRÖM & FINKEL, 2014; RAJENDRAN et. al., 2014).

Para evitar os danos causados pelas EROs, o organismo possui vários mecanismos de defesa, isto é, potenciais de neutralização das ações dos radicais livres, chamados antioxidantes, que dizem respeito a qualquer substância que, presente em baixas concentrações quando comparadas à substrato oxidável, atrasa ou inibe a oxidação deste substrato de maneira eficaz (DIPLOCK et al., 1998).

Deste modo, o organismo conta com um sistema de defesa não enzimático como, por exemplo, a glutationa reduzida (GSH), peptídeos de histidina, proteínas ligadas ao ferro (transferrina e ferritina), compostos provenientes da dieta como o alfa-tocoferol (vitamina E), betacaroteno, ácido ascórbico, e compostos fenólicos (HALLIWELL & GUTTERIDGE, 2007). Além disso, temos também sistema antioxidante endógeno enzimático, que removem cataliticamente as EROs, formado pelas enzimas antioxidantes SOD, que remove o ($O_2^{\bullet}$), convertendo-o em H_2O_2, e a enzima CAT, responsável pela dismutação do H_2O_2 para geração de O_2. A GPx também realiza a remoção de H_2O_2 através da oxidação da GSH, um tripeptídeo que contém um grupo tiol (grupo redutor). Assim há uma reação entre o H_2O_2 e GSH, com a formação de glutationa na forma oxidada (GSSG), que pode ser novamente reduzida a GSH, pela ação da enzima glutationa redutase (GR) (HALLIWELL & GUTTERIDGE, 2007; BÓ et al., 2015). As reações enzimáticas estão representadas na (Figura 2).

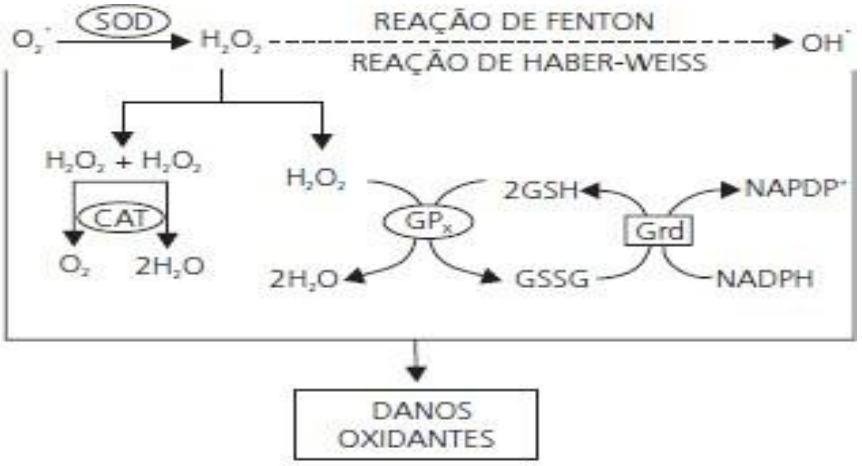

Figura 2: Integração dos sistemas de defesa enzimáticos. (BARBOSA et al., 2010).

Em condições fisiológicas há um equilíbrio entre a produção de EROs e os sistemas de defesas antioxidantes, porém, em certas condições patológicas pode haver o aumento da produção de oxidantes e/ou diminuição dos níveis de antioxidantes, resultando no que chamamos de estresse oxidativo. Este termo se refere ao desequilíbrio entre a capacidade antioxidante e as EROs formadas, podendo resultar em dano aos componentes celulares (HALLIWELL & GUTTERIDGE, 2007; RAJENDRAN, et. al., 2014).

Sabe-se que o estresse oxidativo contribui para o desenvolvimento de doenças neurológicas, devido ao SNC ser particularmente sensível as EROs (CUMURCU et al., 2009; GHANIZADEH e BERK, 2013; SOUSA et al., 2014). A vulnerabilidade do sistema nervoso é decorrente de suas características estruturais e metabólicas como alto consumo de oxigênio, grandes quantidades de cadeias lipídicas insaturadas, abundância de íons metálicos e baixa disponibilidade de enzimas antioxidantes (GU et al., 2008). Neste contexto, o estresse oxidativo poderia funcionar como um gatilho, para o desenvolvimento e/ou progressão de diversas doenças (SALIM, 2014). Assim, alterações que estejam relacionadas tanto ao aumento de geração de EROs, como a diminuição destas poucas defesas já existentes podem explicar, em parte, a fisiopatologia ou surgimento de novos episódios maníacos ou depressivos no TAB.

Estudos prévios da literatura já demonstraram uma diminuição das enzimas SOD, CAT e nos níveis de glutationa em indivíduos com TAB quando comparados a

indivíduos controles, principalmente quando estes se encontravam em algum episódio agudo da doença (maníaco ou depressivo) (AYDEMIR et al., 2014; GHANIZADEH e BERK, 2013). Alterações nos níveis de glutationa também têm sido observadas no sangue de pacientes com TAB I e II e no estado eutímico quando comparado a indivíduos saudáveis (ROSA et al., 2014; SINGH et al., 2018). Outro estudo avaliou dois grupos de pacientes, que apresentavam apenas sintomas maníacos e sem episódios de depressão, sendo que o primeiro grupo apresentava o episódio maníaco pela primeira vez, enquanto o segundo grupo havia tido dois ou mais episódios maníacos. Alterações significativas nos indicadores de estresse oxidativo foram observadas no sangue destes pacientes, ou seja, níveis elevados de oxidantes foram observados de acordo com a gravidade da doença, e não com o número de episódios maníacos (AKARSU et al., 2018).

Considerando que esses achados sustentam a hipótese do envolvimento do estresse oxidativo na neuroprogressão do TAB, torna-se de grande importância estudar compostos que apresentem potencial antioxidante com a finalidade de buscar novos alvos terapêuticos para o tratamento deste distúrbio.

3.3 Ácido Gálico

As plantas produzem uma larga e diversa ordem de componentes orgânicos divididos em metabólitos primários ou secundários. Os metabólitos secundários são de extrema importância para proteção das plantas contra fatores ambientais e bióticos adversos, além destes apresentarem um potencial efeito medicinal para a saúde humana (PENGELLY, 2004).

Dentre os metabólitos secundários, podemos destacar os compostos fenólicos, que apresentam um diversificado grupo de compostos químicos, os quais são geralmente classificados com base no número de átomos de carbono em sua estrutura, como os, fenólicos simples, ácidos fenólicos, acetofenonas, derivados de ácido cinâmico, cumarinas, flavonóides, antocianinas, benzofenonas, xantonas, ligninas, taninos, entre outros, sendo estes os principais subgrupos dos fenólicos naturais (PENGELLY, 2004).

Os ácidos fenólicos são uma das principais classes dos polifenóis, com estrutura química básica de sete átomos de carbono C_6-C_1 (ácidos hidroxibenzóicos), sendo estes os mais simples encontrados na natureza ou C_6-C_3 (ácidos hidroxicinâmicos), que possuem nove átomos de carbono, consistindo de um

anel fenólico e um substituinte carboxila (SOARES, 2002). Esses compostos são sintetizados a partir de duas rotas metabólicas principais: a via do ácido chiquímico e a via do ácido mevalônico, a qual é menos significativa (PENGELLY, 2004; SIAH et al., 2016).

O ácido gálico (3,4,5-trihidróxibenzóico) (Figura 3) é um dos ácidos fenólicos mais abundantes no reino vegetal. É um composto cristalino branco amarelado com massa molecular de 170,12 g/mol, ponto de fusão de 250 °C e solubilidade em água 1,1 % a 20°C (VERMA et al., 2013).

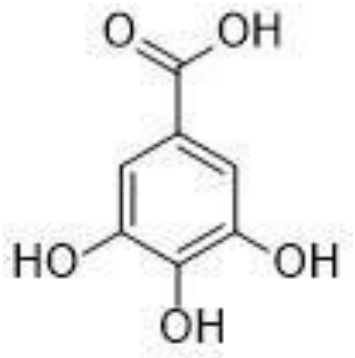

Figura 3: Estrutura química do ácido gálico

A estrutura do ácido gálico consiste de um anel aromático, três grupos hidroxilas e um grupo ácido carboxílico (VERMA et al., 2013). É bem estabelecido na literatura o potencial antioxidante do ácido gálico, o qual pode ser atribuído aos três grupos hidroxilas que estão ligados ao anel aromático. As propriedades redutoras e a estrutura química do ácido gálico desempenham um papel importante na neutralização de radicais livres e quelação de metais de transição, agindo tanto na etapa de iniciação como na propagação do processo oxidativo (SOUSA et al., 2007).

O ácido gálico e seus derivados possuem um número grande de aplicações em vários campos da ciência, com ampla aplicação na indústria alimentícia e farmacêutica (CHOUBEY et al., 2015). Este polifenol é amplamente distribuído em muitas espécies diferentes de plantas superiores, tanto em estado livre ou como moléculas mais complexas, como derivados de ésteres ou polímeros (DAGLIA et al., 2014). Na natureza, o ácido gálico e seus derivados estão presentes em quase todas as partes da planta, como casca, tronco, folha, fruta, raiz e semente ou

estigma de várias plantas farmacologicamente ativas (DAGLIA et al., 2014; CAN et al., 2017).

Eles estão presentes em diferentes concentrações em alimentos comuns, como mirtilos, amora, morango, ameixas, uvas, manga, castanha de caju, avelã, noz, chá e vinho (DAGLIA et al., 2014; PRINCE et al., 2009). É importante ressaltar que estudos recentes mostraram que alimentos ricos em ácidos fenólicos diminuem o risco de depressão (LIU et al., 2016; GROSSO et al., 2016).

Estudos têm demonstrado os efeitos antioxidantes do ácido gálico, ou seja, tratamento com ácido gálico (30 mg/Kg) por 26 dias foi capaz de melhorar danos oxidativos induzidos por injeções de estreptozocina (STZ) intracerebroventriculares (ICV), através da normalização dos níveis de TBARS e conteúdo tiólico, bem como atividade das enzimas antioxidantes GPx, SOD e CAT em estriados de ratos (NAGHIZADEH e MANSOURI, 2015). Administração via oral de ácido gálico nas diferentes concentrações (0,8, 2 e 4 mg/Kg) por 10 dias, melhorou as enzimas antioxidantes no desenvolvimento da depressão (PEMMINATI, 2015). Em outro estudo, o ácido gálico (20 mg/Kg) atenuou o estresse oxidativo da tireóide e o processo inflamatório em ratos albinos machos, induzidos com dicromato de potássio (MOHAMED e TWAB, 2016).

Além disso, alguns estudos indicam claramente a propriedade anti-inflamatória do ácido gálico, devido à sua capacidade de inibir significativamente os mediadores inflamatórios (PANDURANGAN et al., 2015A; PANDURANGAN et al., 2015B; BENSAAD et al., 2017). SIDDIQUI et al (2019) avaliaram os efeitos anti-inflamatório dos compostos ácido gálico e ácido vanílico no modelo de inflamação induzido por lisolecitina e, observaram que ambos os compostos foram capazes de inibirem a desmielinização em neurônios do SNC, assim como promoveram o crescimento de neuritos dos neurônios hipocampais. A administração de lisolecitina também desencadeou respostas inflamatórias, o nível de expressão da proteína ácida fibrilar glial (GFAF) foram significativamente elevados, juntamente com a atividade de outros fatores de transcrição associados a inflamação, tais como, o fator nuclear kappa B (NFkB) e a enzima cicloxigenase 2 (COX-2) e, ambos os compostos reduziram significativamente estas alterações.

Cabe salientar que além da atividade antioxidante e anti-inflamatória, dados da literatura têm demonstrado que o ácido gálico possui outras importantes propriedades terapêuticas tais como, ações antialérgicas, atividades

antimicrobianas, antitumorais, hipoglicemiantes e neuroprotetoras (CHOUBEY et al., 2015; KAHKESHANI et al., 2019). Além disso, é importante ressaltar que estudos prévios também têm demonstrado o efeito antidepressivo do ácido gálico (10 e 20 mg/kg) em um modelo de estresse crônico imprevisível em camundongos (CHHILLAR e DHINGRA, 2012). Esse efeito antidepressivo do ácido gálico tem sido atribuído a sua atividade antioxidante, inibição da enzima monoamina oxidase A (MAO-A) e diminuição nos níveis de corticosterona sérica (CHHILLAR e DHINGRA, 2012). Além disso, CAN et al. (2017) também relataram que o ácido gálico pode aumentar os níveis de serotonina e catecolaminas na fenda sináptica.

Neste contexto, considerando que o ácido gálico possui muitas atividades biológicas que talvez possam proteger, minimizar ou reverter a neuroprogressão em transtornos neurológicos, torna-se relevante avaliar os efeitos deste composto em parâmetros comportamentais e neuroquímicos em situações experimentais como mania e neuroinflamação, a fim de contribuir para a busca de novas terapias que possam beneficiar pacientes com estas alterações cerebrais.

3.4 Modelo Experimental de Mania Induzido por Cetamina

Além de estudos em pacientes, modelos experimentais ampliam e corroboram muitas dessas descobertas em humanos. Mesmo com diferentes graus de validade, modelos animais são relevantes, já que permitem manipulações não factíveis em estudos clínicos (FRIES e MAGALHÃES, 2010). Entretanto, a escassez de modelos animais consistentes é uma das atuais limitações no entendimento da neurobiologia dos transtornos de humor (MANJI et al.; 2001). Atualmente, não existem modelos animais apropriados para o estudo do TAB, uma vez que os modelos atualmente disponíveis não conseguem induzir os dois espectros do TAB no mesmo animal (MACHADO-VIEIRA et al.; 2004), sendo assim, modelos animais de mania têm sido amplamente utilizados para estudar essa condição, uma vez que esta é a característica principal no TAB.

A validação de um modelo animal baseia-se em três critérios: a validade de face, que avalia quanto um modelo é capaz de mimetizar sintomas e/ou comportamentos da doença estudada; a validade de construto considera o quanto o modelo reproduz os mecanismos envolvidos em sua fisiopatologia e a validade preditiva, que refere-se na resposta ao tratamento, ou seja, que o comportamento

induzido no animal seja revertido ou prevenido com agentes terapêuticos eficazes em humanos (FRIES e MAGALHÃES, 2010).

Os modelos animais de mania podem ser induzidos por diferentes estímulos, podendo ser divididos em três tipos: ambientais, genéticos e farmacológicos. Os modelos ambientais, como o de privação de sono, baseiam-se em distúrbio do ciclo cicardiano, dados da literatura demonstram que a privação de sono causa um aumento acentuado na atividade dopaminérgica e concomitante aumento na atividade locomotora (GHOSH et al., 1976; KANAZAWA et al., 2016). Da mesma forma, o aumento nos níveis de dopamina induzidos pela privação de sono é uma fonte de estresse oxidativo no cérebro, que pode estar relacionado ao estabelecimento de sintomas maníacos (BERK et al., 2007; REES et al., 2007). Os modelos genéticos apresentam várias estratégias de investigação para identificar genes determinantes de vulnerabilidade ao TAB (MICHELON e VALLADA, 2005). Diferentes mecanismos genéticos podem estar envolvidos na etiopatogenia do TAB, tais como alterações cromossômicas, heterogeneidade de alelos, heterogeneidade de genes (*loci*), *epistasis*, mutação dinâmica levando ao fenômeno de antecipação, *imprinting* e mutação de genes mitocondriais (MICHELON e VALLADA, 2004; MICHELON e VALLADA, 2005).

Entretanto os modelos farmacológicos têm sido os mais utilizados no estudo da fisiopatologia do TAB (EINAT, 2006; YOUNG et al., 2011), entre eles, podemos citar, o tratamento farmacológico com anfetamina, um psicoestimulante, que atua no SNC, aumentando a liberação e diminuindo a recaptação de dopamina na fenda sináptica e inibindo a sua degradação pela enzima monoamina oxidase (MAO) (FREY et al., 2006; MORETTI et al., 2011), a administração intracerebroventricular de ouabaína, um inibidor específico da Na^+/K^+ATPase, reduzindo a atividade neuronal desta enzima em ratos, análogo ao que ocorre em humanos durante episódios de mania e depressão (BRÜNING et al., 2012; SOUZA et al., 2014) e cetamina (GHEDIM et al., 2012; GAZAL et al., 2014).

A cetamina também tem sido estudada como agente indutor de hiperlocomoção (GHEDIM et al., 2012; GAZAL et al., 2014). Este fármaco é capaz de alterar a neurotransmissão glutamatérgica, uma vez que atua como antagonista não competitivo do receptor N-metil-D-aspartato (NMDA), inibindo a ação do seu neurotransmissor, o glutamato, em seu receptor e gerando assim, alterações no cérebro dos animais, semelhantes às descritas em pacientes com TAB

(KAPCZINSKI e QUEVEDO, 2016). O tratamento com esta substância causou danos oxidativos em córtex, hipocampo e estriados, tais como, o aumento a peroxidação lipídica e redução das enzimas antioxidantes, SOD, CAT e GPx (DEBOM et al., 2016). Em outro estudo, também utilizando cetamina para induzir episódio maníaco, foi observado que esta aumentou os níveis de EROs, peroxidação lipídica e níveis de nitrito, além de diminuir atividade de enzimas antioxidantes (SPOHR et al.; 2019).

Além disso, estudos prévios têm demonstrado os efeitos promissores de compostos naturais, em modelos experimentais de mania induzido por cetamina e, estes resultados nos indicam, que estes compostos poderiam ser empregados como uma terapia complementar associada ao TAB. GAZAL et al (2014) sugerem que a curcumina reduziu os danos oxidativos associados com a fase maníaca do transtorno. DEBOM et al (2016) e SPOHR et al (2019) observaram que o pré-tratamento com extrato de mirtilo foi capaz de prevenir a hiperlocomoção e o estresse oxidativo inzudido por cetamina em ratos. Extrato de amora também melhorou o comportamento e a disfunções neuroquímicas induzidos pela cetamina, demonstrando propriedades neuroprotetoras, bem como, efeitos antioxidantes e antiinflamatórios (CHAVES et al., 2020). Portanto, estes modelos representam ferramentas farmacológicas úteis para avaliar alterações comportamentais e bioquímicas observadas durante o episódio de mania, bem como para auxiliar na busca de novos fármacos com ação mais específica (VALVASSORI et al., 2013).

3.5 Modelo Experimental de Neuroinflamação Induzido por Lipopolissacarídeo (LPS)

Recentemente vários estudos têm relatado que o tratamento com LPS *in vivo* ou *in vitro* causa perda de neurônios e ativação da microglia (WANG et al., 2004; KHAN et al., 2016). O LPS é um glicolípideo, exclusivamente bacteriano, também conhecido como endotoxina altamente tóxica, derivado de bactérias gram negativas (BLUTHÉ et al., 1991). Este é um ligante do receptor toll-like 4 (TLR4), que ao ligar-se a este receptor induz a fosforilação do NF-kB, aumentando drasticamente os níveis de EROs em vários tipos de células, resultando em respostas pró-inflamatórias (CAFÉ-MENDES et al., 2017).

O LPS tem sido descrito como um potente agente neuroinflamatório e, o tratamento com compostos naturais ou isolados tem se mostrado eficaz contra este efeito. Spohr et al (2020) demonstraram que extrato de mirtilo exerceu efeito tipo-

antidepressivo, pois este foi capaz de proteger o cérebro de camundongos, prevenindo alterações no status redox, bem como modulou os níveis de TNF-α induzido por LPS. Dados recentes também mostraram que a endotoxina induziu comportamento tipo-depressivo nos camundongos e, este foi atenuado por tratamento com ácido tânico (30 ou 60 mg/Kg), o LPS também aumentou a peroxidação lipídica e a produção de EROs, o qual foi prevenido pela administração de ácido tânico. Os níveis de TNF-α também aumentaram em córtex cerebral, hipocampo e estriado, sendo que o ácido tânico foi capaz de prevenir esta alteração apenas no córtex cerebral (LUDUVICO et al., 2020).

Em outro estudo foi investigado os efeitos neuroprotetores das antocianinas (extraídas da soja preta) contra a neuroinflamação e neurodegeneração mediada por EROs e induzido por LPS no córtex de camundongos adultos. O tratamento com antocianinas mostrou redução nos níveis de estresse oxidativo, analisados através da ativação da JNK (c-Jun N-terminal cinase) por meio de seu nível de fosforilação (p-JNK), ou seja, as antocianinas reduziram a expressão desta proteína no córtex de camundongos tratados com LPS. Os resultados de imunoblot e morfológico mostraram que as antocianinas reduziram significativamente a neuroinflamação, através da inibição de vários mediadores inflamatórios, como IL-1β e TNF-α e o fator de transcrição NF-kB. Esta também reduziu a ativação de astrócitos e microglia no córtex, conforme indicado por redução da GFAP e a molécula adaptadora ligante de cálcio ionizado 1 (Iba-1) respectivamente (KHAN et al., 2016).

Os resultados de outro estudo mostraram que o alcalóide natural berberina melhorou os déficits cognitivos de memória e aprendizado induzidos por LPS em ratos, por meio da inibição da cascata apoptótica, neuroinflamação e estresse oxidativo (SADRAIE et al., 2019). Entre os modelos de neuroinflamação não manipulados geneticamente, o modelo animal de neuroinflamação induzido com LPS é comumente usado e tem sido útil na avaliação de novos medicamentos, bem como de produtos naturais (CATORCE e GEVORKIAN, 2016; ZAKARIA et al., 2017).

4. Resultados

Os resultados que fazem parte desta tese estão apresentados sob a forma de um artigo e dois manuscritos. As seções materiais e métodos, resultados, discussão e referências encontram-se nos próprios artigos e manuscritos e representam a íntegra deste estudo.

Os itens discussão e conclusões que se encontram no final desta tese apresentam interpretações e comentários gerais sobre os artigos contidos nesse trabalho.

As referências são referentes apenas às citações que aparecem nos itens introdução, revisão de literatura e discussão da tese.

O artigo e os manuscritos estão estruturados de acordo com as revistas as quais foram publicados ou submetidos.

4. 1 Manuscrito I

Therapeutic approaches employing natural compounds and derivatives for treating bipolar disorder: emphasis on experimental models of the manic phase

Encontra-se submetido à revista *Metabolic Brain Disease*

Therapeutic approaches employing natural compounds and derivatives for treating bipolar disorder: emphasis on experimental models of the manic phase

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Abstract

Bipolar disorder (BD) is a complex psychiatric disease characterized by mood swings that include episodes of mania and depression. Given its cyclical nature, BD is especially hard to model; however, the standard practice has been to mimic manic episodes in animal models since investigating these episodes is essential for the diagnosis of the disease. Despite scientific advances, the pathophysiology of BD is not fully understood, and treatment remains limited. Thus, in this review, we explore the therapeutic potential of natural compounds and derivatives against BD, taking into account preclinical and clinical studies. In clinical studies, the potential of these compounds has been described with regard to clinical improvements in symptoms of mania and depression. In animal models, natural compounds and derivatives can modulate many neurochemical pathways involved in the pathophysiology of manic episodes. Thus, although several lines of evidence suggest that consumption of natural compounds represents a strategy to mitigate manic symptoms, additional studies are necessary to confirm the role of these compounds as possible alternative therapies to treat psychiatric conditions.

Keywords: Mania. Bioactive compounds. Natural products. Neuroprotection. Bipolar disorder.

1. Bipolar Disorder

Bipolar disorder (BD) is a severe, chronic, and highly debilitating illness, which affects 1–4% of the world‟s population (Price and Marzani-Nissen 2012; Saundeers and Geddes 2016), and is associated with significant morbidity and mortality risk. BD is the sixth main cause of disability among physical and psychological disorders worldwide, leading to significant economic burden to patients and populations (Young and Dulcis 2015). It is characterized by recurrent pathological mood disturbances that range from extreme elation or mania/hypomania to severe depression. Manic episodes are associated with abnormally elevated mood, irritability, distractibility, psychomotor activation, and reduced need for sleep (Torpy et al. 2009). Hypomania is similar to the manic phase, but the symptoms are less severe than full mania. Depressive episodes are related to the presence of symptoms such as deep sadness, loss of energy, lack of interest in activities, fatigue, and suicidal thoughts, leading to significant suffering and/or impairment in social, professional, and other important facets of life (Mcintyre et al. 2020).

BD can be further subdivided into bipolar disorder type I, which is characterized by episodes resulting from mania and depression, with at least one manic episode throughout life, and bipolar disorder type II, which is characterized by alternations between episodes of hypomania and depression. In addition, cyclothymic disorder type, the mildest form of the disease, involves mood swings between hypomanic and depressive periods (Mcintyre et al. 2020). Males and females are equally affected by type I, whereas type II appears to be more common in women (Burt and Rasgon 2004). According to epidemiological data, BD starts on average between 15 and 44 years of age and has not consistently been associated with sociodemographic factors (Merikangas et al. 2011; Young and Dulcis 2015).

Although BD has been extensively studied, the causes and mechanisms involved in the pathogenesis of this disease are still unknown. Several hypotheses appear to be involved in the pathogenesis of BD, such as genetic and environmental factors, alterations in neurotransmitter signaling, inflammation, and oxidative stress (Sigitova et al. 2017; Andreazza et al. 2008) (Figure 1).

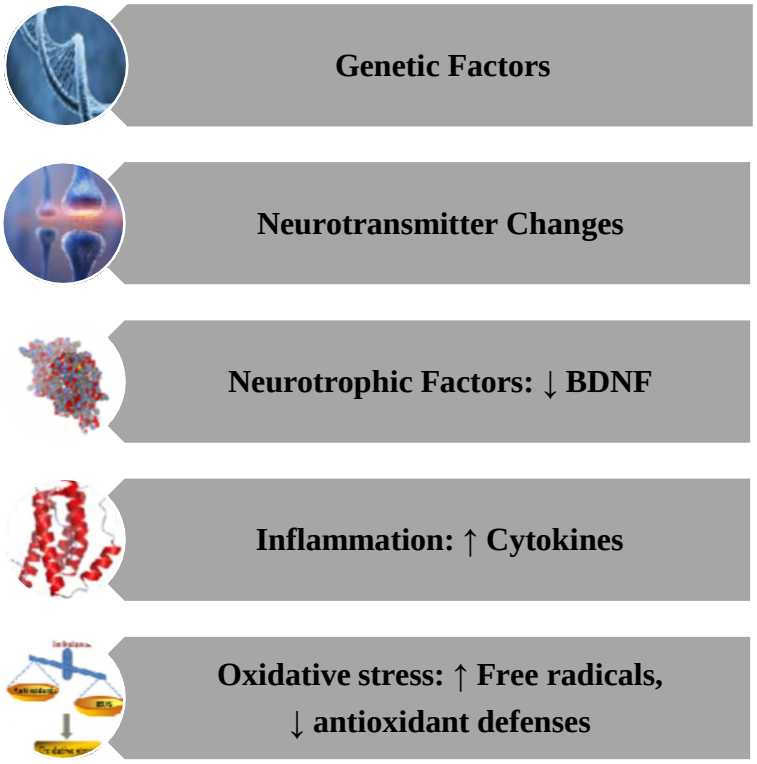

Fig. 1 Factors associated with the pathophysiology of bipolar disorder (BD)

Epidemiological studies have demonstrated that affective disorders are heritable, and that first-degree relatives of affected individuals have a 10-fold increased risk of developing BD compared to relatives of unaffected controls (Smoller and Finn 2003; Michelon and Vallada 2004). In addition, environmental factors such as abuse, mental stress, a significant loss, or some other traumatic event may contribute to or trigger this disease (Aldinger and Schulze 2017).

Previous studies have demonstrated changes in dopaminergic, glutamatergic, noradrenergic, and serotonergic signaling in BD (Shi et al. 2008). In this context, an increase in dopamine levels has been observed in patients during manic episodes. In addition, an increase in the level of norepinephrine in manic individuals and reduced serotonin levels associated with suicidal aggressiveness and attempts have been reported (Ackenheil 2001). Significant enhancement in glutamate concentrations can occur in the prefrontal cortex of bipolar patients (Michael et al. 2003; Dager et al. 2004) suggesting that changes in glutamatergic neurotransmission are also involved in the etiology of this mood disorder.

Brain-derived neurotrophic factor (BDNF) is the most abundant neurotrophin in the brain of adult mammals and plays a central role in synaptic plasticity and neurogenesis (Song et al. 2017). This neurotrophic factor is found throughout the brain with higher abundance in the hippocampus and cerebral cortex, brain regions responsible for the control of mood, emotion, and cognition (Ernfors et al. 1990). Serum BDNF levels are reduced during manic and depressive episodes and recover after treatment for acute mania (Fernandes et al. 2011; Berk et al. 2011; Grande et al. 2012).

In recent years, studies have attempted to identify the mechanisms involved in the inflammatory processes involved in BD (Colpo et al. 2018; Teixeira et al. 2016). Data from the literature have been replicated and been shown to be consistent across studies, including the increase in the levels of pro-inflammatory cytokines in patients, particularly interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α), both in manic and depressive episodes (Munner 2016; Fries 2018). In addition, oxidative stress has also been implicated in the pathophysiology of BD. Oxidative stress is characterized by an imbalance between the production of reactive oxygen species and antioxidant defenses (Jones 2008; Andreazza et al. 2008). Several studies have reported that patients with BD have significant alterations in antioxidant enzymes and lipid peroxidation (Andreazza et al. 2008). In fact, BD patients showed an enzymatic decrease in superoxide dismutase and catalase as well as in glutathione levels when compared to a control group, especially during acute disease episodes (Aydemir et al. 2014; Ghanizadeh and Berk 2013). An increase in lipid peroxidation and a decrease in antioxidant defenses have been described in BD patients (Mansur et al. 2016; Chowdhury et al. 2017), indicating that oxidative stress is involved in neurochemical changes observed in this psychiatric disorder.

Mood stabilizers are the primary treatment for BD. Lithium has been the gold standard for the treatment of BD for several decades (Nolen 2015; Oruch et al. 2014) and is effective for the treatment of acute mania, depressive episodes, and prevention of recurrent manic and depressive episodes (Diniz et al. 2013). It can be administered alone or in combination with other drugs, such as valproate and carbamazepine (Chiu et al. 2013). At therapeutic doses, these medications generally require 1 to 4 weeks before their full effects are observed (Bauer and Mitchner 2004). Moreover, all medications used for treating BD have several adverse effects (Torpy et al. 2009), which contributes negatively to low patient adherence to treatment

(Kasper 2014). Because BD is a chronic illness, continuous treatment is necessary to prevent relapse of manic or depressive symptoms, to improve overall health, and to maximize the quality of life.

In summary, although numerous mechanisms have been proposed to explain the pathophysiology of BD, the contribution of these mechanisms to specific symptomatology of this debilitating psychiatric ailment remains unknown. Additionally, several factors make the therapeutic management of BD complex, including the fluctuation in mood episodes and the effects of these episodes on the patient‟s well-being, treatment non-adherence, and comorbid psychiatric disorders. Thus, in this review, we will explore the therapeutic potential of natural compounds and derivatives in BD with emphasis on episodes of mania, taking into account the results of preclinical and clinical studies.

2. Evaluation of natural compounds and derivatives in patients with bipolar disorder

Accumulating evidence from several decades of research and utilization of several bioactive compounds from both plant and non-plant sources have led to the discovery of potential therapeutic agents. This has created opportunities for advancements in the prevention and treatment of psychiatric diseases. The effects of natural compounds and derivatives have been widely investigated in patients with psychiatric disorders such as major depression; however, little data exist about the beneficial effects of these compounds in patients with BD (Table 1). We will present an overview of advances made in this field over the last several decades, as well as the role of natural compounds and derivatives and their therapeutic potential in clinical studies involving individuals with BD.

2.1 *Omega-3 (ω3) fatty acid*

The benefits of omega-3 (ω3) fatty acids for mood disorders have been reported in the literature. ω3 fatty acids, especially those present in neuronal membranes, are essential for the proper functioning of the central nervous system (CNS). Moreover, ω3 deficiency has been associated with neuroinflammation, a condition present in BD. Accordingly, previous research on eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) has suggested that polyunsaturated fatty acids (PUFAs) may improve brain function in patients with BD (Vesco et al. 2015).

The effectiveness of ω3 fatty acids against manic episodes and other symptoms involved in BD have previously been evaluated in clinical studies. Vesco et al. (2015) demonstrated in a case involving ω3 supplementation (1000 mg in the morning and 1500 mg at night) that this fatty acid can be a safe adjuvant intervention for the treatment of BD in children and adolescents. A 2-year follow-up, beginning from the age of 12, offers hope of a lasting benefit, since a significant and sustained clinical improvement in depression and psychological symptoms was observed (Vesco et al. 2015). In addition, Osher et al. (2005) demonstrated that 8 of the 10 patients who completed at least 1 month of 1.5 to 2 g/day of ω3 supplementation achieved a 50% or greater reduction in Hamilton Rating Scale for Depression (HRSD) scores within 1 month (Osher et al. 2005). Similar results were demonstrated by Frangou et al. (2006), who showed that in a 12-week double-blind study involving individuals with bipolar depression, adjunctive treatment with 1 g/day or 2 g/day of ethyl-eicosapentaenoic acid (ethyl-EPA) significantly improved clinical symptoms compared with that of a placebo (Frangou et al. 2006).

Moreover, in children and adolescents with juvenile BD supplemented with 360 mg/day of EPA and 1560 mg/day of DHA for 6 weeks in an open-label study, the clinician ratings of mania and depression were significantly lower, and global functioning was significantly higher, after supplementation. In fact, a reduction in mania and depression in juvenile BD was associated with long-chain omega-3 polyunsaturated fatty acid supplementation (Clayton et al. 2009).

It should be noted that several studies in the literature have demonstrated the therapeutic potential of ω3 in neuropsychiatric diseases, especially due to its biological effects on the brain. For example, DHA constitutes approximately 12–15% by weight of the total fatty acids in the human brain (Layé et al. 2018; Stoffel and Levant 2018). Thus, this fatty acid might contribute to the maintenance of homeostasis in conditions where there is a disruption in the physicochemical properties of neuronal membranes, as has been reported in BD.

In addition, ω3 is known to be a potent anti-inflammatory agent. Considering that the inflammatory process participates in the pathophysiology of BD, the presence of compounds capable of minimizing neuroinflammation is crucial. A mechanism by which ω3 plays an anti-inflammatory role is through the downregulation of inflammatory gene expression, such as those of cytokines or enzymes involved in the synthesis of eicosanoids. In humans, ω3 consumption is

associated with a lower risk of inflammation-associated neurologic disorders (Layé et al. 2018; Healy-Stoffel and Levant 2018). For this reason, new studies aimed at investigating the neuroprotective effects of ω3 in patients with BD are extremely important and should be encouraged in order to improve the quality of life of patients with this pathology.

2.2 *Inositol*

Inositol is a natural compound found in several foods and is present in all animal tissues, mainly in the heart and brain. This compound constitutes the intracellular phosphatidylinositol, a second messenger system associated with neurotransmitter receptors that include serotonin, dopamine, and glutamate receptors. It is known that soluble inositol phosphate controls the release of Ca^{2+} from intracellular stores. Accordingly, inositol has been reported to be effective for treating patients with depression and has been tested as an alternative for BD patients (Formoso et al. 2019; Schneider 2015; Harwood 2005).

Chengappa et al. (2000) investigated the potential efficacy and safety of inositol in bipolar depression. Men and women with bipolar depression were randomly assigned to receive either 12 g of inositol or D-glucose as a placebo for 6 weeks. The authors found that 50% of patients treated with inositol responded with a 50% or higher reduction in the initial score of the Hamilton Depression Rating Scale and the Clinical Global Improvement scale (Chengappa et al. 2000). In addition, another study evaluated the efficacy of adjunctive inositol (5–20 g/day) for bipolar depression in a placebo-controlled trial study. Seventeen participants with bipolar depression were randomized to receive double-blind inositol or placebo for 6 weeks. The response to inositol was highly variable. Of the nine individuals that received inositol, two showed a worsening of > 50% in HRSD scores without the final treatment, three exhibited no changes, and four had an improvement > 50%, but there was no significant effect between groups. Based on these results, the authors suggested further studies to determine whether inositol can be effective in a subpopulation of patients that do not exhibit agitation or aggressive behavior and whether inositol may be more effective in treatment-resistant patients (Evins et al. 2006).

An important aspect to be taken into account is the reduction in inositol concentrations in the rat brain induced by mood stabilizers, such as lithium and

valproate. Therefore, the use of inositol as an adjuvant in the treatment of BD would be an interesting approach for the management of psychiatric patients. Furthermore, in recent years, antioxidant and anti-inflammatory effects exerted by this compound have been reported, which has increased interest in research studies of inositol in BD, since oxidative stress and inflammation are involved in the pathophysiology of this disorder (Formoso et al. 2018; Schneider 2015; Harwood 2005).

2.3 *N-acetylcysteine (NAC)*

N-acetylcysteine (NAC) is a thiol precursor of cysteine and reduced glutathione. NAC is a source of sulfhydryl groups in cells and a scavenger of free radicals as it interacts with reactive oxygen species (ROS). Considering the antioxidant effects of NAC, many studies have investigated its benefits involving multiple mechanisms of action across neurological disorders, including BD (Deepmala et al. 2015; Slattery et al. 2015; Samuni et al. 2013; Shahripour et al. 2013).

Berk et al. (2011) conducted a study with 132 participants with BD completing an 8-week open label treatment phase with administration of 1 g of NAC twice daily. Treatment with NAC reduced the Bipolar Depression Rating Scale (BDRS) score. However, a placebo arm, which is essential to make definitive conclusions regarding efficacy, was absent in this investigation (Berk et al. 2011). In another randomized, double-blind, multicenter, placebo-controlled study, 75 individuals with BD were treated with 1 g of NAC twice daily adjunctive to usual medication over 24 weeks. NAC treatment significantly improved clinical symptoms (Berk et al. 2008). There was no effect of NAC on time-to-mood episodes and no significant between-group differences in adverse events. Moderated functional outcomes, but not depression, were found in this protocol.

In 2012, Dean and colleagues conducted a randomized, double-blind, placebo-controlled trial study, and they reported that treatment with NAC compared with placebo did not prevent changes in cognitive function tested at baseline and after 6 months to assess changes in cognitive function following administration of either 2000 mg of NAC daily or a placebo. However, this study was conducted with a small sample size and a limited battery of cognitive tests, which limits the data obtained (Dean et al. 2012).

Some researchers developed a series of studies whose objective was to investigate the effects of NAC (500 mg) administration twice daily for 24 weeks in patients with BD. The main findings of the studies were as follows: (1) Six patients in the group treated with NAC (n = 7) showed total remission of depressive and manic symptoms. On the other hand, two patients in the placebo group also showed complete remission of depressive symptoms (n = 7 in the group placebo) (Magalhães et al. 2011a). (2) Eight of the ten participants who received NAC showed alleviation of clinical symptoms of depression, while only one of the seven participants allocated to the placebo showed the same result (Magalhães et al. 2011b). (3) The analysis of this study pointed to an improvement in the group that received NAC for manic symptoms and a worsening of depressive symptoms in the placebo group (Magalhães et al. 2013).

The main mechanisms underlying NAC function that have been investigated are related to glutathione, such as antioxidant properties and modulation of redox-regulated signal transduction associated mainly with its role in the storage and transport of cysteine, regulation of cell proliferation, immune responses, leukotriene and prostaglandin metabolism, detoxification of electrophilic xenobiotics, and synthesis of deoxyribonucleotides. In addition, several applications of NAC have been proposed especially considering its ability to minimize oxidative stress, infections, toxic assault, and inflammatory conditions (Deepmala et al. 2015; Slattery et al. 2015; Samuni et al. 2013; Shahripour et al. 2013). In view of all the effects mediated by NAC in addition to its clinical effects already demonstrated, this natural compound presents itself as a relevant therapeutic alternative for BD.

2.4 *Vitamin D*

1,25(OH)2D3 (vitamin D) has been recognized as a neurosteroid that modulates multiple brain functions and plays a pivotal role in brain development, neurotransmission, neuroprotection, and immunomodulation (Cui et al. 2017). Thus, this vitamin has been the subject of studies investigating many neurological disorders, including BD.

Vitamin D3 supplementation also appears to be a promising natural agent for the treatment of mania. BD patients supplemented with 2000 IU of vitamin D3 exhibited an improvement in mood symptoms in conjunction with their brain neurochemistry (Sikoglu et al. 2015). In recent years, several studies on the

neuroprotective effects of vitamin D have been carried out, including as a strategy for healthy brain aging, since augmenting brain BDNF appears to be a key mechanism through which this vitamin counteracts age-related brain dysfunction (Beckmann et al. 2019).

Changes in serum vitamin D levels in patients with BD have been previously reported in the literature. Altunsoy et al. (2018) showed an important correlation between vitamin D deficiency and acute manic episodes. Unfortunately, this interaction over long periods has not been demonstrated. Therefore, taking into account the data indicating that BD patients may be deficient in vitamin D, along with the promising and beneficial effects of this vitamin, improved studies that aim to investigate the potential of this compound in BD should be encouraged.

2.5 *Free and Easy Wanderer Plus*

Zhang et al. (2007a) demonstrated, through a systematic investigation, a number of beneficial effects of the herbal preparation Free and Easy Wanderer Plus (FEWP), a Chinese herbal medicine (36 g/day for 12 weeks) used as an adjunct to carbamazepine (CBZ) in the treatment of bipolar patients, compared to CBZ monotherapy and placebo. They suggested that adjunctive FEWP has additive beneficial effects in bipolar patients, particularly for those in the depressive phase (Zhang et al. 2007a). The same authors suggested that FEWP monotherapy may also be an effective alternative treatment for depressed conditions after 26 weeks of treatment with FEWP under double-blind conditions. In this study, they demonstrated that in addition to a significantly lower overall follow-up rate at the end point compared to placebo, patients who received FEWP in addition to CBZ showed significantly fewer adverse effects and lower serum CBZ levels than those on placebo. In addition, FEWP (36 g/day for 12 weeks) was used as monotherapy in patients with depression (Zhang et al. 2007b). The results suggest that adjuvant FEWP improves CBZ tolerability upon long-term use, in addition to its use as an effective alternative form of monotherapy for depressive conditions (Zhang et al. 2007b).

Clinical studies on the use of natural products in BD have been carried out in recent years, and it is understood that they are extremely important. However, these studies are limited in their ability to analyze clinical symptoms that are often widely variable. Furthermore, they face an important factor in the impossibility of evaluating

neurochemical parameters. For this reason, studies involving preclinical models are still relevant to the search for new therapeutic alternatives for neuropsychiatric diseases such as BD.

2.6 *Withania somnifera*

Cognitive impairment in patients with BD contributes to inadequate functional recovery. However, despite this knowledge, the mechanisms behind cognitive reduction are still poorly understood, and sometimes end up being neglected during pharmacological treatment (Chengappa et al. 2013). For this reason, Chengappa et al. (2013) investigated the effects of *Withania somnifera* (WSE) extract, which is known to increase body resistance to stress and diseases. Sixty euthymic subjects with DSM-IV bipolar disorder were enrolled in an 8-week, double-blind, placebo-controlled, randomized study of WSE (500 mg/day) as a procognitive agent added adjunctively to medications being used as maintenance treatment for BD. The authors observed that treatment with WSE provided significant benefits for three cognitive tasks: digit span backward, flanker neutral response time, and the social cognition response rating of the Penn Emotional Acuity Test compared with the control group (Chengappa et al. 2013).

Thus, the authors reported that WSE would be an interesting alternative for managing cognitive changes in patients with BD. Corroborating with the beneficial effects observed in this clinical study, preclinical studies with rodents have reported that WSE is able to protect against memory deficits in addition to inducing regeneration of dendritic spines and axons in mice, and that WSE attenuates damage to hippocampal neurons in the CA2 and CA3, decreases hypercortisolemia, and shows procholinergic but not glutamatergic or GABAergic effects in the rat brain (Chengappa et al. 2013). These beneficial effects are possibly associated with some bioactive components found in the WSE extract, such as steroidal lactones, termed glycowithanolides and sitoindosides, as well as Withaferin, which has antioxidant and neuroprotective properties (Chengappa et al. 2013).

Table 1- Clinical studies with natural compounds and derivatives in Bipolar disease (BD).

Compound	Reference	Protocol	Effects
Omega-3 (ω3) fatty acid	Vesco et al. (2015)	1000 mg in the morning and 1500 mg at night for 2 years	Improves clinical depression and psychological symptoms
	Clayton et al. (2009)	360 mg/day of EPA and 1560 mg/day of DHA for 6 weeks	Improves clinical ratings of mania and depression
	Frangou et al. (2006)	1 g/day or 2 g/day for 12 week	Improves clinical symptoms compared with placebo
	Osher et al. (2005)	1.5 to 2 g/day for month	Improves the Hamilton Rating Scale for Depression (HRSD) score
Inositol	Eden Evins et al. (2006)	5–20 g/day for 6 weeks	Improves the Hamilton Rating Scale for Depression (HRSD) scores; no significant effect was found between treated and placebo groups.
	Chengappa et al. (2000)	12 g/day for 6 weeks	Reduces the initial score of the Hamilton Depression Rating Scale and the Clinical Global Improvement scale; no significant effect was found between treated and placebo groups
N-acetylcysteine (NAC)	Dean et al. (2012)	2000 mg daily for 6 months	Did not prevent changes in cognitive function tested
	Magalhães et al. (2011a)	Two NAC (500 mg) capsules twice daily for 24 weeks	Remission of depressive and manic symptoms
	Magalhães et al. (2011b)	Two NAC (500 mg) capsules twice daily for 24 weeks	Improves the clinical symptoms of depression
	Magalhães et al. (2013)	Two NAC (500 mg) capsules twice daily for 24 weeks 1 g twice daily for 8 weeks	Improves manic symptoms Reduces the Bipolar Depression Rating Scale

			(BDRS) score
	Berk et al. (2011) Berk et al. (2008)	1 g twice daily for 24 weeks	Improves clinical symptoms; there was no effect of NAC on time-to-mood episode
Vitamin D	Sikoglu et al. (2015)	2000 IU/day for 8 weeks	Improves mood symptoms and brain neurochemistry changes
Free and Easy Wanderer Plus	Jin Zhang et al. (2005)	36 g/day for 12 weeks	Ameliorates the depressive phase
Withania somnifera	Chengappa et al. 2013	500 mg/day for 8 weeks	Improves cognitive impairment

3. Evaluation of natural compounds and derivatives in experimental models of mania

3.1 Characteristics of manic episodes and experimental models

A manic episode is defined as a distinct period during which patients experience an abnormally and persistently raised, expansive, or irritable mood. These changes must be present for a period of one week or less. During this period, other symptoms are also observed, such as inflated self-esteem, psychomotor agitation, distractibility, involvement in activities with potential for painful consequences, and less need for sleep. The mood disorder is severe enough to cause impairments in occupational or social functioning, or to require hospitalization (Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition - DSM-5).

Although manic episodes and hypomanic episodes have many similar symptoms, mood disturbances in hypomanic episodes are not sufficiently severe to cause pronounced impairment in social or occupational functioning. Mania and hypomania are two distinct types of episodes, but they have the same symptoms; however, mania is more severe than hypomania (Mcintire et al., 2020).

Animal models are essential tools in preclinical research since they promote the advancement of knowledge of the neurobiology of several diseases. However, establishing an adequate model for BD is difficult because of its cyclical nature (Beyer and Freund 2017). It is also worth noting that some symptoms used to diagnose psychiatric illnesses in humans are not possible to assess in animals (Nestler and Hyman 2010). Because manic episodes are decisive for the diagnosis of BD, animal models that mimic this complex behavioral state are widely used to study this disease (Sharma et al. 2016; Beyer and Freund 2017).

It should be noted that several criteria exist for the evaluation of animal models for neuropsychiatric disorders. The animal models must meet these criteria, which demonstrate the relevance of translating experimental findings, in order to be validated. Briefly, animal models must have similarities with the symptoms of the disease (face validity), resemble pathophysiological aspects of the disease (construct validity), and respond to existing clinically successful treatments (predictive validity) (Willner 1984; Nestler and Hyman 2010; Valvassori et al. 2013).

Table 2 describes animal models of mania. It is worth mentioning that this table does not describe all existing mania models, but only those that satisfy the three criteria necessary to be validated (Sharma et al. 2016). Manic-like behavior in animals can be induced by different stimuli and divided into three types: pharmacological, environmental, and genetic. Table 2 displays the neurochemical mechanism that mimics the manic episode of each animal model, the behaviors used to evaluate that episode, and the referenced study. In addition, it should be noted that different tests were performed to assess the behavior of the animals. However, all the authors assessed locomotor behavior, making it possible to examine hyperactivity, one of the symptoms of this disease that can be reproduced in animal models (Ghedim et al. 2012).

Table 2- Animal models of mania.

Animal model	Neurochemical mechanism	Behavioral test	References
Pharmacological			
Psychostimulant	Increase in dopamine (DA) efflux, inhibition of DA reuptake, or DA degradation by the enzyme monoamine oxidase (MAO).	Open-field test.	Frey et al. (2006).
Ouabain	Sodium potassium-activated adenosine triphosphatase (Na^+,K^+-ATPase) inhibitor.	Open-field test.	El-Mallakh et al. (1995).
Ketamine	Non-competitive NMDA glutamate receptor antagonist.	Open-field test.	Ghedim et al. (2012).
Environmental			
Sleep deprivation	Circadian rhythm disturbances.	Open-field test and aggressive behavior.	Benedetti et al. (2008).
Genetic			
Dopamine transporter (DAT)	Inactivation of the DAT causing alterations in DA transmission.	Open-field test.	Giros et al. (1966).
Circadian locomotor output cycle kaput (CLOCK)	Abnormalities in the circadian system.	Intracranial self-stimulation, sucrose preference, Forced swim test, Open-field test, Elevated plus maze test, Reward/Aversion Test, Learned helplessness following inescapable shock.	McClung et al. (2005). Roybal et al. (2007).
Glutamate receptor 6 (GluR6)	GluR6 gene knockout.	Passive avoidance test, Open-field test, Home cage activity, Social interaction, Elevated plus-maze, Forced swim test, Resident-intruder.	Shaltiel et al. (2008).
Extracellular signal-regulated kinase 1 (ERK1)	Disruption of the ERK1 gene.	Elevated plus-maze test, Forced swim test, Exploratory behavior, Long-term home-cage voluntary wheel running activity, Behavioral response to amphetamine, Monitoring the behavioral changes drug-induced.	Engel et al. (2009).
Postsynaptic density protein	Overexpression of SHANK3.	Open-field test, Home-cage activity, Tail suspension test,	Han et al. (2013).

SHANK3		Acoustic startle response and prepulse inhibition, Circadian rhythms, Three chamber test, Grooming, Ultrasonic vocalization, EEG measurement.	
Neuron-specific α-3 subunit (ATP1A3)	Mutation in the neuron-specific NKA α3 isoform.	Open-field test, Forced swim test, Elevated plus-maze test, Light-Dark Box, Head Tracking, Object and Holeboard Exploration.	Kirshenbaum et al. (2011).
		Sucrose Preference Test, Acoustic Habituation, Startle, and PPI, EEG and Electromyographic Recordings of Sleep, Circadian Rhythms, Electrocorticography.	
B-cell lymphoma 2 (Bcl-2)	Bcl-2 heterozygote knockout.	Open-field test, Black/white box test, Forced swim test, Saccharin preference, Elevated plus-maze test, Resident-intruder test, Acute amphetamine response, Amphetamine sensitization.	Lien et al. (2008).

3.2 Evaluation of natural compounds and derivatives in preclinical studies of mania

Experimental preclinical studies have revealed a range of complex psychotropic activity in natural compounds that are potentially beneficial for treating certain psychiatric conditions, such as manic episodes. Because oxidative stress has been associated with the etiology of BD, many experimental studies have focused on the use of antioxidants in the prevention of experimental manic episodes (Figure 2).

3.2.1 Berry fruit extracts

Blueberry and blackberry fruit extracts, which are rich in polyphenols, have been investigated as therapeutic tools in animal models of mania. In a rat model of manic behavior induced by ketamine, Debom et al. (2016) and Spohr et al. (2019) reported that blueberry extract (*Vaccinium virgatum)* (200 mg/kg, administered for 14 days by gavage) prevented the manic-like behavior and attenuated oxidative damage in cerebral cortex, hippocampus, and striatum (Debom et al. 2016, Spohr et al. 2019)

similarly to lithium treatment. In addition, blueberry extract was able to prevent the increase in Na^+, K^+-ATPase, and acetylcholinesterase (AChE) activity induced by ketamine in these brain regions (Spohr et al. 2019). Another similar study, using the mania model induced by ketamine, also showed that blackberry extract (*Rubus* sp.) (200 mg/kg) prevented hyperlocomotion and oxidative brain damage in the cerebral cortex, hippocampus, and striatum (Chaves et al., 2020). In addition, both blueberry and blackberry extracts also modulated the serum and brain IL-6 levels in the ketamine mania model (Debom et al. 2016; Chaves et al. 2020). These beneficial effects of these fruit extracts can be attributed to the presence of a large quantity of anthocyanins, a subset of flavonoids, which are important compounds with antioxidant and anti-inflammatory actions.

Anthocyanins are the most abundant polyphenols in many red, blue, and purple fruits (Smeriglio et al. 2016), and appropriate intake of these phytochemicals is thought to promote health conditions and general well-being (Smeriglio et al. 2016; Fraga et al. 2019), as demonstrated in some studies examining brain health in humans (Boespflug et al. 2018; Whyte et al. 2018). In fact, these red fruit extracts have been shown to modulate pathways involved in BD, and consumption of these extracts could be a useful alternative to prevent or reduce manic episodes, demonstrating its neuroprotective role in the central nervous system as a natural therapeutic approach (Debom et al. 2016, Spohr et al. 2019, Chaves et al. 2020).

3.2.2. Cecropia pachystachya extract

Cecropia pachystachya is a plant that grows in the rainforests of South America and is popularly known in Brazil as embauba. It has been documented in the literature that *Cecropia pachystachya* presents many beneficial actions such as hypoglycemic (Aragão et al. 2010), antidepressant (Gazal et al. 2014), anti-inflammatory, and antioxidant (Pacheco et al. 2014) actions. Gazal et al. (2015) demonstrated that *Cecropia pachystachya* aqueous extract (200 and 400 mg/kg) administered for 14 days prevented hyperlocomotion and oxidative brain damage in the hippocampus and prefrontal cortex in a mania model induced by ketamine. These beneficial effects have been attributed to phytochemical compounds present in the extract used, such as chlorogenic acid, isoorientin, orientin, isovitexin, and isoquercetin (Gazal et al. 2015).

3.2.3 Curcumin

Isolated natural compounds have also been investigated as therapeutic alternatives in different animal models of mania. Gazal et al. (2014) demonstrated that pretreatment with curcumin (20 and 50 mg/kg) by the oral route for 14 days prevented the manic-like behavior caused by ketamine. This phytochemical decreased lipid peroxidation and increased thiol levels and the activity of antioxidant enzymes such as superoxide dismutase and catalase in the brain. The authors concluded that *Curcuma longa* prevented oxidative damage, demonstrating its therapeutic potential in prophylactic interventions during the manic phase (Gazal et al. 2014). Notably, curcumin regulates multiple targets that include transcription factors activated by environmental stressors and pro-inflammatory cytokines, protein kinases, enzymes, growth factors, inflammatory mediators, and anti-apoptotic proteins (Brietzke et al. 2013). Moreover, this compound has been studied in different disorders in preclinical models, such as Alzheimer‟s disease and Parkinson‟s disease, and has demonstrated significant neuroprotective potential (Bassani et al. 2017; Sharma & Nehru 2018).

3.2.4 Quercetin

The antimanic-like effect of quercetin has been demonstrated in studies by Kanazawa and collaborators (2016, 2017). Quercetin, a flavonoid that possesses antioxidant properties and inhibits protein kinase C, prevented manic-like behavior induced by 24 h paradoxical sleep deprivation (PSD) (10 or 40 mg/kg, i.p. during an interval of 24 h) and by methylphenidate (5 mg/kg, i.p.)-induced hyperlocomotion (2.5, 5, 10, and 40 mg/kg, i.p. for 21 days) (Kanazawa et al. 2016, 2017). In both protocols, quercetin was able to protect against oxidative damage in several brain regions. These behavioral and neurochemical effects of quercetin appeared to be correlated with its ability to reduce oxidative stress. In addition, no tolerance was affected after repeated treatment, which is an important issue for the treatment of mania (Kanazawa et al. 2016, 2017). Since an increase in PKC and oxidative stress has been related to mania, compounds with antioxidant effects and/or inhibitory actions on PKC are thought to be important for preventing manic effects. Quercetin exhibits these two properties, which makes it an important target for the study of this pathology (Kanazawa et al. 2016, 2017).

3.2.5 Alpha-Lipoic acid (ALA)

Alpha-lipoic acid (ALA) is a natural compound synthesized in plants and animals in the mitochondrion from octanic acid, and has been identified as a catalytic agent for oxidative decarboxylation of pyruvate and α-ketoglutarate. ALA is also absorbed intact from dietary sources and transiently accumulates in many tissues. It has been described as a potent antioxidant compound and a modulator of inflammatory pathways. Macêdo et al. (2012) used an amphetamine-induced (AMPH) model of mania in mice to investigate the preventive and reversal effects of oral administration of ALA (50 or 100 mg/kg). ALA prevented and reversed AMPH-induced hyperlocomotion and oxidative damage in the brain in both protocols. Moreover, ALA in the reversion model increased BDNF levels in the hippocampus. These findings showed that ALA was effective in reversing and preventing AMPH-induced behavioral and neurochemical damage, possibly by scavenging free radical and lipid peroxidation products. Therefore, this compound demonstrated antimanic-like effects and could be considered an adjunct treatment for BD (Macêdo et al. 2012).

3.2.6 Monoterpenes

Nogoceke et al. (2016) evaluated (R)-(−)-carvone and (S)-(+)-carvone, two types of monoterpene that are present in spearmint (*Mentha spicata*) and caraway (*Carum carvi*) essential oils, respectively. Two protocols of mania were executed in mice: acute and chronic (21 days) carvone (50 and 100 mg/kg, i.p.) treatment in a model induced by methylphenidate (5 mg/kg, i.p.), and acute carvone treatment during sleep deprivation. Acute and chronic treatment with (S)-(+)-carvone (S-100 mg/kg) and (R)-(−)-carvone (R-100 mg/kg) in the methylphenidate model was able to decrease mouse locomotion. S-100 and R-100 also blocked sleep deprivation-induced hyperactivity. The antimanic-like action of both enantiomers of carvone in two different protocols minimized the probability of false-positive results, which was a significant behavioral outcome. The mechanism of action of carvone might be related to the blockade of voltage-gated sodium channels and/or GABAergic activity, which are related to other biological effects of this compound, such as anticonvulsant and antinociceptive actions (Nogoceke et al. 2016).

3.2.7 Omega-3 (ω3) fatty acid

Omega-3 (ω3) is an essential fatty acid derived from docosahexaenoic acid (DHA), which plays an important role in phospholipid composition, and is thus involved in cellular protection (Wall et al. 2010). The effect of ω3 fatty acids has also been investigated in a model of mania induced by methylphenidate (MPD 5 mg/kg, i.p.). Treatment with ω3 and ω3 + lithium + aripiprazole protects against the MPD-induced decrease in succinate dehydrogenase, malate dehydrogenase, and α-ketoglutarate dehydrogenase activity in the brain. Changes in the tricarboxylic acid (TCA) cycle is thought to alter brain metabolism and enhance free radical production, contributing to the physiopathology of manic-like behavior. With regard to AChE activity, an excess of acetylcholine can also disrupt brain mitochondrial activity and stimulate oxidative stress. Interestingly, the activity of these enzymes had a more promising effect in a group treated with ω3 associated with standard drugs for BD. ω3 supplementation can thus be considered in therapies, in combination with atypical antipsychotics and mood stabilizers, in order to optimize the clinical effectiveness of conventional treatment (Arunagiri & Balamurugan 2016).

Cancelier et al. (2016) also investigated ω3 fatty acid treatment in animals subjected to induced mania. Rats were treated with ω3 (0.8 g/kg) by oral gavage, by itself or associated with either lithium (47.5 mg/kg i.p.) or valproate (VPA, 200 mg/kg i.p.) once a day for 14 days. Between days 8 and 15, animals received one daily injection of fenproporex (FEN, 12.5 mg/kg) or vehicle. In the reversal treatment, animals received FEN for 15 days, and between days 8 and 15 received the ω3 by oral gavage, by itself or associated with either lithium or VPA once a day (Cancelier et al. 2016). ω3 treatment co-administered with mood stabilizers prevented and reversed the effects of fenproporex on energy metabolism, as evaluated by succinate dehydrogenase (SHD) activity and mitochondrial respiratory chain complexes (II and IV) in the hippocampus, and by examining behavior parameters. This study reinforces the notion that this natural compound can exert protective effects on energy metabolism through its antioxidant capacity and may be useful for the adjuvant treatment of BD (Cancelier et al. 2016).

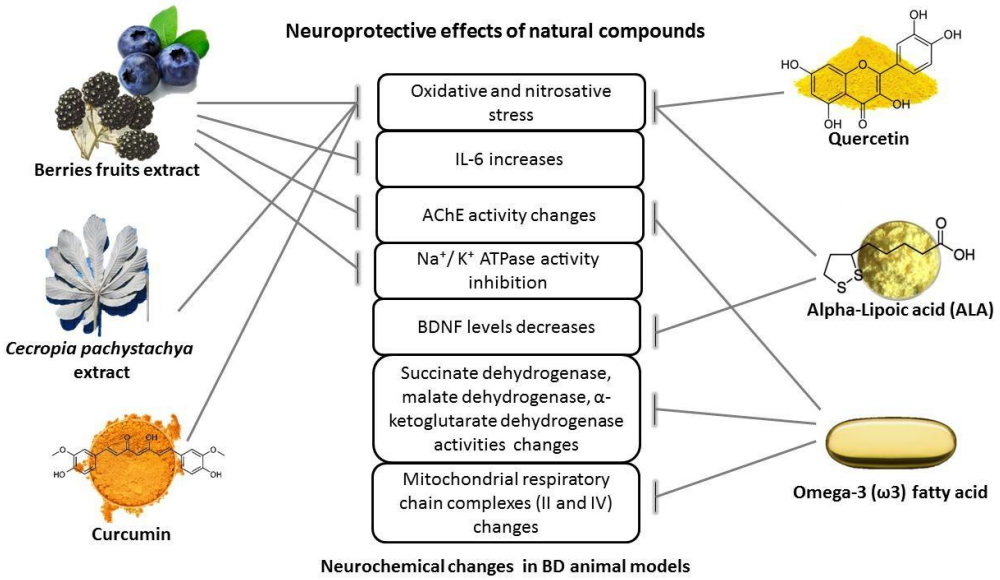

Fig. 2 Neuroprotective effects of natural compounds in neurochemical changes found in mania experimental models

Conclusions

BD is a complex medical condition that usually requires lifelong treatment. Preclinical and clinical studies have confirmed the potential of natural compounds and derivatives in both the prevention and management of BD phases. These compounds are beneficial in alleviating symptoms in patients as well as exerting neuroprotective effects in manic animal models through antioxidant, anti-inflammatory, anticholinesterase, and neurotrophic pathways. Due to the limited effectiveness and safety of conventional drugs used in the treatment of BD, there is an urgent need for more effective, better-tolerated treatments without harmful side effects. Thus, natural compounds and derivatives have emerged as potential neuroprotective agents for BD; however, many aspects remain to be explored both at clinical and preclinical levels, especially during the manic phase.

Funding

We thank the Conselho Nacional de Desenvolvimento Científico e Tecnológico and Fundação de Amparo à Pesquisa do Rio Grande do Sul. This study was also financed in part by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior-Brazil (CAPES - Finance code 001).

Acknowledgments

We would like to thank Editage (www.editage.com) for English language editing.

Conflicts of interest

The authors declare that there are no conflicts of interest.

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4. 2 Artigo I

Gallic acid protects cerebral cortex, hippocampus, and striatum against oxidative damage and cholinergic dysfunction in an experimental model of manic-like behavior: comparison with lithium effects.

Publicado no periódico *International Journal of Developmental Neuroscience*
Doi: 10.1002/jdn.10086

Received: 30 August 2020 | Revised: 1 December 2020 | Accepted: 30 December 2020

DOI: 10.1002/jdn.10086

RESEARCH ARTICLE

INTERNATIONAL JOURNAL OF DEVELOPMENTAL NEUROSCIENCE

WILEY

Gallic acid protects cerebral cortex, hippocampus, and striatum against oxidative damage and cholinergic dysfunction in an experimental model of manic-like behavior: Comparison with lithium effects

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Funding information
This research was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico and Fundação de Amparo à Pesquisa do Rio Grande do Sul (FAPERGS). This study was financed in part by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior—Brasil (CAPES)—Finance code 001. R.M.S is a recipient of the CNPq fellowship (309299/2017-9).

Abstract

Bipolar disorder is characterized by episodes of depression and mania, and oxidative stress has been associated with the observed neurochemical changes in this disease. We evaluated the effects of gallic acid on hyperlocomotion, acetylcholinesterase activity, and oxidative stress in an animal model of ketamine-induced mania. Rats were pretreated orally with vehicle, gallic acid (50 or 100 mg/kg), or lithium (45 mg/kg twice a day) for 14 days. Between days 8 and 14, the animals also received ketamine (25 mg/kg) or saline daily. On the 15th day, hyperlocomotion was assessed, following which the animals were euthanized, and brains were collected. Results showed that ketamine-induced hyperlocomotion and caused oxidative damage by increasing reactive oxygen species levels, lipid peroxidation, and nitrite levels, and decreasing the total thiol content and the activities of catalase, superoxide dismutase, and glutathione peroxidase in the brain. Pretreatment with gallic acid and lithium prevented hyperlocomotion and brain oxidative damage. Further, ketamine increased the acetylcholinesterase activity in the hippocampus and striatum, whereas gallic acid and lithium ameliorated this alteration. Thus, gallic acid may provide effective protection against manic-like behavior by reducing oxidative stress and preventing cholinergic signaling dysfunction in the brain regions involved in emotion regulation.

KEYWORDS

acetylcholinesterase, bipolar disorder, brain, gallic acid, ketamine, oxidative stress

1 | INTRODUCTION

Bipolar disorder (BD) is a chronic disease characterized by recurrent episodes of depression and mania (Carvalho et al., 2020). The exact mechanisms underlying the pathophysiology of BD are not completely understood. Several hypotheses have been postulated and they include genetic factors, inflammation, alterations in neurotransmitters, and oxidative stress (Andreazza et al., 2008; Price & Marzani-Nissen, 2012; Saunders & Geddes, 2016).

Oxidative stress occurs when there is an imbalance between prooxidant levels and antioxidant capacity, causing damage to cellular components such as proteins, lipids, and DNA (Jones, 2008). In fact, increased lipid peroxidation and decreased antioxidant defense have been described in BD patients (Chowdhury et al., 2017; Mansur et al., 2016), indicating that oxidative stress is involved in the neurochemical changes observed in this psychiatric disorder.

Dysfunction of the cholinergic signaling has also been associated with BD pathogenesis (Gibbons et al., 2009). Cholinergic neurotransmission induced by acetylcholine (ACh) occurs through nicotinic and muscarinic receptors, and is mainly terminated by the action of acetylcholinesterase (AChE). Apart from catalytic function, AChE also has potent effects on synaptic development as well as growth and maintenance of neurites (Silman & Sussman, 2005). AChE inhibitors have been used to increase synaptic levels of ACh in neurodegenerative diseases (Galimberti & Scarpini, 2016). However, there is conflicting evidence regarding the efficacy of AChE inhibitors in BD patients (Veronese et al., 2016), and the role of this enzyme in experimental models of mania (Spohr et al., 2019).

Lithium is the main pharmacological agent used for the treatment of BD (Price & Marzani-Nissen, 2012). The mechanism of action of lithium is not well understood. Lithium possesses anti-suicidal properties, and lithium treatment reduces cognitive decline and preserves the volume of the brain regions involved in emotion regulation (Malhi et al., 2013). Interestingly, at cellular levels, lithium inhibits glycogen synthase kinase 3β, regulates neurotransmitter levels, and reduces oxidative stress (Andreazza et al., 2008; Malhi et al., 2013). However, it has been noted that one-third of BD patients do not respond to lithium treatment (Miklowitz & Johnson, 2006). In addition, the side effects commonly associated with lithium use result in a low treatment adherence (Gitlin, 2016). Thus, it is important to explore new therapeutic approaches for managing mood episodes associated with BD.

Gallic acid (3,4,5-trihydroxybenzoic acid) is an important polyphenolic compound found in plants, fruits, and some processed beverages such as wine and green tea (Choubey et al., 2015). Gallic acid has antiviral, antitumor, and neuroprotective properties (Lu et al., 2006; Verma et al., 2013). Of particular importance is its ability to provide efficient protection against oxidative damage by acting as a versatile scavenger of reactive oxygen species (ROS) (Badhani et al., 2015; Marino et al., 2014). Studies have also related the anti-inflammatory property of gallic acid (Bai et al., 2021) and its ability to modulate signaling pathways to neuronal survival and proliferation, such as the Akt-mTOR pathway (Zu et al., 2018).

Considering the complexity of BD, experimental models are of great importance to understand this disease. A previous study showed that glutamatergic system abnormalities are associated with the pathophysiology of BD (Chen et al., 2010). Ketamine is a dissociative anesthetic that acts as a noncompetitive antagonist of the NMDA glutamate receptor. Thus, at non-anesthetic doses, ketamine administration induces manic-like behavior in rats, as demonstrated by hyperlocomotion (Chaves et al., 2020; Debom et al., 2016; Ghedim et al., 2012; Spohr et al., 2019).

Since oxidative stress and cholinergic dysfunction have been associated with BD pathogenesis, this study aimed to investigate the protective effects of gallic acid on hyperlocomotion, brain oxidative stress, and AChE activity in rats with manic-like behavior induced by ketamine.

2 | MATERIALS AND METHODS

2.1 | Animals

Adult male Wistar rats (60 days old, 250–300 g) were obtained from the Central Animal House of the Federal University of Pelotas and housed in standard cages at an ambient temperature of 23 ± 1°C, with 12 hr light/dark cycles and ad libitum access to water and food. All animal procedures were approved by the Committee of Ethics and Animal Experimentation of the institution (CEEA 4609–2015).

2.2 | Animal model of mania and gallic acid treatment

Rats were divided into following groups ($n = 10$ per group): I (saline), II (gallic acid 50 mg/kg), III (gallic acid 100 mg/kg), IV (ketamine 25 mg/kg), V (ketamine 25 mg/kg + gallic acid 50 mg/kg), VI (ketamine 25 mg/kg + gallic acid 100 mg/kg), and VII (ketamine 25 mg/kg + lithium 45 mg/kg twice a day). Rats in groups II, III, V, and VI received gallic acid (Sigma, 98.5% purity) orally. The animals in groups I and IV received the same volume of saline solution while the animals in group VII received lithium. From 8th to 14th day, the animals in groups IV, V, VI, and VII also received ketamine intraperitoneally, while rats in groups I, II, and III received vehicle.

On the 15th day of treatment, the animals in groups IV, V, VI, and VII received a single injection of ketamine, whereas animals in groups I, II, and III received the same volume of saline solution. Thirty minutes later, locomotor activity was assessed in an open field apparatus (Figure 1). The dose of gallic acid and ketamine used were based on previous studies (Debom et al., 2016; Mansouri et al., 2013). In order to follow the principles of the Three Rs (replacement, reduction, and refinement) in relation to animal experimentation, this study did not evaluated the per se effect of lithium. Previous studies using the same animal model of mania showed that lithium per se did not induce changes in behavior and brain oxidative stress parameters (Debom et al., 2016).

2.3 | Open field test

Locomotor behavior was evaluated using the open field test (Spohr et al., 2019). The apparatus consisted of a wooden box measuring 72 × 72 × 33 cm (width × length × height), with the floor divided into 16 equal squares (18 × 18 cm). Locomotion was assessed as the number of quadrants crossed within a period of 5 min. The apparatus was cleaned with 40% of ethanol and dried after each session.

2.4 | Brain tissue preparation

After the locomotor test, the animals were euthanized. Cerebral cortex, striatum, and hippocampus were collected and homogenized in sodium phosphate buffer (pH 7.4) containing KCl (1:10, w/v). The homogenates were centrifuged at 3,500 *g* for 5 min at 4°C and the supernatant was collected for oxidative stress analysis. Protein concentration was measured according to the methods described (Bradford, 1976; Lowry et al., 1951).

2.5 | Brain oxidative stress evaluation

2.5.1 | Reactive oxygen species (ROS) assay

The oxidation of DCFH-DA to fluorescent 2′,7′-dichlorofluorescein (DCF) was used to assess intracellular ROS, which was determined as described by Ali et al. (1992) and expressed as µmol DCF/mg of protein.

2.5.2 | Nitrite levels estimation

Nitrite levels were assessed using the method described by Stuehr and Nathan (1989). Briefly, 50 µl of sulfanilamide in 5% of phosphoric acid was added to 50 µl of the supernatant. After 10 min, samples were mixed with 100 µl of N-(1-naphthyl)ethylenediamine dihydrochloride and incubated for 10 min in dark. Results were expressed as µmol nitrite/mg of protein.

2.5.3 | Thiobarbituric acid reactive substances (TBARS) quantification

TBARS were quantified according to the method described by Esterbauer and Cheeseman (1990). Homogenates were mixed with 15% of trichloroacetic acid and 0.67% of

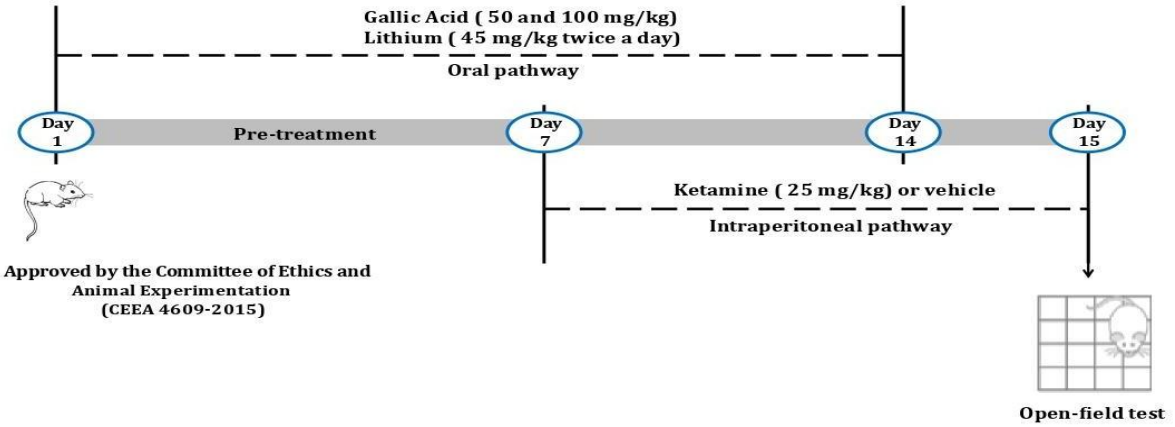

FIGURE 1 Development of the animal model of ketamine-induced mania and treatment protocol using gallic acid (50 or 100 mg/kg) or lithium (45 mg/kg twice a day)

thiobarbituric acid. This mixture was heated for 30 min at 95°C, and then, cooled for 10 min. Results were expressed as nmol TBARS/mg of protein.

2.5.4 | Total sulfhydryl content (SH content) quantification

The SH content was evaluated as described by Aksenov and Markesbery (2001). This method is based on the reduction of 5,5′-Dithiobis(2-nitrobenzoic acid) (DTNB) by thiols, which themselves are oxidized (disulfide) generating a yellow derivative whose absorbance is measured at 412 nm. Results were expressed as nmol TNB/mg of protein.

2.5.5 | Superoxide dismutase (SOD) activity

This assay is based on the inhibition of adrenaline autoxidation in a spectrophotometer at 480 nm. Total SOD activity was measured as described by Misra and Fridovich (1972) and expressed as units/mg of protein.

2.5.6 | Catalase (CAT) activity

CAT activity was assessed using the method described by Aebi (1984). The reduction in the amount of H_2O_2 was monitored for 180 s at 240 nm. The specific activity was expressed as units/mg of protein.

2.5.7 | Glutathione peroxidase (GPx) activity

GPx enzyme activity was measured using a commercial kit (RANSEL®; Randox Lab, Antrim, UK) and expressed as units per mg protein (U/mg of protein).

2.6 | Acetylcholinesterase (AChE) activity

AChE activity was determined as described by Ellman et al. (1961). The reaction system, composed of 10 mM DTNB, 100 mM phosphate buffer, and 15 µl homogenate was incubated for 2 min at 27°C. After incubation, 8 mM acetylthiocholine (AcSCh) was added to the reaction. AChE activity was expressed as µmol AcSCh/h/mg of protein.

2.7 | Statistical analysis

Data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparison test. Results were expressed as mean ± standard error (S.E.M) and considered significant for $p < .05$.

3 | RESULTS

Ketamine-induced hyperlocomotion in rats ($F_{(6,64)} = 29.10$, $p < .001$), as demonstrated by an increased number of crossings in the open field test. Similar to lithium, pretreatment with gallic acid attenuated this behavioral change (Figure 2).

With regard to oxidative stress parameters, our results showed that ketamine administration increased ROS ($F_{(6,38)} = 16.99$, $p < .001$) and TBARS levels ($F_{(6,38)} = 7.66$, $p < .001$) in the cerebral cortex. Gallic acid (both doses) and lithium pretreatment prevented this changes (Figure 3). On the contrary, ketamine decreased the total sulfhydryl content in the cerebral cortex ($F_{(6,35)} = 11.23$, $p < .001$), and gallic acid (100 mg/kg) and lithium pretreatment prevented this alteration (Figure 3). No changes were observed in nitrite levels in the cerebral cortex of all experimental groups evaluated in this study. Ketamine also decreased the SOD ($F_{(6,37)} = 7.76$, $p < .001$) and CAT ($F_{(6,35)} = 6.35$, $p < .001$) activities in the cerebral cortex; gallic acid, and lithium administration prevented these alterations. In addition, the administration of

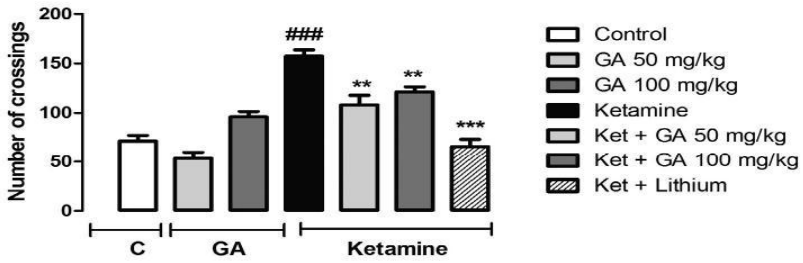

FIGURE 2 Effects of pretreatment with gallic acid (50 or 100 mg/kg) or lithium (45 mg/kg twice a day) on ketamine-induced hyperactivity in rats evaluated using the open field test. Data are expressed as mean ± S.E.M. $^{###}p < .001$ when compared to control group. $^{**}p < .01$ and $^{***}p < .001$ when compared to the vehicle/ketamine group ($n = 9–10$)

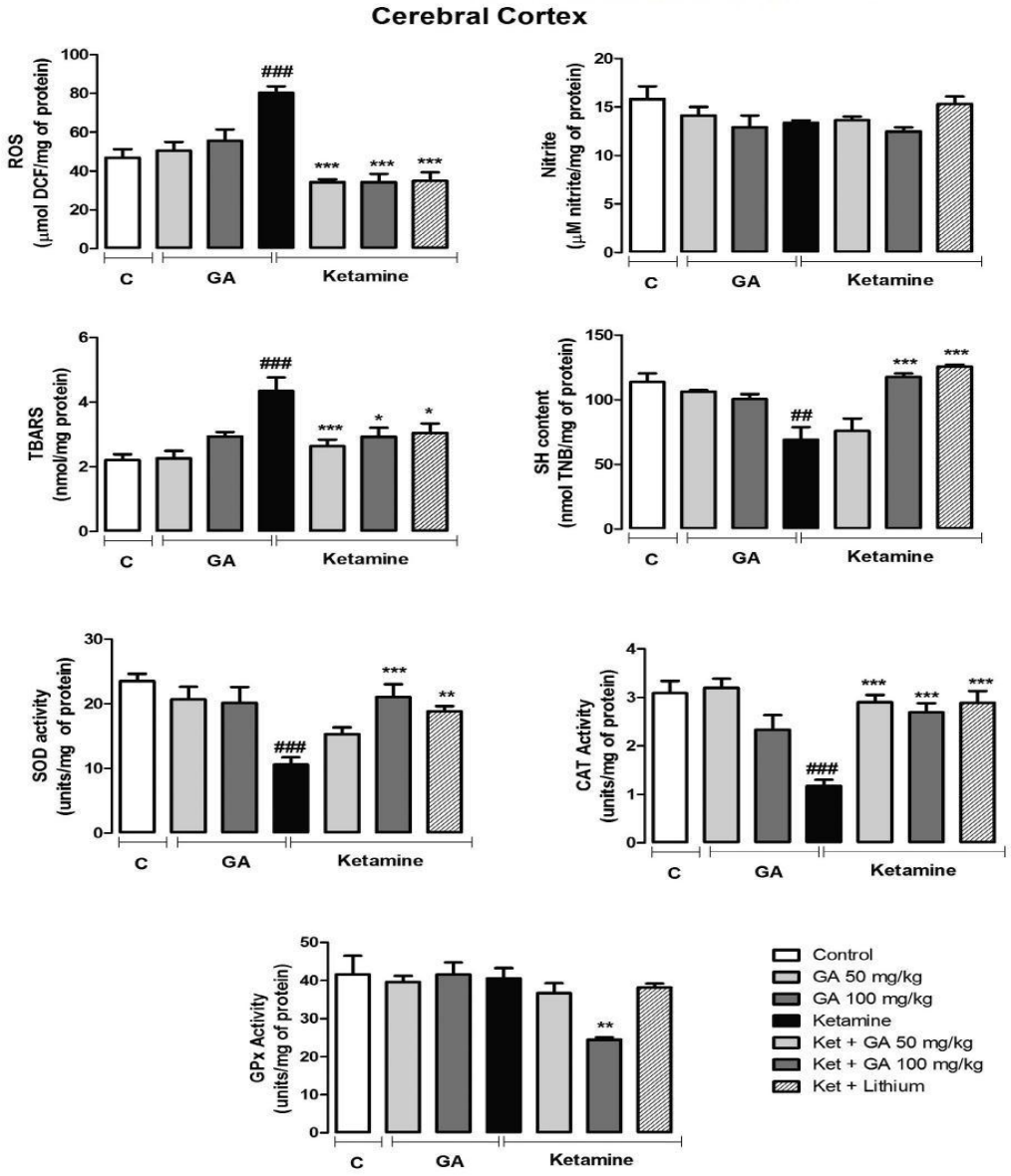

FIGURE 3 Effects of pretreatment with gallic acid (50 or 100 mg/kg) or lithium (45 mg/kg twice a day) on oxidative stress parameters in the cerebral cortex of animals exhibiting manic-like behavior induced by ketamine. Reactive oxygen species (ROS), nitrite levels, thiobarbituric acid reactive substances (TBARS), total thiol content (SH), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities. Data are expressed as mean ± S.E.M. $^{\#\#}p < .01$, and $^{\#\#\#}p < .001$ when compared to control group. $^{*}p < .05$, $^{**}p < .01$ and $^{***}p < .001$ when compared to the vehicle/ketamine group ($n = 5–6$)

ketamine and gallic acid (100 mg/kg) decreased the GPx activity in the cerebral cortex (Figure 3).

In the hippocampus, as shown in Figure 4, ketamine increased ROS levels ($F_{(6,37)} = 10.68$, $p < .001$) and this change was prevented by gallic acid (50 and 100 mg/kg) pretreatment. Similarly, gallic acid (100 mg/kg) and lithium pretreatment prevented the increase in TBARS induced by ketamine administration ($F_{(6,37)} = 6.37$, $p < .001$). We observed a reduction in SOD ($F_{(6,36)} = 6.32$, $p < .05$) and CAT ($F_{(6,36)} = 6.36$, $p < .01$) activities in the hippocampus of ketamine-treated group. Whereas lithium pretreatment prevented the alterations in SOD activity, all treatments were effective in restoring the CAT activity. No changes were observed in nitrite levels, total sulfhydryl content, and GPx activity in the hippocampus.

An increase in ROS ($F_{(6,33)} = 6.33$, $p < .001$), nitrite ($F_{(6,38)} = 9.75$, $p < .001$), and TBARS ($F_{(6,38)} = 13.30$, $p < .001$) were observed in the striatum of ketamine administered animals. Pretreatment with gallic acid and lithium were effective in preventing the aforementioned alterations. In addition, ketamine administration decreased the GPx activity ($F_{(6,33)} = 6{,}33$, $p < .01$); however, gallic acid or lithium pretreatment did not prevented this change in the striatum. No significant changes were observed in total sulfhydryl content, and SOD and CAT activities in the striatum of all experimental groups (Figure 5).

Figure 6 shows that ketamine administration increased the AChE activity in the hippocampus ($F_{(6,34)} = 8.94$, $p < .001$) and striatum ($F_{(6,34)} = 7.53$, $p < .001$). Pretreatment with gallic acid (50 and 100 mg/kg) and lithium were able to prevent these alterations. In the cerebral cortex, no changes in AChE activity were observed in all groups evaluated.

4 | DISCUSSION

In the present study, we showed the neuroprotective effects of gallic acid in an experimental model of manic-like behavior induced by ketamine.

Ketamine alters glutamatergic neurotransmission because it acts as a noncompetitive antagonist of N-methyl-D-aspartate receptor, inducing changes in the rat brain similar to those described in BD patients (Ghedim et al., 2012). In addition, ketamine increases the locomotor activity in animals, and this effect of ketamine is attenuated by lithium treatment (Debom et al., 2016). Our findings showed that ketamine induces manic-like behavior in rats that was evaluated using the open field test. Hyperactivity is a symptom observed in patients during the manic phase of BD (Perry et al., 2016). Interestingly, similar to lithium, treatment with gallic acid decreased the ketamine-induced hyperlocomotion.

BD is associated with abnormalities in the structure and function of the cerebral cortex, hippocampus, and striatum, the brain regions responsible for emotion regulation (Blumberg et al., 2003; Drevets et al., 2008; Tian et al., 2020). Herein, we observed oxidative stress in the brain of manic animals as evidenced by increased TBARS, ROS, and nitrite levels, and decreased thiol content and the activities of antioxidant enzymes such as CAT, SOD, and GPx. Reduction in SOD and CAT activities can lead to an increase in superoxide and hydrogen peroxide levels, which could easily cross the cell membrane and upon reacting with copper or iron ions (Fenton and Haber-Weiss reactions), generate hydroxyl radical (Lee et al., 2020).

The brain is particularly vulnerable to oxidative damage because it utilizes 20% of the total body oxygen and has high levels of polyunsaturated fatty acids, which is one of the main oxidative targets of ROS. In addition, the antioxidant capacity of the brain is limited (Siwek et al., 2013). Alterations in the redox status caused by ketamine could lead to oxidative modification of the brain macromolecules, leading to changes in membrane transport, signal transduction, and brain energy production. This hypothesis is corroborated by previous studies, which demonstrated that oxidative damage plays a critical role in the pathogenesis during the manic phase of BD (De Sousa et al., 2014).

In fact, BD patients in the manic phase have higher serum levels of TBARS compared to healthy controls (Tsai & Huang, 2015). Increased lipid hydroperoxide and malondialdehyde levels, and SOD activity were reported in the BD, while no significant change in glutathione peroxidase activity was observed (Andreazza et al., 2015; Kuloglu et al., 2002). Although findings on oxidative stress parameters in BD are controversial, it is now clear that alterations in redox status may be associated with changes in critical brain signaling that regulates affective and motor functions in this disorder.

Our research group demonstrated that antioxidant compounds prevent hyperlocomotion and protect the brain against oxidative stress in the model of mania (Chaves et al., 2020; Spohr et al., 2019). Gallic acid has received much attention due to its potent free radical scavenging activity and its role as a neuroprotective agent (Can et al., 2017; Mirshekar et al., 2018). In fact, gallic acid effectively protects the brain regions against ketamine-induced oxidative damage by decreasing TBARS, ROS, and nitrite levels, and increasing the activity of antioxidant enzymes. This antioxidant effect of gallic acid may be attributed to its capability to rapidly deactivate a wide variety of ROS and reactive nitrogen species via electron transfer, leading to the activation of enzymatic defenses, and the regulation of mitochondrial dysfunction (Badhani et al., 2015; Marino et al., 2014). Besides, the antioxidant effects of gallic acid could also be mediated through nuclear factor erythroid 2-related factor 2 (Nrf2) (Feng et al., 2018).

Cholinergic dysfunction such as changes in muscarinic receptor have been demonstrated in BD (Sigitova et al., 2017).

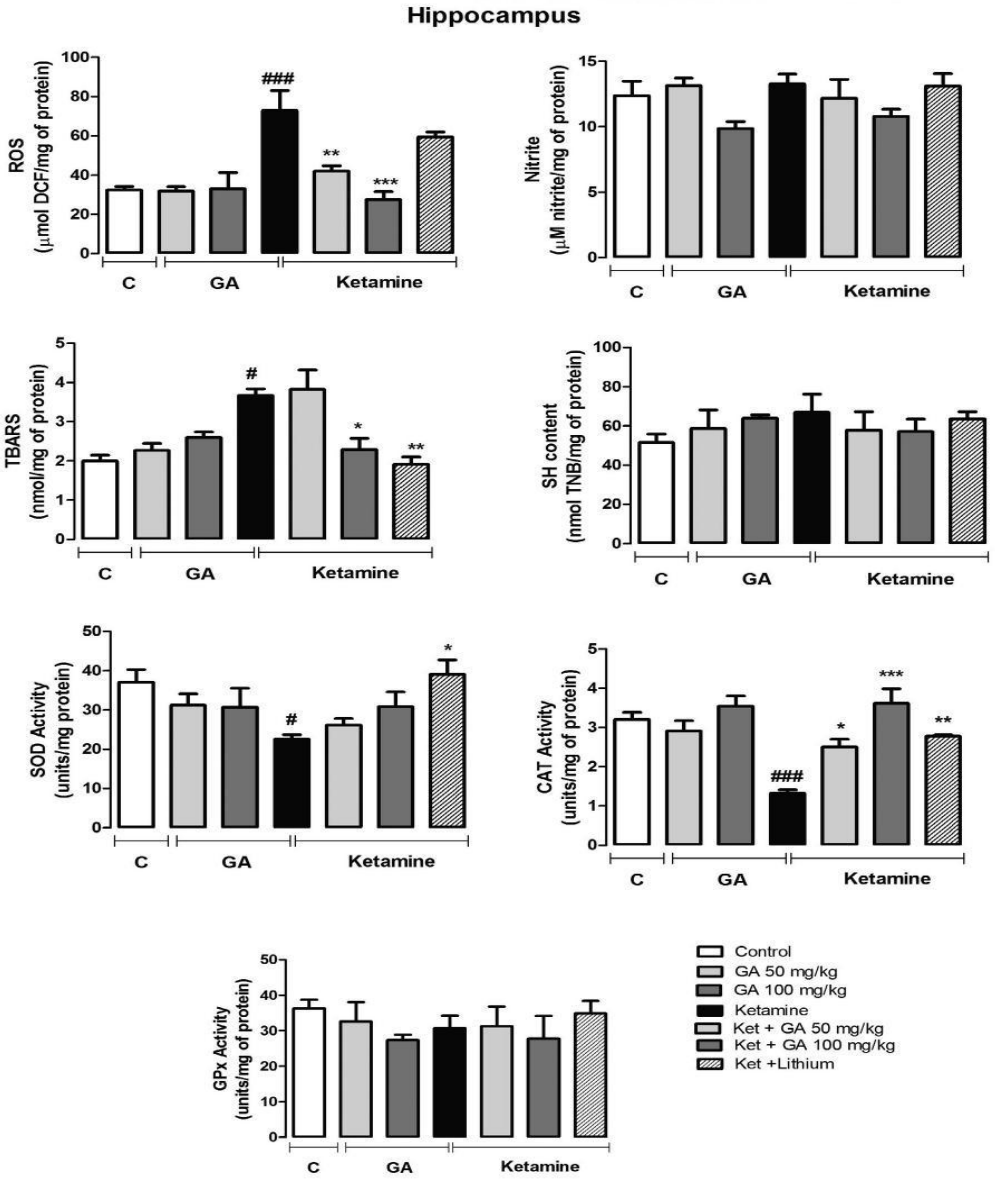

FIGURE 4 Effects of pretreatment with gallic acid (50 or 100 mg/kg) or lithium (45 mg/kg twice a day) on oxidative stress parameters in the hippocampus of animals exhibiting manic-like behavior induced by ketamine. Reactive oxygen species (ROS), nitrite levels, thiobarbituric acid reactive substances (TBARS), total thiol content (SH), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities. Data are expressed as mean ± S.E.M. $^{\#}p < .05$ and $^{\#\#\#}p < .001$ when compared to control group. $^{*}p < .05$, $^{**}p < .01$, and $^{***}p < .001$ when compared to the vehicle/ketamine group ($n = 5–6$)

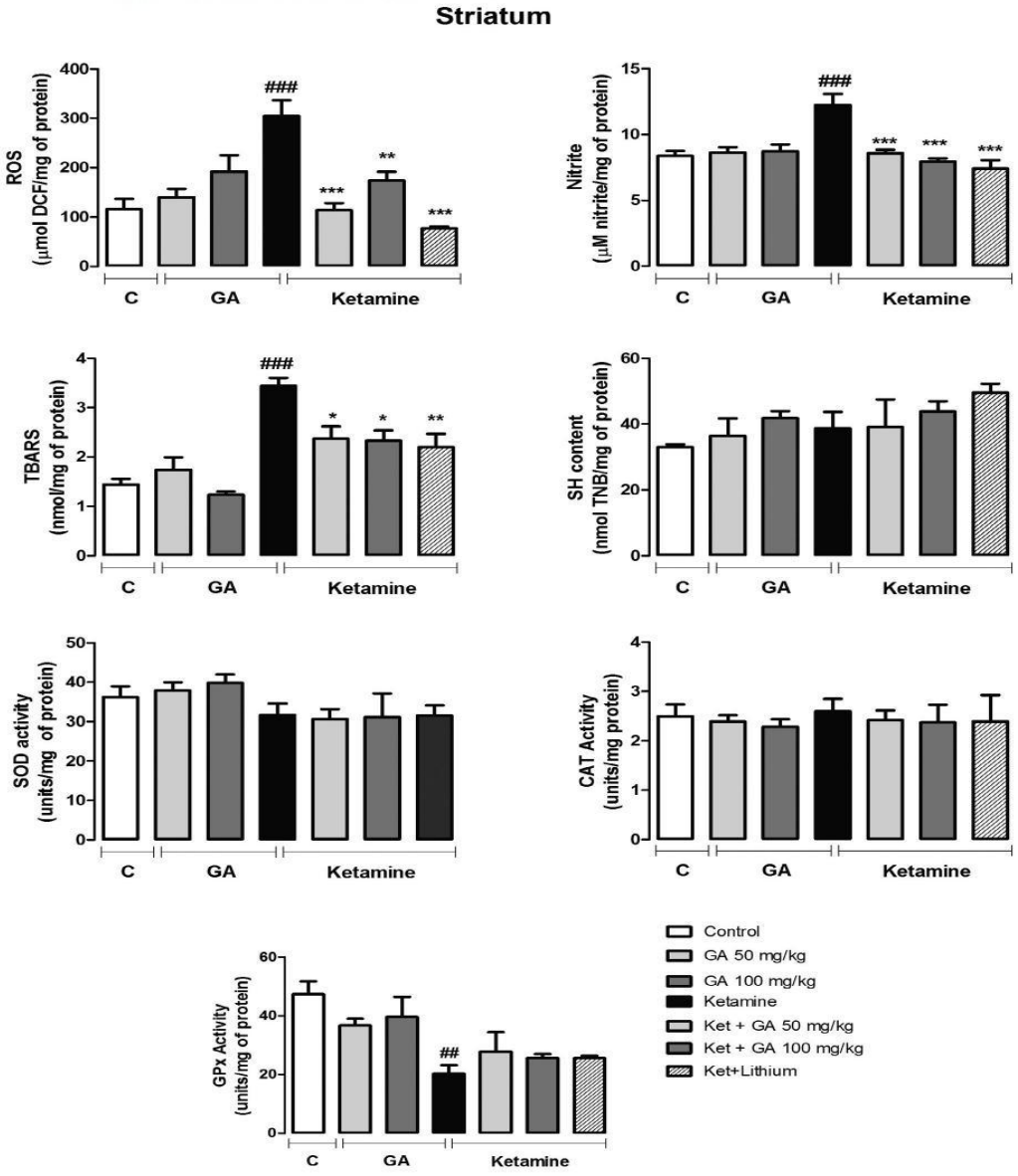

FIGURE 5 Effects of pretreatment with gallic acid (50 or 100 mg/kg) or lithium (45 mg/kg twice a day) on oxidative stress parameters in the striatum of animals exhibiting manic-like behavior induced by ketamine. Reactive oxygen species (ROS), nitrite levels, thiobarbituric acid reactive substances (TBARS), total thiol content (SH), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx) activities. Data are expressed as mean ± S.E.M. $^{\#\#}p < .01$ and $^{\#\#\#}p < .001$ when compared to control group. $^{*}p < .05$, $^{**}p < .01$, and $^{***}p < .001$ when compared to vehicle/ketamine group ($n = 5–6$)

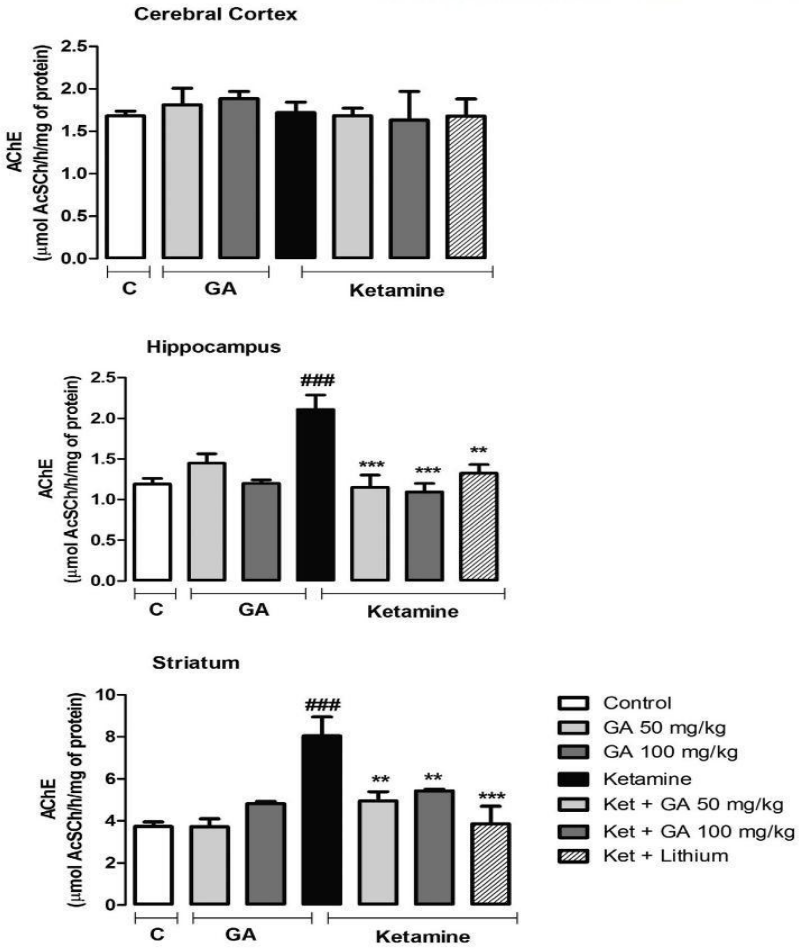

FIGURE 6 Effects of pretreatment with gallic acid (50 or 100 mg/kg) or lithium (45 mg/kg twice a day) on acetylcholinesterase activity in the cerebral cortex, hippocampus, and striatum of animals exhibiting manic-like behavior induced by ketamine. Data are expressed as mean ± S.E.M. $^{###}p < .001$ when compared to control group. $^{**}p < .01$, and $^{***}p < .001$ when compared to the vehicle/ketamine group ($n = 5–6$)

In addition, increasing the cholinergic transmission using muscarinic receptor agonists or AChE inhibitors exacerbates depressive symptoms while alleviates manic symptoms. According to catecholaminergic–cholinergic balance hypothesis of mania and depression, an increase in the central cholinergic function underlies depression, whereas increased activation of catecholamines underlies mania (Van Enkhuizen et al., 2015). In this context, our results showed that ketamine administration increases the AChE activity in the hippocampus and striatum. Similar results were described by Sporh et al. (2019) using the same mania model. An increase in the AChE enzyme activity may lead to a decrease in the levels of ACh, thus, contributing to the occurrence of manic episodes.

Gallic acid prevents ketamine-induced changes in AChE activity in the hippocampus and striatum. Kumar et al. (2019) also showed that gallic acid has a significant inhibitory potential against AChE in vitro. A previous study evaluating the effects of gallic acid on memory in rats showed that administration of gallic acid significantly reduces the activity of this

enzyme (Samad et al., 2019). Yadav et al. (2018) also demonstrated that gallic acid ameliorates psychotic symptoms induced by ketamine (50 mg/kg). The mechanisms involved in these beneficial effects of gallic acid might be through the reduction in dopamine and TNFα levels, increase in GABA levels, and decrease in the AChE activity in the serum and brain of the animals (Yadav et al., 2018).

Considering that lithium is the main pharmacological agent used for the treatment of BD, we used it as a positive control. Lithium was effective in reducing hyperlocomotion, brain oxidative stress, and AChE activity. These results corroborate with others studies from research group (Debom et al., 2016; Spohr et al., 2019). The antioxidant effect of lithium has also been demonstrated in other experimental models of mania as well as in patients with BD (Bengesser et al., 2016; Jornada et al., 2011).

In conclusion, although lithium is effective for the treatment and prevention of recurrent mania and depressive episodes, this drug has many side effects, which is an important factor involved in patient's adherence to the treatment. Since BD is a chronic illness, continuous treatment is necessary to prevent manic or depressive symptoms and to improve the patient's quality of life. Herein, we showed that gallic acid exerts effects similar to those of lithium, suggesting that this natural compound might be effective in preventing brain alterations and improving symptoms associated with mania. Although gallic acid treatment appears free from adverse side effects, further studies are necessary to evaluate the safety and determine the therapeutic dose in humans.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

DATA AVAILABILITY STATEMENT

Supporting data are available from the corresponding author upon reasonable request.

AUTHOR CONTRIBUTIONS

Vânia Machado Recart, Luiza Spohr, and Mayara Sandrielly Pereira Soares: animal model of mania, gallic acid treatment, statistical analysis, and manuscript preparation. Bruna de Mattos, Natália Pontes Bona, Nathalia Stark Pedra, and Fernanda Teixeira: behavioral tests, oxidative stress, acetylcholinesterase assays, and data analysis. Giovana Duzzo Gamaro, Francieli Moro Stefanello, and Roselia Maria Spanevello: experimental design, results interpretation, discussion, and manuscript preparation.

ETHICS APPROVAL STATEMENT

Animal procedures were approved by the Committee of Ethics and Animal Experimentation (CEEA) of the Federal University of Pelotas, Pelotas, RS, Brazil, under protocol number: CEEA 4609–2015.

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How to cite this article: Recart VM, Spohr L, Soares MSP, et al. Gallic acid protects cerebral cortex, hippocampus, and striatum against oxidative damage and cholinergic dysfunction in an experimental model of manic-like behavior: Comparison with lithium effects. *Int J Dev Neurosci*. 2021;00:1–12. https://doi.org/10.1002/jdn.10086

4. 2 Manuscrito II

Gallic acid prevented memory deficits and oxidative damage in brain of mice in a model of neuroinflammation induced by lipopolysaccharide

Em fase de submissão para revista científica.

Gallic acid prevented memory deficits and oxidative damage in brain of mice submitted to lipopolysaccharide exposure

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Abstract

Neuroinflammation is a pathological condition that is associated with many neurological disorders. Here we evaluated the effects of gallic acid on hyperlocomotion, memory and oxidative stress in a model of neuroinflammation induced by lipopolysaccharide (LPS). Male *Swiss* mice were pretreated orally with vehicle or gallic acid (50 or 100 mg/kg) for 14 days. Between days 8 and 14, the animals also received one injection of LPS (250 μg/kg) or saline. In the end of experimental protocol, the animals were submitted to open field and object recognition tests, and after euthanized and brain structures collected. The systemic administration of LPS cause memory deficits and gallic acid in both doses was able to prevent this alteration. No changes were observed in locomotor activity in open field test in any experimental group. LPS also induced oxidative damage, such as an increase in reactive oxygen species and nitrite levels, and a decrease in total thiol content and the activity of the antioxidant enzymes superoxide dismutase, catalase and glutathione peroxidase in cerebral cortex, hippocampus and striatum of mice. Gallic acid also was capable to prevent the alterations in status redox induced by LPS. The neuroprotective effect of gallic acid against neuroinflammation induced by LPS may be attributed to its potent antioxidant and anti-inflammatory properties that specifically modulate many molecular signal transduction pathways including memory mechanisms.

Keywords: neuroinflammation; gallic acid; oxidative stress; lipopolysaccharide

1. Introduction

Gallic acid (GA), 3,4,5-trihydroxybenzoic acid, is a well-known polyphenol compound present in fruits, vegetables, and herbal medicines such as blueberries, strawberries, grapes and also in processed beverages such as red wine and green team (Prince et al., 2009, Daglia et al., 2014). GA have a diverse range of uses as antioxidants in food, cosmetics and pharmaceutical industries (Choubey et al., 2015). The most important pharmacological properties of GA has been attributed to its antioxidant and anti-inflammatory actions (Marino et al., 2014, Badhani et al., 2015, Pandurangan et al., 2015a, Pandurangan et al., 2015b, Bensaad et al., 2017). GA has attracted a great deal of attention in the health field due their acceptable safety and the broad range effects in the prevention and modulation of pathogenic mechanisms of several brain diseases.

It is well stabilized that in brain inflammation, the innate imune response initially protects against central nervous system (CNS) injury. However, long-term dysregulated and chronic inflammation have been associated with neurodegeneration and impaired synaptic plasticity (Simon et al., 2017). Thus, neuroinflammation has been identified as part of the pathogenesis of many brain disorders including the neurodegenerative diseases and psychiatric conditions such as depression and bipolar disorder. Alterations in the brain environment, for example, by infection or neuronal injury, result in microglia and astrocyte activation (Wang et al., 2004; Khan et al., 2016). The activated glial cells release proinflammatory mediators, such as cytokines and reactive oxygen species (ROS). The overproduction of ROS may result in tissue damage leading to a chronic neuroinflammatory process (El-Benna et al., 2016; Drummond, 2011).

One de most important and widely used animal model to induce neuroinflammation is systemic administration of lipopolysaccharide (LPS), an endotoxin from the outer membrane of bacteria. LPS is a potent trigger of inflammation and when administrated peripherally in mice induced many effects in CNS such as microglia and astrocytes activation, as well as cyclooxygenase-2 (COX-2), inducible nitric oxide synthase (iNOS), pro-inflammatory cytokine expression and oxidative stress (Catorce and Gevorkian, 2016, Badshah et al., 2019). Thus, the aim of this study was evaluated the protective effects of gallic acid in memory deficits and oxidative brain damage induced by peripheral administration of LPS in mice.

2. Materials and Methods

2.1 Chemicals

Gallic acid, LPS (*E. coli* strain O55:B5) and dichloro-dihydro-fluorescein diacetate (DCFH-DA) was obtained from Sigma Aldrich Chemical (St. Louis, MO, USA). All others reagents used in the experiments were of analytical grade and the highest purity.

2.2 Animals and ethical procedures

The Committee of Ethics and Animal Experimentation of the Federal University of Pelotas, RS, Brazil (CEEA 17187-2020), approved the animal‟s procedures. Adult male Swiss mice (60 days, 30–45 g) were obtained from the Central Animal House of Federal University of Pelotas. The animals were kept at constant temperature (22 ± 1°C) under the light/dark cycle and free access to water and food. All animal experiments were carried in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

2.3 LPS and gallic acid treatment

Mice were randomly divided into four groups: I- control, II- LPS (250 mg/kg) III- LPS *plus* gallic acid (50 mg/kg) and IV - LPS *plus* gallic acid (100 mg/kg). In the animals of the group I saline was injected as a vehicle for 14 days, while that the animals of the group II, III, and IV saline was injected for 7 days, following LPS injection of the mice at a dose of 250 μg/kg for an additional 7 days (Figure 1). In addition, the animals of the groups I and II received by oral administration saline solution, while that animals of the groups III e VI received orally gallic acid (50 or 100 mg/kg) during 14 days (Figure 1). In the end of treatment period, the animals were submitted to behavioral tasks, and after euthanized and brain collected for biochemical analysis (Khan et al., 2016; Carvalho et al., 2017).

2.4 Behavioral Tests

2.4.1 Open field

Locomotor behavior was evaluated using open-field apparatus. The floor of the arena was divided into 9 equal squares and placed in a sound free room. Mice were placed in the rear left square and allowed to explore freely for 5 min. The total

number of squares crossed with all paws (crossing) and fecal droppings were manually counted; the degree of grooming and rearing was also evaluated. The apparatus was cleaned and dried with a 40% alcohol solution after testing with each mice. This test was carried out to identify motor disabilities, which might influence in memory test performed.

2.4.2 Object recognition

Mice were habituated to the experimental arena 24 h before the test. Objects, made of waterproof plastic, were placed on the sand floor. This test was performed in two stages. During training, mice were placed in the arena with two identical objects (A1 and A2) and allowed to explore for 5 min. The session was valid if mice explored each object for at least 30 s. After 24 hours the training, the animals were submitted to test. For the test, one of the objects was changed to a different, novel object (B). The mice were introduced into the arena for 5 min and allowed to explore freely. The positions of the objects (familiar or novel) were randomly exchanged. Exploration was defined as smelling or touching the object with the nose and/or forelegs. Sitting on or around the object was not considered exploratory behavior. The apparatus and the objects were cleaned and dried with a 40% alcohol solution after each mice. In this task was evaluated the total exploration time (s) in the training and test; Time spent on each object – Training (s) (A1, A2); Time spent on each object – Test (s) (A1 and B); Exploratory preference time for the novel object expressed as a percentage evaluated in the object recognition test (Rossato et al., 2007).

2.5 Oxidative stress parameters

At the end of the experimental protocol, the animals were euthanized by deepening anesthetic using inhaled isoflurane, and the brain was quickly removed for separation of the cerebral cortex, hippocampus and striatum, which were then stored at -80 ° C for subsequent biochemical analyzes. The brain regions were prepared by homogenizing with 20 mM sodium phosphate buffer, pH 7.4 containing 140 mM KCl (1/10 w/v). The homogenates were centrifuged at 2500 x g for 10 min at 4°C. The supernatants were collected and used in oxidative stress analyses.

2.5.1 Reactive oxygen species (ROS) determination

The intracellular ROS levels were analyzed by the oxidation of DCFH-DA to fluorescent dichlorofluorescein (DCF). The intensity of fluorescence emission by DCF was measured at 488/525 nm, 30 min after the addition of DCFH-DA to the medium. The result is expressed in µmol DCF/ mg of protein (Ali et al., 1992).

2.5.2 Nitrite levels

Briefly, sample plus sulfanilamide in 5% phosphoric acid was incubated for 10 min at room temperature. Next, N-(1-naphthyl) ethylenediamine dihydrochloride was added, and the mixture was incubated for 10 min at room temperature while protected from light. A sodium nitrite solution was used as the reference standard, and the results were expressed as µmol NO_2^-/mg of protein (Stuehr and Nathan, 1989).

2.5.3 Total sulfhydryl content (SH)

SH total was measured based on the reduction of DTNB by thiols and in turn becomes oxidized (disulfide) generating a yellow derivative (TNB) whose absorption is measured spectrophotometrically at 412 nm. Results were reported as nmol TNB/mg of protein (Aksenov and Markesbery, 2001).

2.5.4 Thiobarbituric acid reactive substances (TBARS)

Lipid peroxidation was performed using the TBARS test according methodology previous describe. to Samples were mixed with trichloroacetic acid 10 % and thiobarbituric acid 0.67 % and heated in a boiling water bath for 25 min. Results were reported as nmol TBARS/mg of protein. (Esterbauer and Cheeseman, 1990).

2.5.5 Superoxide dismutase (SOD) activity

This method is based on the inhibition of superoxide dependent adrenaline auto-oxidation. The SOD activity was reported as units/mg of protein (Misra and Fridovich, 1972).

2.5.6 Catalase (CAT) activity

CAT activity was based on the methodology previously described. H_2O_2 disappearance was continuously monitored with a spectrophotometer as 240 nm for 90 s. The specific activity was reported as units/mg of protein (Aebi, 1984)

2.5.7 Glutathione S-transferase (GST) activity

GST activity was measured using abuffer containing 1-chloro-2,4-dinitrobenzene (CDNB) as a substrate, glutathione, and 100 mM potassium phosphate buffer (pH 7.0). GST activity was expressed as μmol GS-DNBmin per mg protein (Habig et al., 1974).

2.6 Protein determination

Protein concentration was measured as previously described by Lowry et al. (1951).

2.7 Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey post hoc tests using GraphPad Prism 5.0 (Intuitive Software for Science, São Diego, CA, USA). *P*<0.05 was considered statistically significant. All data were expressed as mean ± standard error of the mean (SEM).

3 Results

3.1 Gallic acid prevent the memory deficits induced by LPS

Figure 2A shows that mice decreased total exploration time in the training *vs.* testing phase of the object recognition test (P<0.05). Yet, in the training phase the mice of LPS group presented a longer exploration time compared to the control group (P<0.01).

During the training phase, the LPS group it explored object A1 for a longer time when compared to the control group (P<0.05) (Figure 2B). During testing, mice exposed to LPS explored the familiar object (A1) more than the mice in the control group (P<0.05) and the animals treated with gallic acid 50 mg/kg (P<0.001) and 100 mg/kg (P<0.001), demonstrated a reduction in exploration time on object the familiar object A1 when compared to animals of the LPS group. Furthermore, the LPS group showed a reduction in the exploration of the new object (B) compared with the control

group (P <0.05) and the pretreatment with gallic acid 100 mg / kg was able to increase the exploration time of the new object (B) when compared to the LPS group (P <0.01) (Figure 2C).

It was possible to demonstrate in figure 2D, that the animals of the LPS group explored significantly more the familiar object (A1) when compared with the new object (B) (P<0.001). In contrast, animals treated with gallic acid 50 mg / kg (P<0.05) and 100 mg / kg (P<0.01) showed a significant increase in the time to explore the new object (B) when compared to the familiar object (A1) (Figure 2D).

Taken together, these findings indicated that LPS impaired object recognition memory (P<0.05) and the pre-treatment with gallic 50 mg/kg (P<0.001) and 100 mg/kg (P<0.001) was able to prevent the memory deficits induced by LPS ($F_{(3,36)}$ = 10.27, Figure 2E). It is important to report that no changes were observed in locomotor activity in any experimental group when compared to the control group ($F_{(3,36)}$ = 2.67, Figure 2F, *P*>0.05) excluding the possibility that the results of the object recognition task are associated with locomotor alterations the mice.

3.2 Gallic acid prevent the increase in ROS levels and CAT and GST activity reduction caused by LPS in the cerebral cortex

As shown in figure 3A, LPS administration increased the ROS levels when compared to the control group in the cerebral cortex. However, the administration of Gallic acid 50 mg / kg (P <0.001) and 100 mg / kg (P <0.001) was able to protect against this increase ($F_{(3,15)}$ = 14.34). In addition, exposure to LPS caused a reduction in SH levels when compared to the control group (P <0.05), but treatment with gallic acid at any dose was able to protect against this reduction (P> 0.05) (Figure 3C) ($F_{(3,15)}$ = 4.23). In relation to nitrites ($F_{(3,12)}$ = 3.73, P> 0.05, figure 3B) and TBARS levels ($F_{(3,16)}$ = 1.65, P>0.05, figure 3D), no changes were observed in any of the experimental groups.

Regarding the activity of the antioxidant enzymes, there was a reduction in the activity of the SOD ($F_{(3,15)}$ = 17.58, P <0.001, figure 6A), CAT ($F_{(3,12)}$ = 8.55, P<0.05, figure 6B) and GST ($F_{(3,14)}$ = 17.27, P <0.05, figure 6C) enzymes in the LPS group when compared to the group control. However, pretreatment with only gallic acid 100 mg/kg was able protect against the reduction in the activity of CAT (P <0.05, figure 6B) and GST (P <0.001, figure 6 C) when compared to the LPS group.

3.3 Gallic acid prevent oxidative damage induced by LPS in hippocampus

In the hippocampus, in figure 4A, it was also possible to observe that the administration of gallic acid 50 mg / kg ($P < 0.05$) and 100 mg / kg ($P < 0.01$) was able to protect against the increase in ROS production induced by LPS in hippocampus ($P<0.001$) ($F_{(3,14)} = 14.41$). LPS also caused an increase in nitrite levels ($P < 0.01$) when compared to the control group. However, pretreatment with gallic acid 100 mg / kg ($P < 0.01$) was able to protect against this increase ($F_{(3,11)} = 11.16$, Figure 4B). Also, as shown in figure 4C, LPS caused a reduction in SH levels compared to the control group ($F_{(3,13)} = 7.10$, $P < 0.01$). However, gallic acid was not able to protect against this reduction in any of the tested doses ($P > 0.05$). In addition, no changes were found regarding lipid peroxidation in any of the experimental groups ($F_{(3,14)} = 0.79$, $P > 0.05$, figure 4D).

In the hippocampus, the activity of the enzymes SOD ($F_{(3,16)} = 8.26$, $P < 0.01$, figure 6D), CAT ($F_{(3,13)} = 11.47$, $P < 0.001$, figure 6E) and GST ($F_{(3,14)} = 7.87$, $P < 0.05$, figure 6F) were significantly reduced in the LPS group when compared to the control group. However, the administration of gallic acid 50 mg / kg ($P < 0.05$) and 100 mg / kg ($P < 0.05$) was able to prevent the reduction of the enzymes SOD and CAT in hippocampus when compared to the LPS group.

3.4 Gallic acid prevent oxidative damage induced by LPS in striatum

As shown in figure 5A, LPS also increased in ROS production in mice striatum ($F_{(3,14)} = 11.70$, $P < 0.001$). In contrast, the administration of gallic acid 100 mg / kg was able to protect against this change ($P < 0.05$). In addition, LPS exposure also caused an increase in nitrite levels ($F_{(3,13)} = 8.20$, $P < 0.01$), but pretreatment with gallic acid 50 mg / kg ($P < 0.01$) and 100 mg / kg ($P < 0.05$) was able to protect the striatum against this increase (Figure 5B). Furthermore, in figure 5D, it can be seen that the administration of LPS caused lipid peroxidation ($F_{(3,15)} = 4.71$, $P < 0.05$) when compared to the control group, and pretreatment with gallic acid in both doses was not able to protect against this damage ($P > 0.05$). As for the SH levels, no significant changes were observed in any of the experimental groups ($F_{(3,18)} = 0.62$, $P > 0.05$).

As shown in figure 6, LPS caused a reduction in the activity of CAT ($F_{(3,14)} = 5.91$, $P < 0.01$, figure 6H) and GST ($F_{(3,12)} = 29.94$, $P < 0.05$, figure 6I) when compared to the control group. However, pretreatment with gallic acid 50 mg / kg ($P < 0.001$) and 100 mg / kg ($P < 0.001$) was able to protect against the reduction in GST activity

caused by LPS (figure 6I). There was no significant change in SOD activity in the striatum of animals in any experimental group ($F_{(3,13)} = 0.93$, $P > 0.05$, figure 6G).

4 Discussion

Several studies have reported that LPS induces neuroinflammation, brain oxidative stress and memory dysfunction in animals. In the present study, we showed the therapeutic potential of gallic acid in prevent memory impairment and oxidative damage induced by systemic LPS administration in mice. Gallic acid is a well-known natural antioxidant and one the most important polyphenolic compounds found in plants, fruits, and in some processed beverages such as wines and green tea (Verma et al., 2013). In addition to antioxidant properties, GA also regulate the wide range biological functions including the anti-inflammatory pathways (Choubey et al., 2015; Kahkeshani et al., 2019).

LPS exposed mice showed a deficit in memory performance in the novel object recognition test. This result corroborated with previous studies from literature that also demonstrated that LPS when administered systemically or centrally affect memory process (Joshi et al., 2014; Abareshi et al., 2016). In fact, LPS has been used to induce neuroinflammation model in rodents. LPS triggers immune responses and induces microglial activation by a Toll-like receptor 4 (TLR4)-dependent pathway accompanied by the production and release of pro-inflammatory cytokines, such as interleukin IL-1β, IL-6 and TNF-α (Yaun et al., 2016; Café-Mendes et al., 2017). IL-1β affects cognitive function and memory and has been showed that intracerebral injection IL-1β receptors antagonist ameliorated LPS-induced cytokines release in the hippocampus (Huang and Sheng, 2010; Frank et al., 2012). Besides, LPS administration also cause an impairment on hippocampal neurogenesis which also has been associated with memory deficits (Valero et al., 2014).

It is well stablished that inflammatory process besides affect memory also contributes to the etiology and progression the many neurological diseases (Albrecht et al., 2016; Kothur et al., 2016). In addition, inflammation and oxidative stress and are closely associate pathophysiological mechanisms that are tightly linked with one another (Biswas, 2016) and both processes are simultaneously found in brain pathologies. The increase of reactive species and consequently oxidative stress can induce inflammation through many pathways such as activation of transcription factor NF-κB and NOD-like receptor protein 3 (NLRP3) inflammasome, an oligomeric

molecular complex associated to the maturation of proinflammatory cytokines like IL-1β and IL-18 (Biswas, 2016).

In fact, previous studies have related that LPS administration besides to induce an increase the proinflammatory cytokines also increase the levels of reactive species (Wang et al., 2004; Khan et al., 2016). Here we also showed that systemic administration of LPS also induce oxidative damage in cerebral cortex, hippocampus and striatum of mice which can be evidenced by increase of ROS, TBARS and nitrite levels and a decrease in SH content as well antioxidant enzymes activities. These findings are similar to other studies from research group which also demonstrated that the administration of LPS alter the status redox in different brain regions (Spohr et al., 2020; Luduvico et al., 2020). This increased in ROS generation can be attributed to activation of the TLR4 receptor by LPS, because this interacts directly with the enzyme NADPH oxidase, which is a major sources of reactive species (Park et al., 2004).

It is well stablished that elevated levels of reactive species cause damage of cellular brain components (Jones, 2008; Tucsek et al., 2011). Considering that LPS induce a liberation of inflammatory cells of reactive species at the site of inflammation, which may subsequently induce the expression of genes of inflammatory mediators, is plausible suggest that brain oxidative damage may be considered to have an important role in deleterious effects of LPS on memory as observed in this study.

Cerebral cortex, hippocampus and striatum are brain regions targets of pathogenic process involved in many neurological diseases such as neurodegenerative and psychiatric. Our results showed that the systemic administration of LPS induced an increase in ROS levels in all structures evaluated, however, in relation to TBARS levels, a lipid peroxidation marker; we observed that administration of LPS caused an increase only in the striatum. In addition, about total thiol content, a marker for protein damage since sulfhydryl compounds are sensitive to free radicals, LPS induced a reduction of this marker only in hippocampus and cerebral cortex. It is important to discuss that this selective vulnerability of brain regions to oxidative stress induced by LPS, can be a result of many factors associated to high demand for ROS/RNS as signaling molecules, inflammatory response, low energy generation, mitochondrial dysfunction, as well calcium dysregulation and glutamate hyperactivity (Whang et al., 2010).

In addition, LPS induced a decrease of antioxidant enzymes activities in all brain structures evaluated. The SOD enzyme is responsible to catalyze the dismutation of the superoxide radical into oxygen and hydrogen peroxide and CAT is responsible for decomposition of hydrogen peroxide to water and oxygen (Gebicka and Krych-Madej, 2019) and GST detoxifes xenobiotics and oxidative stress products, using glutathione as a substrate (Dasari et al., 2018). In fact, this imbalance between free radical production and decrease in antioxidant defenses has been described as crucial pathways involved in neural damage in many pathological conditions.

Another important aspect to be discussed is that in hippocampus and striatum, LPS increased the nitrite levels, which is associated to production of nitric oxide (NO) production. The enzyme responsible for the high-output production of NO, nitric oxide synthase (iNOS), in human monocytes or macrophages is most readily observed in patients with infectious or inflammatory diseases (MacMicking et al., 1997). Evidence suggests that the inducible form of NOS (iNOS) in response to LPS (Weinberg et al., 1995) and this enzyme is a critical element of the NF-κB pathway and can generate NO.

In our study we also showed that gallic acid 50 and 100 mg/kg was effective in prevent memory deficits and brain oxidative damage induced by LPS systemic administration in mice. Gallic acid have a potent antioxidant effects which can be attributed to its capacity to rapidly deactivate a wide variety of ROS and reactive nitrogen species via electron transfer, leading to the activation of enzymatic defenses and the regulation of mitochondrial dysfunction (Marino et al., 2014, Badhani et al., 2015, Lu et al., 2006). The upregulation of antioxidant mechanisms by gallic acid has been previously reported in others experimental conditions (Nabavi et al., 2012; Mansouri et al., 2013, Yang et al., 2015).

Gallic acid is a well-known natural compound that is basically a secondary polyphenolic metabolite. The benefits of gallic acid on brain and cognitive functions are well documented (Nabavi et al., 2016). The polyphenols to exert interesting properties, especially regarding neuroprotection, relies on their ability to cross the blood-brain barrier (BBB) (Carecho et al., 2020). The mechanisms by which phenolic metabolites permeate the BBB are still unknown (Schaffer et al., 2011) may be by interacting with ABC transporters (such as P-glycoprotein), responsible for regulating the entry of these molecules (Campos-Bedolla et al., 2014).

In addition, gallic acid is involved in others signaling pathways including the regulation of inflammatory mechanisms (Kahkeshani et al., 2019). The particular importance has been showed that gallic acid could inhibit LPS-induced inflammatory response in macrophages via TLR4/NF-κB pathway (Huang et al., 2016) and LPS-induced glial cell activation, neuroinflamation and programed cell deaths of nigrostriatal dopaminergic neurons of the brain (Liu et al., 2019) suggesting that the beneficial effects of the gallic acid in this study can be attributed to antioxidant but also anti-inflammatory properties.

5 Conclusion

Our findings showed that the pre-treatment with GA 50 mg/kg and 100 mg/kg was effective to prevent memory dysfunctions and oxidative damage induced by LPS administration in mice. Considering that gallic acid have a broad range of beneficial effects in prevention and/or management of several brain disorders associated with acceptable safety and stability profiles, we suggested that GA may be a promisor therapeutic potential for treatment of diseases associated with neuroinflammation.

Acknowledgments

This research was supported by the Conselho Nacional de Desenvolvimento Científico e Tecnológico and Fundação de Amparo à Pesquisa do Rio Grande do Sul. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior- Brasil (CAPES) – Finance code 001.

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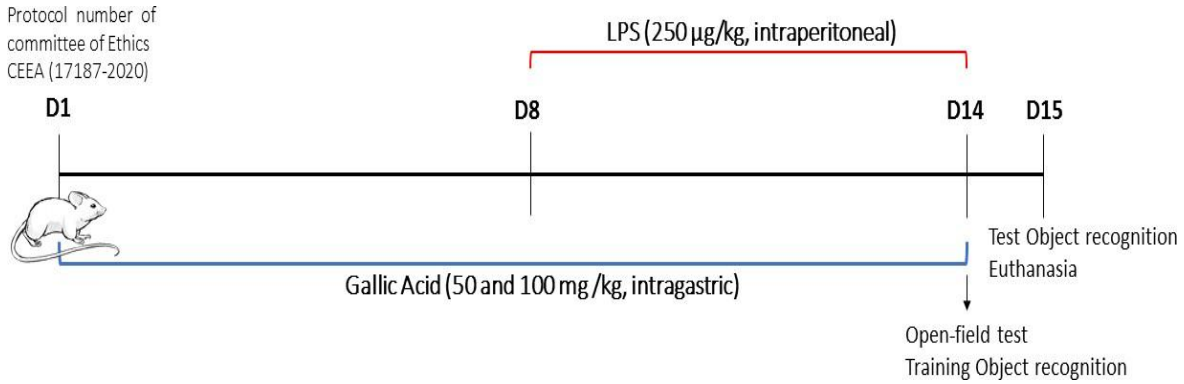

Figure 1 – Scheme of experimental protocol. Male *Swiss* mice were pretreated orally with vehicle or gallic acid (50 or 100 mg/kg) for 14 days. Between days 8 and 14, the animals also received LPS injection (250 µg/kg) or saline. In the end of experimental protocol, the mice were submitted to open field and object recognition tests to evaluate locomotion and memory respectively.

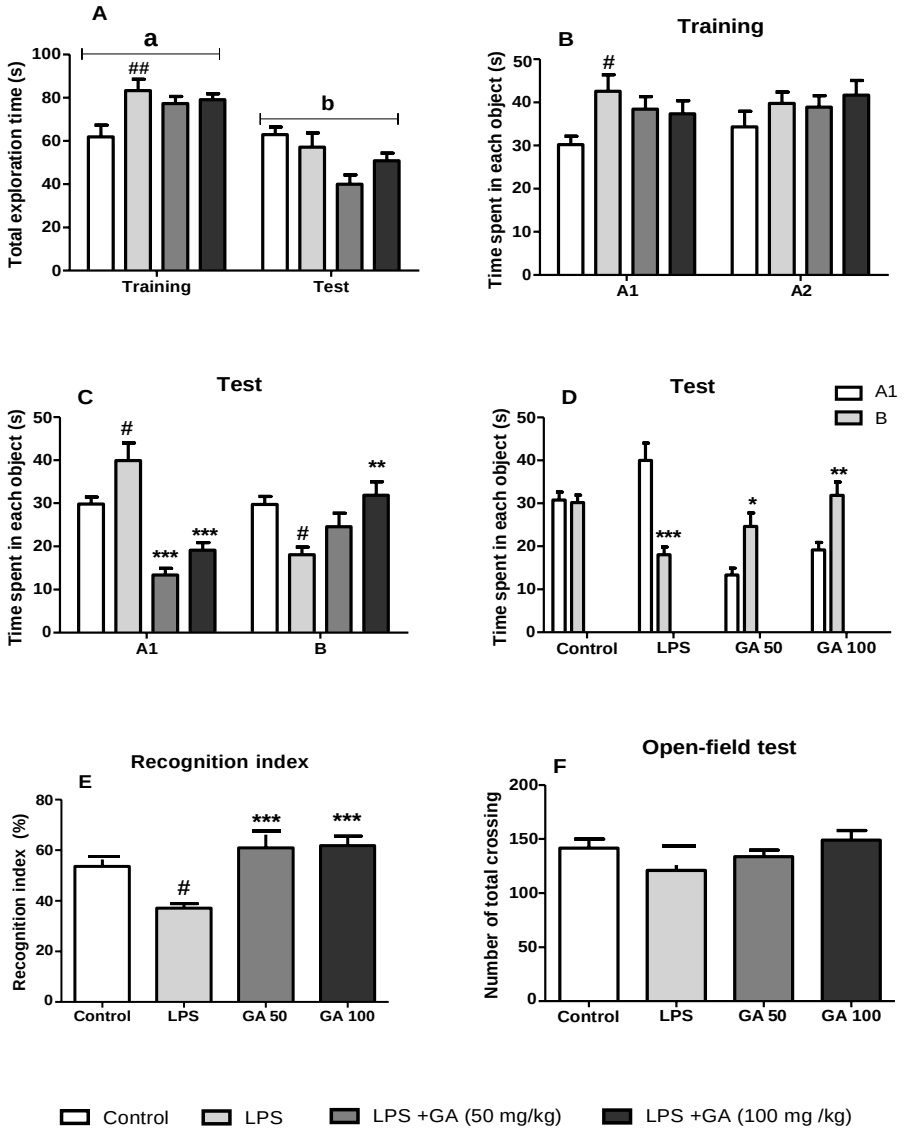

Figure 2 – Effect of Gallic Acid (50 and 100 mg /kg) memory in mice treated with LPS (250 µg/kg). (**A**) Total exploration time (s); (**B**) Time spent on each object – Training (s); (**C**) Time spent on each object – Test (s); (**D**) Total time spent during the test session on each object; **(E)** Exploratory preference time for the novel object expressed as a percentage evaluated in the object recognition test; (**F**) Crossing in the open field-test. Two-way ANOVA and post-hoc Bonferroni multiple comparisons test were used for A–D. One-way ANOVA, and post-hoc Tukey's multiple comparisons tests were performed for E-F. A1 and A2 report the familiar object and B refers to new objet. Bars represent mean and ± SEM. $^{\#}$ P<0.05; $^{\#\#}$ P<0.01 compared to vehicle/saline group. *****P < 0.05; ******P < 0.01; *******P < 0.001, compared to vehicle/LPS group (n = 9–10 per group). In the figure 2A, **a** and **b** denote a significant difference between training and test *P < 0.05.

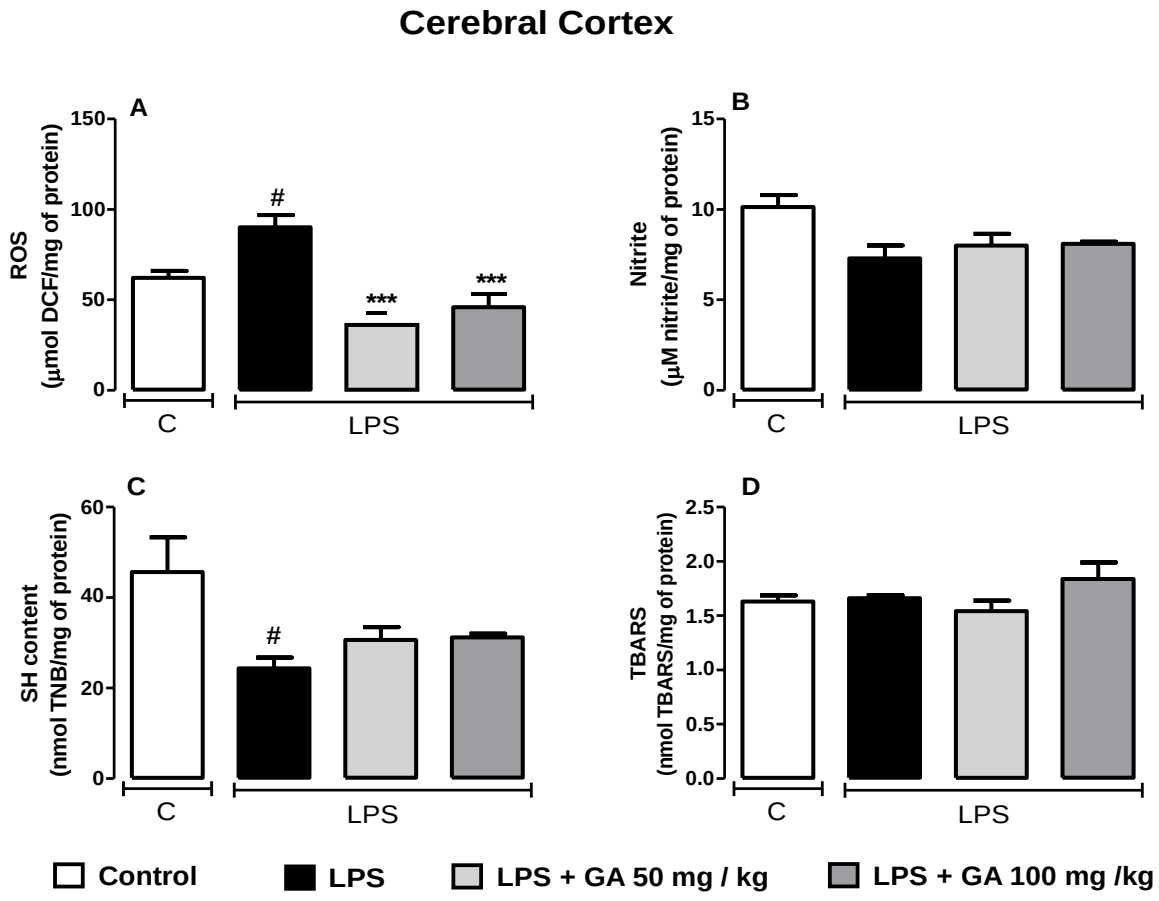

Figure 3 - Effect of gallic acid (50 and 100 mg/kg, p.o.) pretreatment on levels of reactive oxygen species (ROS) **(A),** nitrite **(B),** total thiol content (SH) **(C)** and thiobarbituric acid reactive substances (TBARS) **(D)** in cerebral cortex of mice treated with LPS (250 µg/kg). Data are reported as mean ± S.E.M. # P<0.05 compared to vehicle/saline group. ***P<0.01 compared to LPS group (n=4–5).

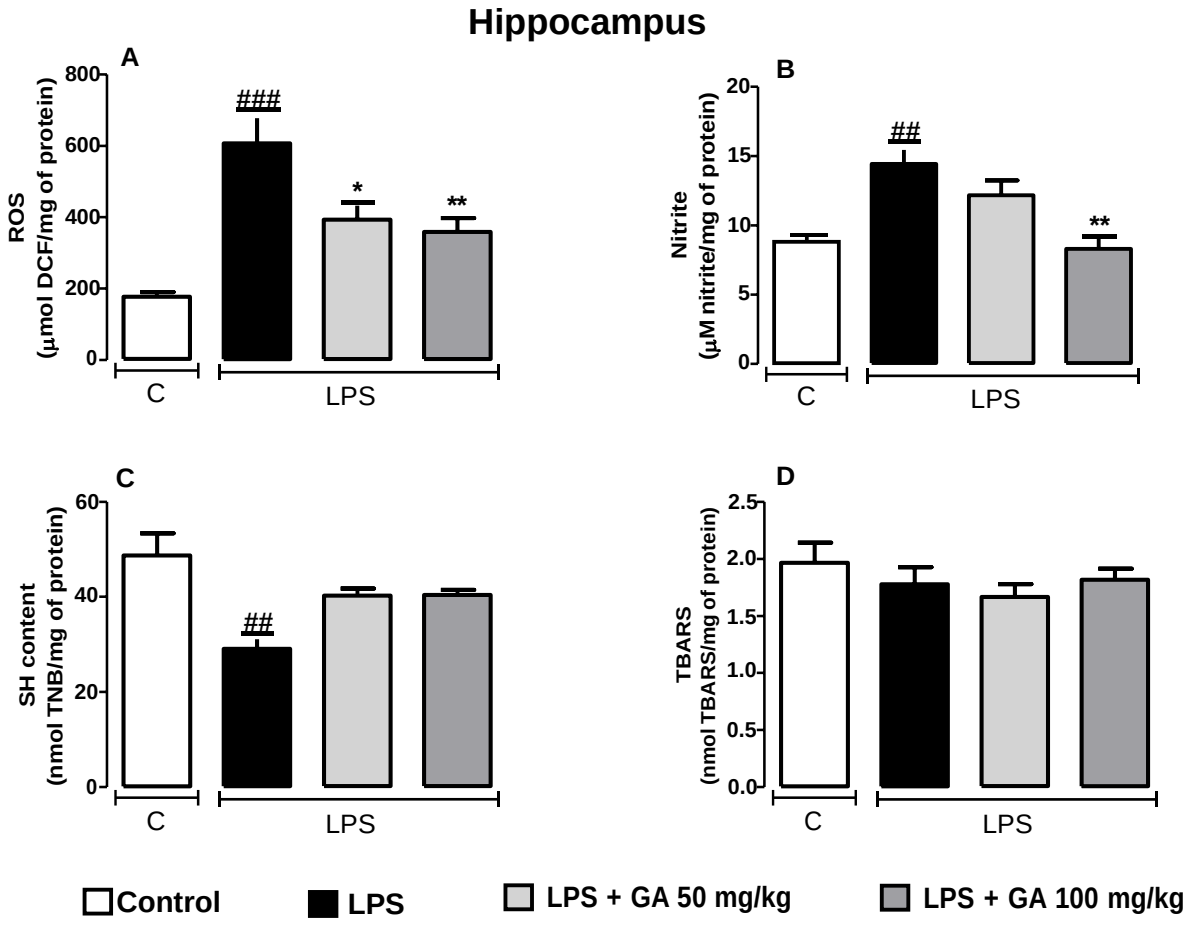

Figure 4 - Effect of gallic acid (50 and 100 mg/kg, p.o.) pretreatment on levels of reactive oxygen species (ROS) **(A),** nitrite **(B),** total thiol content (SH) **(C)** and thiobarbituric acid reactive substances (TBARS) **(D)** in hippocampus of mice treated with LPS (250 µg/kg). Data are reported as mean ± S.E.M. $^{\#\#}$P<0.01 and P<0.001 compared to vehicle/saline group. *P<0.05 and **P<0.01 compared to vehicle/LPS group (n=4–5).

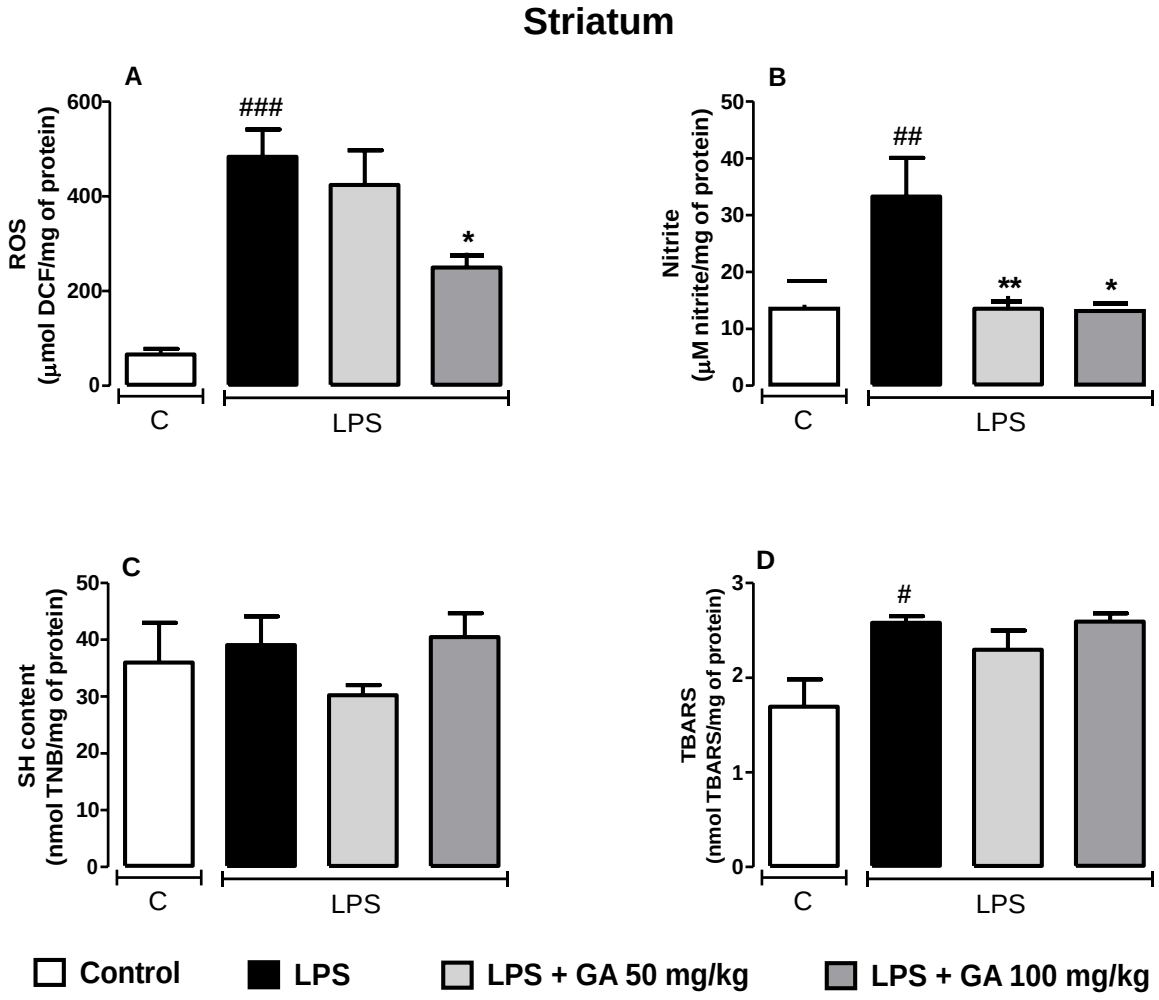

Figure 5 – Effect of gallic acid (50 and 100 mg/kg, p.o.) pretreatment on levels of reactive oxygen species (ROS) **(A),** nitrite **(B),** total thiol content (SH) **(C)** and thiobarbituric acid reactive substances (TBARS) **(D)** in striatum of mice treated with LPS (250 µg/kg).Data are reported as mean ± S.E.M. $^{\#}P<0.05$; $^{\#\#}P<0.01$ and $^{\#\#\#}P<0.001$ compared to vehicle/saline group. *P<0.05 and **P<0.01 compared to vehicle/LPS group (n=4–5).

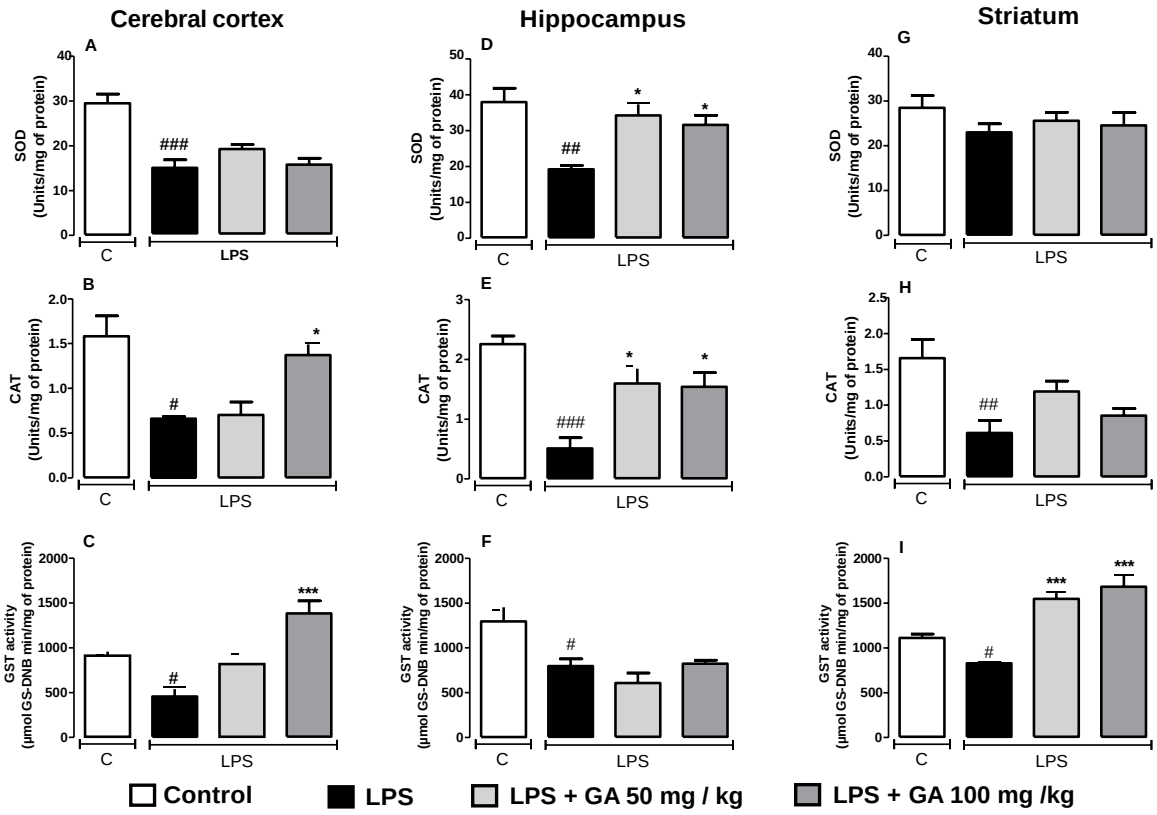

Figure 6 - Effect of gallic acid (50 and 100 mg/kg, p.o.) on superoxide dismutase (SOD), catalase (CAT) and glutathione S-transferase (GST) activities in cerebral cortex **(A-C),** hippocampus **(D-F)** and striatum **(G – I**) of mice treated with LPS (250 µg/kg). Data are reported as mean ± S.E.M. $^{\#}P<0.05$; $^{\#\#}$ P<0.01; $^{\#\#\#}$ P<0.001 compared to vehicle/saline group. *P<0.05;**P<0.01; ***P<0.001 compared to vehicle/LPS group (n=4–5).

5 Discussão

O TAB é uma condição psiquiátrica grave, crônica e altamente debilitante (MERIKANGAS et al., 2011). Além disso, o TAB é a sexta causa de incapacidade entre as condições médicas e psiquiátricas (YOUNG e DULCIS, 2015). Este se caracteriza por oscilações de humor, que incluem episódios de mania e depressão. Embora seja um transtorno amplamente estudado, os exatos mecanismos fisiopatológicos envolvidos ainda são pouco elucidados. Várias hipóteses têm sido descritas, tais como, fatores genéticos, alteração nos níveis de neurotransmissores, fator neurotrófico, inflamação e estresse oxidativo (SIGITOVA et al., 2016). O tratamento farmacológico para o TAB ainda é limitado e apresenta muitos efeitos colaterais os quais contribuem negativamente para a baixa aderência ao tratamento por parte dos pacientes. Desta forma, a busca por terapias mais eficazes tem sido extremamente importante para melhorar a qualidade de vida destes pacientes.

Nos últimos anos, o efeito dos compostos naturais e seus derivados, têm sido amplamente investigados para a prevenção e o tratamento de doenças psiquiátricas, entre elas, o TAB, que se trata de uma patologia complexa, multifatorial, cujo tratamento atual é feito com fármacos, que, porém, muitas vezes, apresentam efeitos adversos. Em conjunto com a grande diferenciação da sintomática, de acordo com cada paciente, e da dificuldade em se estabelecer uma dosagem específica sem efeitos indesejáveis, a pesquisa por tratamentos alternativos torna-se necessária (LAFER e NERY, 2011). Assim o objetivo desta tese foi fazer uma revisão de literatura sobre os avanços do potencial terapêutico dos compostos naturais e derivados frente ao TAB, bem como avaliar o efeito neuroprotetor do ácido gálico em dois modelos experimentais que mimetizam mecanismos patológicos associados a esta desordem psiquiátrica: mania e neuroinflamação.

Em relação ao manuscrito de revisão, o objetivo deste trabalho foi analisar o papel dos compostos naturais e suas propriedades terapêuticas em estudos clínicos e pré-clínicos de TAB. Considerando inicialmente os estudos clínicos, existem poucos trabalhos avaliando os efeitos benéficos desses compostos em pacientes com TAB. Dentre os compostos testados pode-se destacar o uso dos ácidos graxos ômega-3 (VESCO et al., 2015), inositol (FORMOSO et al., 2019; SCHNEIDER, 2015 CHENGAPPA et al., 2000), N-acetilcisteína (NAC) (MAGALHÃES et al., 2011a) vitamina D (SIKOGLU et al. 2015); fitoterápico a base de ervas medicinais (ZHANG et al., 2007a) e o extrato de *Withania somnifera* (WSE) (CHENGAPPA et al., 2013).

Embora as doses bem como o tempo de tratamento foram diferentes entre os compostos utilizados, todos eles demonstram alguma eficácia clínica nos pacientes incluindo melhora dos déficits cognitivos, sintomas depressivos e maníacos.

Em relação aos estudos pré-clínicos, sabe-se que existe uma dificuldade muito grande em estabelecer um modelo adequado para TAB, devido à natureza cíclica desta doença (BEYER E FREUND, 2017). Entretanto, como os episódios maníacos são decisivos para o diagnóstico de TAB, modelos animais que mimetizam este estado comportamental são amplamente usados para estudar essa doença (SHARMA et al. 2016; BEYER e FREUND 2017). Desta forma, os estudos pré-clínicos descritos na literatura têm avaliado o potencial terapêutico de compostos naturais frente principalmente a modelos experimentais de mania.

Considerando que o estresse oxidativo é um dos mecanismos associado à etiologia do TAB, a maioria dos estudos experimentais descritos tem focado no potencial antioxidante dos compostos naturais na prevenção do comportamento tipo maníaco, o qual pode ser observado nos animais através da hiperlocomoção dos mesmos, bem como no efeito neuroprotetor em regiões cerebrais associadas com cognição e emoção. Dentre os principais compostos avaliados nestes estudos pode-se citar o extrato da planta *Cecropia pachystachya*, popularmente conhecida como embaúba no Brasil (GAZAL et al. 2015). O extrato de *Cecropia pachystachya* foi eficaz em prevenir a hiperlocomoção e danos oxidativos em hipocampo e córtex pré-frontal em animais submetidos a um modelo de mania induzido por cetamina. Outro estudo demonstrou que o extrato de mirtilo tem efeitos muito similares ao fármaco padrão litío e foi capaz de prevenir o comportamento tipo-maníaco, danos oxidativos e alterações nas enzimas Na^+K^+-ATPase e AChE em córtex cerebral, hipocampo e estriado de ratos (DEBOM et al., 2016; SPOHR et al., 2019). Efeitos similares foram obtidos com o uso de extrato de amora (CHAVES et al., 2020). Além disso, esses extratos de frutos foram capazes de modular os níveis séricos e cerebrais de IL-6 em animais em um modelo de mania induzido por cetamina (DEBOM et al., 2016; CHAVES et al., 2020). Os efeitos benéficos desses extratos naturais, podem ser atribuídos a sua rica composição em polifenóis caracterizadas por sua potente ação antioxidante e antiinflamatória.

Outros compostos naturais isolados também foram estudados e demonstraram resultados promissores. Dentre estes compostos, pode-se destacar a curcumina que foi capaz de prevenir o comportamento tipo-maníaco causado pela

cetamina (GAZAL et al. 2014) e a quercetina que preveniu o comportamento tipo-maníaco e o estresse oxidativo no cérebro induzido por um modelo de mania através da privação de sono em camundongos (KANAZAWA et al. 2016, 2017). O ácido alfa-lipóico (ALA) além de prevenir a hiperlocomoção e os danos oxidativos cerebrais foi capaz de aumentar os níveis de BDNF no hipocampo em um modelo experimental de mania induzido por anfetamina (MACÊDO et al. 2012). Além disso, cabe destacar o potencial terapêutico tipo-antimaníaco (diminuindo a atividade locomotora dos animais) da carvona, um monoterpeno presente em óleo essencial de hortelã e cominho, em dois diferentes modelos de mania (induzido por metilfenidato e outro por privação de sono) (NOGOCEKE et al., 2016).

O ácido graxo essencial ômega-3 já teve sua eficácia testada em estudos clínicos de TAB. Em um modelo animal de mania induzida por metilfenidato foi demonstrado que um dos mecanismos terapêuticos associados ao uso do ômega-3 podem estar relacionado ao aumento do metabolismo energético cerebral. Além disso, foi observado um efeito promissor na atividade da AChE no grupo de animais tratados com ômega-3 associados a medicamentos padrão para o TAB, sugerindo assim que a suplementação poderia ser utilizada juntamente com antipsicóticos atípicos e estabilizadores de humor, a fim de otimizar a eficácia clínica do tratamento convencional (ARUNAGIRI & BALAMURUGAN 2016).

Em conclusão, o manuscrito de revisão demonstrou que tanto estudos pré-clínicos e clínicos têm confirmado o potencial dos compostos naturais e derivados na prevenção e no manejo das fases de TAB. Esses compostos podem ser benéficos no alívio dos sintomas em pacientes e também exercem efeitos neuroprotetores em modelos animais de mania por meio de vias antioxidantes, antiinflamatórias, anticolinesterásicas, metabólicas e neurotróficas.

Desta forma, considerando que compostos naturais têm sido apontados como potenciais agentes neuroprotetores para TAB outro objetivo desta tese foi avaliar os efeitos do ácido gálico em um modelo experimental de mania.

Os modelos animais de mania podem ser induzidos por diferentes estímulos e, neste trabalho, utilizamos a substância cetamina, que vem sendo estudada como um agente indutor de hiperlocomoção (GHEDIM et al., 2012; GAZAL et al., 2014), pois altera a neurotransmissão glutamatérgica, devido a sua atuação como antagonista não competitivo do receptor NMDA, gerando alterações no cérebro dos

animais, semelhantes com as descritas em pacientes bipolares (GHEDIM et al., 2012).

Primeiramente, os resultados deste estudo demonstraram que a administração de cetamina na dose de 25 mg/Kg, foi capaz de induzir um comportamento tipo-maníaco nos ratos, observado através do aumento da atividade locomotora dos animais. A hiperatividade é um sintoma observado nos pacientes durante o episódio maníaco (PERRY et al., 2016). Entretanto, o tratamento com ácido gálico foi efetivo em reduzir a hiperlocomoção induzida pela cetamina, similar aos efeitos do fármaco padrão lítio. Corroborando com esses resultados DEBOM et al. (2016) e SPOHR et al. (2019) também observaram um aumento na atividade locomotora dos ratos utilizando este mesmo modelo experimental.

Os resultados do artigo 1 também demonstraram que cetamina foi capaz de induzir um aumento de TBARS, EROs e níveis de nitritos, bem como, a diminuição de conteúdo tiólico e a atividade de enzimas antioxidantes, tais como, CAT, SOD e GPx em córtex cerebral, hipocampo e estriado, os quais são regiões cerebrais responsáveis pela regulação da emoção (DREVETS et al., 2008). Os danos oxidativos ocorrem em situações onde existe um desequilíbrio entre a produção de radicais e a capacidade de removê-los pelos sistemas antioxidantes, seja enzimáticos ou não-enzimáticos, causando assim, um acúmulo dessas espécies reativas, que irão causar danos às biomoléculas, como as proteínas, os lipídeos e o DNA (HALLIWELL & GUTTERIDGE, 2007; RAJENDRAN, et. al., 2014). De fato, estudos prévios também demonstraram que estresse oxidativo desempenha um papel crítico na patogênese do TAB, durante episódio maníaco (SOUSA et al., 2014). AKARSU et al. (2018) também observaram níveis elevados de oxidantes no sangue de pacientes com TAB, de acordo com a gravidade da doença. Alterações significativas nos níveis de TBARS, um indicador de peroxidação lipídica, foram demonstradas em pacientes em estado maníaco, quando comparados com os controles saudáveis (TSAI e HUANG, 2015).

O tratamento com ácido gálico nas doses de 50 e 100 mg/kg foi eficaz em proteger as estruturas cerebrais contra danos oxidativos induzidos pelo modelo experimental de comportamento tipo-maníaco, pois este polifenol diminuiu os níveis de TBARS, EROs e nitritos, assim como, aumentou o conteúdo tiólico total e a atividade das enzimas antioxidantes. O efeito benéfico deste composto tem sido

atribuído, principalmente, pela sua potente atividade antioxidante, devido a sua habilidade de sequestrar e reduzir a formação de espécies reativas.

Nesse mesmo artigo, também foi demonstrado um aumento na atividade da enzima AChE em hipocampo e estriado dos animais tratados com cetamina. Resultados semelhantes foram descritos por SPOHR et al. (2019) usando este mesmo modelo experimental. Alterações na atividade desta enzima, estão associadas à diversas patologias, inclusive com o TAB (SIGITOVA et al., 2016; SPOHR et al., 2019). O aumento da atividade da AChE, pode causar uma diminuição nos níveis do neurotransmissor ACh, pois esta é responsável pela hidrólise dessa molécula em acetato e colina, contribuindo assim, para a ocorrência de episódios maníacos (SIGITOVA et al., 2016). O ácido gálico foi capaz de prevenir alterações na atividade da AChE em ambas as estruturas cerebrais. Corroborando com esses dados, SAMAD et al. (2019) também avaliaram os efeitos do ácido gálico contra alterações na memória de ratos e, demonstraram que, a administração deste composto, reduziu significativamente a atividade da AChE.

Neste estudo, também foi utilizado o lítio como controle positivo, pois este trata-se do principal fármaco no tratamento de pacientes com TAB. Este medicamento mostrou-se efetivo ao reduzir a hiperlocomoção e os danos oxidativos presentes nas estruturas cerebrais, bem como, alterações na atividade da AChE. Esses achados estão de acordo com a literatura, que tem demonstrado que o lítio participa na regulação de diferentes mecanismos envolvidos na resposta ao estresse oxidativo, tanto em outros modelos de mania, como em pacientes bipolares (JORNADA et al., 2011; BENGESSER et al., 2016).

A partir dos dados obtidos no artigo 1 pode-se concluir que o ácido gálico foi eficaz em prevenir o comportamento maníaco, dano oxidativo e disfunções colinérgicas em estruturas cerebrais envolvidas na regulação da emoção em um modelo experimental de mania. Outro aspecto importante a ser mencionado é que o TAB é uma doença crônica e sabe-se que o tratamento contínuo é necessário para prevenir sintomas maníacos ou depressivos e melhorar a qualidade de vida dos pacientes. Assim, nossos resultados demonstraram que o ácido gálico tem efeitos semelhantes ao tratamento com lítio, sugerindo que esse composto natural pode ser eficaz na melhora dos sintomas associados à mania. Embora o ácido gálico pareça geralmente isento de efeitos colaterais relevantes, são necessários estudos para avaliar a segurança de uma dose terapêutica em humanos.

Considerando que a neuroinflamação é uma condição envolvida na patogênese de diversas doenças psiquiátricas, incluindo o TAB, o manuscrito II teve como objetivo, avaliar os efeitos neuroprotetores do ácido gálico em um modelo de neuroinflamação induzido por LPS. O LPS é um glicolipídeo, derivado de bactérias gram-negativas e, considerado um ativador potente da resposta imune inata (BLUTHÉ et al., 1991). Este liga-se ao TLR4, induzindo assim a fosforilação do NF-kB e, consequentemente, elevando drasticamente os níveis de citocinas e de EROs, resultando em respostas pró-inflamatórias (CAFÉ-MENDES et al., 2017).

Prévios estudos da literatura têm demonstrado que a administração de LPS induz neuroinflamação, estresse oxidativo no cérebro e déficits de memória em animais. Neste trabalho, observou-se que animais expostos ao LPS apresentaram um prejuízo na memória, o qual pode ser evidenciado pelo teste de reconhecimento de objetos. Esses achados corroboram com estudos anteriores que também demonstraram que o LPS quando administrado sistemicamente ou centralmente afeta o processo de memória (JOSHI et al., 2014; ABARESHI et al., 2016). O LPS desencadeia respostas imunes e induz a ativação microglial por uma via dependente do receptor Toll-like 4 (TLR4), acompanhada pela produção e liberação de citocinas pró-inflamatórias, como interleucina IL-1β, IL-6 e TNF-α (YUAN et al., 2016). O aumento da IL-1β bem como o prejuízo na neurogênese hipocampal induzidos pelo LPS podem ser mecanismos associados, ao menos em parte, com os déficits de memória observados nos animais (HUANG E SHENG, 2010; FRANK et al., 2012; VALERO et al., 2014).

O sistema antioxidante desempenha um papel importante na regulação da ação inflamatória (KURUTAS, 2016), pois as EROs interferem no processo inflamatório, uma vez que se sabe que a liberação excessiva de EROs causa danos aos tecidos, intensificando a reação e pode levar a um processo inflamatório crônico, ou seja, a produção exacerbada de EROs tem sido demonstrada em várias doenças de natureza inflamatória (EL-BENNA et al., 2016; DRUMMOND, 2011).

A administração de LPS além de induzir um aumento das citocinas pró-inflamatórias também aumenta os níveis de espécies reativas (WANG et al., 2004; KHAN et al., 2016). Os resultados do manuscrito II demonstram que LPS quando administrado sistematicamente também induziu dano oxidativo no córtex cerebral, hipocampo e estriado de camundongos, o que pode ser evidenciado pelo aumento dos níveis de EROS, TBARS e nitritos e uma diminuição no conteúdo de SH, bem

como na atividade das enzimas antioxidantes. Esses achados são semelhantes a outros estudos do grupo de pesquisa que também demonstraram que a administração de LPS altera o status redox em diferentes regiões do cérebro (SPOHR et al., 2020; LUDUVICO et al., 2020). ZAREZADEH et al. (2017) também demonstram que os níveis de GSH e enzimas antioxidantes diminuíram significativamente nos cérebros dos animais expostos ao LPS. Esse aumento na geração de ROS pode ser atribuído à ativação do receptor TLR4 pelo LPS, pois este interage diretamente com a enzima NADPH oxidase, que é uma das principais fontes de espécies reativas (PARK et al., 2004).

O tratamento com ácido gálico mostrou-se efetivo em reverter os déficits de memória e proteger regiões cerebrais contra a produção de EROs, danos aos lipídeos, proteínas e a redução do sistema de defesa antioxidante. Uma variedade de antioxidantes naturais, com capacidade de eliminar os radicais livres e proteger as células de danos oxidativos, têm sido alvo de exaustivas investigações, entre eles, o ácido gálico, um polifenol com importantes potencialidades terapêuticas, as quais podem ser atribuídas, principalmente, pela sua capacidade antioxidante e antiinflamatória (CHOUBEY et al., 2015; KAHKESHANI et al., 2019). Em consonância, LU et al. (2006) demonstraram que derivados de ácido gálico apresentam propriedades neuroprotetoras e antioxidantes. Além disso, alguns autores têm destacado também, a sua propriedade antiinflamatória, devido à eficácia deste composto em inibir mediadores inflamatórios (PANDURANGAN et al., 2015a; PANDURANGAN et al., 2015b; BENSAAD et al., 2017).

6 Conclusão

Considerando que compostos naturais são benéficos no alívio dos sintomas em pacientes e também exercem efeitos neuroprotetores em modelos animais que mimetizam episódios maníacos, as terapias a base de compostos naturais podem ser alternativas promissoras no tratamento do TAB.

Nesta tese, foi demonstrado o potencial do ácido gálico em prevenir a hiperlocomoção induzida por cetamina no teste do campo aberto, dano oxidativo e disfunções colinérgicas em estruturas cerebrais envolvidas na regulação da emoção em um modelo experimental de mania. Estes resultados foram semelhantes ao tratamento com o lítio, o fármaco de primeira linha usado no tratamento de pacientes bipolares, sugerindo que este composto natural pode ser eficaz na prevenção da recorrência de novos episódios maníacos.

Visto que o caráter pró-oxidante associado ao ambiente pró-inflamatório parecem estar envolvidos na fisiopatologia do TAB, neste trabalho, também foi avaliado o efeito neuroprotetor do ácido gálico em um modelo de neuroinflamação induzido por LPS. O tratamento com ácido gálico mostrou-se efetivo em reverter os *déficits* de memória e prevenir alterações no *status redox* em regiões cerebrais dos animais, demonstrando que este composto apresenta um potencial terapêutico que pode trazer benefícios para pacientes portadores desta desordem psiquiátrica.

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Anexos

Anexo 1- Carta de parecer do Comitê de Ética em Experimentação Animal 1

UFPel
Comissão de Ética em Experimentação Animal

Pelotas, 14 de setembro de 2015

De: M.V. Dra. Anelize de Oliveira Campello Felix

Presidente da Comissão de Ética em Experimentação Animal (CEEA)

Para: Profa. Dra. Francieli Moro Stefanello

Centro de Ciências Químicas, Farmacêuticas e de Alimentos

Senhora Professora:

A *CEEA* analisou o projeto intitulado: **"Avaliação farmacológica de compostos naturais em modelos animais de transtornos do humor"**, processo nº23110.004609/2015-09 que envolve a utilização de animais pertencentes ao filo Chordata, Subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino, sendo de parecer **FAVORÁVEL** a sua execução, pois está de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA).

Solicitamos, após tomar ciência do parecer, reenviar o processo à *CEEA*.

Salientamos também a necessidade deste projeto ser cadastrado junto ao *COBALTO* para posterior registro no *COCEPE* (código para cadastro nº **CEEA 4609-2015**).

Vigência do Projeto: 15/09/2015 a 31/12/2018
Espécie/Linhagem: *Rattus norvegicus*/Wistar e *Mus musculus*/Swiss
Nº de animais: 312 de cada espécie
Idade: 60 dias
Sexo: Machos
Origem: Biotério Central/Ufpel

M.V. Dra. Anelize de Oliveira Campello Felix
Presidente da CEEA

Ciente em: 05 / 10 /2015

Anexo 2- Carta de parecer do Comitê de Ética em Experimentação Animal 2

UNIVERSIDADE FEDERAL DE PELOTAS

PARECER Nº **97/2020/CEEA/REITORIA**
PROCESSO Nº 23110.017187/2020-91

Certificado

Certificamos que a proposta intitulada **"Potencial terapêutico do ácido gálico em modelo de neuroinflamação em camundongos: avaliação de parâmetros comportamentais, neuroquímicos e sistêmicos."**, registrada com o n° 23110.017187/2020-91**,** sob a responsabilidade de **Roselia Maria Spanevello** - que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica (ou ensino) – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e recebeu parecer **FAVORÁVEL** a sua execução pela Comissão de Ética em Experimentação Animal, em reunião de **07 de agosto de 2020**.

Finalidade	(x) Pesquisa ( ) Ensino
Vigência da autorização	01/09/2020 a 01/09/2023
Espécie/linhagem/raça	*Mus musculus*/Swiss
N° de animais	72
Idade	60 dias
Sexo	Machos
Origem	Biotério Central - UFPel

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M.V. Dra. Anelize de Oliveira Campello Felix

Presidente da CEEA

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