



**UNIVERSIDADE FEDERAL DA FRONTEIRA SUL
CAMPUS CHAPECÓ**

**PROGRAMA DE PÓS GRADUAÇÃO EM CIÊNCIAS BIOMÉDICAS
CURSO DE MESTRADO EM CIÊNCIAS BIOMÉDICAS**

TÁCIO DE OLIVEIRA

**EFEITOS DE EXTRATO E COMPOSTO ATIVO DA *Centella asiatica* EM
COMPORTAMENTO TIPO DEPRESSIVO, ESTRESSE OXIDATIVO E
INFLAMAÇÃO EM RATOS SUBMETIDOS A PRIVAÇÃO MATERNAL**

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Dissertação apresentada ao Curso de Mestrado em Ciências Biomédicas da Universidade Federal da Fronteira Sul (UFFS), como requisito para obtenção do título de Mestre em Ciências Biomédicas, sob orientação da Professora Doutora Zuleide Maria Ignácio, coorientação Professor Doutor Walter Antônio Roman Junior.

CHAPECÓ

2022

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RESUMO

A espécie medicinal *Centella asiatica* e seus princípios ativos isolados (ácido madecássico, madecasoide, madecassosideo) vem sendo estudados no contexto de transtorno depressivo maior (TDM), sendo relacionada a efeitos antidepressivos e com neuroproteção. Vários estudos vêm relatando efeitos anti-inflamatórios, antioxidantes e nootrópicos. O TDM está relacionado com aumento de citocinas inflamatórias e estresse oxidativo no sistema nervoso central (SNC) e periferia. A privação maternal (PM) em ratos filhotes é um modelo animal de reprodução de comportamentos e mecanismos biológicos relacionados ao TDM em humanos. Portanto, este trabalho utilizou ratos submetidos à PM nos primeiros dias de vida e tratados na vida adulta com extrato de *Centella asiatica*, ou ácido madecássico (AM), ou escitalopram (ESC), conforme descrição a seguir: 5 grupos com 10 ratos cada, sendo grupo 1 o controle sem estresse + veículo (controle), grupo 2 PM + veículo (controle negativo), grupo 3 PM + ESC (controle positivo), grupo 4 PM + extrato de *Centella asiatica*, grupo 5 PM + AM. Os animais foram submetidos à PM por 10 dias a partir do segundo dia de nascimento. Aos 60 dias de idade, foram tratados por 14 dias. Ao final do período foram realizados testes comportamentais e realizada eutanásia para aferição dos seguintes parâmetros: mieloperoxidase (MPO) no soro sanguíneo e hipocampo, susbtâncias reativas ao ácido tiobarbitúrico (TBARS) (soro e hipocampo), interleucina-1 β (IL-1 β), interleucina-6 (IL-6) (hipocampo). A PM induziu comportamentos tipo depressivos na vida adulta, bem como aumentou a inflamação e estresse oxidativo. A intervenção com extrato de ESC, AM e *Centella asiatica* induziu reversão dos comportamentos tipo depressivos e proporcionou uma redução significativa nos níveis de MPO no soro nos grupos tratados. O tratamento com AM e ESC conseguiu manter níveis de TBARS no soro e hipocampo iguais aos do grupo controle sem PM. O tratamento com o extrato de *Centella asiatica* não reduziu significativamente os níveis de TBARS, tanto no soro quanto no hipocampo. Com relação à IL1 β , o ESC e o AM revertem os níveis no hipocampo. O extrato de *Centella asiatica* não reduziu significativamente. Todos os tratamentos revertem o aumento de IL-6 no hipocampo. Estes resultados corroboram estudos com outros protocolos, sugerindo um potencial efeito antidepressivo, possivelmente envolvendo redução no estresse oxidativo e inflamação.

Palavras-chave: Transtorno Depressivo Maior, Privação Maternal, Inflamação, Estresse Oxidativo, Ácido Madecássico, *Centella asiatica*.

ABSTRACT

The medicinal species *Centella asiatica* and its isolated active principles (madecassic acid, madecasoid, madecassoside) have been studied in the major depressive disorder (MDD) context, being related to antidepressant and neuroprotective effects. Several studies have reported anti-inflammatory, antioxidant, and nootropic effects. MDD is related to increased inflammatory cytokines and oxidative stress in the central nervous system (CNS) and periphery. Maternal deprivation (PM) in baby rats is an animal model of the reproduction of behaviors and biological mechanisms related to MDD in humans. Therefore, this work used rats submitted to PM in the first days of life and treated in adulthood with *Centella asiatica* extract, or madecassic acid (AM), or escitalopram (ESC), as described below: 5 groups with 10 rats each, being group 1 the stress-free control + vehicle (control), group 2 PM + vehicle (negative control), group 3 PM + ESC (positive control), group 4 PM + *Centella asiatica* extract, group 5 PM + AM. The animals were submitted to the PM for 10 days from the second day of birth. At 60 days of age, they were treated for 14 days. At the end of the period, behavioral tests, and euthanasia were performed to measure the following parameters: myeloperoxidase (MPO) in blood serum and hippocampus, thiobarbituric acid reactive substances (TBARS) (serum and hippocampus), interleukin-1 β (IL-1 β), interleukin-6 (IL-6) (hippocampus). The PM induced depressive-like behaviors in adulthood and increased inflammation and oxidative stress. Intervention with extracts of ESC, AM, and *Centella asiatica* yielded the reversal of depressive-like behaviors and significantly reduced serum MPO levels. Treatment with AM and ESC managed to maintain serum and hippocampal TBARS levels equal to the control group without PM. Treatment with *Centella asiatica* extract did not significantly reduce TBARS levels in serum and hippocampus. Regarding IL1 β , ESC and AM reversed levels in the hippocampus. *Centella asiatica* extract did not decrease substantially. All treatments reversed the increase in IL-6 in the hippocampus. These results corroborate studies with other protocols, suggesting a potential antidepressant effect, possibly involving a reduction in oxidative stress and inflammation.

Keywords: Major Depressive Disorder, Maternal Deprivation, Inflammation, Oxidative Stress, Madecassic Acid, *Centella asiatica*.

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LISTA DE SIGLAS

AM - Ácido Madecássico
AA - Ácido Asiático
BDNF - Fator Neurotrófico Derivado do Cérebro
CAT - Catalase
COX-2 - cicloxigenase 2
CPK - creatinafosfoquinase
DMSO - Dimetilsulfóxido
DNA - Ácido Desoxirribonucleico
EM - Espectrometria de Massa
ERK-1 -
ESC - Escitalopram
GPx - Glutationa Peroxidase
IgE - Imunoglobulina E
IL-1 - Interleucina 1
IL1 β - Interleucina 1 beta
IL-6 - Interleucina 6
iNOS - Óxido Nítrico Sintase Induzida
HPA - Hipotálamo-pituitária-adrenal
HPLC - Cromatografia Líquida de Alta Eficiência
LCE - Labirinto em Cruz Elevada
LPS - Lipopolissacarídeos
MDA - Malondialdeído
MAPK - proteína quinase de ativação da mitose
NF68 - Neurofilamento 68kDa
NF - Natação Forçada
NF-kB - Fator Nuclear Kappa Beta
NGF - Fator de Crescimento do Nervo
PBS - Solução Salina Tamponada com Fosfato
PGE2 - prostaglandina E2
PM - Privação Maternal
SOD - Superóxido Dismutase
TAG - Transtorno de Ansiedade Generalizada

TBARS - Substâncias Reativas ao Ácido Tiobarbitúrico

TCA - Teste de Campo Aberto

TDM - Transtorno Depressivo Maior

TNF - Teste de Natação Forçada

TGO - Transaminase Oxalacética

TGP - Transaminase Pirúvica

TNF- α - Fator de Necrose Tumoral alfa

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1 INTRODUÇÃO

No transtorno depressivo maior (TDM) e doenças neurodegenerativas, um padrão semelhante de liberação de citocinas inflamatórias se repete, a exemplo de interleucinas (IL) como IL-1 β , IL-2, IL-6, TNF- α , sendo que a micróglio é um dos principais constituintes com este fenótipo (KRISHNA et al. 2016). A ativação de células inflamatórias em pacientes com TDM também está associada a aumento de marcadores de estresse oxidativo (SCHMIDT et al., 2018; ZHONG et al., 2019).

Frommberger et al. em 1999 já encontraram níveis elevados de IL-6 no soro de pacientes em episódios de exacerbação de depressão e esquizofrenia (FROMMBERGER et al., 1999). Diversos estudos com inibidores da recaptação da serotonina têm mostrado redução nos níveis em pacientes depressivos após intervenção com sertralina, paroxetina, fluoxetina, escitalopram. Yoshimura et al. em 2009 e 2013 encontraram reduções de IL-6 em pacientes que foram medicados com fluvoxamina, sertralina, e paroxetina (YOSHIMURA et al., 2009; YOSHIMURA et al., 2013). Em um estudo com crianças, foi observado redução nos níveis de TNF- α após uso de fluoxetina, porém o mesmo não ocorreu com a IL-6 e IL-1 β . O mesmo estudo observou que em pacientes com boa resposta clínica ao tratamento, haviam níveis menores de TNF- α , IL-6 e IL-1 β do que pacientes pouco responsivos clinicamente ao tratamento (AMITAI et al., 2016). Já Yoshimura et al., 2013, associou os níveis de IL-6 iniciais mais altos com maior resposta ao tratamento e maior queda da IL-6 após a intervenção (YOSHIMURA et al., 2013).

Em modelo animal, geralmente se utiliza situações estressantes para induzir sintomas depressivos, como privação maternal (PM), isolamento social, ambientação mais escura (ASHKENAZY et al., 2009; IGNÁCIO et al., 2017) e também tratamentos químicos como injeção intraperitoneal de lipopolissacarídeos (LPS) e IL-1 (BLUTHÉ et al., 2000). Foi observado que ratos knockout para o gene IL-6 ou que recebem tratamento farmacológico com inibidores da via NF- κ B têm maior resistência a desenvolver sintomas tipo depressivos (BLUTHÉ et al., 2000; CHOURBAKI et al., 2006; HAYLEY et al., 2008; MONJE et al., 2011).

O estresse oxidativo é outro parâmetro importante no que se refere às doenças. Ele está envolvido em diversas doenças crônicas relacionadas ao envelhecimento, como doença cardiovascular, diabetes, doença pulmonar obstrutiva crônica, doença renal crônica,

sarcopenia, assim como doenças neurodegenerativas e o envelhecimento do organismo (USBERTI et al., 2002; PUCHADES MONTESSA et al., 2009; ROMMER et al., 2016; MASI et al., 2018; BERNABEU-WITTEL et al., 2020; YILMAZ et al., 2020). Na depressão unipolar, foram encontrados níveis elevados de MDA, que expressa a magnitude da peroxidação lipídica proveniente do processo oxidativo (BAJPAI et al., 2014; ALVAREZ-MON et al., 2022). Também foram encontrados níveis baixos em relação aos controles de vitamina C e da enzima antioxidante superóxido dismutase (SOD) (BAJPAI et al., 2014). Estes fatores evidenciam a importância cada vez maior de marcadores oxidativos para auxiliar na identificação e manejo de pacientes com sintomas depressivos.

Um estudo com 178 pares de homens gêmeos de meia-idade mostrou um aumento dos níveis de mieloperoxidase nos pares com TDM ($p < 0,0001$), e identificou um aumento ainda mais evidente entre gêmeos discordantes quanto ao quadro depressivo ($p < 0,0001$) (VACCARINO et al., 2008)

Alguns estudos vêm destacando a espécie medicinal *Centella asiatica* como possibilidade de intervenção e efeito benéfico no TDM (JANA et al., 2010). Estudos in vitro e em roedores indicaram aumento na plasticidade neuronal, incluindo aumento de arborização dendrítica hipocampal (SOUMYANATH et al., 2005; MOHANDAS RAO, MUDDANNA RAO, GURUMADHVA RAO, 2006). Os efeitos aparentemente neuroprotetores envolvem diversos mecanismos moleculares e estruturais, envolvendo também ações benéficas sobre o eixo hipotálamo-pituitária-adrenal (HPA), estresse oxidativo e inflamação (CHEN et al., 2005; LARRIEU et al., 2014).

O extrato de *Centella asiatica* foi capaz de aumentar os níveis hepáticos de SOD, glutationa peroxidase (GPx), ao mesmo tempo que reduziu MDA, transaminase glutâmico oxalacética (TGO) e transaminase glutâmico pirúvica (TGP) em ratos, em doses de 100 a 500mg/kg/dia (ZHAO et al., 2014). Também teve efeito anti-inflamatório, ao inibir a expressão sérica de TNF- α , IL-1 β , IL-6, e IgE, e também inibiu a liberação de óxido nítrico, cicloxigenase-2 (COX-2), atividade de ligação ao DNA do Fator Nuclear Kappa Beta (NF-kB) pós estímulo de lipopolissacáideos (LPS) em macrófagos de ratos tipo RAW264.7 (PARK et al, 2017).

Em um estudo in vitro, os pesquisadores observaram que dois componentes ativos da espécie, os ácido asiático (AA) e ácido madecássico (AM), funcionaram sinergisticamente, proporcionando efeitos semelhantes a fatores de crescimento na diferenciação neuronal (JIANG et al., 2016). Um estudo recente observou que o asiaticosídeo, um componente triterpenóide da *Centella asiatica*, exerceu efeito tipo antidepressivo em camundongos

submetidos a estresse moderado crônico e reduziu a expressão de citocinas inflamatórias (WANG et al., 2020). Os estudos sobre os extratos e compostos ativos da *Centella asiatica* sugerem sua relevância como estratégia farmacológica terapêutica para o TDM, bem como sua função em mecanismos biológicos subjacentes.

2 OBJETIVOS

2.1 OBJETIVO GERAL

- Avaliar o efeito do tratamento com extratos de folhas da espécie *Centella asiatica* e seu composto ativo ácido madecássico sobre comportamentos tipo depressivos, balanço oxidativo e mecanismos pró-inflamatórios em ratos submetidos a estresse de privação maternal nos primeiros dias de vida.

2.2 OBJETIVOS ESPECÍFICOS

- Avaliar comportamentos tipo depressivos em ratos machos submetidos a estresse de privação maternal (PM) nos primeiros dias de vida;
- Avaliar o efeito do estresse de PM em parâmetros de estresse oxidativo e expressão das citocinas pró-inflamatórias no hipocampo e no sangue periférico de ratos;
- Avaliar o efeito do tratamento crônico com extrato hidroalcoólico de *Centella asiatica*, escitalopram e o composto ativo ácido madecássico, nos comportamentos tipo depressivos, estresse oxidativo e inflamação, em ratos que sofreram PM nos primeiros dias de vida.

3 REFERENCIAL TEÓRICO

3.1 *Centella asiatica*

A espécie *Centella asiatica* (L.) Urban é uma planta perene originária da Ásia que cresce em muitas áreas com clima tropical e subtropical no mundo (JIANG et al, 2016). Os triterpenóides pentacíclicos (centelóides) são os ativos mais importantes e abundantes desta planta. Existem dois tipos de triterpenóides pentacíclicos, as saponinas asiaticosídeo e madecassoside (MS), e seus respectivos metabólitos, as agliconas ácido asiático (AA) e ácido

madecássico (AM) (SONGVUT et al., 2019). Os tripterpenos são compostos orgânicos apolares no geral, devido ao número de carbonos da cadeia, contudo radicais carboxila e hidroxila podem gerar uma leve polaridade na molécula (RÍOS et al., 2000). A análise por cromatografia líquida de alta eficiência (HPLC) do extrato mostra as seguintes concentrações: madecassoside $3,10 \pm 4,58\text{mg/ml}$; asiaticosídeo $1,97 \pm 2,65\text{ mg/mL}$; ácido asiático $0,55 \pm 2,29$; ácido madecássico $0,55 \pm 0,89\text{ mg/mL}$ (HANSHIN et al., 2011). Esses dados mostraram que as saponinas foram mais abundantes que as agliconas. No entanto, dependendo da localização de seu crescimento e do método de obtenção do extrato, as proporções de bioativos podem mudar e as saponinas podem variar entre 1 e 8% do extrato seco. Em outro estudo, a ordem dos ativos foi invertida, e as concentrações estimadas foram de ácido madecassico $2,96\text{mg/g}$, madecassosídeo $2,92\text{mg/g}$, ácido asiático $1,29\text{mg/g}$, asiaticosídeo $2,07\text{mg/g}$ (JAMES e DUBERY, 2011). As partes utilizadas para obter o extrato são as folhas e parte aérea (SARI et al., 2018; EMA, 2021)

O extrato obtido por James e Dubery foi viabilizado através da extração por álcool absoluto (100%), tendo eficácia de extração dos compostos madecassosídeo, asiaticosídeo, AM, ácido asiático de 95 a 98% (JAMES e DUBERY, 2011). Já Hashim e colaboradores realizaram a extração através de etanol 36% e água. Nos estudos citados, a concentração que mais se modificou foi a de AM, passando de $0,55\text{mg/ml}$ com extração em etanol 36% para $2,96\text{mg/g}$ com extração etanol 100% (HASHIM et al., 2011; JAMES e DUBERY, 2011). O metanol pode transformar alguns tripterpenos durante o processo de extração, como saponinas que podem se converter em 11-metoxi derivados, e glicosídeos que também podem se modificar (RÍOS et al., 2000), o que pode explicar a diferença de quantidades de ativos entre os diferentes tipos de extratos. Um estudo avaliou diferentes ativos da *Centella asiatica* extraídos por 3 métodos: extrato aquoso, etanólico, etanólico a 50%. Os resultados foram variações de até 15 vezes na quantidade de ativos como rutina, kaempferol, ácido gálico (MOHAMMAD AZMIN e MAT NOR, 2020).

Em humanos foram avaliadas doses orais de 250 e 500mg dia do extrato comercial Eca 233 com 80% de tripterpenos, sendo madecassosídeo e asiaticosídeo na proporção de $1,5(+0,5):1$ respectivamente, não encontrando toxicidade (enzimas hepáticas, hemograma, eletrólitos, creatinina) (WANAKHACHORNKRAI et al., 2013; SONGVUT et al., 2019). A excreção de madecassoside em ratos pela bile foi 7,16% (0-12h), urina 0,25% (0-72h) e fezes (24,68%(0-72h), denotando que a substância se converte em metabólitos no organismo (LENG et al., 2013) Em estudos de farmacocinética de ratos com artrite, após suplementação com madecassosídeo por 7 dias, foi possível ver uma tendência em aumentar a quantidade

AM e reduzir a quantidade de madecassoside no sangue dos ratos com artrite em comparação ao grupo sem artrite, denotando o papel do status inflamatório em possivelmente aumentar a metabolização de madecassósideo em AM (WANG et al., 2014).

O AM é uma molécula apolar, solúvel em Dimetilsulfóxido (DMSO) a 5mg/ml, etanol a 3mg/ml e insolúvel em água (MADECASSIC ACID APEXBIO, 2022; MADECASSIC ACID CAYMANCHEM, 2022). Solventes apolares como diclorometano e clorofórmio são frequentemente usados para o seu isolamento (RÍOS et al., 2000). Abaixo segue a fórmula estrutural do AM.

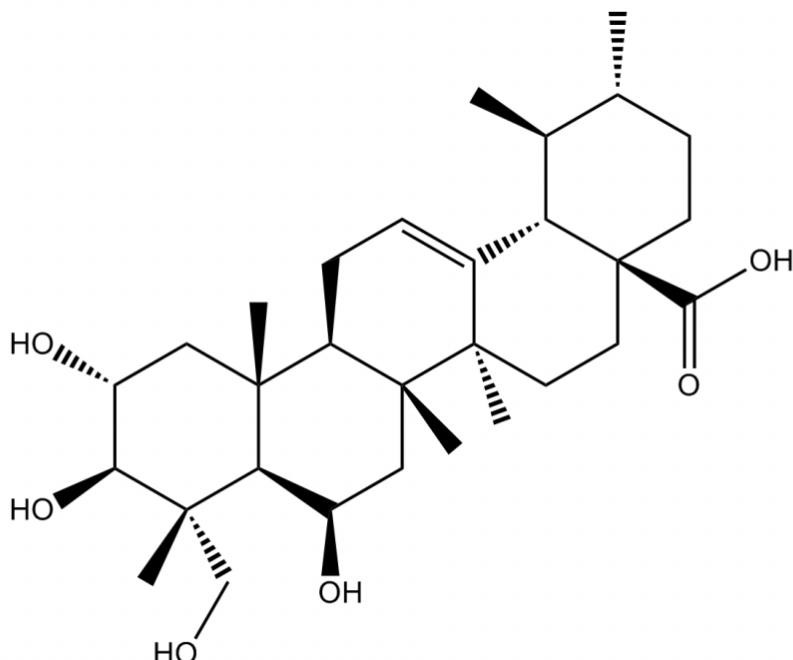


Figura 1: Fórmula estrutural do AM - composto apolar (MADECASSIC ACID APEXBIO, 2022)

Outro grupo de compostos presentes na *Centella asiatica* são os ácidos fenólicos, como o Ácido Clorogênico e seus derivados, como o Ácido Írbico. Este último ocorre praticamente apenas na *Centella asiatica*, e possui atividades potencialmente anti-envelhecimento pela inibição da degradação do colágeno pela collagenase (LONG; STANDER; WYK, 2012) e propriedades altamente potentes de eliminação de radicais livres (ANTOGNINI, 2011; DESAI et al., 2013). Outros grupos de constituintes além dos Ácidos Fenólicos e Terpenóides são os Açúcares Redutores, Alcalóides, Flavonóides, Saponinas, Taninos, Antraquinona, Esteróides e Glicosídeos Cardíacos (DESAI et al., 2013).

Estudos com ratos avaliaram a toxicidade de compostos provenientes da *Centella asiatica*. Chivapat e colaboradores usaram extrato seco diluído em água destilada para gavagem com 10 gramas por quilograma de peso dos ratos por 14 dias, sem encontrar

toxicidade ou efeitos adversos. No mesmo estudo foi avaliado uso crônico por 90 dias com 10, 100 e 1000mg/kg/dia, não obtendo efeitos adversos ou toxicidade (CHIVAPAT et al., 2011). Deshpande avaliou doses de 2000mg/kg/dia por 14 dias e 1000mg/kg/dia por 90 dias, sem encontrar qualquer toxicidade, mantendo parâmetros sanguíneos, TGO e TGP intactos (DESHPANDE et al., 2015).

3.2 TRANSTORNO DEPRESSIVO MAIOR E SUA MIMETIZAÇÃO EM MODELO ANIMAL

O Transtorno Depressivo Maior (TDM) afeta 300 milhões de pessoas em todo o mundo. Contribui para suicídios e morbidade, causando grande perda de qualidade de vida (ORGANIZAÇÃO MUNDIAL DA SAÚDE, 2017; SOLEK et al, 2019; SOUZA MONTEIRO et al., 2019). É resumido pelos sintomas: humor deprimido, interesses diminuídos, apetite interrompido, sono e cognição reduzida (THASE et al., 2012; SOLEK et al, 2019; SOUZA MONTEIRO et al, 2019).

A fisiopatologia do TDM passa pela neuroplasticidade, distúrbios na neurotransmissão e no sistema neuroendócrino, além de alterações na regulação metabólica. Também está relacionado a alterações genéticas-ambientais, microbiota intestinal e o eixo intestino-cérebro (TOLAHUNASE et al, 2018). Uma hipótese importante é a liberação de cortisol, epinefrina e norepinefrina em resposta ao estresse e alterações no eixo hipotálamo-hipófise-adrenal (HPA) (VREEBURG et al, 2009).

Entre os fatores psicossociais, um grande número de evidência destaca que o estresse na infância é um dos fenômenos mais potentes em precipitar a expressão de um fenótipo predisponente ao TDM (IGNÁCIO et al., 2017; LEMOULT et al., 2019). Um aspecto fundamental é que o estresse no início da vida parece estar envolvido na gravidade do transtorno e na pobre resposta aos tratamentos antidepressivos, tanto em humanos (IGNÁCIO et al., 2017; WILLIAMS et al., 2016) quanto em animais submetidos a protocolos de separação maternal (ZHANG et al., 2015). Roedores, submetidos a protocolos de separação maternal nos primeiros dias de vida, apresentaram níveis elevados de ansiedade no labirinto em cruz elevado (LCE) e comportamentos tipo anedônico e depressivo em testes de anedonia (Splash test) e natação forçada (NF), respectivamente (RÉUS et al., 2015; IGNÁCIO et al., 2017). Um aspecto relevante que emerge da utilização do modelo de separação maternal é o

fato de mimetizar uma situação grave de violência, abandono e falta de cuidado em crianças humanas, sendo considerado um dos mais poderosos estressores naturais durante o desenvolvimento (RÉUS et al., 2015; IGNÁCIO et al., 2017).

3.3 *Centella asiatica* E TDM

A teoria do comprometimento neurotrófico de pacientes com TDM, como parte de uma teoria multimodal de causas metabólicas depressivas, tem ganhado destaque nos últimos anos, visto que o fator neurotrófico derivado do cérebro (BDNF) está diminuído no TDM e no Distúrbio Bipolar, recuperando-se com tratamentos medicamentosos (POLYAKOVA et al., 2015). Em um estudo in vitro, os pesquisadores observaram que dois componentes ativos da *Centella asiatica*, AA e AM, funcionaram sinergicamente, proporcionando efeitos semelhantes aos fatores de crescimento neuronal na diferenciação neuronal, aumentando os neurofilamentos que proporcionam um diâmetro regular no axônio, possibilitando uma velocidade normal de condução do impulso nervoso (VERGE et al., 1990; JINAG et al., 2016), caracterizando um efeito neurotrófico. Em um estudo com ratos alimentados com extrato de *Centella asiatica* até o sétimo dia de vida, mostrou aumento no comprimento dendrítico e ramificação em neurônios do *cornu Ammonis* (CA) do hipocampo (área CA3) após 4 a 6 semanas de tratamento, na concentração 4 e 6ml/kg de peso corporal (MOHANDAS RAO; MUDDANNA RAO; GURUMADHVA RAO, 2006). Soumianath e colaboradores observaram que uma linhagem de células de neuroblastoma humano (SH-SY5Y) teve um aumento do ganho de neuritos após exposição ao fator de crescimento do nervo (NGF) mais extrato alcoólico de *Centella asiatica* a 100 µg/mL. No mesmo estudo, ratos submetidos à lesão no nervo ciático tiveram recuperação funcional e regeneração axonal aceleradas em relação aos controles, indicando que os axônios tiveram maior taxa de crescimento. Os parâmetros observados são calibre dos axônios e número de axônios mielinizados (SOUMLYANATH et al, 2005).

Lin e colaboradores, ao realizarem testes clínicos em células de feocromocitoma PC12, avaliaram o efeito de 5 ativos da *Centella asiatica* para diferenciação neuronal. Os seguintes componentes foram isolados: AA, ácido madecássico, madecassoside, quercetina e isoquercetina. Ao cultivar as células por 72 horas, houve crescimento neuronal e aumento da expressão de NF68 (neurofilamento 68kDa), uma proteína de diferenciação neuronal. Notou-se que o ácido asiático e o ácido madecássico sozinhos têm efeitos positivos, mas

reduzidos. Quando usado em sinergismo os resultados foram ainda mais positivos. Em particular, o uso de ácido asiático e madecássico juntos aumentou o NF68 2,5 vezes em comparação com o grupo controle (LIN; JIANG; DING, 2017).

Um estudo recente observou que o AA exerceu um efeito antidepressivo em camundongos submetidos a estresse crônico moderado e reduziu a expressão de citocinas inflamatórias (WANG et al., 2020). Kalshetty et.al., testaram o efeito do asiaticosídeo juntamente com alguns antidepressivos clássicos em roedores submetidos ao estresse crônico pelo método da bulbectomia olfativa. Os roedores foram divididos em grupos que receberam a droga teste, asiaticosídeo, e controles imipramina, desipramina e fluoxetina. Os princípios ativos foram diluídos em água destilada e administrados uma hora antes dos experimentos. Os resultados finais foram que doses de 30mg/kg de asiaticosídeo reduziram significativamente a ingestão alimentar dos roedores. Na dose de 3mg/kg, não houve efeito significativo. Na questão da perda de peso, nenhuma das drogas causou uma mudança significativa. O grau de hiperatividade medido pelo teste de deambulação foi reduzido em todos os tratamentos medicamentosos em relação ao grupo controle, no caso do asiaticosídeo 30,3% na dose de 3m/kg, 51,2% na dose de 10mg/kg e 64,7% na dose de 30mg/kg. kg, sendo esta última dosagem o melhor resultado entre todos os medicamentos testados (KALSHETTY et al, 2012).

Foi realizado um estudo com ratas prenhas suplementadas com extrato de *Centella asiatica* 200mg/kg/dia e seus respectivos filhotes também alimentados com extrato de *Centella asiatica* (20ml/kg/dia) de 7 a 60 dias de idade. As ratas prenhas foram submetidas ao estresse por meio de contenção em tela de arame 45 minutos três vezes/dia do dia 14 ao 21 de prenhez. Aos 60 dias os filhotes foram submetidos ao estudo histológico da área hipocampal no cérebro. O estresse pré-natal teve efeitos adversos nas atividades de aprendizagem e memória dos filhotes (medidas por testes no 45º dia). No grupo de filhotes alimentados com *Centella asiatica*, houve melhora desses parâmetros, paralelamente a um aumento no número de neurônios nas áreas CA3 e CA4 do hipocampo, mas não em CA1 e CA2 (MADHYASTHA et al., 2007). O hipocampo é uma região importante para aprendizado e memorização, e foi encontrada redução em suas áreas CA1 a CA4, giro denteadoo e subículo, em pacientes com TDM recorrente (RODDY et al, 2019)

Um ensaio clínico estudou *Centella asiatica* no Transtorno de Ansiedade Generalizada (TAG). Neste caso, foram incluídos 33 indivíduos, de ambos os sexos, com idade média de 33 anos, que tiveram a inclusão em seu tratamento de 500 mg/cápsula, duas vezes ao dia, após as refeições de extrato hidroetanólico a 70%. Verificou-se que a amostra

que incluiu *Centella asiatica* no tratamento reduziu significativamente o TAG e seus sintomas associados, como estresse e depressão ($p<0,01$). O transtorno de ajustamento e a atenção também foram melhorados sem efeitos colaterais ($p<0,01$) (JANA et al., 2010).

Um estudo avaliou doses de 500 mg 2 vezes ao dia em idosos de extrato seco de *Centella asiatica*, obtendo melhorias no exame mini-mental após 6 meses de uso (TIWARI et al., 2008)

3.4 *Centella asiatica* E INFLAMAÇÃO

Em macrófagos RAW 264.7 de roedores estimulados com lipopolissacarídeos (LPS), uma substância inflamatória, o AM e madecassosídeo foram capazes de reduzir de uma maneira dose dependente a ativação da via Nf-kB, o que provavelmente também possibilitou a redução de Óxido Nítrico (NO), prostaglandina E2 (PGE2), fator de necrose tumoral- α (TNF- α), IL-1, IL-6, Óxido Nítrico Sintase Induzida (iNOS), COX-2. As doses utilizadas foram de 50, 100 e 150 $\mu\text{g}/\text{ml}$ dos dois ativos, sendo que o AM teve um desempenho melhor em inibir a ativação de todos os fatores inflamatórios, com destaque para a dose de 150 $\mu\text{g}/\text{ml}$ (WON et al., 2010).

Outro estudo in vitro com queratinócitos utilizou o extrato comercial Eca 233 (madecassosídeo 52%, asiaticosídeo 41%) nas doses de 0,1m 1, 10 e 100 $\mu\text{g}/\text{ml}$, obtendo redução ou anulando completamente os picos de IL-1 β , TNF- α , PGE2, COX-2, ERK1 (quinase que estimula via Nf-kB) e Nf-kB induzidos por LPS. Os efeitos de redução foram dose dependente, sendo que a dose de 100 $\mu\text{g}/\text{ml}$ teve efeito semelhante a dexametasona para reduzir os parâmetros estudados (MOOLSAP et al., 2020).

Em ratos submetidos a gavagem com extrato de *Centella asiatica* não especificado, foi avaliada a resposta inflamatória contra *Salmonella typhi* (*S. typhi*), uma bactéria que causa frequentemente infecção crônica. Após a gavagem por 14 dias com 125, 250 e 500mg/kg do extrato, foi inoculada em cada rato uma dose intraperitoneal de *S. typhi*. Foi possível observar um aumento de IL-6 a partir de 250mg/kg/dia de gavagem com extrato, muito mais expressivo com a dose de 500mg/kg/dia, evidenciando uma resposta inflamatória mais potente contra a infecção, sugerindo que a imunidade mediada por macrófagos pode ser potencializada ao utilizar a *Centella asiatica* (KERTA BESUNG et al., 2011).

Um estudo onde aplicou-se injeções intradérmicas de mycobacterium butyricum nas patas traseira e rabo de ratos, para simular artrite reumatóide, evidenciou que, isolando os

fibroblastos destes locais, e estimulando com IL-1 β in vitro, com ou sem madecassosídeo (10 e 30 $\mu\text{mol/L}$), há um menor aumento de metaloproteinase 13 e de NF-kB nas células tratadas com madecassosídeo. Os resultados reforçam a via metabólica de inibição de NF-kB, já que as metaloproteinases são sabidamente induzidas por NF-kB (YU et al., 2018).

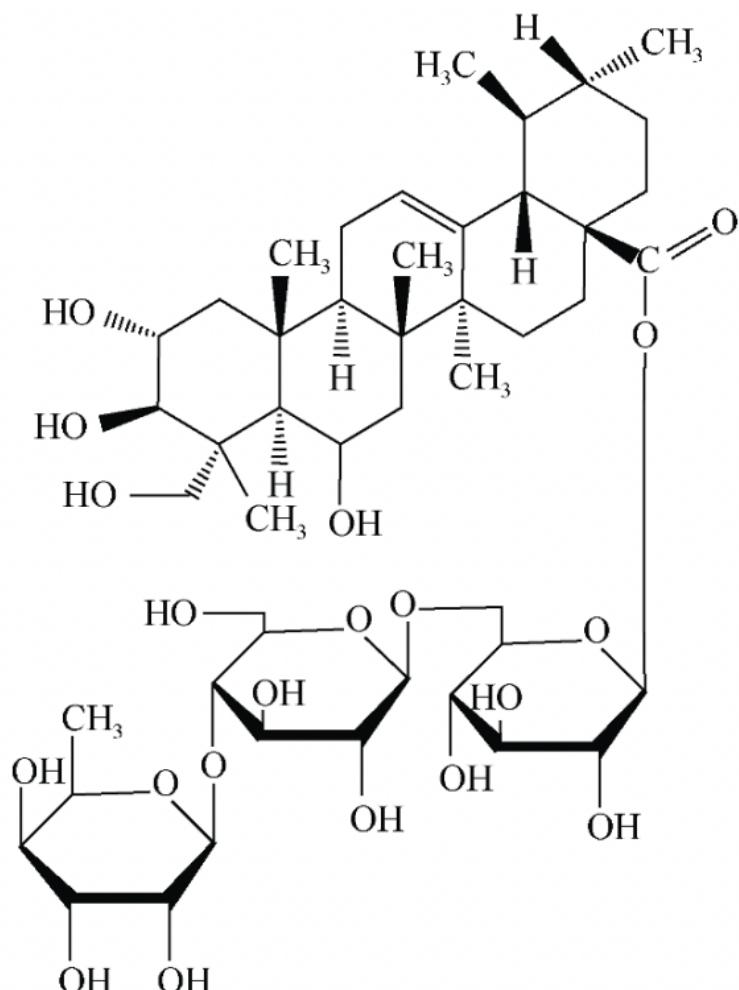


Figura 2: Fórmula Estrutural do madecassosídeo, adaptado de YU et al. (2018)

Uma pesquisa com saliva de crianças com cáries severas, que foi coletada e incubada por 24h com doses de 12,5 a 200 $\mu\text{g/ml}$ de extrato metanólico a 70%, observou uma redução nos níveis de IL-1 β de maneira dose dependente, com a menor redução sendo na dose de 200 $\mu\text{g/ml}$ (LUTHFI et al., 2022)

3.5 *Centella asiatica* E ESTRESSE OXIDATIVO

Um estudo avaliou alguns parâmetros de estresse oxidativo em ratos jovens e idosos tratados ou não com extrato etanólico 50% de *Centella asiatica* por 60 dias. Nos grupos jovens, não ocorreram mudanças dos parâmetros. Já nos ratos idosos tratados com extrato, houve modificações positivas em todos os parâmetros, como menores níveis de MDA (demonstrando redução na peroxidação lipídica) e maiores níveis de enzimas como catalase (CAT), SOD, glutationa, além de maiores quantidades de Vitamina C e Vitamina E no córtex, núcleo estriado, hipocampo, hipotálamo e cerebelo. O aumento de enzimas endógenas demonstra uma maior resposta endógena às agressões oxidativas sofridas devido à idade e uma economia de antioxidantes não enzimáticos que neutralizam a peroxidação lipídica, como a Vitamina C e Vitamina E (SUBATHRA et al., 2005).

Gnanapragasam e colaboradores estudaram perfil oxidativo em ratos após dano ao miocárdio causado por injeção de doxorrubicina. O grupo tratado com extrato aquoso 200mg/kg/dia, uma semana antes e duas semanas após a doxorrubicina, obteve preservação completa de SOD, CAT, GPx, Glutationa S-transferase, LDH, TGO, TGP e CPK, além de manter o peso total e peso cardíaco. No grupo doxorrubicina sem *Centella asiatica*, houve piora importante de todos os marcadores avaliados (GNANAPRAGASAM et al., 2004).

Apesar de poucos estudos, a literatura científica traz evidências de que a espécie medicinal *Centella asiatica*, bem como alguns dos seus compostos ativos, constituem potenciais estratégias de tratamento para o TDM, estando possivelmente relacionados com efeitos em mecanismos biológicos envolvidos no transtorno, tais como o estresse oxidativo e a inflamação.

4 MATERIAIS E MÉTODOS

Trata-se de uma pesquisa quantitativa de cunho experimental desenvolvida em laboratório com parceria entre a Universidade Federal da Fronteira Sul (UFFS) e a Universidade Comunitária da Região de Chapecó (Unochapecó).

4.1 MATERIAL VEGETAL

A coleta de material vegetal de *Centella asiatica* foi realizada em Chapecó (SC, Brasil) ($27^{\circ} 01' 55.14''\text{S}$ e $52^{\circ} 47'29.42''\text{O}$), em outubro de 2021. A identificação botânica foi realizada pelo Prof. Adriano Dias de Oliveira, curador do Herbário da Unochapecó, onde uma exsicata foi depositada (#4930). O restante do material vegetal (folhas) foi desidratado em temperatura ambiente ($\pm 20^{\circ}\text{C}$), pulverizado em moinho de facas (CiemLab®, CE430) selecionado em tamis 425 μm (35 Tyler/Mesh), e estocado ao abrigo da luz e umidade.

4.2 PRODUÇÃO DE EXTRATOS HIDROALCOÓLICOS DE *C. asiatica*

A amostra de *Centella asiatica* foi seca à temperatura ambiente ($25 \pm 5^{\circ}\text{C}$), após sendo Triturada em moinho de facas (Ciemlab®, CE430) e passando pelo processo de seleção em peneira (425 μm ; 35 Tyler/Mesch). Após foi identificada e armazenada ao abrigo da luz. Foi produzido o extrato pela maceração (5 dias) à temperatura ambiente, utilizando folhas moídas desidratadas (500 g) e etanol a 70% (1:20, p/v). Em seguida a solução extrativa passou pela filtração através do funil de Büchnere e sua posterior concentração via evaporador rotatório sob pressão reduzida, liofilização, pesagem e armazenamento a -20°C .

4.3 ANÁLISES QUÍMICAS DE EXTRATOS DE *Centella asiatica*

A infusão direta de fluxo do extrato de *Centella asiatica* foi realizada em um analisador Braker SolariX FT-ICR-MS equipado com fonte de ionização por eletropulverização (ESI), em modo negativo, taxa de fluxo de gás de secagem 3,0 L/min, temperatura do gás de secagem 200 °C, tensão de pulverização de - 0,5 kV, tensão capilar de -

4,5 kV, lente ECD de -10 V, amostra de fluxo de 5 µL/h e taxa de fluxo de gás nebulizador de 0,4 bar. As fragmentações (MS/MS) das amostras foram realizadas usando o método de decomposição induzida por colisão (CID) contra argônio para ativação de íons. O primeiro evento foi um espectro de massa de varredura completa para obter dados sobre íons na faixa de m/z 154 - 2000. O segundo evento de varredura foi um experimento de MS/MS realizado usando uma varredura dependente de dados nas moléculas [MH]⁺ de os compostos de interesse a uma taxa de fluxo de gás de colisão de 30%.

4.4 AQUISIÇÃO DO COMPOSTO ATIVO ÁCIDO MADECÁSSICO

Foi adquirido o AM através da empresa Interprise Instrumentos Analíticos Ltda, localizada na Rua Jardim América, Paulina, SP, sendo fabricado pela empresa Cayman Chemical, com sede nos Estados Unidos, estado do Michigan, na quantidade de 1 grama. O composto conta com pureza >95%, apresentado em forma sólida cristalina.

4.5 PRIVAÇÃO MATERNAL

Os filhotes foram submetidos à privação da mãe por 3 h/dia nos primeiros 10 dias de vida, consistindo na remoção dos filhotes da gaiola. Os controles (sem PM) permaneceram imperturbados na gaiola original com a mãe. O desmame ocorreu no 21º dia de vida, permanecendo após, caixas de 5 animais cada, com ciclo claro/escuro de 12 horas (das 07:00 às 19:00 horas, com luz a partir das 7:00 horas), comida e água *ad libitum*, a 23 ± 1 ° C de temperatura.

4.6 GRUPOS EXPERIMENTAIS E TRATAMENTOS

Protocolo de Privação Maternal: os animais foram submetidos ao protocolo de privação maternal nos dez primeiros dias de vida. Quando atingiram 60 dias de vida, os animais foram submetidos a um tratamento crônico de extrato de *Centella asiatica* e AM por 14 dias pelo método de gavagem. Os animais (60) foram divididos em 6 grupos de 10 animais cada: Controle sem estresse + veículo (Controle sem estresse); PM + veículo (Estresse + Tratamento controle); PM + ESC 10mg/kg (Estresse + Tratamento controle positivo); PM + Extrato de *Centella asiatica* 30mg/kg; PM + AM 10mg/Kg. O controle positivo com ESC foi

escolhido por ser um antidepressivo clássico da classe dos inibidores seletivos da recaptação de serotonina. A figura 1 mostra o desenho experimental do estudo.

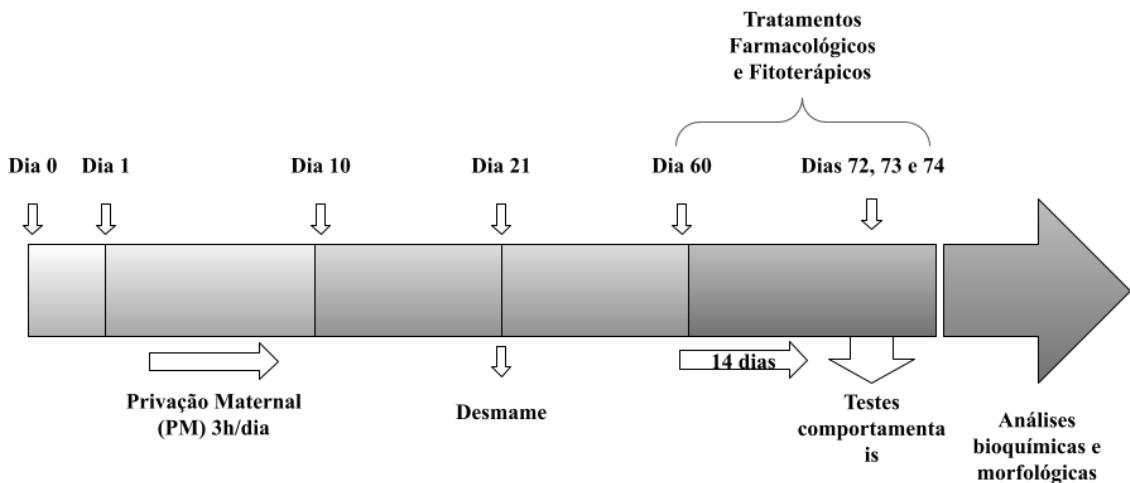


Figura 3: Desenho Experimental

4.7 TESTES COMPORTAMENTAIS

Todos os testes comportamentais foram conduzidos durante um único período do dia, manhã (8:00 - 12:00) ou tarde (13:00 - 18:00), sempre iniciados 60 minutos após cada tratamento. Todos os testes comportamentais foram realizados por um observador cego aos grupos experimentais.

O teste de natação forçada (TNF) avalia o comportamento tipo depressivo (CAN et al., 2012). Os ratos foram individualmente colocados em um cilindro com água à temperatura de 23°C, preenchido com água suficiente para que o animal não consiga apoiar as patas no fundo. Este teste é realizado em dois dias, sendo que no primeiro dia (13^a dia de tratamento farmacológico) os ratos foram forçados a nadar durante 15 minutos (pré-teste), e no segundo dia do teste (14º dia de tratamento farmacológico) os ratos foram forçados a nadar por 5 minutos. Os parâmetros avaliados foram os seguintes: imobilidade, que envolve imobilidade total ou movimentos para manter a cabeça fora da água sem intenção de escapar; mobilidade, que é o tempo que o animal fica nadando e o tempo que fica escalando as paredes do cilindro na tentativa de escapar.

O teste de campo aberto (TCA) avalia a atividade motora (Habr et al., 2011; Réus et al. 2013). Consiste numa caixa de 40 x 60 cm, cercada por três paredes de madeira, uma parede frontal de vidro e assoalho dividido em 9 retângulos iguais por linhas pretas. Foi permitido que os animais explorassem o ambiente por 5 minutos, tempo no qual foram contados os cruzamentos entre as linhas pretas bem como a quantidade de vezes em que o rato ficou apoiado nas patas traseiras a fim de explorar o ambiente.

4.8 COLETA DE TECIDOS

Após o último teste comportamental (natação forçada), os animais foram submetidos à eutanásia por decapitação, sendo coletados o tecido cerebral do hipocampo e sangue para análise dos marcadores. Após a extração do cérebro foi separado o hipocampo como descrito em literatura (PAXINOS e WATSON, 1986). O sangue foi processado e as amostras cerebrais foram armazenadas em freezer -80°C para posterior análise dos parâmetros bioquímicos.

4.9 PARÂMETROS INFLAMATÓRIOS

Os níveis de IL-1 β e IL-6 no hipocampo e sangue foram analisados através de Kits de Elisa. O soro e o hipocampo foram armazenados em ultra-freezer -80° C e foram avaliados os níveis de IL-1 β e IL-6 pelo kit ELISA “Milipore Rat IL-6 ELISA Kit”. Todas as amostras foram testadas em duplicata.

Para avaliar os níveis de IL-1 β e IL-6 pelo kit ELISA no soro dos animais o anticorpo de captura (13 ml, contém 0,1% de Azida de sódio) foi diluído em solução salina tamponada com fosfato (PBS), adicionado a cada poço e deixado durante a noite a 4° C. Em seguida, a placa foi lavada quatro vezes com PBS 0,05% Tween 20% (SIGMA, ST. LOUIS, MO, EUA). A placa foi bloqueada com albumina sérica bovina 1% e incubada por 1 h em temperatura ambiente antes de ser lavada quatro vezes com PBS e Tween 0,05%/20%. As amostras e padrões foram adicionadas, e a placa ficou incubada toda a noite a 4° C. Depois de lavar a placa, um anticorpo de detecção (concentração fornecida pelo fabricante TURER) foi diluído em PBS. A placa foi incubada por 2h à temperatura ambiente. Depois de lavar a placa, a enzima estreptavidina (DUOSET R&D SYSTEMS, MINNEAPOLIS, MN, EUA) foi adicionada, a qual foi incubada novamente durante 30 minutos. Finalmente, um reagente de

cor, fenilenodiamina (Sigma, St. Louis, MO, EUA), foi adicionado a cada poço e a reação se desenvolveu no escuro por 15 min. A reação foi interrompida pela solução de parada H₂SO₄ 1 M para cada poço. A absorbância foi lida em um leitor de placas em comprimentos de onda de 492 nm (EMAX, MOLECULAR DEVICES, MINNEAPOLIS, MN, EUA).

Antes do armazenamento, o hipocampo foi submetido à digestão enzimática com TRIS HCL 50% e armazenado em frascos apropriados (eppendorf). Para o descongelamento, as amostras foram mantidas no frasco de armazenamento, em temperatura ambiente por 150 minutos e agitado em vórtex após. Em seguida foi realizada a centrifugação a 3500 rpm durante 15 minutos, sendo coletado o sobrenadante para as análises. Através do método de Peterson com espectrofotômetro, foi realizada a quantificação de proteínas (PETERSON, 1977). O procedimento da análise dos níveis de IL-1 β e IL-6 pelo kit ELISA “Milipore Rat IL-6 ELISA Kit”, no hipocampo dos animais foi iniciado com a adição de 100 μ L de anticorpo de captura em cada poço da microplaca, sendo incubadas em seguida por toda a noite a 4° C. Pela manhã a placa foi lavada com tampão de lavagem e adicionado 200 μ L diluente, com incubação por 1 hora. O próximo passo foi adicionar 100 μ L de amostra em poços diferentes, 100 μ L dos padrões e controles, submetendo então a mais 2 horas de incubação. Em seguida foi adicionado 100 μ L do anticorpo de detecção, 100 μ L de enzima e 100 μ L de substrato, sendo intercalados com períodos de incubação e lavagem. Por fim, foi adicionada uma solução de parada da reação e realizada a leitura em espectrofotômetro com microplacas no espectro de 450 nm. Os resultados em pg/ml foram calculados considerando a equação da reta obtida através da curva padrão.

4.10 PARÂMETROS OXIDATIVOS

Foram avaliados 2 parâmetros de estresse oxidativo: MPO e formação de TBARS.

A MPO é uma hemeperoxidase presente em leucócitos. Ela reflete o nível de ativação de leucócitos e neutrófilos durante infecções ou estados oxidativos. Na presença de H₂O₂, converte íons cloreto em ácido hipocloroso (HOCl), um potente oxidante para o combate de bactérias. Juntando a amostra com H₂O₂ como agente oxidante, adiciona-se fenol e aminoantipireno, resultando num produto de coloração que pode ser medido a 512nm no espectrofotômetro (SUZUKI et al., 1983; KUSUMA; VASUDH; VANITHA GOWDA, 2009).

A peroxidação lipídica foi avaliada pela interação entre MDA com o ácido tiobarbitúrico que culmina gerando uma coloração rósea, que pode ser medida com

espectrofotômetro com absorbância máxima a 532 a 535nm (ESTERBAUER e CHEESMAN, 1990).

4.11 ANÁLISE ESTATÍSTICA

Os resultados serão analisados através de ANOVA one-way, seguido pelo teste post-hoc de Tukey. Valores de $p < 0,05$ foram considerados estatisticamente significantes. Todas as análises foram realizadas em um computador compatível com IBM PC usando o software Statistica 7.0.

4.12 ASPECTOS ÉTICOS

Esta pesquisa foi aprovada pela Comissão de Ética no Uso de Animais (CEUA), da UNOCHAPECÓ, SC, sob o protocolo 002/CEUA/2021, estando de acordo com as diretrizes do CONCEA (Conselho Nacional de Controle de Experimentação Animal): Diretriz da Prática de Eutanásia do CONCEA e Diretriz Brasileira para o Cuidado e a Utilização de Animais para Fins Científicos e Didáticos.

Foi exigido dos pesquisadores comprovação de capacitação em experimentação com animais. Tanto o método de sacrifício quanto os testes comportamentais já foram bem elucidados na literatura, para evitar sofrimento desnecessário das cobaias, sendo utilizado o número mínimo indicado para o experimento, a fim de fazer uso ético dos animais, sem sacrifícios desnecessários (ALMEIDA; ALMEIDA, 2016; PACHECO; SAAD; TREVIZAN, 2012).

A eutanásia com utilização de anestésicos e outros métodos químicos poderia interferir nos resultados bioquímicos, portanto a decapitação foi o método de escolha, sendo um método mecânico que não interfere nos resultados dos testes bioquímicos, de rápida execução, virtualmente indolor se executado com precisão, e que permite a retirada de estruturas como o Cortex Pré-Frontal, Amígdala, Núcleo Acumbens e Hipocampo sem alterações químicas. (COMISSÃO DE ÉTICA, BIOÉTICA E BEM-ESTAR ANIMAL/CFMV, 2012).

5 RESULTADOS

5.1 DADOS COMPORTAMENTAIS - NATAÇÃO

Os efeitos da PM e dos tratamentos com *Centella asiatica* (30 mg/kg), AM (10 mg/kg) e ESC (10 mg/kg) nos parâmetros comportamentais estão ilustrados na figura 4. A ANOVA de uma via revelou diferença significativa na imobilidade na água ($F=9,4344$ e $p < 0,0001$) e natação ($F=3,1520$ e $p < 0,05$) entre os grupos. O teste Post hoc de Tukey revelou as seguintes diferenças estatísticas: A PM elevou significativamente o tempo de imobilidade com relação ao controle sem PM ($p < 0,005$) e o tratamento com ESC ($p < 0,005$), *Centella asiatica* ($p < 0,001$) e AM ($p < 0,0005$) reverteu o aumento do tempo de imobilidade observado no grupo PM salina. Com relação ao tempo de natação, o teste Post hoc de Tukey evidenciou redução no tempo de natação no grupo PM salina em relação ao grupo sem PM, porém o resultado não alcançou o nível de significância estabelecido ($p < 0,05$). O tempo de natação foi aumentado nos grupos tratados com escitalopram, *Centella asiatica* e AM, porém, o resultado só alcançou o nível de significância adotado no grupo tratado com AM ($p < 0,05$).

Com relação às escaladas no teste de natação, cruzamentos e levantamentos no teste de campo aberto, o teste ANOVA mostrou que não houveram alterações com significância estatística.

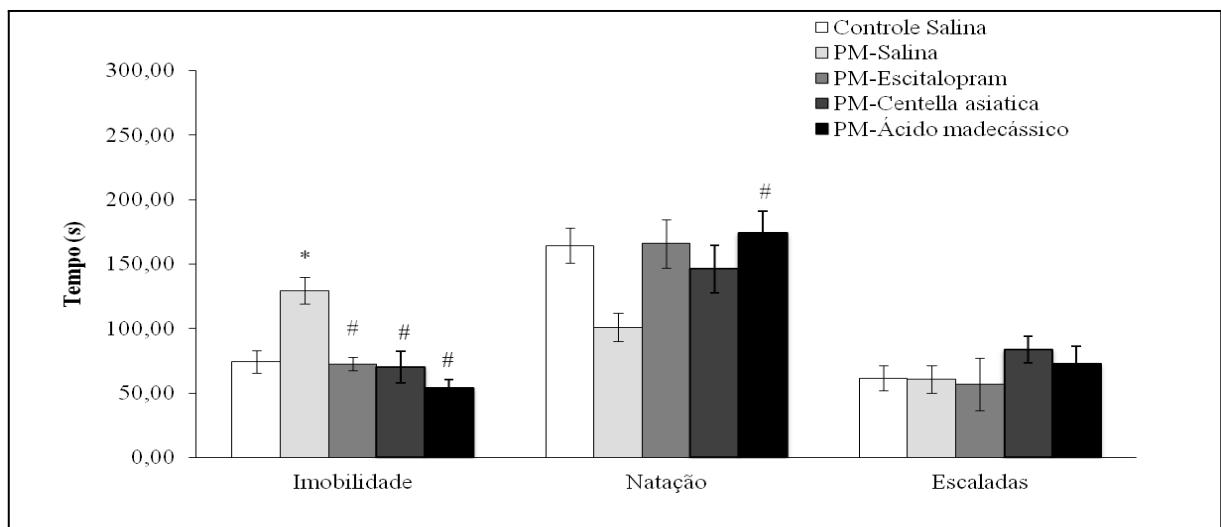


Figura 4 - Efeitos do estresse de PM e dos tratamentos com *C. asiatica* (30 mg/kg), ácido madecássico (10 mg/kg) e escitalopram (10 mg/kg) sobre os parâmetros de mobilidade no teste de natação forçada. Os dados são apresentados como média ± erro padrão da média. *diferente do controle salina ($p < 0,05$); # diferente do PM Salina ($p < 0,05$).

5.2 DADOS COMPORTAMENTAIS - CAMPO ABERTO

Os efeitos da PM e dos tratamentos com *C. asiatica* (30 mg/kg), ácido madecássico (10 mg/kg) e escitalopram (10 mg/kg) nos parâmetros avaliados no teste de campo aberto estão ilustrados na figura 5. No teste de campo aberto a ANOVA de uma via não revelou interação significativa entre os grupos sem estresse e os grupos privados maternalmente. Tanto a PM quanto os tratamentos não induziram alterações significativas na atividade locomotora, avaliada através dos números de cruzamentos ($F=1,89$; $p > 0,05$) e levantamentos ($F=1,14$; $p > 0,05$) no teste do campo aberto.

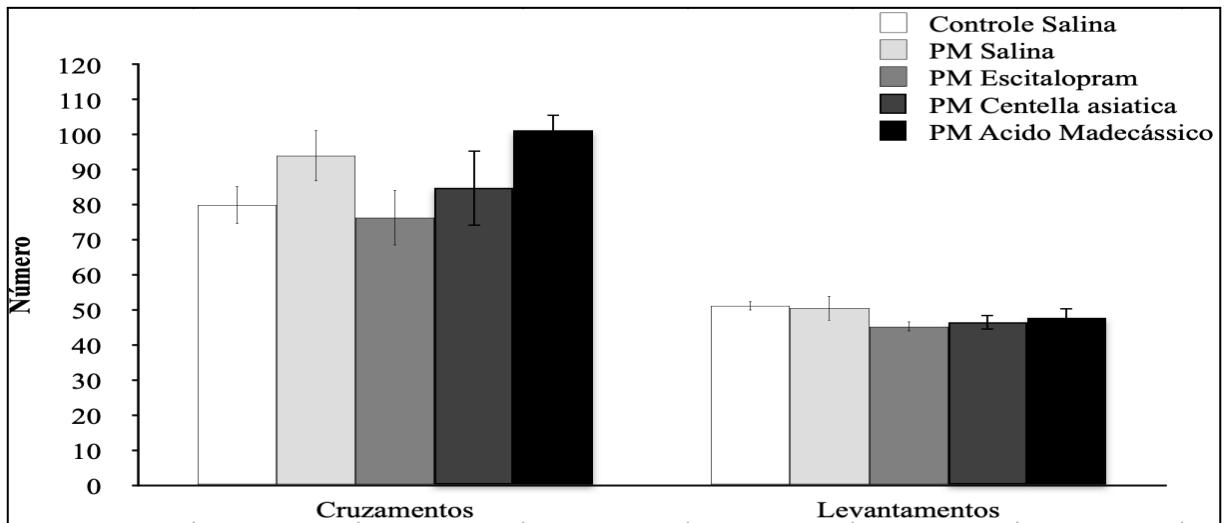


Figura 5 - Efeitos do estresse de PM e dos tratamentos com *C. asiatica* (30 mg/kg), ácido madecássico (10 mg/kg) e escitalopram (10 mg/kg) sobre a os parâmetros de atividade locomotora no campo aberto. Os dados são apresentados como média ± erro padrão da média. Não houve diferença estatística entre os grupos.

5.3 ESTRESSE OXIDATIVO - MPO

Os efeitos da PM e dos tratamentos com *C. asiatica* (30 mg/kg), ácido madecássico (10 mg/kg) e escitalopram (10 mg/kg) nos níveis de MPO no soro e hipocampo estão ilustrados na figura 6. A ANOVA de uma via revelou uma interação significativa nos níveis de MPO ($F=11.2807$ e $p < 0,001$) entre os grupos no soro. O teste Post hoc de Tukey revelou as seguintes diferenças estatísticas: a PM elevou significativamente os níveis de MPO em comparação ao grupo controle ($p < 0,001$), e o tratamento com escitalopram ($p < 0,01$), *C. asiatica* ($p < 0,01$) e ácido madecássico ($p < 0,001$) reverteram o efeito da PM. Em relação ao hipocampo, não foi identificado diferença estatística entre os grupos.

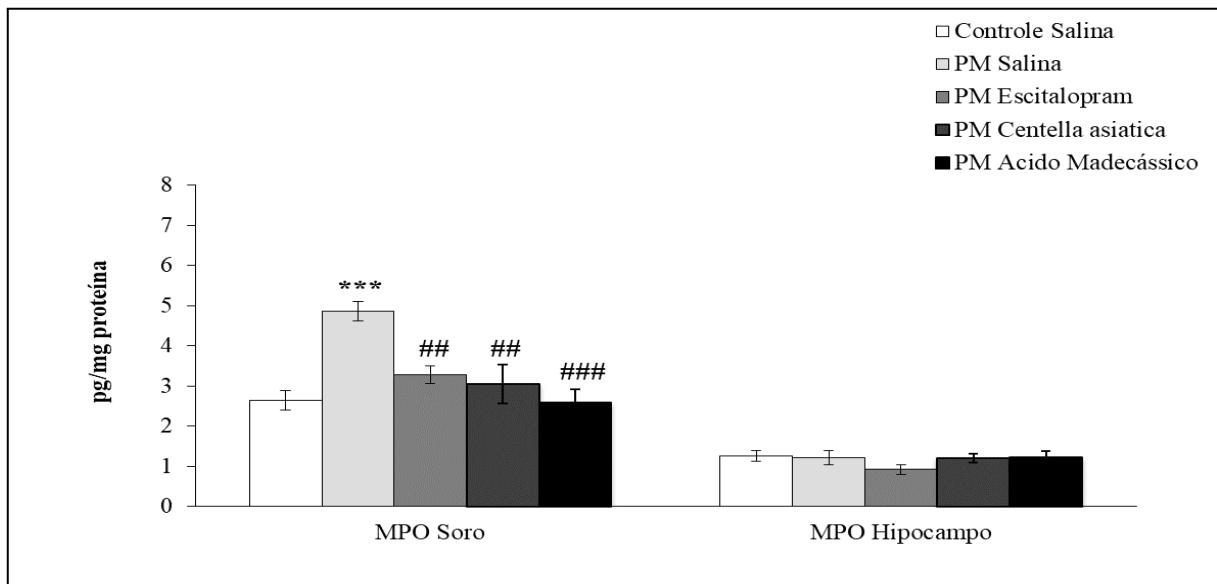


Figura 6 - Efeitos do estresse de PM e dos tratamentos com *C. asiatica* (30 mg/kg), ácido madecássico (10 mg/kg) e escitalopram (10 mg/kg) sobre os níveis de mieloperoxidase (MPO) no soro e hipocampo. Os dados são apresentados como média ± erro padrão da média. ***diferente do Controle Salina ($p < 0,001$); ##diferente do PM Salina ($p < 0,01$); ### diferente do PM Salina ($p < 0,001$).

5.4 ESTRESSE OXIDATIVO - TBARS

Os efeitos da PM e dos tratamentos com *C. asiatica* (30 mg/kg), ácido madecássico (10 mg/kg) e escitalopram (10 mg/kg) nos níveis de TBARS no soro e hipocampo estão ilustrados na figura abaixo 7. A ANOVA de uma via revelou uma interação significativa nos níveis de TBARS ($F=8.2847$ e $p < 0,001$) entre os grupos no soro. O teste Post hoc de Tukey revelou as seguintes diferenças estatísticas: A PM elevou significativamente os níveis de TBARS em comparação ao grupo controle ($p < 0,01$) e o tratamento com escitalopram ($p < 0,05$) e ácido madecássico ($p < 0,05$) revertearam o efeito da PM, porém o grupo com *Centella asiatica* não reverteu esta alteração. Em relação ao hipocampo, a ANOVA de uma via revelou uma interação significativa entre os grupos experimentais ($F=4.77$; $p < 0,01$). O teste de Tukey apontou que a PM aumentou os níveis de TBARS em comparação ao grupo controle ($p < 0,05$). Os tratamentos com escitalopram ($p < 0,01$) e ácido madecássico ($p < 0,05$) revertearam este efeito.

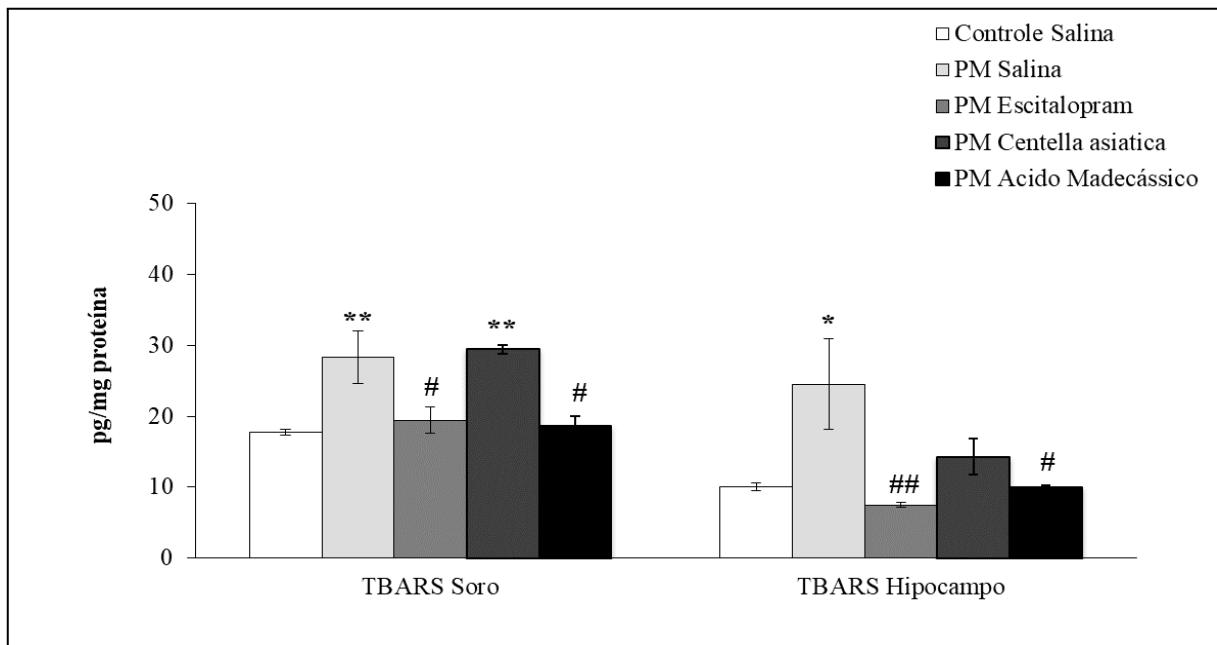


Figura 7 - Efeitos do estresse de PM e dos tratamentos com *C. asiatica* (30 mg/kg), ácido madecássico (10 mg/kg) e escitalopram (10 mg/kg) sobre os níveis de substâncias reativas ao ácido tiobarbitúrico (TBARS) no soro e hipocampo. Os dados são apresentados como média ± erro padrão da média. *diferente do Controle Salina ($p < 0,05$); **diferente do Controle Salina ($p < 0,01$); #diferente do PM Salina ($p < 0,05$); ##diferente do PM Salina ($p < 0,01$).

5.5 INTERLEUCINAS INFLAMATÓRIAS - IL-1 β E IL-6

Os efeitos da PM e dos tratamentos com *Centella asiatica* (30 mg/kg), AM (10 mg/kg) e ESC (10 mg/kg) nos níveis de IL-1 β e IL-6 no hipocampo estão ilustrados na figura 8. A ANOVA de uma via revelou diferença significativa nos níveis de IL-1 β ($F=5,97618$ e $p < 0,01$) e IL-6 ($F=6,05716$ e $p < 0,01$) entre os grupos. O teste Post hoc de Tukey revelou as seguintes diferenças estatísticas: A PM elevou significativamente os níveis de IL-1 β e IL-6 em comparação ao grupo controle ($p < 0,01$) e o tratamento com ESC ($p < 0,01$), *Centella asiatica* ($p < 0,05$) e AM ($p < 0,01$) revertem o efeito da PM para a IL-6. Porém para a IL-1 β apenas ESC ($p < 0,01$) e AM ($p < 0,05$) conseguiram reverte com significância estatística.

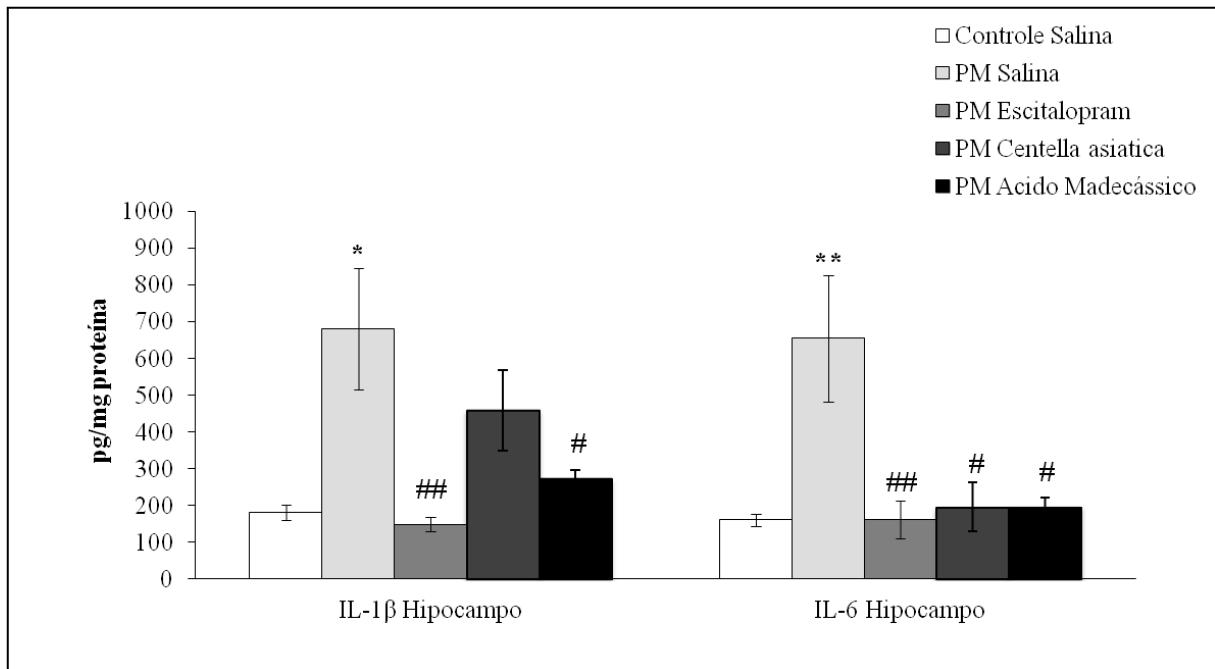


Figura 8 - Efeitos do estresse de PM e dos tratamentos com *C. asiatica* (30 mg/kg), ácido madecássico (10 mg/kg) e escitalopram (10 mg/kg) sobre os níveis de interleucinas inflamatórias (IL-1 β e IL-6) no hipocampo. Os dados são apresentados como média \pm erro padrão da média. *diferente do Controle Salina ($p < 0,05$); **diferente do Controle Salina ($p < 0,01$); #diferente do PM Salina ($p < 0,05$); ##diferente do PM Salina ($p < 0,01$).

6 DISCUSSÃO

6.1 INFLAMAÇÃO

Estudos anteriores já apontaram que o estresse na infância está relacionado a aumento de IL-6 e TNF- α na vida adulta, indicando que o estresse na infância causa uma marca bioquímica pró-inflamatória para a vida toda (GROSSE et al., 2016; D'ACUNTO et al., 2019; COLANTO; MADIGAM e KORCZAK, 2020). Uma meta-análise com 82 estudos sobre TDM em adultos também relacionou aumento de IL-6 e TNF- α no soro sanguíneo (KÖHLER et al., 2017).

Os mecanismos pelos quais a inflamação pode mediar o TDM se relacionam com alterações de neurotransmissores, neurotoxicidade, redução na neurogênese no hipocampo e alterações do eixo HPA (CARROL et al., 1976; STEINER et al., 2011; LUI et al., 2018). Um estudo com 42 indivíduos, 20 depressivos e 22 previamente hígidos, evidenciou aumento do transportador de serotonina associado a aumento de IL-6, IL-1 β , interferon-gama e TNF- α em indivíduos com depressão, evidenciando que nestes indivíduos há um aumento da recaptação da serotonina (TSAO et al., 2006). Mais do que isto, a inflamação foi identificada como causadora do aumento do transportador de serotonina, através da via proteína quinase de ativação da mitose (MAPK) p38, que está envolvida no aumento destes transportadores (ZHU et al., 2006). A metabolização da serotonina também pode estar envolvida no processo de depressão. A IL-1 β tende a ativar enzimas como 2-3-indoleamina-dioxigenase (IDO), 2-3-triptofano-dioxigenase (TDO), as quais metabolizam o triptofano em direção à via da quinurenina, ácido quinurênico, ácido quinolínico (PEDRAZ-PETROZZI et al., 2020). Estes podem estar aumentados em pacientes com TDM, ao passo que o triptofano pode estar reduzido (LUI et al., 2018). Porém há estudos que falharam em encontrar relações entre aumento de enzimas e metabólitos do triptofano e TDM em humanos (MACKAY et al., 2008), ou encontraram níveis aumentados de triptofano e baixos de quinurenina no plasma de pacientes com TDM, resultados totalmente opostos (POMPILI et al., 2019). O ácido quinolínico é um metabólito da via de eliminação do triptofano que é neurotóxico. Foi encontrado aumento de ácido quinolínico na região do giro cingulado anterior do cérebro de pacientes com depressão grave (STEINER et al., 2011). A via da eliminação do triptofano necessita mais estudos para se consolidar como um mecanismo básico do TDM.

A aplicação do modelo de simulação de status depressivo em ratos (PM) foi bem sucedida neste estudo. Dos parâmetros comportamentais, houve aumento da imobilidade na

água e redução do tempo de nado, denotando alterações no comportamento compatíveis com sintomas depressivos (IGNÁCIO et al., 2017). Mais do que isto, foi possível acompanhar alterações metabólicas nos ratos submetidos a PM, como aumento de IL-1 β , IL-6 no hipocampo, MPO no soro, TBARS no soro e hipocampo, resultados estes que estão em concordância com estudos anteriores de PM em ratos (WON et al., 2010; MOOLSAP et al., 2020).

6.2 EIXO HPA

A ativação do eixo HPA em situações estressantes ou depressão é um mecanismo bem estudado. Pacientes com TDM têm níveis de cortisol livre urinário aumentados (CARROL et al., 1976). Um estudo com mulheres sob risco de depressão ou com diagnóstico de depressão, evidenciou níveis de cortisol salivar basal maiores que as mulheres controle, e uma resposta de cortisol a estímulo de estresse bem superior nos grupos depressão e risco de depressão, comparada ao grupo controle (DIENES; HAZEL e HAMMEN, 2012). O hipocampo exerce um papel fundamental em exercer feedback negativo aos pulsos de cortisol liberados pela glândula adrenal (CARROL et al., 1976). Uma meta-análise avaliou estudos de volume do hipocampo em pacientes depressivos, encontrando atrofia hipocampal nestes pacientes, o que pode reduzir a capacidade do hipocampo em exercer o feedback negativo aos pulsos de cortisol da glândula adrenal SANTOS et al., 2018). Em cultura de células de hipocampo de ratos, o ácido quinolínico foi capaz de exercer ação neurotóxica para os elementos pós-sinápticos (KHASPEKOV et a., 1989). Em 64 pacientes com histórico de tentativa de suicídio vesus grupo controle, foi possível ver um desvio dos metabólitos da via da quinurenina em direção ao ácido quinolínico, sendo que níveis de ácido quinurênico se mantiveram normais. (ERHARDT et a., 2013). Estes dados nos permitem fazer uma conexão entre a via do ácido quinolínico com neurotoxicidade ao hipocampo, que está aumentada em pacientes com depressão grave e que têm níveis de interleucinas pró-inflamatórias aumentado, assim como ativação do eixo HPA acima do normal, evidenciando uma falta de feedback por parte do hipocampo fechando este círculo.

6.3 MPO NO CÉREBRO

No hipocampo não houve alteração nos níveis de MPO em nenhum dos grupos deste estudo. Maki e colaboradores denotaram que a expressão de MPO em tecido cerebral humano

normal foi virtualmente zero. Já no cérebro humano com Doença de Alzheimer, a MPO se expressa generosamente. No mesmo estudo foi avaliado cérebro de ratos com gene APP23 que induz deposição de placa amilóide, denotando alta concentração de MPO no córtex frontal, mas discreta expressão no hipocampo (MAKI et al, 2009). Um estudo observou a variação da MPO no cérebro após parada cardíaca induzida por 8 minutos de asfixia em ratos. Foi observado um aumento de MPO em torno de 100% no cérebro em comparação ao grupo sem asfixia (XIAO et al., 2002). Um estudo com ratos com esclerose múltipla demonstrou que existe um aumento da MPO em regiões desmielinizadas do córtex (GRAY et al., 2008). Um estudo com córtex frontal humano demonstrou que existem níveis detectáveis porém baixos de MPO, e no caso de Doença de Alzheimer, os níveis sobem de maneira significativa (GREEN et al., 2004). Matsuo e colaboradores utilizaram isquemia cerebral aguda em ratos para demonstrar a elevação da MPO no cérebro, entretanto no grupo que recebeu anticorpos monoclonais anti-neutrófilos a MPO foi completamente inibida, evidenciando que a MPO é liberada no cérebro através dos neutrófilos recrutados em processos inflamatórios agudos (MATSUO et al., 1994). Estes estudos demonstram uma importante presença de MPO em doenças neurodegenerativas e agudas no cérebro, sendo que há aumento de MPO em estados inflamatórios agudos (isquemia cerebral) e crônico degenerativos (alzheimer), provavelmente por recrutamento de células de defesa. Uma explicação para não haver aumento de MPO no cérebro neste estudo pode ser devido ao processo de neuroinflamação do TDM não ser potente o suficiente para recrutar neutrófilos.

6.4 ESTRESSE OXIDATIVO

Estudos anteriores já demonstraram redução de marcadores inflamatórios, a exemplo de IL-1 β , TNF- α , PGE2, COX-2, ERK1 e Nf- κ B após suplementação com diferentes tipos de extrato de *Centella asiatica* ou seus principais princípios ativos isolados (madecassosídeo, AM, asiaticosídeo, ácido asiático). Este estudo observou uma redução significativa de IL-1 β e IL-6 após tratamento com escitalopram, AM e extrato hidroalcólico de *Centella asiatica* no hipocampo dos ratos submetidos a estresse de PM. Os resultados deste estudo estão corroborando as evidências da literatura científica (WON et al., 2010). Um estudo *in vitro* com simulação da barreira hematoencefálica comprovou que o madecassosídeo, ácido asiático e asiaticosídeo são capazes de passar pela barreira hematoencefálica em alta concentração, exercendo efeito citoprotetor contra estresse oxidativo (HANAPI et al., 2021). Um estudo comparou as quantidades no sangue com os diferentes tecidos de ratos alimentados com

vegetais que continham diversos triterpenos, como AM e ácido asiático. O estudo evidenciou que após 8 semanas de alimentação os níveis no cérebro, coração, rins, fígado, cólon e bexiga são sempre maiores que no plasma sanguíneo, evidenciando que há acúmulo nos tecidos e que há passagem pela barreira hematoencefálica (YIN et al., 2012). Os dados mostram que a metabolização dos compostos no organismo favorece sua deposição nos tecidos, e pelo fato de ser uma substância apolar e portanto lipossolúvel, possibilita a deposição em tecidos gordurosos, no interstício e interior das células, necessitando mais estudos para especificar essas localizações.

Um estudo com idosos com TDM versus controle, encontrou níveis de carbonilação de proteínas e óxido nítrico, dois parâmetros que medem o grau de estresse oxidativo, aumentados nos pacientes com TDM, ao passo que níveis de enzimas antioxidantes como SOD e glutationa estavam depletados em relação ao controle (DA SILVA et al., 2019).

Houve também redução da peroxidação lipídica nos ratos em que houve intervenção, denotada pela redução dos níveis de MPO nos 3 grupos de intervenção terapêutica, com resultado mais potente no grupo AM em comparação ao escitalopram. Já para TBARS, o aumento importante que se deu no soro e no sangue foi quase completamente neutralizado nos grupos tratados com escitalopram e AM. No grupo *Centella asiatica*, houve neutralização importante no hipocampo, mas não no soro, podendo estar relacionada à quantidade de AM do extrato hidroalcoólico ser menor que a quantidade de AM que foi suplementada isoladamente. Como o AM é substância apolar, a menor quantidade de AM do extrato hidroalcoólico poderia ser compensada pelo acúmulo no cérebro, e com o acúmulo as duas doses diferentes de AM conseguiriam exercer funções igualadas a partir de alguns dias de suplementação. Já no soro, como não ocorre acúmulo, ou ocorre um acúmulo de dose muito menor, a quantidade pequena de AM contida no extrato hidroalcoólico pode não conseguir induzir o efeito anti-inflamatório e antioxidante que a dose isolada mais concentrada, o que corrobora com os resultados deste estudo (YIN et al., 2012).

7 CONCLUSÃO

O estresse de privação maternal nos primeiros dias de vida culminou em comportamentos tipo depressivos na vida adulta. Paralelamente, os animais submetidos à privação maternal apresentaram aumento dos níveis de interleucinas no hipocampo, indicando aumento da inflamação. Adicionalmente, o estresse de privação maternal induziu aumento de MPO no soro e de TBARS no soro e hipocampo dos animais na vida adulta. Os tratamentos com escitalopram, extrato de *Centella asiatica* e ácido madecássico reduziram significativamente os comportamentos tipo depressivos na vida adulta. Todos os tratamentos reduziram significativamente os níveis de MPO no soro. Com relação aos níveis de TBARS, o escitalopram e o ácido madecássico reduziram significativamente os níveis, tanto no soro quanto no hipocampo. Entretanto, o extrato de *Centella asiatica* não conseguiu reverter ou reduzir significativamente os níveis de TBARS. Com relação aos marcadores inflamatórios, todos os tratamentos reverteram os níveis de IL-6 no hipocampo. Com relação aos níveis de IL-1 β , os tratamentos com escitalopram e ácido madecássico reverteram os níveis aumentados pelo estresse, porém o extrato de *Centella asiatica* não conseguiu reduzir significativamente. Esses resultados corroboram resultados da literatura científica a partir de outros protocolos, sugerindo que a espécie medicinal *Centella asiatica*, principalmente através do composto ativo ácido madecássico apresenta potencial efeito antidepressivo, relacionado, pelo menos em parte, aos efeitos antioxidantes e anti-inflamatórios periféricos e centrais.

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Abstract: Major depressive disorder (MDD) is one of the most prevalent and debilitating psychiatric disorders, with a large number of patients not showing an effective therapeutic response to available treatments. Several biopsychosocial factors, such as stress in childhood and throughout life, and factors related to biological aging, may increase the susceptibility to MDD development. Included in critical biological processes related to aging and underlying biological mechanisms associated with MDD is the shortening of telomeres and changes in telomerase activity. This comprehensive review discusses studies that assessed the length of telomeres or telomerase activity and function in peripheral blood cells and brain tissues of MDD individuals. Also, results from *in vitro* protocols and animal models of stress and depressive-like behaviors were included. We also expand our discussion to include the role of telomere biology as it relates to other relevant biological mechanisms, such as the hypothalamic-pituitary-adrenal (HPA) axis, oxidative stress, inflammation, genetics, and epigenetic changes. In the text and the discussion, conflicting results in the literature were observed, especially considering the size of telomeres in the central nervous system, on which there are different protocols with divergent results in the literature. Finally, the context of this review is considering cell signaling, transcription factors, and neurotransmission, which are involved in MDD and can be underlying to senescence, telomere shortening, and telomerase functions.

Keywords: inflammation; major depressive disorder; senescence; telomere; telomerase.

Introduction

Data from the World Health Organization (WHO) have shown that major depressive disorder (MDD) affects more than 300 million people worldwide and contributes to the highest percentage of disability. In addition, MDD is known to be the leading cause of suicide deaths, contributing to 800,000 suicides per year (World Health

Organization 2017). In addition to affecting thousands of people of different ages worldwide, MDD does not have an effective treatment for a large number of patients yet (Souza-Monteiro et al. 2019) and causes immense losses to patients' quality of life (Solek et al. 2019). The disorder is involved with a complex heterogeneity of biological processes which, together with behavioral expressions, are the target of studies in a variety of protocols, including humans and animal models. Possibly as a consequence of the heterogeneity of factors, responses to treatments are still quite inconsistent (Belmaker and Agam 2008; Halaris 2017). On top of that, MDD presents comorbidity with several psychiatric disorders, and some comorbidities appear to be involved in the poor response to treatments with classic antidepressants or treatment-resistant depression (TRD) (De Carlo et al. 2016). In addition to psychiatric disorders, individuals affected have increased risk for developing chronic diseases (Halaris 2017), which are more common in the elderly and include diabetes, cardiovascular diseases, and neurodegenerative diseases (Kinser and Lyon 2013; Luca et al. 2013). Accordingly, some authors suggest that MDD is associated with the accelerated aging processes (Kinser and Lyon 2013; Luca et al. 2013).

The hypothesis of accelerated aging in individuals with MDD suggests that the shortening of telomeres, a condition that accompanies normal cell aging, happens in a faster pace in patients (Muneer and Minhas 2019). Some studies not only evidence a reduction of telomere lengths in samples of MDD patients (Lindqvist et al. 2015), but also an inverse correlation between its length and duration of the depressive symptoms (Verhoeven et al. 2014a). Although the causal relationship is still unknown, evidence proposes that shorter telomeres may be risk markers, predisposing individuals to MDD (Gotlib et al. 2015). MDD has also been associated with changes in the function of telomerase, the enzyme responsible for the stability and maintenance of the size of the telomeres (Szebeni et al. 2014). It is also important to highlight that oxidative stress and inflammation, conditions potentially related to senescence and MDD, among other disorders, are actively involved with shortening of telomeres (Barnes et al. 2019).

This review summarizes research evidence on the relationship between MDD and telomere shortening, as well as with the function of telomerase. Also, we review evidence from relevant biological mechanisms potentially underlying this association, including oxidative stress, inflammation, genetics, epigenetic, neurotransmission, and cell signaling. This review also lists some studies demonstrating pharmacological and

non-pharmacological therapies involving telomere shortening and MDD.

Finally, conflicting results in the literature were observed, especially considering the size of telomeres in tissues of the central nervous system, where there are more divergences in the studies.

This work is a narrative review, for which we searched for original articles that published relevant evidence on telomere shortening or telomerase function in MDD in humans or depressive-like behaviors in animal models. It was decided to include the most significant number of relevant researches in the area, considering the scarcity of study protocols involving the subject and also the great need for elucidation on the mechanisms of aging and MDD.

In addition to original research obtained from databases such as PubMed and Google scholar, bibliographic reviews were considered to extract the context from perception and argumentation of other authors and an additional way of searching for original works that did not appear in the search strategies in the databases.

All cited references were read by two or more authors who participated in this review. The purpose of reading by more than one author was to verify the studies' relevance and extract critical data that an author could overlook.

Telomere and telomerase

The telomere is a heterochromatin structure that consists of repetitions of nitrogenous bases (TTAGGG) associated with non-histone proteins found at the chromosomal ends (Zhang et al. 2007). One of the main functions of this nucleoprotein complex is the protection of genetic material, avoiding the shortening of the chromosome through replication and stressful processes to DNA (Mattson and Klapper 2001; Patrick and Weng 2019). This telomere function protects the cell from mutations, senescence, and apoptosis. In addition to the repetitions of double-strand (ds) bases that connect with the rest of the chromosome, a circular structure called T-loop is also found at the end of the telomere, where the guanine-rich 3' chain stands out, forming a single chain, single-strand (ss) of DNA. This occurs with the help of a multiprotein complex associated with the telomere, called shelterin, whose proteins work together to regulate homeostasis and maintain the stability of the telomere (Palm and de Lange 2008).

The proteins that make up the complex (Figure 1) are the telomeric repeat-binding factors 1 and 2, encoded by the *TERF1* and *TERF2* genes, respectively, *TERF1*-interacting nuclear factor 2 (TIN2 or TIN2), protection of telomeres protein 1 (POT1), repressor activator protein 1

(Rap1), and tripeptidyl peptidase 1 (TPP1) (Kim-Cohen et al. 2006; Smith et al. 2020). TERF1 and TERF2 are linked to the double chain, and TERF2 is the one that produces the angle in the chain that will give rise to the Loop. TIN2 makes the connection between TERF1, TERF2, and TPP1. TPP1, in turn, acts by binding to POT1, which binds to the ss in the Loop region and is responsible for telomerase binding for the telomere elongation (Kanoh 2017). Rap1 binds to TERF2 to reduce its affinity to ds and consequently prevents the generation of bonds with non-telomeric parts of the DNA, increasing the specificity of TERF1 to the TTAGGG sequence. Finally, there is another structure completing the T-Loop, the D-Loop, where the 3' chain is separated from the ds and reconnects to it later. This leaves a gap in the 5' chain where the 3' chain of the telomere end is inserted to complete the stabilization of the T-loop structure (Lange 2018). Another function of TERF1, TERF2, and Rap1 is to negatively regulate the access of telomerase, avoiding excessive stretching of the telomeres (Kanoh 2017). TERF1 and TIN2 form a bridge that helps to stabilize TERF2 (Lange 2018). The lack of any component of the shelterin complex enables the gradual loss of telomere DNA, predisposing the cell to failure in its ability to divide and ultimately resulting in senescence and apoptosis (Kanoh 2017). When lacking TERF2, the complex binds to the telomere Mre11/Rad50/Nbs1 (MRN), which activates ataxia-telangiectasia mutated (ATM), a protein kinase that initiates the DNA-damage response (DDR), which interrupts the cell cycle and leads to accumulation of DNA damage and apoptosis. Of note, two main theories are used to explain how TERF2 inhibits ATM kinase: the first would be to prevent the binding of ATM kinase to chromatin due to the compression that TERF2 causes in it; the second would be through the direct link between TERF2 and ATM kinase. TERF2 also inhibits the Ku70/80 pathway, which is activated when there is a double-strand break (DSB), forming a complex called DNA-dependent protein kinase (DNA-PK) via the so-called non-homologous end joining (NHEJ) (Lange 2018; Zhang et al. 2007).

Telomerase is one of the main ribonucleic enzymes that help maintain the telomere, effectively preventing its shortening. This enzyme consists of two units, one of RNA, the telomerase RNA component (TERC), which serves as the basis for DNA synthesis and the other which contains a catalytic reverse transcriptase domain for DNA synthesis, the telomerase reverse transcriptase (TERT) (Smith et al. 2020).

During the development of the CNS, telomerase activity remains high in progenitor neuronal cells. Activity drops as these cells differentiate, with this fall in activity occurring in other post-mitotic cells of different tissues

(Jacobs 2013; Mattson and Klapper 2001), as well. In contrast, in stem cells, telomerase is active to different degrees, depending on the cell type. Despite its activity in these cells, this enzyme does not seem to be active at a sufficient level to definitively prevent telomere shortening after mitosis, limiting itself to decreasing only the shortening speed of daughter stem cell telomeres, which concerns somatic cells. In male germ cells, telomerase starts with high activity and gradually decays as spermatogenesis occurs. On the other hand, telomere length gradually increases as enzymatic activity decreases, denoting a negative feedback relationship for telomerase activity as the telomere increases (Ozturk 2015; Perini et al. 2008).

Telomere and central nervous system

The CNS tissue consists of two cell groups: post-mitotic neurons and glial cells. Glial cells are made up of astrocytes (responsible for support and nutrition), microglia (macrophagic cells), oligodendrocytes (which form the myelin sheath in the CNS), and ependymal cells (which form the simple lining of the ventricles and the central canal of the spinal cord) (Zhang et al. 2007). Neurons, unlike the vast majority of cells in organic tissues, cannot replicate (Zhang et al. 2007).

During the development of the nervous system, telomerase is necessary to build the ends of the chromosome. TERT levels remain high at the beginning of postnatal development and appear to decrease gradually during the period of synaptogenesis and natural cell death (Mattson and Klapper 2001). Functional telomeres are essential in the early stages of brain development and for adult neurogenesis. Although some authors found that telomeric functions are not critical to neuronal maturation (Lobanova et al. 2017), evidence suggests that the drop in telomerase activity is strongly linked to neurodegenerative diseases, psychiatric disorders, and other aggravating factors (Liu et al. 2018).

Neurons are cells with high metabolic demand and levels of oxidative stress, processes that lead to aging, senescence, and shortening of telomeres (Zhang et al. 2007). Many of these stressors can lead to neuronal DNA damage and cell death, with the ATM and p53 pathways being the major mediators in these situations (Pan et al. 2014). The neuronal aging process affects the cell cycle, with mitochondrial dysfunction inducing cerebrovascular changes and increasing inflammatory processes, genomic

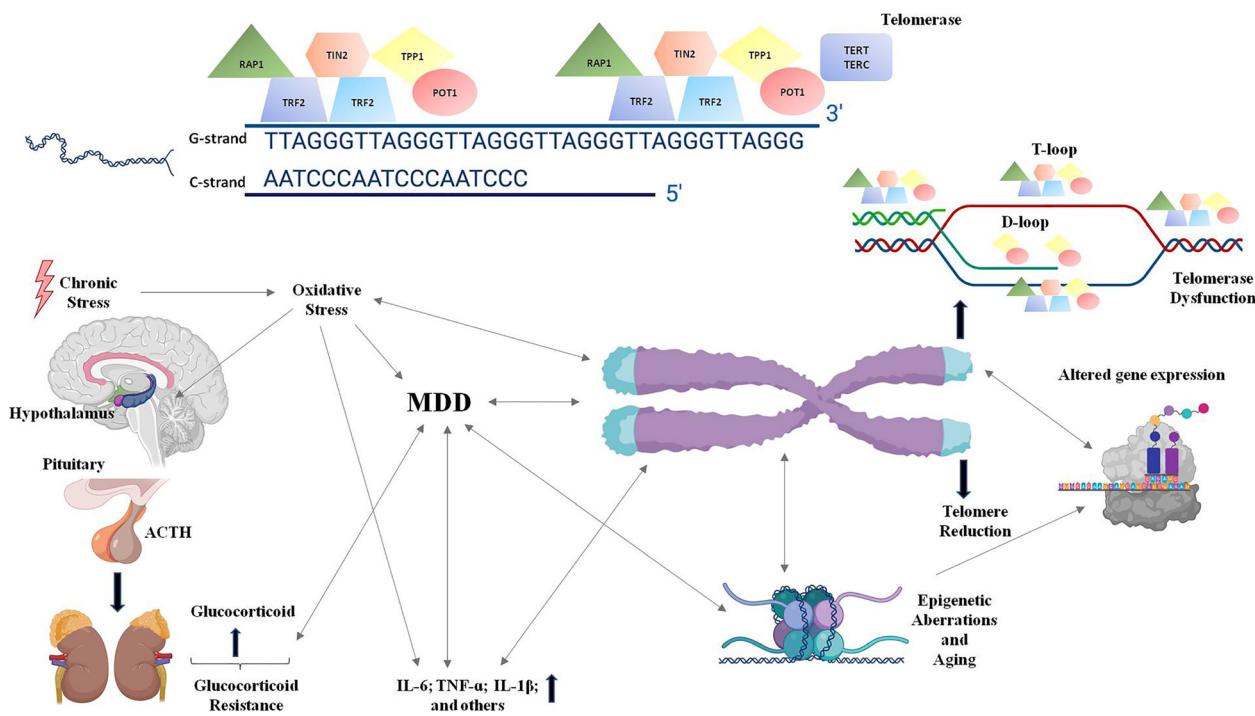


Figure 1: Chronic stress and the shortening of telomeres and changes in telomerase function.

Chronic stress in the beginning and throughout life is involved with a series of biological changes, which are connected with the shortening of telomeres and changes in telomerase function. Among these mechanisms is the hypothalamus-pituitary-adrenal (HPA) axis, mainly with a chronic increase in the release of glucocorticoids and a possible process of glucocorticoid resistance. Telomere and telomerase dysfunctions are linked to these changes, to the increase in peripheral inflammation and neuroinflammation, epigenetic changes, and the expression of genes related to the arsenal of biological mechanisms underlying to major depressive disorder (MDD) and senescence. At the top of the figure are telomeric proteins that participate in the increase in telomeres and telomerase activity. Rap1à repressor activator protein 1; TIN2 à TERF1-interacting nuclear factor 2; TPP1 à tripeptidyl peptidase 1; TRF1 and 2 à telomeric repeat-binding factor 1 and 2; POT1 à protection of telomeres protein 1; TERC à telomerase RNA component; TERT à telomerase reverse transcriptase. Images were extracted from BioRender app.

instability, and shortening of telomeres (Elkahloun and Saavedra 2020).

In conditions of oxidative stress, toxicity, and inflammation, an increase in the telomerase activity in astrocytes, neurons, and microglia has been observed, suggesting that it may be related to DNA repair and chromatin remodeling (Masutomi et al. 2005). Evidence suggests that the role of telomerase in the nervous system occurs not only during its development but also in cell survival and anti-apoptotic mechanism against cytotoxic agents (Chung et al. 2005; Mattson et al. 2001). A recent study on neural tissue in elderly mice provides evidence that the shortening of telomeres in the CNS increases with brain age and occurs regardless of cell replication. The authors also found that the shortening of telomeres in the CNS in elderly animals was not accompanied by changes in telomerase activity. This evidence suggests that physiological processes, which accompany aging, such as oxidative stress, inflammation, and other mechanisms that cause DNA damage, in addition to the lack of action of telomerase, may be involved in

the shortening of telomeres in elderly post-mitotic neurons (Ain et al. 2018).

Telomere and major depressive disorder

Aging has consequences for the brain and predisposes individuals to several diseases related to cell senescence, such as neurological diseases, coronary heart disease, type 2 diabetes, and obesity (Manoliu et al. 2018; Verhoeven et al. 2014b). Any neurological or psychiatric conditions promote an exposure to aging factors (Cole et al. 2019), which can culminate in brain physiological changes. These alterations can be seen macroscopically, including enlargement of the ventricles, cortical thinning, and accumulation of hyperintense white masses (Fotenos et al. 2005), and also microscopically, including axonal loss, synaptic suppression, mitochondrial changes,

modification in the number of glial cells, altered gene expression, shortened telomeres, dysfunctional calcium signaling pathways, and epigenetic changes (Fjell et al. 2014). The reduction of the neurotrophic brain factor (BDNF) associated with MDD, together with dementia in elderly individuals, strengthens the arguments that characterize MDD as a premature aging syndrome (Nunes et al. 2018).

Telomere shortening is associated with several psychiatric disorders, cognitive impairments, and chronic conditions related to the stress excess and aging (Price et al. 2013). Studies concerning MDD pathophysiology indicate that neuroplasticity is a physiological phenomenon that is altered in patients (Liew et al. 2006; Player et al. 2013), with alterations in neurotransmission mechanisms (Autry and Monteggia 2012; Bocchio-Chiavetto et al. 2010; Martinowich and Lu 2008), neuroendocrine (Vreeburg et al. 2009), immune, and metabolic regulation (Cai et al. 2013). Moreover, the gut-microbiota-brain axis and the gene-environment interaction are physiological processes that may be altered in MDD (Tolahunase et al. 2018). Indeed, a recent cross-sectional study proposed a protocol to investigate the complex relationship between inflammation, telomere length, gut microbiota, and psychiatric conditions (Manchia et al. 2020).

In recent years, several studies have investigated the interaction between telomere length and the pathophysiology of MDD, as well as the underlying cellular senescence process. Tables 1 and 2 summarize the main findings of the involvement of telomeres and telomerase in MDD individuals and preclinical studies, respectively. Overall, results regarding the relationship between MDD and telomeric length vary significantly between studies. Findings such as those by Simon et al. (2006), Hartmann et al. (2010), Wikgren et al. (2012), and Shalev et al. (2014), in addition to a meta-analysis carried out by Lin et al. (2016), bring evidence of a significant shortening of telomeres in MDD patients, while other studies, such as that of Zhang et al. (2010) and Rius-Ottenheim et al. (2012), showed no association between MDD and telomere length. However, these cohort and case-control studies have variations between the population samples analyzed, including differences in ethnicity, age, sex, medication use, duration and severity of MDD, among other variables, making it difficult to establish a standard of analysis that allows defining effectively what the relationship between MDD and telomere length is (Needham et al. 2015). Consistent with these arguments, a study with a large sample of MDD patients assessed over six years found that the shortening of telomeres in peripheral blood was not significantly different between controls and patients at baseline, but

telomeres were significantly shorter in depressive patients when evaluated at the end of six years. It is also important to note that telomeres were significantly shorter in patients with more severe conditions of the disorder (Verhoeven et al. 2016). A previous study also pointed out that the most severe and chronic conditions in MDD individuals are significantly related to shorter telomeres (Verhoeven et al. 2014a). Accordingly, evidence suggests that telomere shortening may be a process related to an accumulation of stress. In fact, a study showed that adolescents with MDD and without antidepressant treatment had shorter salivary telomeres when compared to control individuals. In addition to shorter telomeres, the researchers noted that the right hippocampal volume was lower in MDD adolescent individuals; however, no direct relationship was found between hippocampal volume and the size of telomeres at the beginning of MDD (Henje Bloom et al. 2015).

On the other hand, in a series of studies, the authors observed that telomere size was not different in the hippocampus and nucleus accumbens, while it was greater in the PFC and amygdala of mice submitted to chronic stress and with depressive-like behaviors. The authors observed that telomere length was shorter in peripheral blood mononuclear cells (PBMCs) from MDD subjects and animals with depressive-like behaviors in the same series of studies. The authors suggest that the increase in telomeres in the PFC and amygdala may be related to specific situations of stress and MDD (Liu et al. 2020).

The conflicting results in the literature may be related to different protocols and the scarcity of studies to elucidate the role of telomere size in neurons and glial cells in the CNS. Thus, these conflicting results need to be discussed with caution and suggestions for new protocols so that the objective function of telomeres in the CNS of MDD individuals can be elucidated.

Changes in telomerase activity have also been found in postmortem brain of MDD patients. A study conducted on oligodendrocytes of the cerebral white matter observed that oxidative stress is related to changes in the mechanisms of maintenance and protection of telomeric length, such as telomerase (Szebeni et al. 2014). It is also important to note that several forms of stress are capable of activating neurochemical cascades, which lead to telomeres degradation (Monaghan 2014). Considering the brain areas, it was found that changes in TERT of patients with MDD are related to telomeric shortening in the hippocampal region (Souza-Monteiro et al. 2019). Other authors have noted that stress can accelerate telomere shortening in brain areas susceptible to MDD. In MDD patients, shorter telomeres were found in the hippocampus, dorsolateral prefrontal cortex, and substantia nigra. In addition to the shortening

Table 1: Studies in MDD patients – telomeres size and telomerase activity.

Patients	N	Studied Cells	Treatment	Main physiological findings	Authors
Adolescent participants (13–18 years)	117 54 with MDD and 63 controls	Saliva	Not applicable	Significantly shorter salivary TL and hippocampal volume were observed in the depressed.	Henje Bloom et al. (2015)
Caucasians, both sexes, mean age 49 years	54 MDD and 20 controls	Leukocytes	Antidepressant drugs in different doses and a single strategy with ECT	Shorter TL in the MDD group as a whole, with no difference between treatment groups or the severity of the disorder.	Hartmann et al. (2010).
Northern Sweden patients, both sexes, aged 21–87 years	91 MDD and 451 controls	Leukocytes	Uninformed	Shorter TL in MDD, associated with hypocortisolemia and high family burden of affective disorders	Wikgren et al. (2012)
MDD, bipolar disorder and schizophrenia individuals	10 MDD, 10 bipolar, 10 schizophrenia, 10 controls	Postmortem tissues of the dorsolateral prefrontal cortex, hippocampus, amygdala, nucleus accumbens and substantia nigra	Uninformed	Shorter TL in the MDD hippocampus. Changes in the expression of genes involved in neuroprotection and stress response. The expression of CRH, GPR37, and HSPA2 was reduced, while the expression of FKBP5, PPARD, and PPARG was increased in the hippocampus of MDD.	Mamdani et al. (2015)
USA individuals, both sexes, aged 19 to 86.	12 MDD and 12 controls	Oligodendrocytes and astrocytes of postmortem brain tissue	Not applicable	Reduced expression of telomerase and antioxidant enzymes SOD1, SOD2, GPX1, and CAT genes associated with shorter telomeres.	Szeberi et al. (2014)
Caucasian, pregnant women, with perinatal depression and >18 years old	25 MDD and four baby blues	Buccal mucosa	Not applicable	Childhood stress was associated with DNA methylation, perinatal depression, and shorter TL.	Robakis et al. (2020)
US and low-income women with a 12-week-old baby	48 mother-baby dyads. Different stressful stimuli on newborns.	Buccal mucosa	Not applicable	Greater correlation between reactivity to cortisol and TL shortening in MDD mothers' babies.	Nelson et al. (2018)
Girls 10–14 years old and MDD mothers' daughters Individuals of both sexes aged 25–69 years	50 from MDD mothers and 47 controls	Buccal mucosa	Not applicable	Shorter TL in MDD mothers' daughters	Gotlib et al. (2015)
MDD individuals aged 19–50 years	18 MDD and 17 controls	Leukocytes	Not applicable	Greater accumulation of depressive episodes was associated with increased oxidative stress and shorter TL.	Wolkowitz et al. (2011)
	58 MDD 29 MDD + yoga and MDD without yoga	Peripheral blood	Yoga for 12 weeks	Reduced MDD scores, increased telomerase activity, and BDNF levels, in parallel to reduced cortisol levels, inflammatory, and DNA damage markers in MDD with yoga therapy	Tolahunase et al. (2018)
Individuals of both sexes, with an average age of 34 years	20 MDD and 18 controls	Leukocytes	Sertirline for eight weeks	Baseline telomerase activity was significantly reduced in MDD. Sertirline treatment did not alter telomerase activity.	Wolkowitz et al. (2012)
	17 MDD and 16 controls	Leukocytes	Not applicable		Teyssier et al. (2012)

Table 1: (continued)

Patients	N	Studied Cells	Treatment	Main physiological findings	Authors
Female individuals diagnosed with MDD				The OGG1, p16 ^{Ink4a} , and STMN1 genes were upregulated. p16 ^{Ink4a} , and STMN1 showed a direct correlation with MDD.	
Patients diagnosed with cardiac diseases, among others, together with MDD	948 patients with coronary disease, 743 of them without MDD, 205 with MD	Leukocytes	Not applicable	Shorter TL in patients with MDD. Stronger social connections, physical activity, and better quality of sleep were associated with longer telomeres in the two groups.	Puterman et al. (2013)
2981 participants	1095 with current MDD, 802 with remitted MDD, 510 healthy controls	Leucocytes	Not applicable	Shorter TL in patients with MDD current or remitted, and it was cumulative proportionally to the severity and duration of the disease	Verhoeven et al. (2014a).
2981 participants between 18 and 65 years	2292 current or remitted depression or anxiety disorders, and 644 control	Leucocytes	Not applicable	MDD and anxiety individuals had shorter TL, compared to control group in the beginning and at six years after, but the attrition rates were the same in both groups	Verhoeven et al. (2016).
132 elderly people with an average age of 69.7 years. Caregivers of patients with dementia + adversity in childhood (ELS)	58 caregivers of patients with dementia 74 controls	T cells and monocytes	Not applicable	ELS was associated with shorter TL, increased IL-6, and TNF- α . ELS + caregiver had a stronger effect on the increase in TNF- α . ELS and caregivers had a significant increase in depression scores.	Kiecolt-Glaser et al. (2011)
Impatient with mean age 39.5 ± 3.3.	50 MDD and 50 healthy control	PBMCs	Not applicable	Shorter telomeres in MDD patients	Liu et al. (2020)

MDD → major depressive disorder; TL → telomere; ELS → early life stress; ECT → electroconvulsive therapy; CRH → corticotropin-releasing hormone; GPR37 → G protein-coupled receptor 37; HSPA2 → heat shock protein family A (Hsp70) Member 2; FKBP5 → FK506 binding protein 5; PPARD → peroxisome proliferator activated receptor delta; PPARG → peroxisome proliferator activated receptor gamma; SOD → superoxide dismutase; GPX1 → glutathione peroxidase 1; CAT → catalase; OGG1, p16^{Ink4a} → cyclin-dependent kinase inhibitor 2A, STMN1 → stathmin 1; OGG1 → 8-oxoguanine DNA glycosylase; BDNF → brain-derived neurotrophic factor; TNF- α → tumor necrosis factor alpha; IL-6 → interleukin 6; PBMCs → peripheral blood mononuclear cells.

Table 2: Evidence about telomere or telomerase activity reduction in animal models of depression.

Animals	Evaluated tissue or cell	Induction of depressive-like behavior or behavioral protocol	Behavioral response	Analyzed biological mechanisms	Authors
Male Swiss mice	Hippocampus, striatum, and prefrontal cortex	LPS i.p. FST or SPT.	The administration of LPS induced immobility in the FST and anhedonic behavior in the SPT. EO treatment reduced depressive-like behaviors in LPS animals.	The depressive-like behavior was accompanied by a reduction in the cerebral expression of TERT, an increase in lipid peroxidation, and a reduction in markers of mature neurons in the hippocampus, striatum, and prefrontal cortex. These impairments were reversed by the EO treatment.	Souza-Monteiro et al. (2019).
C57BL/6 mice	Primary hippocampal neurons cultures and hippocampal tissue of mice	CMS TST, FST and SPT	Mice submitted to CMS showed depressive-like behavior in immobility and anhedonia tests. Fluoxetine treatment reversed depressive-like behaviors.	CMS was related to reduced telomerase activity and TERT expression in the hippocampus. Fluoxetine reverted the changes in telomerase and TERT. Inhibition of telomerase increased depressive behaviors and impaired hippocampal neurogenesis.	Zhou et al. (2011).
Male Wis-far rats	Leukocytes and cells from liver and hippocampus	CMS FST and SPT	The group exposed to CMS exhibited decreased sucrose intake and increased immobility time in the FST	Increased expression of TERT reversed depressive behaviors and impaired neurogenesis.	Xie et al. (2017)
Male FSL and FRL rats	Hippocampal cells	Unreported	Unreported	Rats exposed to CMS exhibited reduced telomerase activity and shortening of telomeres in the liver, impaired oxidative balance, and reduced levels of 5-HT and corticosterone in the serum. Hippocampal telomeres were shorter in FLS rats. TERT and BDNF was also reduced in the FLS hippocampus, as well as telomerase activity. The treatment with lithium managed to return TERT expression and telomerase activity to normal levels.	Wei et al. (2015)
Male C57BL/6 mice	PBMCs, hippocampus, amygdale, prefrontal cortex, nucleus accumbens, paraventricular nucleus	CMS, SPT, FST	Mice submitted to CMS showed depressive-like behavior in immobility and anhedonia tests.	Telomeres in mice with depressive behavior were shorter in PBMCs. No size change in nucleus accumbens, hippocampus, and paraventricular.	Liu et al. (2020)
				Longer telomeres in the prefrontal cortex and amygdala.	

FST → forced swimming test; SP → splash test; SPT → sucrose preference test; TST → tail suspension test; EO → *Euterpe oleracea*; LPS → lipopolysaccharide; CMS → chronic mild stress; FSL → flinders sensitive line (genetic rat model of depression-like behavior); FRL → flinders resistant line (depression-like behavior control); TERT → telomerase reverse transcriptase; 5-HT → serotonin; PBMCs → peripheral blood mononuclear cells.

of telomeres, research has shown changes in the hippocampus mRNA genes involved in neuroprotection and stress response (Mamdani et al. 2015). Of note, the hippocampus is a brain area of the limbic system, which has neurogenesis in the adult brain (Eriksson et al. 1998) and is involved in the pathophysiology of MDD (Warner-Schmidt and Duman 2006). The hippocampal volume of MDD patients has been shown to be reduced and also related to a decrease in telomeric length (Eriksson et al. 1998; Souza-Monteiro et al. 2019). The decrease in telomeric length in the hippocampus of MDD patients may be a result of glial cell divisions. However, there is evidence that non-replicating neurons may be reduced telomeres due to stress and other cellular changes (Von Zglinicki 2002; Baruch-Eliyahu et al. 2019).

Interestingly, pre-clinical studies have found that the expression of TERT in the hippocampus is strongly related to antidepressant effects. Authors have found that chronic stress in mice, in addition to inducing depressive behaviors, also reduced the expression of TERT and telomerase activity in the hippocampus. Furthermore, intra-hippocampal infusion of TERT increased adult neurogenesis and reduced depressive behaviors in animals

(Zhou et al. 2011). In animals with depressive behaviors, there was a decrease in the expression of TERT in the liver and hippocampus (Xie et al. 2017), in addition to a significant decrease between 25 and 50% in the striatum and prefrontal cortex, respectively. This reduction may be inherent to MDD and related to long-term deletions, which seem to be involved in the premature aging of neurons and in increasing the susceptibility to aging diseases (Souza-Monteiro et al. 2019) (Figure 2).

It is also possible that telomerase promotes cell survival through the actions of BDNF (Martinowich et al. 2007). Accordingly, BDNF levels have been associated with impairments in neuroplasticity (Fu et al. 2002; Nunes et al. 2018) and premature aging in MDD individuals (Nunes et al. 2018). Besides, several forms of stress are capable of activating neurochemical cascades, which lead to the degradation of telomeres (Monaghan 2014).

Considering many possible pathways by which MDD interferes with telomere length to promote its shortening, and the divergence that still exists between studies, the need to conduct more controlled studies is evident, seeking to establish more clearly how the pathophysiology of MDD changes telomeric dynamics. Furthermore, it is important

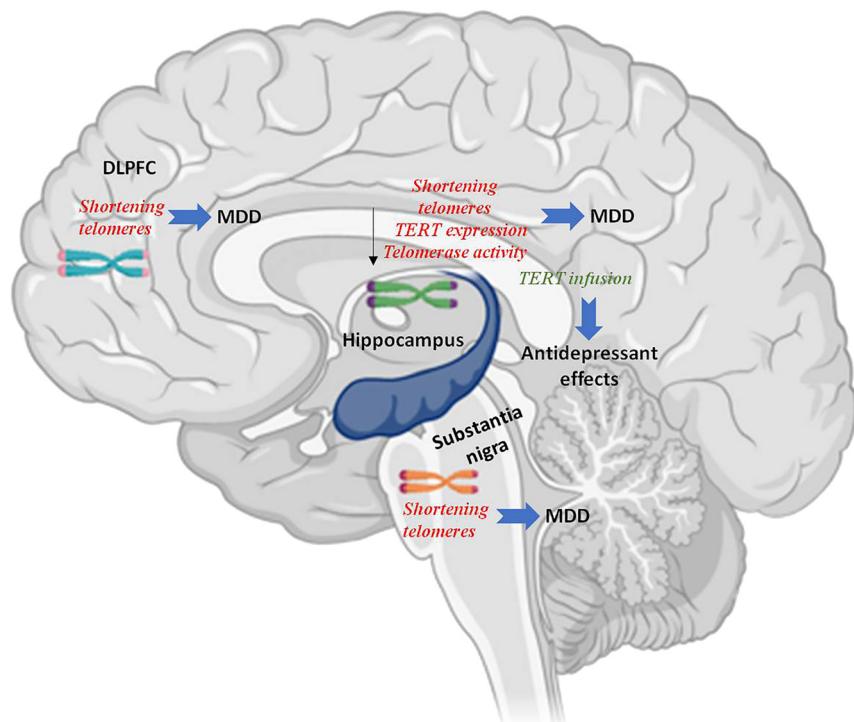


Figure 2: Brain areas involved in telomeres changes and major depressive disorder (MDD) pathophysiology.

Brain areas involved in telomeres changes and major depressive disorder (MDD) pathophysiology. In the dorsolateral prefrontal cortex (DLPFC), hippocampus, and substantia nigra shortening of telomeres are associated with MDD. In the hippocampus reduced TERT expression and telomerase activity are correlated with stress and depressive behavior in experimental models. On the other hand, the infusion of TERT promotes antidepressant-like effects. Images were extracted from BioRender app.

to consider studies in animal models, in which it is possible to investigate the potential macro and microstructural mechanisms in CNS and other organic systems, in addition to the interaction of these mechanisms with telomeric variation and MDD.

Telomere, genes, and MDD

Studies in elderly individuals observed a correlation between genes associated with oxidative stress and biomarkers of aging, including shortening of telomeres. The authors suggested that the impaired metabolism of metals, such as copper and iron, as well as reduced antioxidant enzyme activity, appear to be targeting or interacting with changes in mitochondrial function, transcriptional factors, and attrition of telomeres. This interaction may culminate in an increase in pro-inflammatory cytokines and damage in other mechanisms that can lead to apoptosis and senescence (Starr et al. 2008).

Genetic mechanisms may mediate the reduction of telomeres in MDD. For instance, studies have shown an association between telomere shortening and a high-activity allele related to a polymorphism in the promoter region of the monoamine oxidase A (*MAOA*) gene. Further, studies by the same authors showed that this association was related to MDD (Lung et al. 2005, 2007). Another study looking at genetic polymorphism for *MAOA* also found an association between candidate alleles for psychiatric disorders with shorter telomeres in young individuals (Speck-Hernández et al. 2015).

Some researchers observed a shortening of telomeres in oligodendrocytes from the temporal and prefrontal cortices of postmortem MDD individuals, which was in parallel to a reduction in the expression of telomerase genes and antioxidant enzymes superoxide dismutase (SOD), SOD1 and SOD2, glutathione peroxidase 1 (GPx1) and catalase (CAT) (Szebeni et al. 2014). These results suggest that impairments in the antioxidant defenses and lack of telomerase action may be involved in telomere shortening and white matter abnormalities in MDD patients (Szebeni et al. 2014). Another postmortem study found that MDD individuals had shorter telomeres in the dorsolateral prefrontal cortex compared to the substantia nigra, suggesting a different aging pace across different brain regions. In the hippocampal tissue, genes responsible for the response to stress and neuroprotection were less expressed in MDD, a fact that was associated to the reduction in telomere length. Specifically, isolated genes were those encoding FK506 binding protein-5 (FKBP5), corticotropin-releasing hormone (CRH), heat-shock 70 kDa

protein-2 (HSPA2) peroxisome proliferator-activated receptor-alpha (PPAR α), retinoid X receptor-alpha (PPARG), and G-protein coupled receptor 37 (GPR37). Overall, their findings support the hypothesis that the hippocampal cell aging is accelerating due to stress in MDD patients (Mamdani et al. 2015).

The expression of genes related to telomere dysfunction markers and cellular senescence has been shown to be increased in leukocytes from MDD women, specifically OGG1, P16^{Ink4a}, and STMN1 (Teyssier et al. 2012). Specifically, the p16^{Ink4a} is the principal member of the Ink4 family of CDK inhibitors, showing antiproliferative activity (Romagosa et al. 2011). The STMN1 gene encodes stathmin, a protein that integrates intracellular signals, participating in the regulation of microtubule clustering and activity and, thus, influencing synaptic reorganization and plasticity (Rubin and Atweh 2004; Uchida and Shumyatsky 2015). Another study in patients with suicidal ideation found a significantly increased expression of stathmin mRNA before starting antidepressant treatment (Belzeaux et al. 2019). As for the OGG1 gene, it encodes the DNA glycosylase I enzyme, which initiates the base excision repair (BER) pathway and selectively cleaves the main oxidized base, 8-hydroxy-29-deoxyguanine (8-oxoG), of the telomeres (David et al. 2007). Of note, repeated base oxidation in telomeres is a crucial factor for telomeric reduction and loss (Fouquerel et al. 2019). The increase of these proteins in MDD likely has a compensatory function to reduce oxidative damage to the DNA and, consequently, reduce cellular damage induced by the shortening of telomeres (which is possibly occurring chronically in the disorder). Thus, increased expression of these genes may be a key marker of oxidative damage to DNA and telomeric reduction in MDD. In this sense, it is also important to consider the work of Herbet et al. (2017), in which the authors observed that the OGG1 mRNA levels were significantly higher in the hippocampus of rats subjected to chronic stress, in parallel with an increase in oxidative stress.

Telomere, epigenetics, and MDD

The processes underlying epigenetics constitute changes in the function genes without altering the DNA sequence. It can be defined as a reprogramming of the gene's action in certain situations (López-Maury et al. 2018). The main mechanisms that culminate in epigenetic changes involve chemical modifications of DNA and histones, structural changes in chromatin, as well as changes in the action of non-coding RNAs. These changes in the mechanisms and

processes associated with the function of DNA alter its function, implying a deviation in the course of gene expression and resulting in phenotypic changes (Weinhold 2006). Although epigenetic changes are crucial during embryonic development, as well as in other periods of life, epigenetic alterations can lead to diseases or psychiatric disorders (Ignácio et al. 2014; López-Maury et al. 2018). Other studies go further, showing evidence that epigenetic changes from long-term stress can be transmitted to future generations, highlighting their potential intergenerational epigenetic transmission (Chan et al. 2020; Heard and Martienssen 2014).

Some studies suggest that epigenetic changes may lead to a shortening of telomeres. On the other hand, telomere shortening appears to predispose to variations in gene activity from changes in chromatin condensation levels near the region that has suffered shortening (Starkweather et al. 2014). A wide variety of sites with epigenetic alterations, as broad changes in DNA methylation, have been associated with childhood adversities, attachment difficulties, and perinatal depression. Accordingly, childhood adversities have also been related to shortening of telomeres in buccal epithelial cells (Robakis et al. 2020).

Studies in humans and animal models have shown that stress and adversity in early life are major villains in the onset of MDD (Ignácio et al. 2017a, 2017b; Phillips et al. 2005), other psychiatric disorders (Green et al. 2010), and chronic diseases (Rich-Edwards et al. 2010; Scott et al. 2012). Several biological mechanisms seem to emerge from early life stress (ELS), suggesting a pleiotropic effect and implying interactions with each other, culminating in allostatic load to trigger MDD (Ignácio et al. 2017a; Scheuer et al. 2018). These mechanisms may be associated with the shortening of telomeres, which may also be the target of ELS (Drury et al. 2012; Kananen et al. 2010). In fact, individuals with post-traumatic stress disorder had shorter telomeres of leukocytes in adulthood, but telomere shortening was significant only in individuals who suffered multiple traumas in childhood (O'Donovan et al. 2011). A relevant aspect that deserves further studies is the fact that the shortening of telomeres occurs in childhood and is related to the time of exposure of children to institutional care (Drury et al. 2012). Moreover, a recent study also showed that epigenetic aging, which may be associated with shortening of telomeres, was significantly higher in more severe cases with depression and in MDD individuals who suffered childhood trauma (Han et al. 2018).

Among the negative sequelae associated with epigenetic variations are the dysregulation of the HPA axis, availability and function of serotonin, and an increase in inflammatory cytokines. These physiological changes have

all been associated to shortening of telomeres, developmental impairments, and MDD (Casavant et al. 2019). Recent studies also highlight that there is an interrelation between stress and erosion of telomeres, as well as the role of telomerase and psychiatric disorders (Epel and Prather 2018).

Moderate to high antenatal or postnatal stress is associated with some impairment in the child's development, which can lead to chronic diseases, psychiatric disorders, and cognitive deficits (Gelaye et al. 2016). Although the evidence on biological mechanisms is still scarce, some studies have observed epigenetic changes, as well as in the function of the HPA axis, with indications of functional variations in brain regions involved with emotional and cognitive functions, such as the hippocampus (Bleker et al. 2019; Brunton 2013; Weinstock 2005).

In a systematic review, the authors point out that stress from premature birth and its consequences, such as distance from the mother, pain, among other factors inherent to the condition, is associated with epigenetic changes and reduced telomere length. In the same study, the authors found that polymorphic variants of several genes associated with HPA axis function, inflammation, neurotransmission, and growth factors were correlated with more epigenetic changes and greater behavioral impairments in childhood (Casavant et al. 2019).

Although chronic childhood stress is one of the biggest villains for epigenetic changes and telomere shortening, some studies have shown that the accumulation of stress in adulthood is also a factor involved in telomeric reduction. However, these authors also found that the accumulation of stress in childhood resulted in a more significant decrease in telomeres than chronic stress in adult life (Mayer et al. 2019). Accordingly, previous studies had already observed in the elderly that chronic adult stress, specifically in those who were also subjected to childhood adversity, culminated in shorter telomeres in peripheral blood mononuclear cells (PBMCs) parallel to increased pro-inflammatory cytokines and more depressive symptoms (Kiecolt-Glaser et al. 2011).

Telomere, HPA axis, and MDD

Chronic stress promotes greater activation of the HPA axis, leading to an increase in the release of glucocorticoids (Bellavance and Rivest 2014). Acute and chronic stress are important etiologic factors of MDD (Wikgren et al. 2012). The HPA axis is the main neuroendocrine axis involved in the stress response and has been linked to telomere shortening (Gotlib et al. 2015; Nelson et al. 2018). A

dysregulation of the HPA axis has been reported in MDD patients, with hypersecretion of the CRH by the hypothalamic paraventricular nucleus (Pruessner et al. 2003), impaired negative feedback of the HPA axis (Malhi and Mann 2018), enlarged adrenal glands, hypercortisolemia, and decreased suppression of cortisol by dexamethasone (Pruessner et al. 2003). It was also observed that the hypersensitivity of the HPA axis leads to the release of cortisol in response to low levels of stress, as well as maintenance of chronically high levels of cortisol (Dean and Keshavan 2017). About 40–60% of MDD patients experience hypercortisolemia or other disorders of the HPA axis, with changes in the circadian cycle of cortisol release (Keller et al. 2017).

In vitro studies have shown that high levels of cortisol accelerate the telomere shortening process (Vartak et al. 2014). Through studies with T lymphocytes exposed to cortisol, it was observed that cortisol influences telomeric length and cell senescence by reducing telomerase activity, resulting in a decrease in telomeric length (Choi et al. 2008).

High levels of glucocorticoids are also known to affect the medial prefrontal cerebral cortex, the hippocampus, and the amygdala (Dean and Keshavan 2017). In the medial prefrontal cortex, emotions are processed from regions such as amygdala, and a reduced activity in this area promotes the inadequate processing of negative affect (Cerqueira et al. 2005). In the hippocampus, high levels of cortisol impair the ability of this region to adapt to environmental changes, compromising adaptation and learning (Alfarez et al. 2002). Chronic stress decreases, over time, the projections from the basolateral amygdala to the medial prefrontal cortex and increases the excitability of the amygdala, resulting in an increased reactivity to stress and decreasing cognitive processing (Duvarci and Paré 2007). In MDD patients a telomeric loss in the hippocampus has been observed, a condition that may be related to the various mechanisms involved with its pathogenesis, including HPA axis dysfunction (Souza-Monteiro et al. 2019).

In a meta-analysis, Jiang et al. (2019) observed that baseline cortisol levels are not correlated with telomeric length. In contrast, a significant association was observed between increased levels of salivary cortisol, psychosocial stressors, and shortening of telomeres. Moreover, studies also highlight that females have greater physiological responses to stress than males, with some researchers suggesting that women show a greater impact on telomeric length in response to psychosocial stress than men (Chopra et al. 2009).

One study found a greater correlation between reactivity to cortisol and telomeric shortening in children. In adults, no significant correlation has been observed between reactivity to cortisol and telomeric length (Nelson et al. 2018). Some studies have been carried out to understand the influence of MDD during pregnancy and the impacts on the child (Valsamakis et al. 2019). In addition, maternal psychological characteristics can act as protective or risk factors for children. Maternal stress during pregnancy has been associated with shorter telomeres in childhood and higher reactivity to cortisol in stressful situations for children (Gotlib et al. 2015). On the other hand, women with a greater willingness to pay attention (mindfulness) and showing less depressive symptoms have greater flexibility in HPA axis responses, as well as a faster recovery of responses to cortisol. The children of mindfulness mothers also had lower levels of cortisol when exposed to stressful situations in childhood (Laurent et al. 2017; Nelson et al. 2018).

Glucocorticoids exert pleiotropic effects, which can promote an important adaptation of the organism under stress conditions but can also lead to dysregulation of homeostasis, contributing to the triggering of diseases and premature death (Wolkowitz et al. 2009). Some studies have suggested that the state of hypercortisolemia, which accompanies chronic stress in many situations, may be the result of the establishment of a condition of resistance to glucocorticoids (Ignácio et al. 2019; Wolkowitz et al. 2009). Still, different tissues of the body may respond differently, creating relative conditions between hyper and hypocortisolemia and making it difficult to establish a specific and effective condition (Ebrecht et al. 2000; Lu and Cidlowski 2006). Both conditions have been observed frequently in MDD and represent a decrease in quality of life when compared with individuals with normal HPA axis functionality (Maripuu et al. 2014).

Since cortisol has anti-inflammatory activity, the hypothesis of hypocortisolism would be justified by the increase in serum levels of pro-inflammatory molecules (TNF- α , IL-1 β , among others) in MDD individuals, thus contributing to the manifestation of depressive symptoms. On the other hand, high levels of circulating cortisol and pro-inflammatory molecules seem to be inherent to the desensitization of leukocytes to cortisol (Pace et al. 2007), considering that sensitivity of these receptors is inversely related to the levels of TNF- α (Fitzgerald et al. 2006).

Hypocortisolism seems to be a characteristic of atypical depression, in which situation hypersomnia, weight gain, and other metabolic disorders predominate (Lyndon et al. 2017). In this context, some researchers found a

positive correlation between hypocortisolism and reduced telomeres in MDD individuals. It is interesting to note that most patients were older individuals and also had a significant increase in some inflammatory markers (Wikgren et al. 2012). On the other hand, the hypothesis of hypercortisolemia is justified by the phenotypic changes resulting from excess of cortisol in several organs and by the increase in cortisol signaling in MDD individuals (Wolkowitz et al. 2009). The hippocampal atrophy observed in MDD subjects due to excess of cortisol is quite prominent (Lucassen et al. 2006). Some researchers observed that patients with psychotic depression, a condition closely related to high levels of cortisol, were associated with lower volumes of the hippocampus and the subcallosus region of the anterior cingulate cortex (Bijanki et al. 2014). More recent studies have also shown an association with increased levels of cortisol and cortical atrophy in MDD individuals (Lebedeva et al. 2018). These studies did not analyze the size of the telomeres, but an *in vitro* study with T cells found that the exposure of these cells to high levels of hydrocortisone, comparable to the levels of cortisol in the body during stress, resulted in a significant reduction in telomerase activity, in parallel to a reduction in the transcription of hTERT, the catalytic component of the enzyme (Choi et al. 2008).

Bearing in mind that MDD in many cases is related to a deregulation of the HPA axis (Keller et al. 2017) and that cortisol acts in an anti-inflammatory way (Cain and Cidlowski 2017), it is important to understand the effects of the neuroimmune system in situations of high glucocorticoid release due to exposure to stressors, as observed in depressive individuals, and its relationship with cell aging.

Aging, which has telomeric shortening among its causes, is related to the dysregulation of the immune and neuroimmune systems (Fonken et al. 2018a), favoring an environment with neuroinflammation (Rawji et al. 2016). High sensitivity to microglia (primed microglia) was observed in elderly mice (Huang et al. 2008), a condition characterized by an expression of molecules similar to that of activated microglia (Frank et al. 2007). Another study noted that the microglia of elderly rats was sensitized, and also it showed exaggerated expression of pro-inflammatory cytokines after immune challenge with lipopolysaccharide (LPS) (Frank et al. 2010). Still, it was observed that the sensitization of the microglia happens preferentially in the white mass, in comparison with the gray one (Hart et al. 2012). Another factor to be considered is that some regions of the aged CNS, such as the hippocampus, are more vulnerable, but not all regions have sensitization of the microglia (Grabert et al. 2016).

Some studies have observed that cell aging works by altering the circadian cycle of glucocorticoid, with older mice having high levels of corticosterone in the hippocampus during the inactive phase of the circadian cycle. Although glucocorticoids are normally anti-inflammatory, high levels of glucocorticoids in the aged CNS are a critical factor involved in preparing for neuroinflammation (Barrientos et al. 2015). Still, in rodents, corticosteroids are an indication from which stress initiates inflammatory responses (Frank et al. 2013). The administration of exogenous glucocorticoids in rodents has been shown to lead to neuroinflammation (Fonken et al. 2018b).

Neuroinflammatory activation may also occur through resistance to glucocorticoids. It was observed that after the interruption of stress, peripheral macrophages became hyposensitive to glucocorticoids, increasing inflammatory responses (Avitsur et al. 2001). It is believed that the same can happen with microglia (Fonken et al. 2018a). The hyposensitization of glucocorticoid receptors was detected in brain macrophages after stress conditions and is dependent on the release of cortisol from the adrenal glands (Niraula et al. 2018). However, the microglia isolated from animals exposed to chronic stress did not show resistance to glucocorticoids (Wohleb et al. 2018). These results indicate that changes in brain glucocorticoid receptors still lack studies intending to unravel the mechanisms and biological interrelationships involved (Barrientos et al. 2015).

Telomere, oxidative and nitrosative stress, and MDD

Oxidative and nitrosative stress includes conditions resulting from changes in the balance between the release of reactive oxygen (ROS) and nitrogen (RNS) species, respectively, and the amount of biological antioxidant molecules, such as vitamins A, C, and E, flavonoids, as well as the activity of endogenous antioxidant enzymes, such as SOD, CAT, and GPx (Luca et al. 2013; Smith et al. 2013). ROS may be released as byproducts of oxidative respiration or during redox cycling events involving environmental toxins and Fenton reactions involving heavy metals (Jackson and Bartek 2009).

In vitro high levels of oxidative stress have been associated with double-strand breaks (DSB) in the telomeric region. This phenomenon does not occur under moderate oxidative stress, which indicates that oxidative damage to the telomere only accelerates shortening if there is no enzymatic repair of the chain (Von Zglinicki 2002).

The brain is one of the tissues with the highest demands for oxygen due to a high metabolic rate. The accumulation of oxidative stress in the brain tissue is capable of causing direct damage to neuronal DNA, altering nitrogenous bases. The most susceptible base is guanine, which is predominant in telomeres (Cooke et al. 2003). The increase in lipid peroxidation has also been associated with a deficit in the lipid bilayer that surrounds neuronal cells and organelles, altering neuronal permeability and fluidity, which can lead to the toxicity and cell death (Catala 2011, 2012).

Gene expression of the antioxidant enzymes SOD1, SOD2, GPx1, and CAT has been shown to be significantly reduced in post-mortem white matter of MDD individuals. A significant reduction in the size of telomeres and the expression of the TERT gene has been observed in oligodendrocytes, but not in the astrocytes, from the same individuals (Szebeni et al. 2014). It has been suggested that astrocytes have a greater capacity for the balance of oxidative stress, considering that they have a greater intracellular expression of reduced glutathione (GSH) and also lower iron storage when compared to oligodendroglia (Juurlink et al. 1998). Therefore, the evidence in these studies highlights oxidative stress as a relevant mechanism intertwining the shortening of telomeres in nervous system cells of patients with MDD.

Another study investigated oxidative stress markers in the leukocyte telomeres of patients with MDD and found that the size of the telomeres was inversely correlated with exposure to depressive symptoms throughout life, corresponding to approximately seven years of accelerated cell age. In this same study, the researchers showed that telomere shortening was inversely correlated with oxidative stress, both in MDD and in control subjects (Wolkowitz et al. 2011). Of note, the reduction of antioxidant enzymes, such as GPx, CAT, and SOD, was listed among factors responsible for increased oxidative stress and, consequently, brain aging (Cornejo and von Bernhardi 2016).

The relationship between oxidative stress and shortening of telomeres is also demonstrated in studies investigating other chronic diseases, which are often associated with MDD. Studies conducted on patients with Parkinson's disease (PD) observed a reduction in telomeres length in leukocytes, which was associated with greater expression of carbonylated proteins (Watfa et al. 2011). Hypertension reduces the expression of the terminal restriction fragment (TRF) of the telomeres and this characteristic was proportional to the higher index of oxidative stress or insulin resistance in hypertensive patients (Demissie et al. 2006). Another study noted that diabetes mellitus 2 (DM2) is

directly related to the shortening of the telomere fraction in leukocytes. The authors also observed that a variant allele of uncoupling protein 2 UCP2, which determines a lesser expression of protein mRNA, was correlated with the increase in oxidative stress and with shorter telomeres. UCP2 is involved with pancreatic insulin function and participates in the negative regulation of ROS mitochondrial overproduction (Salpea et al. 2010). Although these studies are not directly related to MDD, it is important to note that oxidative stress is involved and appears to be a relevant factor in shortening telomeres. It should be noted that these diseases are comorbid in many MDD patients, in addition to the fact that many pathophysiological mechanisms involved are also related to chronic metabolic diseases (Penninx and Lange 2018).

Telomere, inflammation, neuroinflammation, and MDD

Inflammation can induce cell senescence, just as cell senescence can induce more inflammation. Cultures of different senescent cells with short telomeres have been shown to present increased TNF- α , IL-6, NF- κ B levels, a pattern that was named "Associated Senescent Secretory Phenotype (SASP)" (Kordinas et al. 2016). Inflammatory cytokines can induce oxidative and nitrosative processes as ROS and RNS are released by macrophages and neutrophils at inflammation sites. Conversely, ROS are primarily responsible for recruiting leukocytes in the early stages of inflammation, in addition to increasing vascular permeability and inducing angiogenesis. If there is no efficient balance between the ROS produced and antioxidant substances, oxidative stress is established, which, if maintained chronically, leads to chronic inflammation (Chelombitko 2018).

Neuroinflammation is closely related to the activation of microglia, breakdown of the blood-brain barrier, production of cytokines and chemokines, infiltration of peripheral immune cells, and the presence of T-autoreactive cells. This process leads to the loss of myelin and can cause fragmentation of axons (DiSabato et al. 2016). Aged microglia have impaired branching and are suggested as a key process involved in age-related neurodegeneration in brain regions such as the hippocampus (Choi et al. 2007), in addition to increasing the expression of inflammatory cytokines, such as TNF- α , IL-1 β , and IL-6 (Sierra et al. 2007), and reducing anti-inflammatory cytokines, such as IL-10 and IL-4 (Maher et al. 2005).

Several stressors have been shown to induce the expression of pro-inflammatory cytokines in brain regions such as the hippocampus and hypothalamus (Johnson et al. 2005). Still, stress sensitizes the preparation of the neuroimmune phenotype, leading to an increase in inflammatory markers and microglial activation for a long time. This process has been associated with neuronal degeneration (De Pablos et al. 2014; Frank et al. 2018) and neuroinflammation, both of which are related to MDD (Fonken et al. 2018a,b).

The pro-inflammatory challenge through LPS and the anti-inflammatory stimulus through IL-4 were observed in primary cells cultured from mouse microglia. The challenge through LPS reduced the expression of genes associated with mitochondrial biogenesis, cell cycle, and the telomere complex. On the other hand, the stimulus through IL-4 upregulated the expression of genes associated with energy metabolism, mitochondrial biogenesis, as well as tumor suppressor genes. In the same series of studies, although the effect of chronic stress was more modest, the exposure of mice to three different situations, such as subchronic cerebral ischemia, a model of Alzheimer's disease and chronic stress, induced an effect similar to what happened in the pro-inflammatory challenge by LPS in primary microglia cultivation (Kronenberg et al. 2017). Thus, we can see how microglia with an inflammatory pattern have a reduction in the functionality of their telomeres, the same pattern found in the aged brain that predisposes to depressive conditions after acute or chronic aggression (DiSabato et al. 2016). These findings lead to the theory that the inflammatory stimulus of the CNS leads to the aging of the microglia through telomeric dysfunction, and these factors together seem to predispose individuals to MDD.

In caregivers of patients with Alzheimer's disease, higher levels of depressive symptoms, shorter telomeres, signs of immunological senescence, and increased levels of TNF- α were observed in peripheral blood mononuclear cells. Evidence holds up that smaller telomeres from chronic stress are involved with accelerated aging of the immune system, dysregulation of inflammation, and, consequently, with MDD (Damjanovic et al. 2007; Verhoeven et al. 2014a).

The time of exposure of patients to depressive symptoms seems to be a relevant factor involved in the increase of inflammation and shortening of telomeres. Wolkowitz et al. (2011) observed that in individuals exposed to depressive symptoms for more than 9.2 years during their lifetime, the length of the telomeres was 281 base pairs shorter than in the control group. In the same studies, the researchers found an inverse correlation

between IL-6 levels and telomere lengths in patients' peripheral blood.

In a study carried out in young adults, a positive correlation between the increase in C-reactive protein (CRP) and a decrease in telomere length was found in blood samples from MDD women. However, in men, telomere reduction was associated with a significant increase in CRP, without a correlation with depression. Interestingly, in women without depression, increased CRP was not associated with telomeric reduction (Shin et al. 2019).

The interrelationship between inflammation, telomeric reduction, and MDD also seems to be inherent in the lack of some mineral micronutrients. Serum selenium levels outside the range of 82–85 µg/L, both up and down, were related to an increased risk for depressive symptoms in young adults (Conner et al. 2015). Low plasma selenium concentration was associated with increased IL-6 and CRP (Wang et al. 2018). In a study with 3194 individuals over 45 years old, it was observed that selenium supplementation in the diet provided an increase in telomere length (Shu et al. 2020). In light of the crossing of this information, it is possible to make a relationship between inadequate selenium intake, chronic inflammation, depression, and telomeric reduction. This evidence is important, but further studies are needed, both in humans and in animal protocols, for research purposes interrelating inflammatory biological mechanisms and markers, telomeres size, MDD, and the relationship with selenium levels.

Telomeric attrition has been observed to increase in cell cultures in magnesium-deficient media. The structure and integrity of telomeric chromatin are related to magnesium, since the nuclear layer, a structure in which more than 50% of the telomeres are found, depends on Mg²⁺ to stabilize itself. The reduction of lamina B1 is necessary to drive cellular senescence (Maguire et al. 2018). Mg²⁺ deficiency is related to increased TNF- α , IL-1, IL-6, CRP, fibrinogen, intracellular adhesion molecule-1, cellular-vascular adhesion molecule-1, conditions that seem to underlie inflammation low-grade chronic (Nielsen 2018). Several studies, including both rodents and humans, demonstrate the inverse relationship between serum levels and magnesium intake with MDD (Wang et al. 2018). Some studies have shown that supplementation with Mg²⁺ improve depressive symptoms. Among several relevant mechanisms related to MDD, such as the HPA axis and oxidative stress, Mg²⁺ seems to interfere with inflammatory markers and mechanisms, reducing inflammation (Wang et al. 2018). Crossing these analyzes, we can have perspectives for future studies relating to Mg²⁺, telomeres, inflammation, and MDD.

Telomere, neurotransmission, and MDD

Several genes related to neurotransmission influence individual sensitivity to environmental conditions, thus participating in the regulation of behavior through phenotypic expressions after environmental exposures (Belsky and Pluess 2009). In this context, studies have shown that children with some genotypes related to serotonin (5-HT) and dopamine presented telomeric shortening when exposed to stressful conditions (Brody et al. 2014; Sales et al. 2014).

The interaction of 5-HT with different CNS receptors regulates emotional functions and behavior. The serotonin transporter (SERT) is a membrane component that reuptakes the neurotransmitters released in the synaptic cleft, thus participating in the control of the functions performed by 5-HT (Homberg and Lesch 2011). In mice lacking or with low SERT expression, more expressive responses to stress are observed, including anxiety and depressive-like behaviors and increased ACTH levels (Li 2006), suggesting a positive correlation between the low expression phenotype of SERT and susceptibility for mood disorders development (Fox et al. 2009; Thomason et al. 2010). It is also important to note that the short polymorphic variant (S) of the 5-HTT gene for SERT (5-HTTLPR) is associated with more epigenetic aberrations, less expression of the serotonin transporter, and development of MDD, especially in individuals who suffered adversity early in life (Ignácio et al. 2014).

Although the mechanism by which the low expression of SERT is related to the erosion of telomeres is not clear, it is believed that in these cases the greater emotional sensitivity to environmental stimuli mimics negative adverse psychological conditions that lead to a telomeric reduction (Li et al. 2014). In these studies, the authors observed that in healthy and homozygous individuals for the short variant (SS) of the 5-HTTLPR region, leukocyte telomeres were significantly shorter than in individuals homozygous for the long variant (LL) and in heterozygous individuals (LS). This evidence suggests that telomere shortening may be one of the pathophysiological processes involved in the S 5-HTTLPR variant, which predisposes to behavioral impairments when interacting with adverse conditions throughout life.

Another gene involved in emotional responses to environmental conditions is the one that encodes the oxytocin receptor (OXTR). In cases where individuals have the polymorphic variant in the rs53576 region with the G allele, they are more sensitive to the social environment (Olff et al. 2013), including negative conditions, in which

they express greater emotional dysfunction (Bradley et al. 2013) and anti-social behavior (Smearman et al. 2015). A study demonstrated that homozygous GG adolescents in the rs53576 region, when they lack emotional support and they have parental conflicts, greater telomeric shortening are found. Also, an increase in the scores of chronic rabbies for more than five years and an association of this behavior with shorter telomeres was observed in these individuals (Smearman et al. 2016).

A meta-analysis found that variants of the monoamine oxidase A (MAOA) gene, which controls the levels of the neurotransmitters norepinephrine, dopamine, and 5-HT at synaptic termination (Duncan et al. 2012), are strongly related to the psychological stress and the development of psychiatric disorders (Kim-Cohen et al. 2006). Some researchers have observed an association between the polymorphic variant of MAOA, MAOA-uVNTR, and shorter telomeres in healthy young individuals (Speck-Hernández et al. 2015). Of note, the MAOA-uVNTR variant is associated with greater expression of the MAOA enzyme and, therefore, to a greater reduction in synaptic monoamine levels. It is important to note that this variant is related to an increase in suicide attempts in male MDD individuals (Lung et al. 2011).

Telomere, neural signaling, neural plasticity, and MDD

Neuronal plasticity is linked to several neurotrophic factors, such as the BDNF (Germann 2020), neurotrophin-3 (NT-3), neurotrophin-4/5 (NT-4/5), the nerve growth factor (NGF) (Duman 2004), and the insulin-like growth factor 1 (IGF-1) (Levada and Troyan 2017). Neurotrophic factors are important in the regulation of neurogenesis and neurodegeneration, in addition to interfering in synaptic transmission, in modulating the activity of different neurons, and in the formation of memory (Szczęsny et al. 2013).

The function of BDNF is mediated by the interaction with the TrkB receptor, belonging to the tyrosine kinase receptor (Trk) family, and with the pan75 neurotrophin receptor (Lee and Kim 2010). The neurotrophic hypothesis of MDD reports a decrease in BDNF levels, which is directly related to neurogenesis and the number of dendritic buds in a synapse (Duman 2002). Numerous studies provide evidence of a reduction in BDNF expression in brain regions involved with MDD, such as the hippocampus and prefrontal cortex, in addition to a differential regulation between structures involved in psychiatric disorders (Amidfar et al. 2017).

In the autopsy of untreated depressive patients, lower levels of BDNF were found in the hippocampus and the bloodstream (Li et al. 2008). From the treatment with antidepressants, there was an increase in serum BDNF levels (Groves 2007) and an increase in neurogenesis in the hippocampus, the latter related to changes in behavior observed after pharmacological treatment (Warner-Schmidt and Duman 2006). Still, BDNF has antioxidant and anti-inflammatory effects, which contribute to its neuroprotective functions (Joosten and Houweling 2004), in addition to increasing cell survival in the presence of telomerase activity (Fu et al. 2002).

In an *in vitro* study with hippocampal cells, it was found that BDNF increases telomerase activity through activation of Akt kinase (Fu et al. 2002). This regulation is related to the increase in resistance to apoptosis (Zhang et al. 2007). Some studies hold up that some portions of telomerase may be related to functions beyond the maintenance of telomeres (Bradshaw et al. 2005). For example, TRF2 has been correlated with chromatin remodeling, in the regulation of genes that control the differentiation of neurons, and even with gene regions that encode factors involved in neurogenesis (Zhang et al. 2007).

IGF-1 is a neurotrophic protein with pleiotropic effects, involved in cell growth and proliferation, as well as in the control of apoptosis (Levada and Troyan 2020). Together with the other neurotrophic factors, its expression is related to changes in the hippocampus, prefrontal cortex, and amygdala of depressed individuals (Mitschelen et al. 2011). Besides, the scientific literature provides evidence that IGF-1 can act as an antidepressant, anti-inflammatory, and neuroprotective factor (Szczęsny et al. 2013).

The decrease in IGF-1 levels is related to neuronal loss, inhibition of stem cell differentiation into neurons, and reduction of gray matter in the hippocampus, in the striated nucleus (Lichtenwalner et al. 2001), and the dentate gyrus (Morel et al. 2017). Furthermore, it was observed that prenatal stress alters IGF-1 signaling and decreases the phosphorylation of IGF-1 receptors in the prefrontal cortex, hippocampus, and olfactory bulb, favoring depressive behaviors in adult mice (Basta-Kaim et al. 2014). In animal models of rodents males exposed to social isolation and with depressive-like behaviors, low levels of the IGF-1 receptor and 5-HT were also found (Das et al. 2015).

IGF-1 increases BDNF synthesis and activity (Park et al. 2011), leads to an increase in the modulation of serotonergic neurotransmission (Hoshaw et al. 2008), and promotes an increase in telomerase activity (Fu et al. 2002). IGF-1 also acts to decrease neuroinflammation, which has been suggested as a relevant feature in the

pathophysiology of MDD (Jeon and Kim 2018). The reduction of neuroinflammation seems to occur, at least in part, through the suppression of inflammatory cytokines, such as IL-1 β , TNF- α , among others (Puzik et al. 2012), and increased expression of anti-inflammatory molecules, such as IL-10 and BDNF (Park et al. 2011), favoring the M2 phenotype of microglia, related to repair and regeneration (Labandeira-Garcia et al. 2017). However, apparently paradoxical, in a large group of individuals with an acute depressive episode, a considerable increase in peripheral IGF-1 levels was observed, which after treatment with antidepressants reduced systemically (Kopczak et al. 2015). Some authors suggest that the increase in levels may be justified by the decreased sensitivity of IGF-1 receptors in the brain due to neuroinflammation (Tu et al. 2016).

Some evidence suggests a positive correlation between lower levels of IGF-1 signaling and longevity (Arai et al. 2001; Bonafè et al. 2003). A meta-analysis also brought evidence of a correlation between the decrease in circulating levels of IGF-1 and an improvement in other biomarkers of aging (Lettieri-Barbato et al. 2016). Other studies bring inverse results, showing that an increase in IGF-1 is related to lower mortality (Brugts et al. 2008). Regarding the length of telomeres, studies have shown that the increase in IGF-1 levels is related to an increase in telomeric length in leukocytes of elderly individuals and also in younger people (Barbieri et al. 2009; Yeap et al. 2020). Considering the conflicting results in the scientific literature regarding the function of IGF-1, longevity, and MDD, further studies are needed, both in humans and animals, which can analyze depressive behaviors, other biological mechanisms related to the disorder, and telomere length.

Some reports have shown that in untreated depressive individuals, there is a decrease in serum levels of NGF, which can result from high levels of cortisol (Martino et al. 2013). Its levels are altered in the prefrontal cortex, amygdala, and hippocampus (Hellweg et al. 2002). The reduction in NGF levels may be related to the high production of inflammatory cytokines in response to stress.

Although studies on the relationship of NGF and telomere size are lacking, a recent study has shown that treating mice or primary hippocampal cell cultures with a substance that increases TERT expression also elevates NGF and BDNF expression, in addition to other markers and growth pathways, neural differentiation, and neurogenesis. The authors suggest that these neuronal functions of TERT are non-canonical functions of the protein. It is likely that the mechanisms of TERT, apparently beneficial on neuronal plasticity, involve increased expression of factors and transcriptional pathways, as

well as anti-apoptotic, antioxidant and protective mitochondrial functions (Baruch-Eliyahu et al. 2019).

Telomere and antidepressants

Considering that traditional treatments for MDD include the use of antidepressant drugs, some studies seek to analyze the effect of using these drugs on molecular pathways. Studies on amitriptyline, a tricyclic class (TCA) antidepressant, have observed DNA damage and oxidative stress in *in vitro* studies using dopaminergic cell lines. In the same series of studies, the researchers showed the loss of neurons of the pars compacta of the substantia nigra in mice subchronically systemically treated with amitriptyline. Also, animals expressed abnormal motor behaviors, similar to find in Parkinson's disease (Lee et al. 2015). Other *in vitro* studies with hippocampal cell lines have found that amitriptyline causes an oxidative imbalance, genomic instability, and changes in shelterin complex proteins. These changes seem inherent, at least partially, to the loss of function of Klotho, a protein that exerts a neuroprotective effect, based on the oxidative balance, among other mechanisms (Mytych et al. 2019). On the other hand, another *in vitro* study using hippocampal cell lineage revealed that the joint treatment of TCA class antidepressants, amitriptyline, imipramine, and fluoxetine, a selective serotonin reuptake inhibitor (SSRI), increases the expression of components of shelterin, TRF1, and TRF2, making it possible to decrease damage to telomeres and increase cell proliferation, a result that was not observed after the isolated administration of one of these drugs (Solek et al. 2019). However, it is interesting to note that fluoxetine treatment in mice subjected to chronic stress reversed depressive-like behavior, TERT reduction, and telomerase activity reduction (Zhou et al. 2011).

Some findings have observed an increase in telomerase activity in PBMCs from untreated MDD patients compared to healthy controls. This high telomerase activity was noticed mainly in patients who did not show a good response to treatment with sertraline, an SSRI, for eight weeks. In the same study, the researchers observed that patients, who had low enzyme activity before treatment and who had increased telomerase actions during treatment with antidepressants, had a better antidepressant response from pharmacological therapy (Wolkowitz et al. 2012). Although still speculative, the authors argue that the high baseline telomerase activity in MDD individuals may reflect a compensatory biological mechanism for the reduction of telomeres and other physiological damage in these patients. A study in patients with bipolar disorder

(BD) showed that telomerase activity in leukocytes was negatively associated with improvement of the depressive symptoms after lithium treatment. A suggestion from the authors' discussions is that the reduction in telomerase activity may represent a reduction in cell stress with the clinical improvement established by the treatment (Soeiro-de-Souza et al. 2014). Other studies provide evidence that long-term treatment with lithium in BD patients is correlated with longer telomeric length in leukocytes and also that the better antidepressant therapeutic response to lithium has been associated with longer telomeres (Martinsson et al. 2013). In a study with a genetic animal model of depressive behavior, the authors observed that the treatment with lithium reversed the reduction in TERT expression, as well as in the activity of telomerase in the hippocampus of animals with depressive behavior. In the same report, there was an increase in the expression of β -catenin, which is known to increase the expression of TERT (Wei et al. 2015). It is also suggested that among other complex actions of lithium are its functions in mitochondrial activities, with a reduction in the effects of oxidative stress on the size of telomeres (Squassina et al. 2017).

Telomere, MDD, and other therapeutic strategies

Going beyond pharmacological treatment, some strategies, such as nutritional supplementation, physical exercises, and other practices that reduce stress and some biological mechanisms inherent to stress and cell aging, have been investigated (Figure 3). In this sense, a study through a systematic review highlights that supplementation of nutrients and healthy lifestyles, such as meditation and regular physical activity, among others, are factors involved in improving telomerase activity and function (Deng et al. 2016).

Some researchers have shown that an increase in the levels of the polyunsaturated fatty acid (PUFA) omega 3 (*n*-3) or a reduction in the rate of *n*-6: *n*-3 PUFA have been associated with longer telomeres in leukocytes and reduction of inflammatory and oxidative markers. The same authors also suggest that supplementation of *n*-3 PUFA reduces telomere attrition and improves mood in more depressed individuals (Kiecolt-Glaser et al. 2013). Other studies showed that a larger diet intake of *n*-6 PUFAs was associated with shorter telomeres (Cassidy et al. 2010).

It is important to note that the *n*-3 and MDD association is shown in other studies, which suggest a benefit of *n*-3 PUFA in reducing depression (Appleton et al. 2010). A

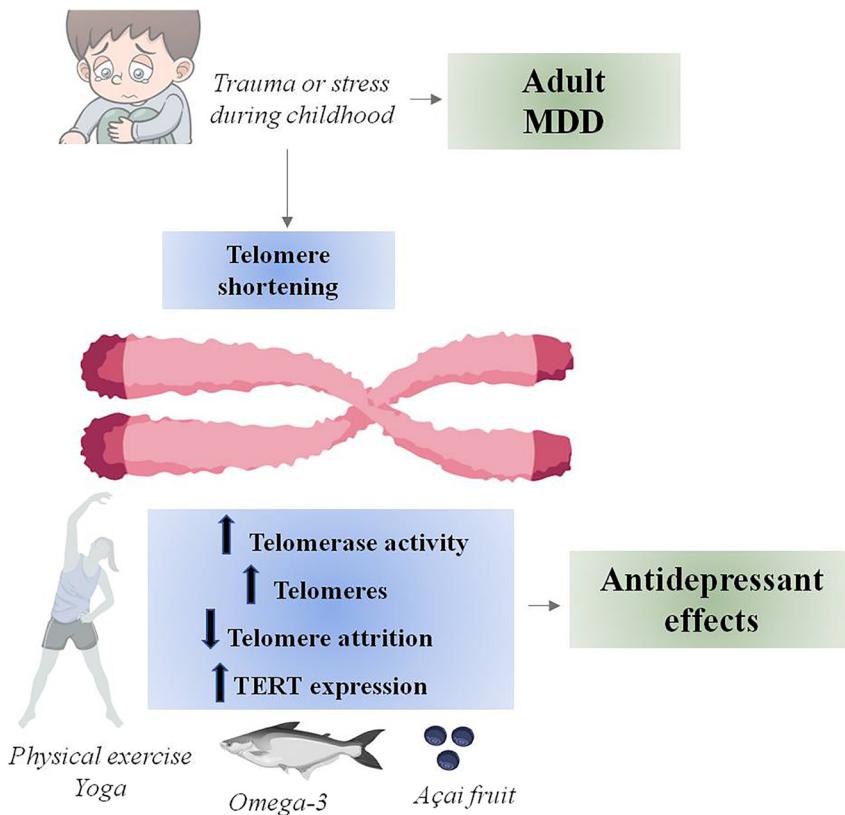


Figure 3: Negative and positive environmental factors involved in telomeres modulation.

Trauma or childhood stress is associated with telomere shortening. Also, when individuals are exposed to early life traumas and adult stress events, telomeres changes are reported. These effects are correlated with major depressive disorder (MDD) development. In another way, a healthy life-based in practical exercise and healthy food consumption improves telomeres and depressive symptoms. For example, yoga practice increases telomeres and telomerase activity; omega-3 increases telomeres and decreases telomere attrition; açaí fruit juice increases TERT expression and reduces depressive behavior in experimental studies. Images were extracted from BioRender app and pinclipart.

meta-analysis also found that *n*-3 supplements were effective in helping to treat MDD. The authors argue that *n*-3 supplementation reduces some inflammatory cytokines such as IL-1 β , IL-2, IL-6, TNF- α , and IFN- α . Besides, *n*-3 seems to be related to increased serotonergic and dopaminergic neurotransmission in the CNS (Liao et al. 2019).

A recent study highlights that researches with specificities for the inclusion of *n*-3 in the clinical practice of MDD individuals are still lacking. However, what became more evident was that *n*-3 supplementation seems to favor MDD individuals with higher levels of inflammatory markers (Guu et al. 2020). Considering that inflammation is one of the biological phenomena strongly associated with the shortening of telomeres and MDD, new studies become relevant, in the sense of detailing the biological processes involved, as well as the interaction between these mechanisms and the specific individuals that can be benefited.

In a study with mice, the use of açaí juice (*Euterpe oleracea*) reduced depressive-like behaviors, prevented

lipid oxidation, and increased the expression of TERT mRNA in the hippocampus, striatum, and prefrontal cortex, regions directly related with the depressive condition. There was also a protective effect of hippocampal neurons against neuronal loss seen in aging and depressive conditions (Souza-Monteiro et al. 2019).

The medicinal species *Centella asiatica* is known to have neuroprotective properties. In addition to beneficial cognitive effects, few studies have also found antidepressant effects (Chandrika and Prasad Kumarab 2015; Lokanathan et al. 2016). Triterpenes are the most abundant compounds in the plant (Chandrika and Prasad Kumarab 2015). Interestingly, a recent *in vitro* study found that *C. asiatica* triterpenes composition significantly increased telomerase activity (Tsoukalas et al. 2019). Thus, more *in vitro* and *in vivo* studies are essential to unveil the effects on telomeric function and possible correlation with depressive behaviors.

In addition, the practice of yoga for 12 weeks in patients with MDD reduced depression scores and

promoted an increase in telomerase activity in peripheral blood cells, which paralleled a reduction in oxidative stress, cortisol, and inflammatory markers, and an increase in BDNF levels. These results suggest that the practice of yoga reduces depressive symptoms, at least in part, through the improvement of biological mechanisms related to cell stress and neuronal plasticity (Tolahanase et al. 2018).

Studies in humans and animal protocols provide several pieces of evidence about the benefits of physical exercises in reducing depressive symptoms and other psychiatric disorders. The reduction in depressive symptoms is related, at least in part, to the effects of physical exercise on oxidative, inflammatory processes and the HPA axis, possibly interfering in biological processes inherent to resistance to glucocorticoids and low-grade chronic inflammation (for a review, see: Ignácio et al. 2019). These biological phenomena are related to the function of telomeres, as already described in the previous topics. Therefore, it is important to consider studies that have analyzed the effects of physical exercise on telomere size and, more specifically, the relationship between physical exercise, MDD, and telomere size. Accordingly, the relationship between physical exercise and increased telomeres was highlighted in a systematic review, which also suggests that the increase in telomeres from physical activity is related to the well-being and longevity of individuals (Arsenis et al. 2017). In a large sample of twin subjects, it was observed that physical activity and healthier lifestyle habits with higher leisure activity were associated to significantly longer telomeres. In the same series of studies, the authors found that in a smaller group of twins, whose pairs were discordant in terms of activity levels, the most active individuals had significantly larger sizes of telomeres (Cherkas 2008). Finally, a study with mice showed that spontaneous and frequent physical activity, both in the short and long term, increased the activity of cardiac telomerase and the expression of TRF2 and TERT proteins and, in parallel, reduced the expression of pro-apoptotic markers, as well as cardiomyopathy induced in animals (Werner et al. 2008).

When correlated with chronic stress, a characteristic of depressive patients, physical activity at a frequency of three times a week, promoted a decrease in telomere shortening in peripheral blood of postmenopausal women, submitted to chronic stress (Puterman et al. 2010). It should also be noted that in MDD patients, in addition to stress control, there is the influence of the so-called multisystem resilience, which was characterized by engaging in regular physical activity, social support connections, and quality of sleep. Higher multisystem resilience in MDD individuals

was positively correlated with longer telomeres length (Puterman et al. 2013).

Discussion and future directions

The studies summarized in this review provide several pieces of evidence that suggest that MDD is involved with telomeric dysfunction, as seen both in terms of telomere length and in enzymatic mechanisms responsible for maintaining the stability of telomeres. Although some studies bring conflicting results, most of the findings point to an association between MDD and reduction in the size of telomeres, predominantly in peripheral blood cells of patients.

Interestingly, the most relevant mechanisms associated with MDD are also the main ones involved in the shortening of telomeres and in the impairment of telomerase enzyme activity. The main pathophysiological processes associated with MDD and impaired telomere function, such as changes in the HPA axis, oxidative stress, and neuroinflammation, as well as some epigenetic changes, are also pivotal in the regulation of biological senescence (Figure 1). Of note, changes in the HPA axis, oxidative stress, and neuroinflammation are conditions associated with the severity of MDD. Stress conditions in early life are also involved in chronic diseases that are more prevalent in old age.

It is important to note that epigenetic aging, which also appears to be related to telomere dysfunction, is a biological event strongly associated with severe depression and childhood trauma in MDD individuals. Epigenetic aging has been evidenced both in the peripheral blood of patients and in the postmortem brain tissue of MDD individuals. Also, it is one of the phenomena related to biological senescence (Han et al. 2018). Similarly, some studies show that MDD is a syndrome of premature age, which can be accompanied by impaired physical conditions and premature brain aging (Muneer and Minhas 2019). Consistent with these observations and suggestions, some authors point out that the shortening of telomeres is characterized as an indicator of biological or cellular age, rather than chronological age, considering that it is inherent to several factors which may be present at the early and throughout life and predispose to chronic diseases or psychiatric disorders (Verhoeven et al. 2016). Other researchers point out that the relationship between the history of MDD and molecular damage related to neuronal plasticity, and also associated with advancing age, are factors that support the hypothesis that the disorder is characterized as a premature aging syndrome (Nunes et al. 2018).

Although current studies cannot yet point to a causal relationship between telomere shortening, associated physiological mechanisms, and MDD, an important aspect to consider is that stress and the chronicity of stressful situations can lead to a vicious cycle in which each condition associated with both senescence and MDD may increase the shortening of telomeres, which, in turn, may amplify the network of underlying pathophysiological mechanisms (Verhoeven et al. 2014b).

From another angle, although with limited scientific support of only a few studies, the literature already has some evidence that the shortening of telomeres can be reversed or reduced by some pharmacological therapies and, mainly, improving the quality of life of MDD individuals, through other therapies, nutritional supplementation, and physical exercises, among others. The exact mechanisms inherent to the conditions that improve the structure and function of telomeres are not still known, but some studies in mice have shown that restoring telomerase function can be an important factor that can reduce damage, for example, neurodegeneration (Jaskelioff et al. 2011). Finally, some data in the literature indicate that the physiological mechanisms involved in chronic stress, telomere dysfunction, MDD, and senescence are mechanisms that can be reversed or mitigated based on pharmacological or other therapeutic strategies that improve quality of life.

As mentioned in some topics, it is crucial to highlight the conflicting results in the scientific literature. Divergent results can be attributed to the few studies, different protocols, and lack of repetition of protocols in different contexts, especially considering the telomeric function in the CNS. These observations point to the need for more studies that can compare different contexts and protocols to evolve in knowledge about the contexts and interacting biological mechanisms and, consequently, in the elucidation of the function of telomeres in the CNS and MDD.

All in all, these data indicate that further studies, considering the mechanisms triggered by therapeutic strategies already known, in addition to translational research with direct interventions in the functional mechanisms of telomeres, are relevant and should be considered for future investigations.

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