UNIVERSIDADE FEDERAL DE PELOTAS
Centro de Ciências Químicas, Farmacêuticas e de Alimentos
Programa de Pós-Graduação em Bioquímica e Bioprospecção

TESE

Potencial da 7-cloro-4-(fenilselanil)quinolina como uma estratégia terapêutica inovadora para a depressão associada ao comprometimento de memória

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Pelotas, 2023

Universidade Federal de Pelotas / Sistema de Bibliotecas
Catalogação na Publicação

O48p Oliveira, Renata Leivas de

Potencial da 7-cloro-4-(fenilselanil)quinolina como uma estratégia terapêutica inovadora para a depressão associada ao comprometimento de memória / Renata Leivas de Oliveira ; Cristiane Luchese, orientadora ; Ethel Antunes Wilhelm, coorientadora. — Pelotas, 2023.

144 f. : il.

Tese (Doutorado) — Bioquímica e Bioprospecção, Centro de Ciências Químicas, Farmacêuticas e de Alimentos, Universidade Federal de Pelotas, 2023.

1. Alzheimer. 2. Eixo hipotálamo-pituitária-adrenal. 3. Estresse oxidativo. 4. Neuroinflamação. 5. Neuroplasticidade. I. Luchese, Cristiane, orient. II. Wilhelm, Ethel Antunes, coorient. III. Título.

CDD : 616.8983

Elaborada por Ubirajara Buddin Cruz CRB: 10/901

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Tese apresentada ao Programa de Pós-Graduação em Bioquímica e Bioprospecção do Centro de Ciências Químicas, Farmacêuticas e de Alimentos da Universidade Federal de Pelotas, como requisito parcial à obtenção do título de Doutora em Ciências com ênfase em Bioquímica e Bioprospecção.

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Pelotas, 2023

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Tese aprovada, como requisito parcial, para obtenção do grau de Doutora em Ciências com ênfase em Bioquímica e Bioprospecção, no Programa de Pós-Graduação em Bioquímica e Bioprospecção, Centro de Ciências Químicas, Farmacêuticas e de Alimentos, Universidade Federal de Pelotas.

Data da Defesa: 26/05/2023

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Agradecimentos

Em especial, sou grata a Deus por me guiar, me iluminar, me dar tranquilidade e colocar pessoas tão especiais ao meu lado para que eu pudesse seguir em frente com os meus objetivos e não desanimar com as dificuldades.

A meus padrinhos, Fernanda e Edemilton e ao meu irmão Pedro, meu infinito agradecimento por sempre acreditaram em minha capacidade e nunca me deixarem desanimar. Vocês sempre souberam me dizer as palavras certas nos momentos em que eu precisava de motivação. Obrigada pelo amor incondicional.

Ao Guilherme, por ser tão importante na minha vida e por estar sempre a meu lado me incentivando. Devido a seu companheirismo, amizade, compreensão, apoio e amor, esta etapa pode ser concluída com maior tranquilidade. Obrigada por estar sempre ao meu lado compartilhando nossos sonhos e correndo atrás dos nossos objetivos.

A minha segunda família, Rosi, Beto e Alice, por todo incentivo, amor, cuidado e por não medirem esforços para nos ajudar. Vocês são grandes exemplos na minha vida.

A minha orientadora, Cristiane e coorientadora, Ethel, pelo suporte e estímulo constante, me ensinando e me guiando durante toda a minha formação acadêmica. Obrigada por todo carinho e amizade. Vocês foram essenciais na minha trajetória.

A todos os meus colegas do LaFarBio, que de alguma forma contribuíram para a realização deste trabalho. Em especial a Mikaela (minha amiga de longa data), a Ane, a Karline, a Jaini e a Caren aos quais eu considero mais que colegas. Obrigada por dividir comigo as angústias e as alegrias durante um longo tempo. Foi bom poder dividir tantos momentos e contar com vocês.

A Universidade Federal de Pelotas (UFPel) e ao Programa de Pós-graduação em Bioquímica e Bioprospecção por me proporcionar a oportunidade de realizar este estudo, e à CAPES pela concessão da bolsa. A todos os professores do Programa de Pós-Graduação em Bioquímica e Bioprospecção. Ao Biotério Central da UFPel e aos funcionários pelo cuidado e comprometimento com os animais disponibilizados.

Enfim agradeço, a todos que de uma forma ou de outra colaboraram para a realização deste trabalho.

Resumo

DE OLIVEIRA, Renata Leivas. **Potencial da 7-cloro-4-(fenilselanil)quinolina como uma estratégia terapêutica inovadora para a depressão associada ao comprometimento de memória.** Orientadora: Cristiane Luchese. 2023. 144f. Tese (Doutorado em Ciências com ênfase em Bioquímica e Bioprospecção) - Centro de Ciências Químicas, Farmacêuticas e de Alimentos, Universidade Federal de Pelotas, Pelotas, 2023.

Embora o comprometimento da memória seja o principal sintoma de pacientes com doença de Alzheimer (DA), a depressão é um transtorno neuropsiquiátrico comum nessa doença. Atualmente, não existe um único tratamento capaz de reduzir os sintomas dessas doenças quando associadas. Portanto, a busca por uma nova alternativa terapêutica capaz de amenizar a comorbidade em questão é de extrema importância. A 7-cloro-4-(fenilselanil)quinolina (4-PSQ), tem se destacado como um alvo promissor para o tratamento de transtornos que afetam o sistema nervoso central. Assim, o objetivo principal desta tese é investigar o possível efeito farmacológico da 4-PSQ na comorbidade depressão e DA em dois modelos experimentais em camundongos machos, bem como os mecanismos envolvidos no efeito desta molécula. No primeiro estudo foi avaliado a atividade farmacológica da 4-PSQ (10 mg/kg, dose única) no comportamento do tipo-depressivo associado ao comprometimento de memória induzido por estresse de restrição agudo (ARS, do inglês *acute restraint stress*). Posteriormente, levando em consideração que o peptídeo β-amiloide (βa) é uma marca neuropatológica da DA e é capaz de induzir tanto comprometimento de memória como depressão, no segundo estudo avaliou-se o tratamento com 4-PSQ (1mg/kg) durante 7 dias, no comportamento do tipo-depressivo associado ao comprometimento da memória em um modelo de DA induzido pelo peptídeo βa (fragmento 25-35). O efeito do tipo-antidepressivo da 4-PSQ foi confirmado por meio da sua avaliação no teste da suspensão da cauda, teste do nado forçado e teste da borrifagem de sacarose em ambos os protocolos experimentais. Adicionalmente, a 4-PSQ demonstrou efeito contra o comprometimento de memória através dos testes do labirinto em Y, da esquiva inibitória e teste do reconhecimento de objetos. Ao final dos protocolos

experimentais, o córtex pré-frontal e o hipocampo dos camundongos foram removidos para determinar o efeito da 4-PSQ em modular o estresse oxidativo, através da avaliação dos níveis de espécies reativas, peroxidação lipídica e a atividade das enzimas antioxidantes (superóxido dismutase, glutationa peroxidase e glutationa redutase). Adicionalmente, a 4-PSQ foi capaz de modular o sistema colinérgico por meio da redução da atividade da enzima acetilcolinesterase. Além disso, a 4-PSQ reestabeleceu os níveis do fator nuclear kappa B (NF-κB, do inglês *nuclear factor kappa B*), fator de necrose tumoral alfa (TNF-α, do inglês *tumor necrosis factor alpha*), interleucina (IL)1β, IL-6, IL-18, IL-33, proteína ácida fibrilar glial (GFPA, do inglês *Glial Fibrillary Acidic Protein*), fosfatidilinositol-3-quinase (PI3K, do inglês *phosphatidylinositol-3-kinase*) e proteína quinase B (PKB/AKT, do inglês *protein kinase B*), demonstrando a sua capacidade de modular a neuroinflamação, neurogênese e a neuroplasticidade. Ademais, a 4-PSQ foi capaz de modular o sistema monoaminérgico e as alterações no eixo hipotálamo-pituitária-adrenal (HPA), via reestabelecimento das isoformas da enzima monoamino oxidase (MAO)-A e B e redução dos níveis plasmáticos de corticosterona, respectivamente. Estima-se que os resultados desta pesquisa possam contribuir para um melhor esclarecimento dos mecanismos pelo qual a 4-PSQ exerce seus efeitos em diferentes protocolos experimentais e assim torna-se uma alternativa terapêutica multialvo para o tratamento da comorbidade depressão e DA.

Palavras-chave: Alzheimer. Eixo hipotálamo-pituitária-adrenal. Estresse oxidativo. Neuroinflamação. Neuroplasticidade.

Abstract

DE OLIVEIRA, Renata Leivas. **Potential of 7-chloro-4-(phenylselanyl)quinoline as an innovative therapeutic strategy for depression associated with memory impairment.** Advisor: Cristiane Luchese. 2023. 144f. Thesis (Doctorate in Science with an emphasis on Biochemistry and Bioprospecting) - Center for Chemical, Pharmaceutical and Food Sciences, Federal University of Pelotas, Pelotas, 2023.

Although memory impairment is the main symptom of patients with Alzheimer's disease (AD), depression is a common neuropsychiatric disorder in this disease. Currently, there is no single treatment capable of reducing the symptoms of these diseases when associated. Therefore, the search for a new therapeutic alternative capable of alleviating the comorbidity in question is extremely important. 7-chloro-4-(phenylselanyl)quinoline (4-PSQ) has emerged as a promising target for the treatment of disorders that affect the central nervous system. Thus, the main objective of this thesis is to investigate the possible pharmacological effect of 4-PSQ on the comorbidity of depression and AD in two experimental models in male mice, as well as the mechanisms involved in the effect of this molecule. In the first study, the pharmacological activity of 4-PSQ (10 mg/kg, single dose) was evaluated on depressive-like behavior associated with memory impairment induced by acute restraint stress (ARS). Subsequently, taking into account that the β-Amyloid peptide (Aβ) is a neuropathological hallmark of AD and is capable of inducing both memory impairment and depression, the second study evaluated treatment with 4-PSQ (1mg/kg) for 7 days, on depressive-like behavior associated with memory impairment in a model of AD induced by Aβ peptide (fragment 25-35). The antidepressant-like effect of 4-PSQ was confirmed through its evaluation in the tail suspension test, forced swimming test and splash test in both experimental protocols. Additionally, 4-PSQ demonstrated an effect against memory impairment through the Y-maze test, the inhibitory avoidance test, and the object recognition test. At the end of the experimental protocols, the prefrontal cortex and the hippocampus of the mice were removed to determine the effect of 4-PSQ in modulating oxidative stress, through the evaluation of the levels of reactive species, lipid peroxidation and the activity of antioxidant enzymes (superoxide dismutase, glutathione peroxidase

and glutathione reductase). Additionally, 4-PSQ was able to modulate the cholinergic system by reducing the activity of the acetylcholinesterase enzyme. In addition, 4-PSQ reestablished levels of nuclear factor kappa B (NF-κB), tumor necrosis factor alpha (TNF-α), interleukin (IL) 1β, IL-6, IL-18, IL-33, glial fibrillary acidic protein (GFPA), phosphatidylinositol-3-kinase (PI3K) and protein kinase B (PKB/AKT), demonstrating its ability to modulate neuroinflammation, neurogenesis and neuroplasticity. Furthermore, 4-PSQ was able to modulate the monoaminergic system and alterations in the hypothalamic-pituitary-adrenal (HPA) axis, via the reestablishment of the isoforms of the enzyme monoamine oxidase (MAO)-A and B and reduction of plasmatic levels of corticosterone, respectively. It is estimated that the results of this research may contribute to a better understanding of the mechanisms through which 4-PSQ exerts its effects in different experimental protocols and thus becomes a multi-target therapeutic alternative for the treatment of comorbid depression and AD.

Keywords: Alzheimer. Hypothalamic-pituitary-adrenal axis. Oxidative stress. Neuroinflammation. Neuroplasticity.

Lista de Figuras

Revisão Bibliográfica

Artigo

Manuscrito

Lista de Tabelas

Revisão Bibliográfica

Artigo

Lista de abreviaturas e siglas

4-PSQ	7-cloro-4-(fenilselanil)quinolina
OMS	Organização Mundial da Saúde
SNC	Sistema Nervoso Central
DA	Doença de Alzheimer
ARS	Estresse de restrição agudo -do inglês *acute restraint stress*
MAO	Monoamino Oxidase
5-HT	Serotonina - do inglês *5-hydroxytryptamine*
βa	Beta-amiloide
NF-κB	Fator nuclear kappa B - do inglês *nuclear factor kappa B*
TNF-α	Fator de necrose tumoral-alfa - do inglês *tumor necrosis factor alpha*
NMDA	N-metil D-aspartato
GFPA	Proteína ácida fibrilar glial - do inglês *Glial Fibrillary Acidic Protein*
PI3K	Fosfatidilinositol-3-quinase - do inglês *phosphatidylinositol-3-kinase*
PKB/AKT	Proteína quinase B - do inglês *protein kinase B*
DSM-V	Manual Diagnóstico e Estatística de Transtornos Mentais
PPA	Proteína precursora amiloide
FAD	Dinucleotideo de flavina adenina – do inglês *Flavin Adenine Dinucleotide*
ChAT	Colina acetiltransferase - do inglês *Choline acetyl transferase*
H_2O_2	Peróxido de Hidrogênio
Ach	Acetilcolina
RS	Especies reativas – do inglês *reactive species*
CRH	Hormônio liberador de corticotropina - do inglês *corticotropin-releasing hormone*
ACTH	Hormônio adrenocorticotrópico - do inglês *adrenocorticotropic hormone*
DNA	Ácido desoxirribonucleico do inglês *Deoxyribonucleic acid*
GSSH	Glutationa oxidada
GSH	Glutationa reduzida
GC	Glicocorticoides

O_2	Oxigênio
H_2O	Água
IDO	Indolamina-2,3-dioxigenase
FAD	Cofator dinucleotídeo de flavina e adenina – do inglês *Flavin adenine dinucleotide*
$OH^{\bullet}$	Radical hidroxila
GSK-3βk	Glicogênio sintase quinase 3 beta- do inglês *Glycogen synthase kinase 3 beta*
AChE	Acetilcolinesterase
FDA	*Food and Drug Administration*
ISRN	Inibidor seletivo da recaptação de serotonina e de noradrenalina
ANVISA	Agência nacional de vigilância sanitária
GPx	Glutationa Peroxidase
ADH	Aldeído desidrogenasse
iMAO	Inibidor da Monoamino Oxidase
ISRS	Inibidor seletivo da receptação de serotonina
IL	Interleucina
HPA	Eixo hipotálamo-pituitária-adrenal – do inglês *hypothalamic-pituitary-adrenal*
FST	Teste do nado forçado - do inglês *forced swimming test*
TST	Teste da suspensão da cauda - do inglês *tail suspension test*
SPT	Teste da borrifagem de sacarose - do inglês *splash test*

SUMÁRIO

1. INTRODUÇÃO

A 4-PSQ, quimicamente denominado 7-cloro-4-(fenilselanil)quinolina é uma quinolina funcionalizada com um grupamento organoselênio que tem se destacado devido aos seus efeitos farmacológicos significativos. Anteriormente, em estudos pré-clínicos a 4-PSQ mostrou efeito em distúrbios que afetam o sistema nervoso central (SNC), reduzindo sintomas neuropsiquiátricos, como ansiedade e depressão em diferentes modelos experimentais (PALTIAN et al., 2020; REIS et al., 2017; RODRIGUES et al., 2021), prevenção de comprometimento da memória e déficit cognitivo (BARTH et al., 2019; DUARTE et al., 2017; PINZ et al., 2018), bem como atenuação da comorbidade de doença de Alzheimer (DA), depressão e ansiedade em camundongos (PINZ et al., 2018). Além disso, foi evidenciado o efeito anti-DA da 4-PSQ em camundongos (PINZ et al., 2018), bem como sua ação na melhora da memória de ratos idosos (BARTH et al., 2019).

Há um número crescente de estudos demonstrando os efeitos da 4-PSQ em diferentes modelos animais; e os mecanismos relacionados estes efeitos incluem sua ação antioxidante (DUARTE et al., 2017; LUCHESE et al., 2020; PINZ et al., 2016; VOGT et al., 2018), efeito anti-inflamatório (PINZ et al., 2016; SILVA et al., 2017), bem como sua capacidade de modular os sistemas serotoninérgicos, nitrérgicos, glutamatérgicos e colinérgicos, evidenciando a característica multialvo dessa molécula (BARTH et al., 2019; PALTIAN et al., 2020; PINZ et al., 2016, 2018; REIS et al., 2017; SILVA et al., 2017; VOGT et al., 2018a). Ademais, este composto demonstrou ausência de toxicidade em modelos pré-clínicos (REIS et al., 2017; SALGUEIRO et al., 2017), modulação da plasticidade sináptica através do aumento dos níveis de moléculas de adesão celular neural e níveis de polisialiltransferase em estruturas cerebrais de ratos idosos (BARTH et al., 2019), além de reduzir a captação de glutamato no cérebro de camundongos (REIS et al., 2017). Assim, persistem razões importantes para ampliar o estudo dos efeitos da 4-PSQ principalmente em doenças multifatoriais que afetam o SNC, bem como a relação entre estas doenças.

A depressão é uma doença neuropsiquiátrica que tem como principal característica o humor deprimido persistente e a falta de interesse ou prazer em atividades agradáveis (OTTE et al., 2016) . Segundo a Organização Mundial da Saúde (OMS), cerca de 264 milhões de indivíduos são acometidos por este

transtorno (WORLD HEALTH ORGANIZATION, 2023). Sabe-se que a depressão é um transtorno multifatorial no qual diversas etiologias podem estar envolvidas, porém acredita-se que fatores genéticos e ambientais, como o estresse, são considerados seus maiores determinantes (OTTE et al., 2016). O estresse promove um desequilíbrio na homeostase corporal, resultando em alterações fisiológicas, patológicas e/ou cognitivas que afetam principalmente o SNC (MARIOTTI, 2015).

Paralelamente, a depressão pode se desenvolver como consequência de algumas condições, como na DA (HEUN et al., 2013). Além disso, embora o comprometimento de memória seja um dos principais sintomas em pacientes com DA, a presença de outras complicações neuropsiquiátricas (como depressão) também acomete estes pacientes (GALTS et al., 2019). Nesse contexto, a depressão é considerada uma das comorbidades mais prevalentes em pacientes com DA, sendo responsável por afetar cerca de 50% desses indivíduos, estando ligada a uma alta carga social, médica e econômica (CHI et al., 2014; ROMANO et al., 2015). Em consonância com isso, a alta incidência de depressão em indivíduos com DA está associada a um alto risco de morbidade e mortalidade (STECK; COOPER; ORGETA, 2018).

A DA é uma doença neurodegenerativa, que se caracteriza por disfunção cognitiva e perda neuronal em regiões cerebrais envolvidas na memória e comportamentos emocionais (BLUM; BUÉE, 2019). As principais características neuropatológicas da DA incluem a deposição de peptídeo β-amiloide (βa) em regiões do cérebro, acompanhada pela presença de emaranhados neurofibrilares intracelulares, compostos de proteína *tau* hiperfosforilada (JACK et al., 2018).

Os mecanismos moleculares relacionados à díade depressão e DA não são totalmente compreendidos, no entanto, há evidências que tanto o estresse como a neurotoxicidade do peptídeo βa são capazes de induzir alterações comportamentais e neuroquímicas semelhantes às encontradas em pacientes com esta comorbidade (KLENEROVÁ et al., 2007; NAMEKAWA et al., 2013). Nessas circunstâncias, pesquisas para elucidar os mecanismos envolvidos na depressão e no comprometimento de memória/DA têm despertado o interesse para novos estudos. Diante disso, foi demonstrado que em pacientes com depressão e DA ocorre uma desregulação do eixo hipotálamo-hipófise-adrenal

(HPA), promovendo aumento dos níveis de glicocorticoides, como o cortisol (SIERKSMA et al., 2010). Além disso, outros mecanismos fisiopatológicos são comuns na depressão e na DA, incluindo anormalidades no metabolismo de neurotransmissores, estresse oxidativo, neurodegeneração, disfunção da função neuronal, neuroinflamação e alterações na neurogênese e neuroplasticidade (HUANG et al., 2019; MAES et al., 2011; SELKOE, 2005; SELKOE; SCHENK, 2003). Ainda, foi demonstrado que o prejuízo na neurotransmissão colinérgica é um dos mecanismos que contribui para a disfunção cognitiva na DA associada à depressão (SELKOE; SCHENK, 2003).

Adicionalmente, algumas moléculas de sinalização celular estão envolvidas na progressão da depressão associada a DA. Dentre elas, destaca-se a via da fosfatidilinositol-3-quinase (PI3K, do inglês *phosphatidylinositol-3-kinase*) e proteína quinase B (PKB/AKT, do inglês *protein kinase B*), na qual, quando ativada, é responsável por regular a sobrevivência neuronal e a neuroplasticidade, funções importantes para a comorbidade em questão (LI et al., 2015). Paralelamente, o fator nuclear kappa B (NF-κB, do inglês *nuclear factor kappa B*) pode ser ativado diante diferentes estímulos, como respostas inflamatórias e de estresse, regulando assim a expressão de citocinas e marcadores inflamatórias (OECKINGHAUS; GHOSH, 2009). Portanto, é de grande relevância avaliar esses mecanismos, visto que podem atuar como alvos potenciais no desenvolvimento de um tratamento para esta comorbidade.

Dada a etiologia multifatorial da comorbidade, depressão e DA, e ainda, o fato de que os medicamentos combinados para esta comorbidade causam diferentes efeitos adversos, moléculas multifuncionais com duas ou mais atividades biológicas complementares podem representar um importante avanço para o tratamento dessas doenças. Diante disso, a molécula 4-PSQ surge como um agente promissor para o tratamento de doenças que afetam o SNC, incluindo DA e depressão (PINZ et al., 2016; RODRIGUES et al., 2021) . Assim, persistem razões importantes para estender o estudo do efeito da 4-PSQ. Diante disso, o objetivo principal da seguinte tese foi determinar a atividade farmacológica da 4-PSQ na comorbidade, depressão e comprometimento de memória/DA, bem como os mecanismos que medeiam os efeitos da 4-PSQ em diferentes modelos experimentais em camundongos.

2. REFERENCIAL TEÓRICO

2.1 Depressão

A depressão é considerada um transtorno psiquiátrico incapacitante, associado frequentemente à alta morbimortalidade em todo o mundo, sendo responsável por 30% da carga global de doenças (WORLD HEALTH ORGANIZATION, 2023). Segundo a American Psychiatry Association, (2022) a depressão afeta uma em cada seis pessoas (16,6%) em algum momento da sua vida. Ainda, os sintomas de depressão podem variar de leves a graves e podem incluir alterações somáticas e cognitivas, estando associada com um alto índice de suicídio (MCKENNA et al., 2005).

Este transtorno caracteriza-se por alterações psicológicas, comportamentais e fisiológicas que afetam negativamente a qualidade de vida do seu portador (WORLD HEALTH ORGANIZATION, 2023). Para abordagens clínicas, os sintomas depressivos são diagnosticados por meio de critérios descritos no Manual Diagnóstico e Estatística de Transtornos Mentais (DSM-V). Segundo o DSM-V para o diagnóstico de depressão os pacientes devem apresentar por um período mínimo de duas semanas, cinco ou mais dos seguintes sintomas, descritos na tabela 1, sendo que na maior parte do tempo os dois primeiros sintomas devem estar presentes (DSM-V, 2014). A anedonia é definida como a perda do interesse e capacidade de sentir prazer em relação a estímulos prazerosos, sendo considerada um sintoma central e decisivo para diagnosticar indivíduos com depressão (FEIGHNER et al., 1972).

Acredita-se que diversos fatores podem predispor um indivíduo ao desenvolvimento de depressão, como fatores genéticos, psicológicos, bioquímicos e ambientais, bem como as suas interações (AMERICAN PSYCHIATRY ASSOCIATION, 2020). Evidências sugerem que o principal fator ambiental que desempenha um importante papel na etiologia da depressão é o estresse (OTTE et al., 2016). De acordo com isto, o envolvimento do estresse em pacientes depressivos compreende a hiperativação do eixo HPA, desencadeando diversas reações ao organismo que promovem alterações comportamentais e mudanças neuroquímicas associadas a doença (MAYBERG, 2009).

Tabela 1. Critérios para o diagnóstico da depressão segundo o DSM-V.

Critérios diagnósticos de depressão	
1.	**Humor deprimido;**
2.	**Anedonia;**
3.	Alteração no peso;
4.	Distúrbio do sono;
5.	Retardo ou agitação psicomotora;
6.	Fadiga ou perda de energia;
7.	Sentimento de culpa;
8.	Dificuldade de concentração;
9.	Pensamentos recorrentes de morte ou suicídio.

Fonte: Adaptado de DSM-V.

Além disso, o avanço no estudo da neurobiologia da depressão elucidou outros mecanismos implicados em sua fisiopatologia, como a disfunção monoaminérgica, o estresse oxidativo, a neuroinflamação, bem como alterações na neuroplasticidade e neurogênese (LIU et al., 2015; MILLER; HAROON; FELGER, 2017; NESTLER et al., 2002; OTTE et al., 2016). Entretanto, apesar dos avanços na compreensão da neurobiologia da depressão, nenhum mecanismo estabelecido pode explicar todas os aspectos desta doença, mas podem se complementar. Frente a isto, a depressão não deve ser considerada um transtorno único, com apenas uma causa, e sim como uma síndrome heterogênea composta de inúmeros fatores causadores e de fisiopatologias distintas.

2.2 Doença de Alzheimer

A DA é um distúrbio neurodegenerativo, no qual caracteriza-se clinicamente por afetar funções cognitivas, resultando em falha sináptica e destruição neuronal em regiões cerebrais importantes levando ao comprometimento da memória e outras habilidades cognitivas (VILLEMAGNE et al., 2017). De acordo com a World Health Organization (2023), a DA é a forma mais comum de demência e pode contribuir com 60% destes casos. Além disso, este distúrbio é responsável por afetar 50 milhões de pessoas mundialmente

(BROOKMEYER et al., 2018), sendo o Brasil o segundo país com maior prevalência (NICHOLS et al., 2019).

O envelhecimento é considerado um dos fatores de risco que predispõe o desenvolvimento da DA, com a sua prevalência crescendo acentuadamente, estando presente em 3% de indivíduos entre 65 e 74 anos de idade, 17% das pessoas na faixa etária de 75 a 84 anos e 32% na população com idade acima de 85 anos (ASSOCIATION ALZHEIMER'S, 2016, 2020, 2023). Além disso, fatores genéticos e ambientais também estão fortemente associados ao desenvolvimento da DA (DORSZEWSKA et al., 2016). Sendo assim, a DA também é considerada uma doença multifatorial e heterogênea, uma vez que diversos fatores exercem papéis significativos na sua patogênese (SINGH et al., 2013).

A principal característica neuropatológica da DA é o depósito extracelular de placas senis contendo o peptídeo βa em regiões cerebrais, incluindo o córtex cerebral, o hipocampo, o córtex entorrinal e o estriado ventral. As placas senis são formadas através da clivagem proteolítica da proteína precursora amiloide (PPA), que é uma proteína altamente expressa na membrana dos neurônios (DI PAOLO; KIM, 2011). Como pode ser observado na Figura 1, a PPA pode ser clivada por duas vias, via amiloidogênica e via não amiloidogênica. Na via não amiloidogênica, o PPA é clivado por meio da ação da enzima α-secretase resultando apenas em um fragmento solúvel. Quando clivada pelas enzimas β-secretase e γ-secretase na via amiloidogênica, resulta na produção do fragmento de peptídeos beta amiloide (βa), que ao se acumular no espaço extracelular se agregam (formando oligômeros) e posteriormente formam as placas senis, que se depositam nos tecidos neuronais causando neurodegeneração (KARCH; CRUCHAGA; GOATE, 2014).

As placas contendo o peptídeo βa nas regiões cerebrais corticais e límbicas causam efeitos neurotóxicos aos neurônios e parecem induzir disfunções sinápticas e dendríticas, contribuindo para a sintomatologia da DA (SELKOE, 2005). Além disso, a neurotoxicidade do peptídeo βa ocasiona dano aos neurônios por meio da ativação da micróglia e dos astrócitos, resultando em uma resposta neuroinflamatória, estresse oxidativo e dano celular (MEDEIROS et al., 2007).

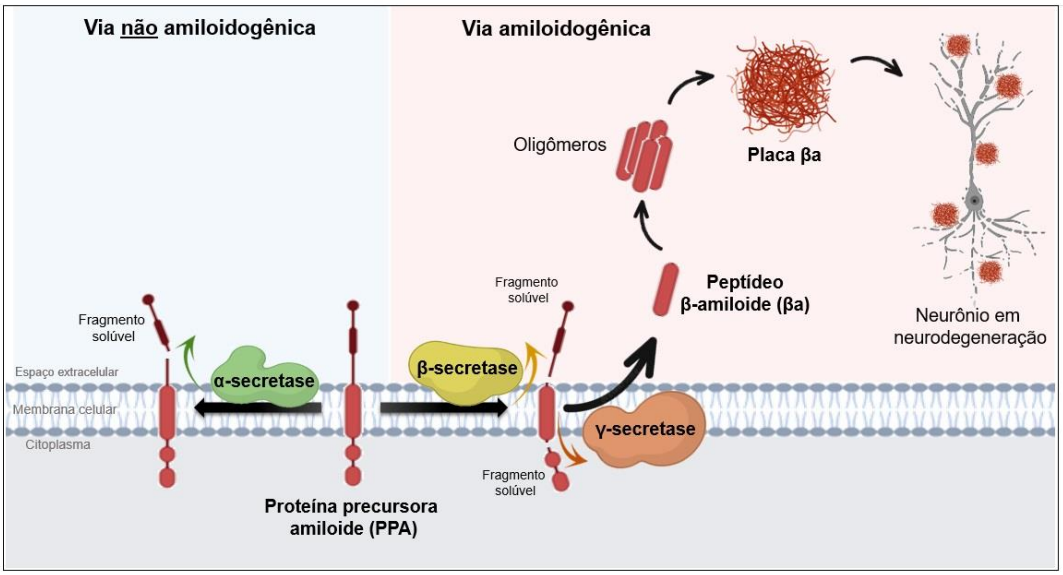

Figura 1. Processamento da PPA através da via não amiloidogênica e amiloidogênica, com formação de placas amiloides que se depositam nos neurônios, causando neurodegeneração. A plataforma BioRender foi utilizada para criação desta figura (https://biorender.com). Autoria própria.

Evidências sugerem a existência de outra marca neuropatológica na DA, no qual inclui a presença de emaranhados neurofibrilares intracelulares formados pela proteína *tau* hiperfosforilada (KENT; SPIRES-JONES; DURRANT, 2020). A proteína *tau* é responsável por regular os microtúbulos. Porém, a hiperfosforilação desta proteína têm um impacto negativo sobre a estabilidade dos microtúbulos, comprometendo a viabilidade dos neurônios (DOMISE et al., 2016). Isto pode ocorrer em decorrência do aumento nos níveis do peptídeo βa no cérebro e da ativação microglial, levando a alterações na ação de quinases e fosfatases, desencadeando uma hiperfosforilação da proteína *tau.* Quando ocorre a hiperfosforilação desta proteína, ela se dissocia dos microtúbulos e se agrega sob a forma de filamentos helicoidais pareados insolúveis, formando os emaranhados neurofibrilares, levando à morte neuronal e à perda progressiva da função neuronal (OLSSON et al., 2016), conforme demonstrado na figura 2.

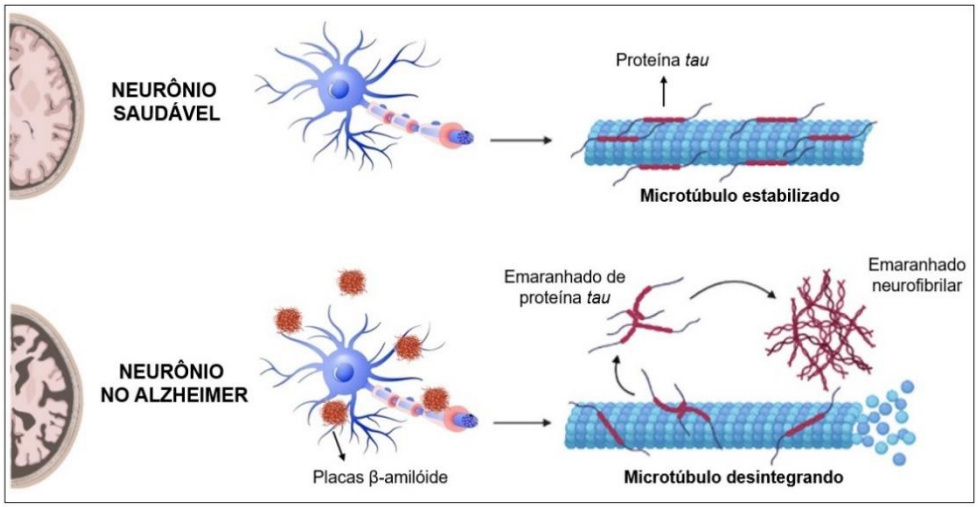

Figura 2. Formação de emaranhados neurofibrilares intracelulares. No neurônio saudável o microtúbulo é estabilizado pela proteína *tau*. Nos neurônios na DA a presença de placas senis formadas de peptídeo βa induz a hiperfosforilação da proteína *tau* e formação de emaranhados neurofibrilares, que conduzem a lesão neuronal. Adaptado de (BARRON et al., 2017). A plataforma BioRender foi utilizada para criação desta figura (https://biorender.com). Autoria própria.

No entanto, existem outros mecanismos que podem ser incluídos na patogênese da DA. É evidenciado que a disfunção do sistema colinérgico é um dos mecanismos que contribuem para a disfunção cognitiva observada na DA. De fato, isto ocorre devido a uma a redução nos níveis do neurotransmissor acetilcolina (ACh) e na atividade da colina acetiltransferase (ChAT) (enzima responsável pela síntese de ACh nos neurônios colinérgicos) no cérebro destes indivíduos (LU et al., 2013; SCHLIEBS, 2005). Adicionalmente, sugere-se que outros mecanismos também podem levar à neurodegeneração, como o estresse oxidativo, a neuroinflamação, a resistência à insulina, as anormalidades cerebrovasculares e as alterações na neuroplasticidade e neurogênese (DAFSARI; JESSEN, 2020). De acordo com o que foi exposto, levando em consideração a etiologia multifatorial da DA, um dos principais desafios em relação à esta doença incluem a falta de biomarcadores confiáveis para seu diagnóstico precoce, bem como a identificação de alvos moleculares potenciais para o desenvolvimento de estratégicas profiláticas e terapêuticas contra o amplo espectro sintomático da DA (WANG et al., 2018a).

2.3 Comorbidade depressão e doença de Alzheimer

A relação entre a depressão e a DA vem sendo estudada, tendo em vista que é considerada complexa e não compreendida de forma conclusiva (DINIZ et al., 2014; SANTOS; BECKMAN; FERREIRA, 2016). Embora o comprometimento de memória seja uma das principais características clínicas da DA, frequentemente estes pacientes são acometidos por outras complicações neuropsiquiátricas, principalmente a depressão (GALTS et al., 2019). Estes sintomas ocasionam um grande impacto aos pacientes com DA, e estão associados a uma rápida progressão para quadros graves de demência (PETERS et al., 2015).

Neste contexto, um dos fatores limitantes para aprofundar o estudo desta comorbidade é o diagnóstico destas doenças quando associadas, isso porque muitos dos sintomas são os mesmos para as duas doenças, como apatia, perda de interesse em atividades antes prazerosas, retraimento social e isolamento, problemas cognitivos e pensamento confuso (CHI et al., 2014). Além disso, está comorbidade causa grande impacto na qualidade de vida dos seus portadores e a busca por estratégias de tratamento é considerada extremamente desafiadora (CHI et al., 2014).

Entre os fatores que podem predispor os indivíduos a comorbidade depressão e DA, está o estresse, que é um dos principais fatores ambientais associados ao desenvolvimento de depressão (OTTE et al., 2016), e geralmente está acompanhado por comprometimento de memória (MARAZZITI et al., 2010; MURROUGH et al., 2011). O estresse é definido como uma resposta do organismo frente a um estímulo estressor, ocasionando uma cascata de reações que levam a distúrbios no equilíbrio fisiológico normal, podendo resultar em alterações patológicas (MARIOTTI, 2015). De fato, eventos altamente estressantes causam alterações fisiológicas, psicológicas e/ou cognitivas, com um grande impacto prejudicial sobre o SNC (LINTHORST; REUL, 2008).

Acredita-se que o papel causal do estresse na depressão e na DA é suportado pela hiperativação do eixo HPA, no qual desencadeia a síntese e secreção exacerbada de glicocorticoides (GC), como o cortisol (SPIERS et al., 2015). Neste contexto, o desequilíbrio do eixo HPA com elevação sustentada de cortisol, resulta em efeitos deletérios na estrutura e função de importantes regiões cerebrais (MCEWEN; GIANAROS, 2010). De acordo com isto, sabe-se

que a depressão está relacionada com uma redução no volume hipocampal (LUPIEN et al., 2009), estrutura extremamente relacionada com a função cognitiva e o comprometimento de memória, que são sintomas característicos na DA (COLCIAGO et al., 2015).

O hipocampo é uma importante estrutura cerebral do sistema límbico que desempenha um papel essencial na resposta do cérebro ao estresse (DRANOVSKY; HEN, 2006). Isto é possível, pois esta estrutura apresenta alta densidade de receptores de GC. Desta maneira, o aumento prolongado de cortisol é capaz de induzir atrofia hipocampal, contribuindo para o desenvolvimento do comprometimento cognitivo (BUTTERS et al., 2008). Assim, tem-se a hipótese de que a exposição ao estresse e/ou a depressão leva a alterações no hipocampo, contribuindo para o desenvolvimento de DA (BYERS; YAFFE, 2011; FRODL; O'KEANE, 2013).

Outra hipótese que liga a depressão a DA, implica no aumento da produção do peptídeo βa, associada à altos níveis de GC (BYERS; YAFFE, 2011). Frente a isto, estudos utilizando modelos de DA demonstram que a prolongada liberação de GC induz alterações na clivagem da PPA e da enzima β-secretase, facilitando a produção do peptídeo βa (GREEN et al., 2006). De acordo com isto, a neurotoxicidade do peptídeo βa no cérebro está associada a sintomas depressivos, apoiando a hipótese de que os sintomas neuropsiquiátricos podem representar uma manifestação precoce na DA (DONOVAN et al., 2018). Portanto, pode-se confirmar que há uma estreita relação entre a depressão e a DA. Adicionalmente, além destas alterações neuroquímicas que fazem a interligação da depressão com a DA, outros mecanismos são semelhantes as duas doenças e podem contribuir para o desenvolvimento desta comorbidade.

Considerando que o diagnóstico precoce da DA é extremamente difícil, entender os mecanismos subjacentes às comorbidades psiquiátricas e à DA são de grande importância. Por sua vez, se a ocorrência de depressão antecede o desenvolvimento de DA, isso facilitaria o diagnóstico (DAFSARI; JESSEN, 2020; SANTOS; BECKMAN; FERREIRA, 2016). Entretanto existem discrepâncias no que diz respeito a esta comorbidade, tendo em vista que há relatos que a depressão pode ser um fator de risco ou um sintoma prodrômico a DA, no entanto isto ainda veem sendo discutido (DAFSARI; JESSEN, 2020; SANTOS;

BECKMAN; FERREIRA, 2016). Curiosamente, alguns estudos argumentaram que essas hipóteses podem ser simultaneamente verdadeiras e que várias vias e mecanismos conectam a depressão e a DA (BUTTERS et al., 2008b; GREEN et al., 2003).

2.4 Mecanismos envolvidos na depressão associada ao comprometimento de memória

2.4.1 Eixo hipotálamo-pituitária-adrenal (HPA)

Estudos clínicos relatam que a hiperativação do eixo HPA com aumento nos níveis de GC circulantes são encontradas tanto em pacientes depressivos quanto naqueles com DA (CARACI et al., 2010; MARQUES; SILVERMAN; STERNBERG, 2009; POPP et al., 2015). Assim, a ativação do eixo HPA frente a estímulos estressores ao organismo, estimula a secreção do hormônio liberador de corticotropina (CRH, do inglês *corticotropin-releasing hormone*) do núcleo paraventricular do hipotálamo, e a sua ação estimula a hipófise a liberar o hormônio adrenocorticotrópico (ACTH, do inglês *adrenocorticotropic hormone*). O ACTH, por sua vez, estimula a liberação de GCs, como o cortisol (corticosterona em roedores), pelo córtex da glândula adrenal. Este processo é normalmente controlado por meio de uma retroalimentação negativa, no qual os GCs suprimem a liberação de ACTH e CRH, controlando assim a ativação do eixo HPA (JOSEPH; GOLDEN, 2016) (Figura 3). Esta resposta é considerada parte do funcionamento biológico adequado do organismo, no qual o cortisol secretado pode exercer seus efeitos metabólicos (MAYBERG, 2009).

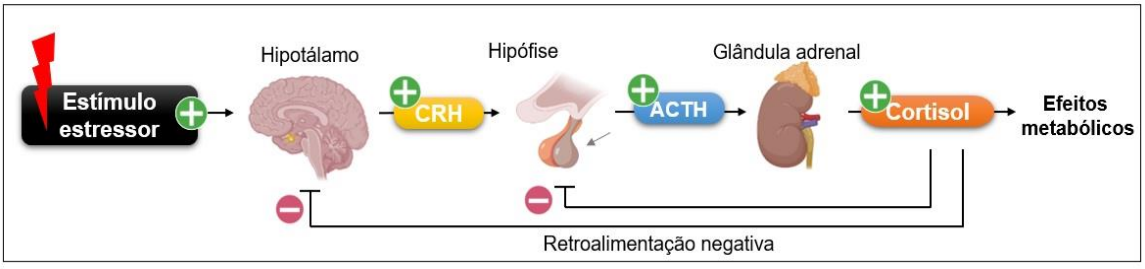

Figura 3. Ativação do eixo hipotálamo-pituitária-adrenal (HPA) resultando na secreção de cortisol. A plataforma BioRender foi utilizada para criação desta figura (https://biorender.com). Autoria própria.

Porém, quando a exposição a estímulos estressores é intensa ou persistente o organismo perde a capacidade de se adaptar, resultando em desregulação hormonal e problemas neurotóxicos (VILELA; JURUENA, 2014). Como resultado pode haver uma desregulação do mecanismo que retorna esse sistema hormonal ao normal (retroalimentação negativa), ocasionando um aumento crônico de cortisol no organismo, devido a desregulação do eixo HPA (JOSEPH; GOLDEN, 2017). Entre os fatores que estão implicados na hiperativação do eixo HPA está o estresse e a neurotoxicidade do peptídeo βa (JAYASINGH CHELLAMMAL et al., 2019). Sendo assim, em pacientes com depressão e DA, acredita-se que o sistema de retroalimentação negativa esteja comprometido. Diante disso, o aumento exacerbado desses GCs é responsável por danificar as células neuronais, principalmente do hipocampo (devido à alta densidade de receptores de GCs nesta região), induzindo prejuízo de memória e alterações emocionais, sintomas encontrados em pacientes com depressão e DA (BYERS; YAFFE, 2011; LUPIEN et al., 2009).

2.4.2 Estresse oxidativo e defesas antioxidantes

Evidências indicam que além do mecanismo relatado anteriormente, o estresse oxidativo é considerado um dos principais mecanismos envolvidos na etiologia da depressão e da DA (MANOHARAN et al., 2016). O estresse oxidativo é um distúrbio metabólico no qual ocorre um desequilíbrio entre a produção celular de espécies reativas (RS, do inglês *reactive species*) e das defesas antioxidantes, causado pelo excesso de RS no organismo (CHE et al., 2015a). Em consequência do excesso de RS, pode ocorrer danos as células por meio de reações de oxi-redução em proteínas, lipídeos e ácido desoxirribonucleico (DNA), podendo induzir morte celular (CAMPOS; LEME, 2018). Um dos fundamentos para a associação entre o estresse oxidativo e aos transtornos que afetam o SNC é o fato do cérebro ser vulnerável a danos oxidativos, devido a sua alta taxa de consumo de oxigênio, alto teor lipídico e baixa concentração de enzimas antioxidantes (MANOHARAN et al., 2016). De acordo com isto, o desequilíbrio constante na homeostase do organismo resultante do aumento do estresse oxidativo, pode induzir sinergicamente a dano nos tecidos,

neuroinflamação, neurodegeneração e apoptose neuronal, e assim contribuir para o desenvolvimento de diversas doenças, como a depressão e DA (BAKUNINA; PARIANTE; ZUNSZAIN, 2015)

Em contrapartida, estes pacientes têm um sistema antioxidante prejudicado (BALMUS et al., 2016). As enzimas antioxidantes endógenas são responsáveis por inibirem a formação de RS e/ou removerem os radicais livres e seus precursores (KODYDKOVÁ et al., 2009). O excesso de RS promove o processo de peroxidação lipídica, que é uma das principais consequências de dano neuronal (NIKI, 2012). Assim, a deficiência dessas enzimas antioxidantes também está implicada na etiologia das doenças neuropsiquiátricas e neurodegenerativas (LANG; BORGWARDT; LANG, 2013).

Evidências indicam que o estresse é capaz de tornar o cérebro vulnerável a danos oxidativos, por meio da ativação de vias intracelulares envolvidas na formação de RS e, consequentemente, produzir mudanças comportamentais relacionadas a distúrbios neuropsiquiátricos (CHE et al., 2015). Nesse sentido, há uma relação direta entre o estresse, a hiperativação do eixo HPA e o aumento dos parâmetros oxidativos, no qual os GCs atuam favorecendo o aumento da taxa metabólica em conjunto com as catecolaminas, e por sua vez neste processo, ocorre um aumento na produção de RS (BALMUS et al., 2016; KV et al., 2018). Paralelamente, a neurotoxicidade do peptídeo βa também contribui para a produção exacerbada de RS e produtos da peroxidação lipídica, induzindo ao dano mitocondrial, e consequentemente a morte celular (CHAUHAN; CHAUHAN, 2006). De acordo com isto, compreender os mecanismos pelo qual o estresse oxidativo atua nestas patologias são de importante relevância.

2.4.3 Neuroinflamação

Como já mencionado anteriormente, sabe-se que a depressão e a DA compartilham uma etiologia neuroinflamatória intimamente ligada (AMANI; SHOKOUHI; SALARI, 2019; MAES et al., 2011). A neuroinflamação se caracteriza pela ativação do sistema imunológico inato frente a estímulos nocivos ao SNC (HEPPNER; RANSOHOFF; BECHER, 2015). A micróglia é a principal célula imunológica do SNC, responsável pela liberação de moléculas pró-inflamatórias (PRINZ; PRILLER, 2014) e sua ativação está implicada na

incidência e progressão da DA (LENG; EDISON, 2021) e da depressão (YIRMIYA; RIMMERMAN; RESHEF, 2015). Um fator de transcrição responsável por regular os processos neuroinflamatórios mediados pela micróglia é o NF-κB (DRESSELHAUS; MEFFERT, 2019). Este fator de transcrição pode ver ativado por diferentes estímulos, como estresse, presença de RS e respostas inflamatórias (BALMUS et al., 2016; SIOMEK, 2012). Quando ativado, modula a transcrição de diversos genes pró-inflamatórios, contribuindo para o desenvolvimento do processo neuroinflamatório (JOPE et al., 2017). Em vista desses mecanismos, a ativação do NF-κB também está fortemente relacionada ao desenvolvimento de comportamentos depressivos e comprometimento da memória (CAVIEDES et al., 2017; JHA et al., 2019)

Além disso, diversos fatores exógenos e endógenos que afetam a homeostase corporal, como infecções, envelhecimento e estresse, podem induzir a ativação microglial e contribuir para as patologias do SNC (YANG; ZHOU, 2019). Adicionalmente, as citocinas pro-inflamatórias podem interagir com diferentes mecanismos, como através do metabolismo de monoaminas, da plasticidade neuronal e da função endócrina, resultando em distúrbios ao SNC (YANG; ZHOU, 2019). Frente a isto, uma vasta gama de estudos clínicos estabeleceu que níveis aumentados de citocinas e mediadores pró-inflamatórios derivados da micróglia, como as interleucinas (IL)-6, IL-12, I-18 e o fator de necrose tumoral-alfa (TNF-α, do inglês *tumor necrosis factor alpha*) se correlacionam com a depressão e a DA (DARWEESH et al., 2018; FELGER; LOTRICH, 2013).

Acredita-se que o fator chave que leva a neuroinflamação na DA é a exposição da micróglia a neurotoxicidade do peptídeo βa, que desencadeia um aumento na produção de citocinas pró-inflamatórias (LENG; EDISON, 2021). A exposição ao peptídeo βa acompanhada de processos neuroinflamatórios, além de induzir sintomas semelhantes aos encontrados em pacientes com DA, pode desencadear comportamento semelhantes a depressão em modelos animais (LEDO et al., 2013). Sendo assim, estas descobertas sugerem que a neurotoxicidade do βa podem fornecer uma ligação mecanicista entre a depressão, a DA e a neuroinflamação. Reciprocamente, a neuroinflamação mediada por citocinas pró-inflamatórias pode resultar na ativação da via amiloidogênica e induzir um aumento nos níveis de βa, culminando num ciclo

patológico vicioso (GRIFFIN et al., 1998). Esta via de sinalização depende da ativação do fator de transcrição NF-κB, no qual além de ocasionar um aumento na produção de citocinas, também é capaz de aumentar os níveis de βa por meio da produção e clivagem da PPA (ZHANG; JIANG, 2015). Portanto, o peptídeo βa é considerado tanto uma causa contribuinte, como uma consequência da neuroinflamação (LENG; EDISON, 2021).

Ademais, tem sido relatado que a produção aumentada de citocinas pró-inflamatórias podem modular a disponibilidade de neurotransmissores e aumentar a produção de RS, contribuindo para a disfunção e morte neuronal na DA (ZHANG; JIANG, 2015). De acordo com isto, evidências apontam que processos neuroinflamatórios estão relacionados com a redução na síntese de importantes neurotransmissores, como a serotonina (5-HT, do inglês *5-hydroxytryptamine*) e dopamina (MILLER; RAISON, 2016). Isto ocorre em virtude da produção exacerbada de citocinas pró-inflamatórias que induzem a expressão microglial da indolamina-2,3-dioxigenase (IDO), enzima responsável pela conversão do triptofano (aminoácido precursor da biossíntese de 5-HT) em quinurenina. Assim, a redução da disponibilidade deste aminoácido pode prejudicar a síntese de 5-HT (DANTZER et al., 2008) e contribuir para o desenvolvimento de depressão, tendo em vista que a redução de neurotransmissores, principalmente a 5-HT, produz sintomas semelhantes aos da depressão em modelos animais (DANTZER et al., 2008) e alterações de humor em humanos (RUHÉ; MASON; SCHENE, 2007). Além da redução da biodisponibilidade de 5-HT, a superativação da IDO pode aumentar a produção da quinuremina, que em condições inflamatórias, pode gerar metabólitos neurotóxicos, os quais têm sido implicados também no desenvolvimento da DA (KINCSES; TOLDI; VÉCSEI, 2010).

Tais dados correspondentes, no entanto, ainda são insuficientes para determinar se a ativação microglial é uma causa ou uma consequência de outros aspectos da patologia destas doenças. Neste sentido, com o papel da ativação microglial sendo considerado um mecanismo que relaciona a depressão e a DA (Figura 4), alternativas terapêuticas com ação anti-inflamatória podem contribuir para a descoberta de um novo tratamento para esta comorbidade (AMANI; SHOKOUHI; SALARI, 2019).

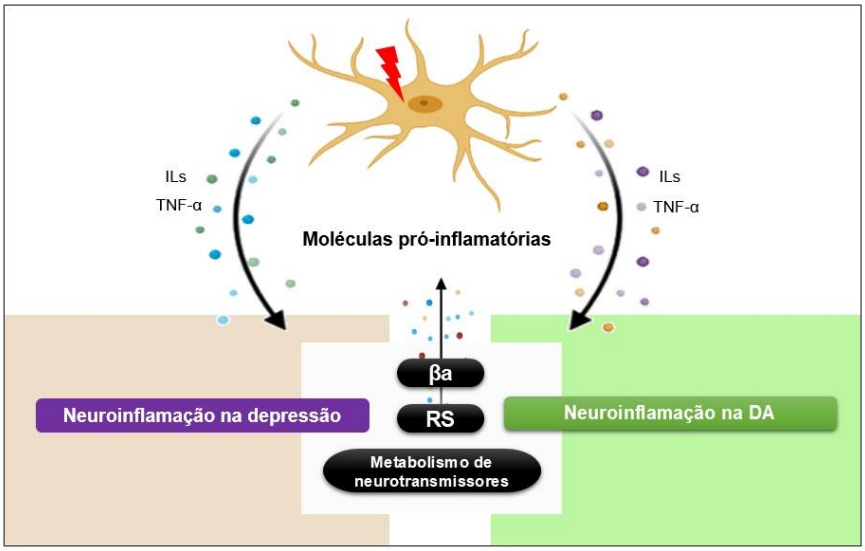

Figura 4. Resumo do mecanismo que relaciona a neuroinflamação a depressão e a DA. Autoria própria.

2.4.4 Via monoaminérgica

Adicionalmente, o sistema monoaminérgico tem sido estudado na depressão e na DA (BEHL et al., 2021; OTTE et al., 2016; VERMEIREN et al., 2014). Esta hipótese baseia-se na redução da disponibilidade de monoaminas na fenda sináptica, como a 5-HT, dopamina e/ou noradrenalina, ou mesmo de receptores de tais neurotransmissores ineficientes (OTTE et al., 2016). De fato, níveis diminuídos destes neurotransmissores no SNC, particularmente em pacientes deprimidos com DA (VERMEIREN et al., 2014), podem desempenhar um papel importante na deterioração da memória e outras funções cognitivas (BUTTERS et al., 2008).

Neste sentido, a deficiência destes neurotransmissores pode ocorrer pela excessiva atividade da enzima monoaminaoxidase (MAO) ou pela deficiência dos seus precursores (FINBERG; RABEY, 2016). A MAO é a enzima do sistema monoaminérgico responsável por regular os níveis de neurotransmissores monoaminérgicos, por meio de sua desaminação oxidativa, regulando assim a disponibilidade dessas monoaminas nas sinapses (FINBERG; RABEY, 2016). Esta enzima atua catalisando a desaminação oxidativa de uma série de monoaminas gerando aldeído e amônia e, quando o cofator dinucleotídeo de flavina e adenina (FAD) é reoxidado pelo oxigênio (O_2), produz peróxido de

hidrogênio (H_2O_2). Já o aldeído formado é rapidamente metabolizado pela enzima aldeído desidrogenase (ADH) a metabólitos ácidos.

O H_2O_2 formado geralmente é inativado por enzimas antioxidantes como a catalase e a glutationa peroxidase (GPx). Entretanto, quando há um aumento na atividade da MAO, consequentemente há um aumento do H_2O_2 e esse por sua vez pode ser convertido por íons ferro (Fe^{2+}) em radicais hidroxila (OH°), que são altamente reativos. Estes radicais provocam efeitos deletérios que podem levar ao dano e morte neuronal (Figura 5) (YOUDIM; EDMONDSON; TIPTON, 2006).

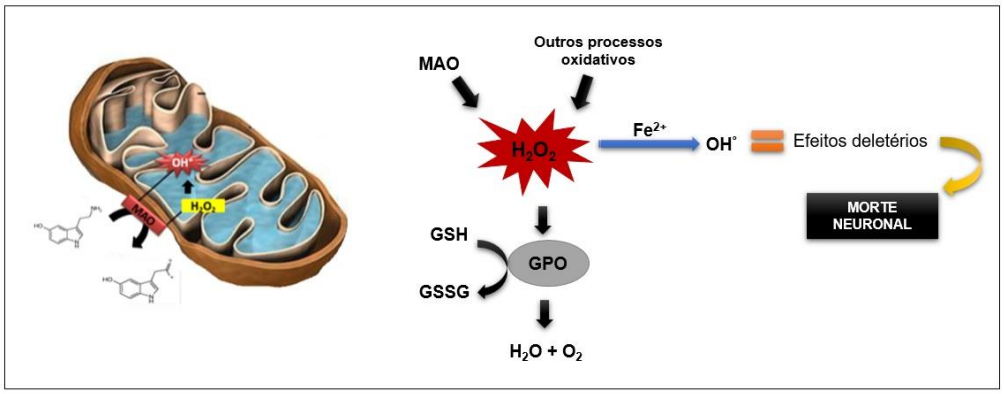

Figura 5. Mecanismo de neurotoxicidade induzido pelo peróxido de hidrogênio. MAO: monoamina oxidase, GSH: glutationa reduzida; GSSH: glutationa oxidada; H_2O: água, O_2: oxigênio, Fe^{2+}: íons ferro, OH°: radicais hidroxila, H_2O_2: peróxido de hidrogênio, GPx: glutationa peroxidase (Adaptado de YOUDIM; EDMONDSON; TIPTON, 2006). Autoria própria.

Vários destes produtos são considerados tóxicos em altas concentrações e contribuem para a patologia e/ou sintomas de alguns distúrbios psiquiátricos e neurodegenerativos (BILICI et al., 2001). Dessa forma, esses mecanismos estabelecem os fundamentos teóricos para a implicação da MAO na fisiopatologia de distúrbios neurodegenerativos, como na DA, e os distúrbios psiquiátricos e neurológicos, como a depressão (DANIELCZYK et al., 1988).

Ademais, essa enzima existe em duas isoformas enzimáticas, MAO-A e MAO-B, que diferem em sua especificidade de substrato e inibidor e em sua distribuição nos tecidos (SHIH; CHEN; RIDD, 1999). Os inibidores seletivos da isoforma MAO-A atuam como antidepressivos potentes, enquanto os inibidores MAO-B são úteis em distúrbios neurodegenerativos, como na DA (SAURA et al.,

1994). Assim, a regulação do sistema monoaminérgico via isoforma MAO-A e MAO-B pode ser valiosa para a terapia da comorbidade da depressão e DA

2.4.5 Via de sinalização celular fosfatidilinositol-3-quinase (PI3K)/ proteína quinase B (AKT)

Além dos mecanismos anteriormente relatados, algumas moléculas de sinalização celular também estão envolvidas na progressão da depressão associada a DA. A fim de se adaptar a eventos estressantes e o desequilíbrio na homeostase corporal, as vias de transdução de sinal intracelular são alteradas para promover a sobrevivência neuronal e a neuroplasticidade (BEGNI; RIVA; CATTANEO, 2017). Dentre essas vias de sinalização, destaca-se a PI3K/AKT, que está envolvida na sobrevivência neuronal, neuroplasticidade, aprendizagem, memória e depressão (QI et al., 2016 YANG et al., 2008).

A enzima PI3K é ativada na presença de fatores de crescimento, hormônios, citocinas e/ou neurotransmissores. Seus produtos lipídicos atuam como segundos mensageiros, uma vez que ativam proteínas como a AKT, sendo o efetor de PI3K para as respostas celulares (BEAULIEU, 2012). A AKT, quando ativada, regula negativamente a atividade da enzima glicogênio sintase quinase 3 beta (GSK-3β) (BEAULIEU; GAINETDINOV; CARON, 2009). Esta regulação é de extrema importância, tendo em vista que a ativação da GSK-3β regula negativamente a viabilidade neuronal (FRAME; COHEN; BIONDI, 2001). Sendo assim, a inibição da GSK-3β promovida pela AKT é importante para a sobrevivência das células neuronais. Portanto, a modulação da via PI3K/AKT é considerada crítica nos processos de neuroplasticidade e neurogênese, funções importantes para a díade de depressão e DA (LI et al., 2015).

Além disso, a via PI3K/AKT também desempenha um papel importante no controle do metabolismo do peptídeo βa e na fosforilação da proteína *tau* na DA (HOJIN; KOH, 2016; YANG et al., 2020), demonstraram que a morte neuronal induzida pela neurotoxicidade de βa está associada a inibição da via PI3K/AKT e consequentemente ativação da enzima GSK-3β. Confirmando esta teoria, moléculas com capacidade de ativar a via PI3K/AKT, como por exemplo a donepezila (fármaco utilizado para o tratamento da DA), apresentam efeitos neuroprotetores contra a lesão induzida por βa (NOH et al., 2009). Além disso,

estudos indicam que o peptídeo βa é capaz de ativar a sinalização GSK-3β, contribuindo para o processamento anormal da PPA e aumentando a hiperfosforilação de *tau* diretamente associada a falha sináptica aos sintomas clínicos da DA, incluindo a depressão (DENG et al., 2014)

Levando em consideração o papel significativo da modulação da via de sinalização PI3K/AKT/GSK-3β nos mecanismos subjacentes à depressão e na DA, torna-se interessante estender os estudos sobre esta via.

2.4.6 Sistema colinérgico

A disfunção do sistema colinérgico é uma das características mais marcantes da DA (LU et al., 2018), e estudos mais recentes tem implicado essa via também em transtornos depressivos (FITZGERALD et al., 2020). As alterações no sistema colinérgico são caracterizadas principalmente pela redução nos níveis do neurotransmissor ACh. Este neurotransmissor é sintetizado por meio da enzima ChAT, a partir da colina e da acetilcoenzima A nos neurônios colinérgicos. A ACh, após sintetizada, é liberada na fenda sináptica e então interage com seus receptores muscarínicos e/ou nicotínicos para exercer suas ações (WILSON, 2010).

Por outro lado, a enzima acetilcolinesterase (AChE) é a enzima do sistema colinérgico responsável por hidrolisar o neurotransmissor ACh e, assim, encerrar a transmissão colinérgica após a sua ligação aos seus respectivos receptores, impedindo seu acúmulo nas sinapses e evitando a produção de estímulos contínuos (MOKRANI et al., 2019). Portanto, a estimulação da atividade da AChE resulta em uma redução na disponibilidade da ACh, uma enzima responsável por manter o processamento das sinapses e a modulação da cognição e da memória (DREVER; RIEDEL; PLATT, 2011).

Em paralelo, já foi demonstrado que a AChE também possui funções não colinérgicas, como através da sua interação com o peptídeo βa por meio de seu sítio aniônico periférico, modificando as suas propriedades bioquímicas e aumentando a neurotoxicidade do peptídeo βa (HOU et al., 2014). Com isso, a AChE é capaz de promover a agregação de βa e formar complexos estáveis e fortemente ligados que são mais tóxicos do que as placas amiloides, além de favorecer a sua deposição nas células neuronais (GUPTA; MOHAN, 2014). De acordo com isto, a estimulação dos receptores colinérgicos através da inibição

da AChE pode deslocar o processamento da PPA em direção a via não amiloidogênica, resultando na redução da liberação de βa (SCHLIEBS, 2005). Em consonância, terapias que visam a inibicão da enzima AChE podem estar relacionadas com o processo de geração dos componentes não amiloidogênicos (substratos solúveis) (MUÑOZ-TORRERO; CAMPS, 2006).

Paralelamente a isto, as alterações neuroquímicas no sistema colinérgico também são relacionadas a transtornos de humor (FITZGERALD et al., 2020). Neste contexto, sabe-se que os principais antidepressivos agem através do aumento dos níveis sinápticos de neurotransmissores monoaminérgicos (MARATHE et al., 2018). Em paralelo, a enzima ACh é capaz de interagir com os neurotransmissores monoaminérgicos no cérebro, tendo em vista que a sua distribuição cerebral é sobreposta a estas monoaminas (RHO; STOREY, 2001). Além disso, os receptores da ACh e das monoaminas podem ser encontrados nos mesmos neurônios (ROBBINS, 2005). Desta forma, a ACh pode não apenas interagir com o sistema monoaminérgico, mas também compartilhar propriedades funcionais com eles, incluindo a regulação do humor (MINCES et al., 2017). De acordo com isto, um estudo de estresse crônico em roedores descobriu que os inibidores da colinesterase reverteram o comprometimento cognitivo induzido por estresse e apresentam efeitos semelhantes aos dos medicamentos antidepressivos neste modelo (PAPP et al., 2016).

Portanto, de acordo com estas observações são evidenciadas as interações existentes entre o sistema colinérgico e a patologia da DA e da depressão. Com base nisso, torna-se importante a investigação da enzima AChE em pesquisas que visam estudar a DA e a depressão e/ou desenvolver possíveis alternativas terapêuticas para estas patologias (ABHISHEK; SHITAL, 2018; FITZGERALD et al., 2020).

2.5 Busca por novas terapias para a comorbidade depressão e DA

2.5.1 Tratamentos existentes

Na última década, as estratégias de tratamento para a DA, aprovadas pela *Food and Drug Administration* (FDA), foram destinadas, principalmente, em

melhorar a neurotransmissão colinérgica no cérebro. Essas abordagens incluem o uso de inibidores da AChE, tais como a rivastigmina, a donepezila e a galantamina (WEINREB et al., 2016). Outra estratégia terapêutica utilizada em pacientes com DA é a memantina, um fármaco antagonista não competitivo de receptores de glutamato do subtipo N-metil D-Aspartato (NMDA), que age promovendo a neuroproteção contra a excitotoxicidade glutamatérgica presente na DA (DOMINGUEZ et al., 2011). Porém, estes medicamentos apresentam eficácia moderada, efeitos secundários indesejáveis (falta de apetite, perda de peso, náuseas, diarreia, insônia e redução da frequência cardíaca), além de não impedir a neurodegeneração (ALI et al., 2015).

Atualmente ocorreu um grande avanço nas pesquisas científicas em relação a terapias efetivas na regressão da progressão da DA. Nos últimos anos, o FDA aprovou um novo tratamento para a DA, com o intuito de retardar a progressão da doença ao invés de aliviar apenas os sintomas (CAVAZZONI, 2021). O aducanumabe é um anticorpo monoclonal que tem como alvo o peptídeo βa, facilitando a sua eliminação, e consequentemente reduzindo a formação de placas amiloides, modificando o curso da doença. Mesmo sendo considerado um medicamento em fase experimental e com custo elevado, o aducanumabe é, até o momento, uma esperança para os pacientes com DA e seus cuidadores (BERKELEY, 2021; CAVAZZONI, 2022).

Paralelamente, os principais tratamentos utilizados na clínica para a depressão agem principalmente na modulação do sistema monoaminérgico, devido a hipofunção deste sistema estar associada a depressão (LIU; ZHAO; GUO, 2018). Os antidepressivos são classificados em diferentes classes, como os inibidores da monoamina oxidase (iMAO), tricíclicos, inibidores seletivos da recaptação de serotonina (ISRS) e de noradrenalina (ISRN) ou inibidores de receptação de serotonina e noradrenalina, além dos antidepressivos atípicos. No entanto, estes antidepressivos apresentam efeitos adversos significantes, eficácia limitada e início lento de ação terapêutica (MCINTYRE et al., 2014). Mais recentemente, a Agência Nacional de Vigilância Sanitária (ANVISA) aprovou um novo tratamento para a depressão indicado a pacientes resistentes aos antidepressivos existentes. Trata-se de uma formulação intranasal de cloridrato de escetamina (Spravato®), no qual age nos receptores de glutamato - NMDA, ajudando a restaurar as conexões sinápticas. Esta medicação pode ser

administrada apenas em hospitais e clínicas autorizadas, sob a supervisão de um profissional de saúde. Seus efeitos são rápidos, reduzindo os sintomas em até 24 horas depois da primeira inalação. Como comparação, os fármacos atuais levam mais de três semanas para promover alguma melhora, e cerca de um terço dos portadores do transtorno não responde a eles (MOORE et al., 2022).

Os fármacos disponíveis para o tratamento da depressão associada à DA são os mesmos utilizados para tratar a depressão. Há evidências clínicas de que os antidepressivos disponíveis não são totalmente eficazes na depressão associada à DA e, portanto, há uma necessidade urgente de compreender os mecanismos neurobiológicos subjacente a esta comorbidade para o desenvolvimento de uma estratégia terapêutica realmente efetiva (CASSANO et al., 2019; GALTS et al., 2019). Até o momento, nenhum medicamento foi aprovado pelo FDA para o tratamento de sintomas depressivos na DA. Neste contexto, os antidepressivos da classe dos ISRS (principalmente a sertralina e mirtazapina) são os medicamentos mais prescritos para pacientes com depressão e DA, tendo em vista que apresentam um risco menor de causar interações com outros medicamentos e seus efeitos adversos são toleráveis (ALZHEIMER'S ASSOCIATION, 2020). Embora, existem evidências que a sua eficácia nestes pacientes é controversa. Entretanto, um número substancial de estudos relatou que os ISRS não foram eficazes em reduzir a depressão em pacientes com DA (AN et al., 2017; BANERJEE et al., 2013; GALTS et al., 2019).

Frente a isto, os efeitos adversos dos antidepressivos são somados aos dos fármacos utilizados para o tratamento da DA, e como consequência ocorre uma redução na qualidade de vida do paciente acometido com esta comorbidade (KETTUNEN et al., 2019). Os principais efeitos adversos da combinação destes agentes farmacológicos são reações gastrointestinais, náuseas, vômitos, dor abdominal, diarreia, dores de cabeça, ansiedade, boca seca, perda de apetite e tontura (BURKE et al., 2019). Dessa forma, torna-se prioridade a busca por uma ferramenta que seja capaz de elucidar os mecanismos fisiopatológicos destas doenças, bem como encontrar estratégias efetivas para mitigar a depressão na DA, à medida que a prevalência entre estas doenças cresce exponencialmente.

2.5.2 7-cloro-4-(fenilselanil)quinolina (4-PSQ)

Levando em consideração a etiologia multifatorial da comorbidade depressão e DA, e ainda, os efeitos adversos da utilização de medicamentos combinados, moléculas multifuncionais com duas ou mais atividades biológicas complementares podem representar um avanço importante para o tratamento destas doenças. Diante deste contexto, nosso grupo de pesquisa tem buscado moléculas multialvo direcionadas ao tratamento de alterações que afetam o SNC. Tendo em vista os estudos de prospecção de novas drogas, o uso de agentes contento o núcleo quinolina para o tratamento de doenças vêm recebendo atenção em nossas pesquisas. Assim, várias pesquisas demonstram que a fração quinolina é o farmacóforo de muitas drogas anti-DA, tais como a tacrina, o clioquinol, o azul de metileno, os derivados da berberina e o PMS1339 (FREEMAN; DAWSON, 1991; JIANG et al., 2011; MANCINO et al., 2009; OZ; LORKE; PETROIANU, 2009). Em paralelo, o selênio é um micro nutriente essencial para o nosso organismo no qual desempenha um papel crítico o funcionamento adequado de várias selenoproteínas envolvidas nas defesas antioxidantes do cérebro (SHER, 2000). Dado seu papel neuroprotetor na função neuronal, estudos recentes investigaram uma relação entre os níveis de selênio na prevenção do início e na progressão da DA e na depressão (VARIKASUVU et al., 2019; WANG et al., 2018b).

Com essa hipótese, a 4-PSQ (Figura 6), um derivado de quinolina contendo um grupo organoselênio, tem sido extensivamente estudado por nosso grupo de pesquisa. Anteriormente, foi demonstrado que a 4-PSQ teve efeito em distúrbios que afetam o SNC, por meio da redução de sintomas neuropsiquiátricos, como ansiedade e depressão (PALTIAN et al., 2020; REIS et al., 2017; RODRIGUES et al., 2021), prevenção do comprometimento da memória e do déficit cognitivo (BARTH et al., 2019; DUARTE et al., 2017; PINZ et al., 2018), bem como atenuação do estresse oxidativo cerebral (LUCHESE et al., 2020; VOGT et al., 2018). Adicionalmente, foi evidenciado o efeito anti-DA da 4-PSQ em camundongos (PINZ et al., 2018), bem como sua ação em melhorar a memória de ratos envelhecidos (BARTH et al., 2019). Cabe ressaltar ainda que os mecanismos relacionados aos efeitos da 4-PSQ incluem a modulação da atividade da AChE, a neuroinflamação e a neuroplasticidade, bem como seu efeito antioxidante, evidenciando a característica multifuncional desta

molécula (BARTH et al., 2019; LEMOS et al., 2021; PINZ et al., 2016; PINZ et al., 2018). Ademais, verificou-se que a 4-PSQ apresentou efeito na comorbidade DA, depressão e ansiedade em camundongos (PINZ et al., 2018).

Ainda, estudos anteriores realizados no nosso grupo de pesquisa mostraram que o grupo fenilselanil presente na estrutura da quinolina é crítico para a atividade antioxidante da 4-PSQ em um modelo de estresse oxidativo cerebral induzido por nitroprussiato de sódio (VOGT et al., 2018). Nesse contexto, demonstramos a importância do selênio nos efeitos positivos da 4-PSQ. Além disso, de FREITAS COUTO e colaboradores (2019) demonstraram que a 4-PSQ foi capaz de restaurar os níveis de selênio na cabeça e no corpo de moscas *Drosophila melanogaster* em um modelo de doença de Parkinson, prevenindo os danos causados neste modelo. Os autores sugeriram que a 4-PSQ foi capaz de penetrar na barreira hematoencefálica, uma vez que foram observados níveis elevados de selênio nas cabeças das moscas após o tratamento com 4-PSQ (DE FREITAS COUTO et al., 2019). Além disso, BARTH e colaboradores (2021) demonstraram que o tratamento com 4-PSQ restaurou os níveis plasmáticos de selênio em ratos idosos, contribuindo assim para a restauração dos danos causados pelo envelhecimento. Portanto, sugere-se que a 4-PSQ seja capaz de atingir a corrente sanguínea após sua absorção (BARTH et al., 2021). Dessa forma, considerando que transtornos complexos, como depressão e a DA, têm maior probabilidade de serem atenuados por meio da modulação simultânea de múltiplos alvos, a versatilidade da 4-PSQ nos levou a considerar seu uso como uma possível estratégia terapêutica para a comorbidade em questão, e para isto é de suma importância a realização de estudos experimentais para a descoberta das alterações neuroquímicas associadas a comorbidade depressão e DA.

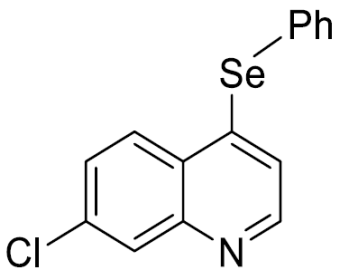

Figura 6. Estrutura química da 7-cloro-4-(fenilselanil)quinolina (4-PSQ)

3. OBJETIVOS

3.1 Objetivo geral

Investigar os possíveis efeitos neuroprotetores da molécula 4-PSQ na comorbidade depressão e comprometimento de memória/DA em diferentes modelos experimentais em camundongos, bem como investigar os possíveis mecanismos pelos quais o composto exerce seu efeito.

3.2 Objetivos específicos

a) Investigar o efeito da molécula 4-PSQ na comorbidade depressão e comprometimento de memória em um modelo de estresse de restrição agudo (ARS, do inglês *acute restraint stress*) em camundongos:
   - Avaliar o comportamento do tipo-depressivo e anedônico induzido pelo ARS, bem como o possível efeito do tipo-antidepressivo da 4-PSQ em camundongos;
   - Verificar o efeito terapêutico da 4-PSQ em atenuar o comprometimento de memória promovido pelo ARS em camundongos;
   - Avaliar a hiperatividade do eixo HPA na comorbidade depressão e comprometimento de memória induzida por ARS, bem como investigar o efeito da 4-PSQ em modular este eixo;
   - Investigar o envolvimento do estresse oxidativo na comorbidade depressão e comprometimento de memória em estruturas cerebrais de camundongos estressados, bem como a capacidade da 4-PSQ em modular esta via;
   - Avaliar o envolvimento do sistema monoaminérgico na comorbidade depressão e comprometimento de memória em estruturas cerebrais de roedores submetidos ao ARS, bem como investigar o efeito da 4-PSQ em modular este eixo;
   - Estabelecer uma relação entre moléculas de sinalização celular envolvidas na neuroplasticidade e neurogênese com a comorbidade depressão e comprometimento de memória em

estruturas cerebrais de roedores submetidos ao ARS, bem como o efeito do tratamento com 4-PSQ nestas moléculas;

- Investigar a possível modulação da neuroinflamação através do tratamento com 4-PSQ na comorbidade depressão e comprometimento de memória promovido pelo ARS em estruturas cerebrais de camundongos;
- Determinar o envolvimento do sistema colinérgico, por meio na avaliação da atividade da enzima AChE, na comorbidade depressão e comprometimento de memória em estruturas cerebrais de camundongos estressados, bem como o efeito do tratamento com 4-PSQ neste sistema.

b) Estudar o efeito da molécula 4-PSQ na comorbidade depressão e comprometimento de memória em um modelo de DA induzida pelo peptídeo βa em camundongos:
    - Verificar o possível efeito neuroprotetor da 4-PSQ em atenuar o comprometimento de memória promovido por βa em camundongos;
    - Avaliar a ação da 4-PSQ em atenuar o comportamento tipo-depressivo no modelo de DA induzido por βa em camundongos;
    - Verificar o efeito da 4-PSQ em modular as alterações do eixo HPA induzida por βa na comorbidade depressão e comprometimento de memória/DA em camundongos;
    - Investigar a capacidade da 4-PSQ em modular o estresse oxidativo cerebral promovido por βa na comorbidade depressão e comprometimento de memória/DA em camundongos;
    - Avaliar o envolvimento do sistema colinérgico na comorbidade depressão e comprometimento de memória/DA em camundongos induzidos com βa, bem como o efeito do tratamento com 4-PSQ neste sistema;
    - Avaliar a possível modulação da neuroinflamação através do tratamento com 4-PSQ na comorbidade depressão e comprometimento de memória em um modelo de DA induzido por βa em estruturas cerebrais de camundongos.

4. **CAPITULOS**

Os resultados que fazem parte dessa tese estão apresentados sob a forma de 1 artigo científico e 1 manuscrito. As seções materiais e métodos, resultados, discussão e referências encontram-se no artigo e no manuscrito, representando a íntegra desse estudo. O artigo está estruturado conforme a revista no qual está publicado e o manuscrito está estruturado de acordo com revista na qual foi submetido.

4.1 Artigo

Prospecting for a quinoline containing selenium for comorbidities depression and memory impairment induced by restriction stress in mice

O artigo científico encontra-se publicado na revista *Psychopharmacology*

Psychopharmacology
https://doi.org/10.1007/s00213-021-06039-8

ORIGINAL INVESTIGATION

Prospecting for a quinoline containing selenium for comorbidities depression and memory impairment induced by restriction stress in mice

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Received: 24 June 2021 / Accepted: 29 November 2021

Abstract

Rationale Depression is often associated with memory impairment, a clinical feature of Alzheimer's disease (AD), but no effective treatment is available. 7-Chloro-4-(phenylselanyl) quinoline (4-PSQ) has been studied in experimental models of diseases that affect the central nervous system.

Objectives The pharmacological activity of 4-PSQ in depressive-like behavior associated with memory impairment induced by acute restraint stress (ARS) in male Swiss mice was evaluated.

Methods ARS is an unavoidable stress model that was applied for a period of 240 min. Ten minutes after ARS, animals were intragastrically treated with canola oil (10 ml/kg) or 4-PSQ (10 mg/kg) or positive controls (paroxetine or donepezil) (10 mg/kg). Then, after 30 min, mice were submitted to behavioral tests. Corticosterone levels were evaluated in plasma and oxidative stress parameters; monoamine oxidase (MAO)-A and MAO -B isoform activity; mRNA expression levels of kappa nuclear factor B (NF-κB); interleukin (IL)-1β, IL-18, and IL-33; phosphatidylinositol-se-kinase (PI3K); protein kinase B (AKT2), as well as acetylcholinesterase activity were evaluated in the prefrontal cortex and hippocampus.

Results 4-PSQ attenuated the depressive-like behavior, self-care, and memory impairment caused by ARS. Based on the evidence, we believe that effects of 4-PSQ may be associated, at least in part, with the attenuation of HPA axis activation, attenuation of alterations in the monoaminergic system, modulation of oxidative stress, reestablishment of AChE activity, modulation of the PI3K/AKT2 pathway, and reduction of neuroinflammation.

Conclusions These results suggested that 4-PSQ exhibited an antidepressant-like effect and attenuated the memory impairment induced by ARS, and it is a promising molecule to treat these comorbidities.

Keywords Corticosterone · Alzheimer's disease · Oxidative stress · Neuroinflammation · Neuroplasticity · Acute restraint stress · Central nervous system

Abbreviations

4-PSQ	7-Chloro-4-(phenylselanyl) quinoline
AD	Alzheimer's disease
ARS	Acute restraint stress
CNS	Central nervous system
NF-κB	Kappa nuclear factor B
PI3K	Phosphatidylinositol-3-kinase
AKT2	Protein kinase B-2

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Published online: 11 January 2022

AChE	Acetylcholinesterase
Ach	Acetylcholine
AcSCh	Acetylthiocholine
HPA	Hypothalamic–pituitary–adrenal
OFT	Open field test
SPT	Splash test
TST	Tail suspension test
FST	Forced swimming test
RS	Reactive species
TBARS	Thiobarbituric acid reactive species
SOD	Superoxide dismutase
GPx	Glutathione peroxidase
GR	Glutathione reductase
S1	Supernatant fractions
MDA	Malondialdehyde
GSH	Glutathione
GSSH	Oxidized glutathione
NADPH	Nicotinamide adenine dinucleotide phosphate
Pi	Inorganic phosphate
NMR	Nuclear magnetic resonance
DCHF-DA	Dichlorofluorescein diacetate
DCF	Fluorescent dichlorofluorescein
ANOVA	Analysis of variance
COVID-19	Coronavirus disease
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
MAO	Monoamine oxidase
IL	Interleukin
BBB	Blood–brain barrier
ORT	Object recognition task
STM	Short-term memory
LTM	Long-term memory
MP	Mitochondrial pellet

Introduction

Depression is a neuropsychiatric disease whose main characteristics are persistent depressed mood and anhedonia (Anisman et al. 2008). According to the World Health Organization (2021), about 264 million individuals are affected by this disorder (Miret et al. 2013). Stress has been the main factor associated with the development of depression, promoting an imbalance in body homeostasis, resulting in physiological, pathological, and/or cognitive changes that mainly affect the central nervous system (CNS) (Linthorst and Reul 2008; Mariotti 2015). In addition, relatively high rates of stress (81.9%) are associated with the impact of the Coronavirus 2019 disease pandemic (COVID-19), affecting the mental health of the population (Xiong et al. 2020).

In parallel, depression can develop as a consequence of some conditions, such as Alzheimer's disease (AD) (Heun et al. 2013). In fact, stress (mainly psychosocial) and depression are usually accompanied by memory impairment, which is one of the most important clinical characteristics of patients with AD (Marazziti et al. 2010; Murrough et al. 2011). In this context, depression is considered one of the most prevalent comorbidities in AD, being responsible for affecting about 50% of patients, and associated with a great social, medical, and economic burden (Chi et al. 2014; Romano et al. 2015). An animal model of acute restraint stress (ARS) is commonly used to induce behavior related to depression in rodents, which can induce behavioral and neurochemical changes similar to those found in patients with this disorder (Klenerová et al. 2007). In addition, studies report that ARS may cause memory impairment in rodents and can be useful to assess the comorbidities of depression and memory impairment/AD (Baker and Kim 2002; Walesiuk et al. 2005; Nagata et al. 2009; Li et al. 2012). Indeed, molecular mechanisms related to the depression and memory impairment/AD dyad are considered complex and still poorly understood. Under these circumstances, research to elucidate the mechanisms involved in depression and memory impairment/DA has attracted the researchers' interest.

It has been shown that in patients with depression and memory impairment/AD, there is a deregulation of the hypothalamic–pituitary–adrenal (HPA) axis, promoting an increase in the glucocorticoid levels, such as cortisol (corticosterone in rodents) (Sierksma et al. 2010). Other common neuronal pathways in depression and memory impairment/AD include dysfunction in the monoaminergic system, oxidative stress, neuroinflammatory processes, and altered neuroplasticity (Caraci et al. 2010; Sierksma et al. 2010; Maes et al. 2011). In addition, some cell signaling molecules are involved in the progression of depression associated with memory impairment/AD. Among them is the phosphatidylinositol-3-kinase (PI3K)/protein kinase B (AKT) pathway that when activated is responsible for regulating neuronal survival and neuroplasticity, which are important functions for the comorbidity in question (Li et al. 2015a). In parallel, the nuclear factor kappa B (NF-κB) can be activated in the face of different stimuli, such as inflammatory and stress responses, thus regulating the expression of inflammatory cytokines (Oeckinghaus and Ghosh 2009). Therefore, it is highly relevant to evaluate these mechanisms, as they can act as potential targets in the development of a treatment for depression associated with memory impairment/AD.

Given the multifactorial etiology of comorbidities, depression, and memory impairment/AD, besides the adverse effects of using combined drugs, multifunctional molecules with two or more complementary biological activities may represent an important advance for the treatment of these conditions. In this context, 7-chloro-4-(phenylselanyl) quinoline (4-PSQ), a quinoline functionalized with an organoselenium group, has been outstanding,

given that it demonstrated several pharmacological effects. Previously, 4-PSQ showed an effect on disorders that affect the CNS in preclinical models, by reducing neuropsychiatric symptoms, such as anxiety and depression (Reis et al. 2017; Paltian et al. 2020; Rodrigues et al. 2021), prevention of memory impairment, and cognitive deficit (Duarte et al. 2017; Pinz et al. 2018; Barth et al. 2019), as well as attenuation of AD comorbidity and anxiety in mice (Pinz et al. 2018). Additionally, the anti-AD effect of 4-PSQ in mice was evidenced (Pinz et al. 2018), as well as its action in improving the memory of aged rats (Barth et al. 2019). It should also be noted that the mechanisms related to the effects of 4-PSQ include its antioxidant action (Pinz et al. 2016; Duarte et al. 2017; Vogt et al. 2018; Luchese et al. 2020), anti-inflammatory effect (Pinz et al. 2016; Silva et al. 2017), as well as its ability to modulate the serotonergic, nitrergic, glutamatergic, and cholinergic systems, evidencing the characteristic multi-target of this molecule (Pinz et al. 2016, 2018; Reis et al. 2017; Silva et al. 2017; Vogt et al. 2018; Barth et al. 2019; Paltian et al. 2020). Furthermore, this compound has already demonstrated the absence of toxicity in mice (Reis et al. 2017; Salgueiro et al. 2017), modulation of synaptic plasticity through increased levels of neural cell adhesion molecules, and levels of polysialyltransferase in brain structures of elderly rats (Barth et al. 2019), besides reducing glutamate uptake in the brain of mice (Reis et al. 2017). Bearing in mind that this molecule presents various promising activities, the objective of this study was to determine the pharmacological activity of 4-PSQ in the dyad of depression and memory impairment induced by ARS in mice, as well as the mechanisms involved in these processes.

Materials and methods

Animals and ethical approval

The experiments were conducted using male Swiss mice (25–35 g). Animals were maintained in standard cages placed in rooms at a controlled temperature (22 ± 2 °C) and humidity (75 ± 5%), with free access to water and food, under a 12 h light/dark cycle (with lights on at 7:00 a.m.). The experiments were approved by the Committee on Care and Use of Experimental Animal Resources, Federal University of Pelotas, Brazil (CEEA 28,008–2019), following the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978). All behavioral tests were conducted during the light period of the light/dark cycle. Every effort was made to minimize the number of animals used and their discomfort.

Drugs

4-PSQ (Fig. 1a) was prepared and characterized in our laboratory using the method previously described by Duarte et al. (2017). Analysis of the ^{1}H nuclear magnetic resonance (NMR) and ^{13}C NMR spectra showed analytical and spectroscopic data in full agreement with its assigned structure. The chemical purity of 4-PSQ (99.9%) was determined by gas chromatography-mass spectrometry. Paroxetine (antidepressant, selective serotonin reuptake inhibitor) and donepezil (acetylcholinesterase inhibitor, used in the treatment of AD) were used as positive controls. All drugs were diluted in canola oil and administered at a constant volume of 10 ml/kg of body weight. All other chemicals were of analytical grade and obtained from standard commercial suppliers.

Acute restraint stress procedure

The animals were submitted to physical restraint as previously reported by Sousa et al. (2018). Mice were subjected to immobilization for 240 min using an individual rodent restraint device made of fenestrated Plexiglas, restraining physical movement. During the period of exposure to stress, the animals were deprived of water and food. Behavioral tests were performed 40 min after removal of the animals from the restraint in order to avoid non-specific motor effects because of restricted movement.

Experimental design

In the experimental protocol (Fig. 1b), the animals were randomly divided into six experimental groups (seven animals/group): (I) control (non-stressed), (II) 4-PSQ (non-stressed), (III) control—ARS, (IV) ARS + 4-PSQ, (V) ARS + Paroxetine and (VI) ARS + Donepezil. In stressed animals, the ARS protocol was performed for 240 min and non-stressed animals continued to have access to water and food during the same period. After the restriction, stressed animals were kept under standard environmental conditions (22 ± 1 °C) with free access to water and food for 10 min to readjust movements. Afterwards, animals in groups I and III received canola oil (vehicle of the compounds, 10 ml/kg, intragastrically (i.g.)), groups II and IV received 4-PSQ (10 mg/kg, i.g.), group V received paroxetine (positive control, 10 mg/kg, i.g.), and group VI received donepezil (positive control, 10 mg/kg, i.g.). After 30 min of treatments, the animals were subjected to behavioral tasks: open field test (OFT), splash test (SPT), tail suspension test (TST), forced swimming test (FST), and Y-maze task. All behavioral tests were evaluated in independent groups of animals. After the behavioral assessment, the mice were anesthetized (isoflurane inhalation) before blood collection by cardiac puncture (Parasuraman et al. 2010). Subsequently, the animals were euthanized

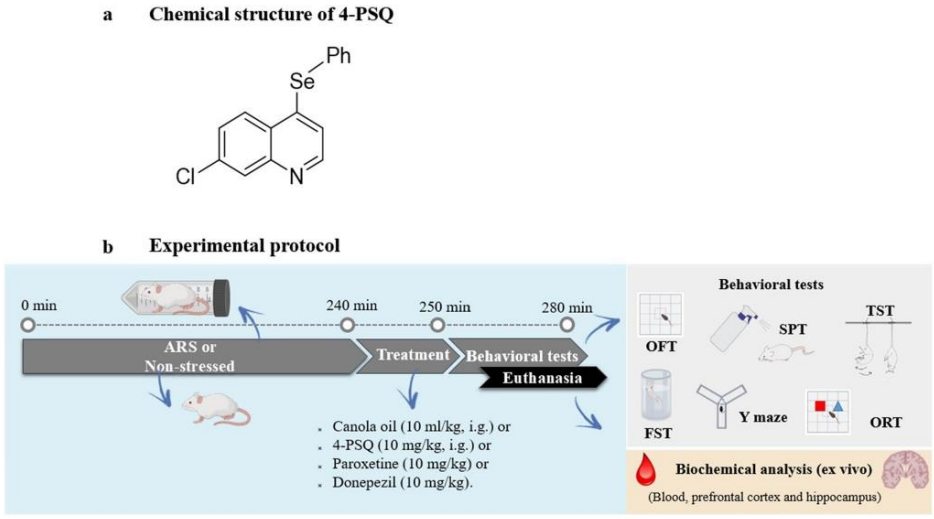

Fig. 1 **a** Chemical structure of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) and **b** Scheme of the experimental protocol. Initially, the stressed animals were subjected to acute restraint stress (ARS) for 240 min and the non-stressed animals continued to have access to water and feed during the same period. Then, the stressed animals were removed for 10 min to readjust their movements, and then all received canola oil (10 ml/kg) or 4-PSQ (10 mg/kg, i.g.) or paroxetine (10 mg/kg, i.g.) or donepezil (10 mg/kg, i.g.) intragastrically (i.g.). After 30 min of treatment, the animals were subjected to behavioral tests: open field test (OFT), splash test (SPT), tail suspension test (TST), forced swimming test (FST), Y-maze task, and object recognition test (ORT). After the behavioral assessment, the animals were sacrificed for biochemical analyses (ex vivo)

to remove brain structures, prefrontal cortex, and hippocampus, for biochemical analysis. The dose, vehicle, and administration time/route of 4-PSQ treatment were based on previous studies (Pesarico et al. 2015; Sousa et al. 2018; Casaril et al. 2019; Domingues et al. 2019; Martini et al. 2019; Pregardier Klann et al. 2020), in order to minimize the number of animals, a dose–response curve was not used in the present study.

Behavioral tests

All behavioral tests were scored by a blinded observer; i.e., the researcher does not know to which treatment the mouse was submitted.

OFT

OFT evaluated the general locomotor and exploratory behavior of mice in order to exclude any psychomotor alterations (Walsh and Cummins 1976). The open field was made of plywood and surrounded by 30 cm-high walls. Nine squares (3 rows of 3) marked by masking tape, divided the open field into 45 cm long and 45 cm wide sections. Thirty minutes after treatments, each animal was placed at the center of the open field and observed for 4 min to record the locomotor (number of segments crossed with the four paws) and exploratory (number of rearings on the hind limbs) activities.

SPT

The grooming behavior of mice was observed as a measurement of motivational and self-care activities, which are considered to be reduced in depressive patients. This method consists of spraying a 10% sucrose solution on the back of mice in their home cage. The sucrose solution dirties the coat and induces grooming behavior (nose/face, head, and body) due to its viscosity. The total grooming time is assessed over a period of 5 min (Freitas et al. 2013).

TST

TST was conducted as described by Steru et al. (1985). Mice were suspended 50 cm above the ground by an adhesive tape placed approximately 1 cm from the tip of the animal's tail. Mice were considered immobile only when they hung passively and completely motionless. Immobility time was

manually recorded during a 6-min period (Kaster et al. 2005).

FST

FST was conducted using the method described by Porsolt et al. (1977). In this test, mice were individually forced to swim in an open cylindrical container (10 cm in diameter and 25 cm in height), containing 19 cm of water at 25 ± 1 °C. The duration of immobility was scored during a 6-min period (Kaster et al. 2005). Each mouse was considered immobile when floating motionless or making only those movements necessary to keep its head above the water.

Y-maze task The Y-maze task was performed as described by Sarter et al. (1988) and it was used as a measure of working memory. The Y-maze apparatus consisted of a three-armed horizontal maze (40 cm long and 3 cm wide with walls 12 cm high) in which the three arms at 120° angles to each other radiate out from a central point. Briefly, mice were initially placed within one arm (A), and the arm entry sequence (e.g., ABCCAB, where letters indicate arm codes) and the number of arm entries were recorded in an 8-min period. Alternation was determined from successive entries into the three arms on overlapping triplet sets in which three different arms are entered. An actual alternation was defined as entries into all three arms consecutively (i.e., ABC, BCA, or CAB but not ABA). An entry was defined as placing all four paws within the boundaries of the arm.

Object recognition task Object recognition task (ORT) was used to assess the short-term (STM) and long-term (LTM) memories of mice in an open field apparatus, according to Lueptow (2017) with some modifications. Initially, the animals were submitted to a habituation session in the absence of objects, for 10 min (day 1). Subsequently, four objects were used: A1, A2, B, and C. Each object had the following color patterns: blue, red, and yellow. All objects were made of plastic, measuring 10 × 10 cm (length × height). On the second day of the experimental protocol, the ARS and treatments were performed (according to item 2.4) before the training session. During the training, 24 h after the habituation session (day 2), the animals were placed in the arena containing two identical objects (objects A1 and A2) for 5 min. Object exploration was defined as sniffing, touching the object with the nose, or pointing the nose towards the object from a distance shorter than 2 cm. The STM of mice was evaluated 1 h after training in the presence of a familiar object (A1) and a new object (B), and the total time spent in exploring each object was determined for 5 min to measure the learning and recognition memory. The LTM was performed 24 h after training (day 3), where mice were placed to explore a familiar object (A1) and a new object (C) for 5 min and the total time spent in exploring each object was determined. In order to analyze the cognitive performance, the exploratory preference was calculated and data were expressed as percentage as follows: training = (A2 / (A1 + A2)) × 100; STM = (B / (A1 + B)) × 100; LTM = (C / (A1 + C)) × 100).

Biochemical analysis

Tissue processing

Mice were anesthetized with isoflurane and blood samples collected from the heart ventricle, using heparin as an anticoagulant. Blood samples were centrifuged at 900 × *g* for 15 min to obtain plasma. Plasma was used to determine corticosterone levels. Prefrontal cortex and hippocampus were removed and immediately homogenized in cold 50 mM Tris–HCl, pH 7.4 (1/10, weight (w)/volume(v)). The homogenates were centrifuged at 900 × *g* at 4 °C for 10 min and supernatant fractions (S1) were used to determine reactive species (RS) levels, thiobarbituric acid reactive species (TBARS) levels, and activity of superoxide dismutase (SOD), glutathione peroxidase (GPx), and glutathione reductase (GR). For the determination of the activity of acetylcholinesterase (AChE), brain structures of mice were homogenized in 0.25 M sucrose buffer (1/10, w/v) and centrifuged at 900 × *g* at 4 °C for 10 min. Additionally, the prefrontal cortex and hippocampus were separated for mRNA extraction and expression of NF-kB, IL-1β, IL-18, IL-33, PI3K, and AKT2. For this, the samples were immediately processed and adequately stored (− 80 °C) until the mRNA expression levels were evaluated.

Measuring the activity of the monoamine oxidase (MAO) enzyme preparation of brain mitochondria was performed. For this, the prefrontal cortex and the hippocampus were removed and homogenized with an isolation medium (Na_2PO_4/KH_2PO_4 isotonized with sucrose, pH 7.4) (1:4, w/v). Then, the homogenate was centrifuged at 900 × g at 4 °C for 5 min. The supernatant was centrifuged at 12.500 × *g* for 15 min. The mitochondrial pellet was then washed once with an isolation medium and centrifuged under the same conditions. Finally, the mitochondrial pellet (MP) was reconstituted in a buffer solution (Na_2PO_4/KH_2PO_4 isotonized with KCl, pH 7.4) and stored in aliquots.

Plasma corticosterone level

Corticosterone levels were estimated by the fluorescence method previously described by Zenker and Bernstein (1958). Briefly, aliquots of plasma were incubated with chloroform and centrifuged for 5 min at 900 × *g*, followed by the addition of 0.1 M NaOH and another round of centrifugation. After the addition of the fluorescence reagent

(H_2SO_4 and ethanol 50%), samples were centrifuged (5 min at 900× *g*) and incubated at room temperature for 2 h. After that, the fluorescence intensity emission was recorded at 540 nm (with 257 nm excitation) and corticosterone levels were expressed as ng corticosterone/ml plasma.

MAO activity assay

MAO activity was determined according to Krajl (1965), with some modifications. An aliquot of MP of each sample was incubated at 37 °C for 5 min in a medium containing buffer solution and specific inhibitors, selegiline (a MAO-B inhibitor, 250 nM) or clorgiline (a MAO-A inhibitor, 250 nM). Then, kynuramine dihydrobromide was added to the reaction mixture (90 µM (MAO-A) and 60 µM (MAO-B)) as substrate. Samples were then incubated at 37 °C for 30 min. After incubation, the reaction was terminated by adding 10% trichloroacetic acid (TCA). After cooling and centrifugation at 3000×g for 15 min, an aliquot of the supernatant was added to 1 M NaOH. The fluorescence intensity was detected spectrofluorimetrically with excitation at 315 nm and emission at 380 nm (Shimadzu RF-5301 PC). Clorgiline (100 nM) and selegiline (100 nM) were used as positive controls in MAO-A and MAO-B assays, respectively. The concentration of 4-hydroxyquinoline (4-OH quinoline) was estimated from a corresponding standard fluorescence curve of 4-OH quinoline. MAO activity was expressed as nmol 4-OH quinoline/mg protein/min.

Oxidative parameters

Samples of the prefrontal cortex and hippocampus were collected to determine RS and TBARS levels. These measurements were performed to evaluate the effect of 4-PSQ on the modulation of cerebral oxidative stress.

RS levels were determined by a spectrofluorimetric method, using a 2′,7′ dichlorofluorescein diacetate (DCHF-DA) assay (Loetchutinat et al. 2005). Briefly, an aliquot of S1 was incubated with 1 mM DCHF-DA and 10 mM Tris–HCl pH 7.4. The oxidation of DCFH-DA to fluorescent dichlorofluorescein (DCF) is measured for the detection of intracellular RS. The DCF fluorescence intensity emission was recorded at 520 nm (with 480 nm excitation) (Shimadzu RF-5301 PC). RS levels were expressed as units of fluorescence.

TBARS content was used as a marker of lipid peroxidation (Ohkawa et al. 1979). An aliquot of S1 was added to the reaction mixture containing: thiobarbituric acid (0.8%), sodium dodecyl sulfate (8.1%), and acetic acid (pH 3.4) and incubated at 95 °C for 2 h. The absorbance was measured at 532 nm in a spectrophotometer (Shimadzu RF-5301 PC). Results were reported as nmol malondialdehyde (MDA)/mg protein.

Antioxidant enzymes

SOD activity was assayed spectrophotometrically as described by Misra and Fridovich (1972). This method is based on the capacity of SOD to inhibit the autoxidation of epinephrine. Briefly, S1 was diluted 1:10 (v/v) to determine SOD activity. An S1 aliquot was added to a 0.05 M Na_2CO_3 buffer and the enzymatic reaction was started by adding the epinephrine. The color reaction was measured at 480 nm (Shimadzu RF-5301 PC spectrophotometer). One unit of enzyme was defined as the amount of enzyme required to inhibit the rate of epinephrine autoxidation by 50% at 37 °C. Results were expressed as units U SOD/mg protein.

GPx activity was evaluated spectrophotometrically (Shimadzu RF-5301 PC) using the method described by Wendel (1981) which involves monitoring the dismutation of H_2O_2 in the presence of S1 at 340 nm. S1 was added to a system composed of reduced glutathione (GSH)/ nicotinamide adenine dinucleotide phosphate (NADPH)/ GR, and the enzymatic reaction was initiated by the addition of H_2O_2. In this assay, the enzyme activity is indirectly measured by NADPH decay. H_2O_2 is reduced and generates oxidized glutathione (GSSG) from GSH. GSSG is regenerated back to GSH by the GR present in the analysis medium at the expense of NADPH. Enzymatic activity was expressed as nmol NADPH/min/mg protein.

GR activity was determined spectrophotometrically by a method described by Carlberg and Mannervik (1985). In this assay, GSSG is reduced by GR at the expense of NADPH consumption, which follows at 340 nm (Shimadzu RF-5301 PC spectrophotometer). An aliquot of S1 was added to the system containing 0.15 M potassium phosphate buffer, pH 7.0, 1.5 mM ethylene diamine tetra-acetic acid, 0.15 mM NADPH. After the basal reading, the substrate (20 mM GSSG) was added to assess the activity in GR. Enzymatic activity was expressed as nmol NADPH/min/mg protein.

RNA extraction and expression of NF-kB, PI3K, and AKT2 by real-time PCR

Total mRNA was extracted from 50 to 100 mg of prefrontal cortex and hippocampus tissue using TRIzol reagent (Invitrogen™, Carlsbad, USA) followed by DNase treatment with DNase I Amplification Grade (Invitrogen™, Carlsbad, USA) in order to ensure minimum DNA contamination of the samples. The total RNA isolated was quantified and its purity (260/280 and 260/230 ratios) was examined by a NanoVuel spectrophotometer (GE, Fairfield, CT, USA).

The cDNA synthesis was performed using a High-Capacity cDNA Reverse Transcription kit (Applied Biosystems™, UK), according to the manufacturer's protocol. For reverse transcription, 1 µg of total RNA was used in a reaction volume of 20 µl. The amplification was performed

with GoTaq® qPCR Master Mix (Promega, Madison, WI) using the LightCycler® 96 Real-Time PCR System (Roche Molecular Systems Inc., CA, USA) and the sequences of primers used are indicated in Table 1. The qPCR conditions were as follows: 10 min at 95 °C to activate the hot-start Taq polymerase, followed by 35 cycles of denaturation for 15 s at 95 °C, primer annealing for 60 s at 60 °C, and extension for 30 s at 72 °C (fluorescence signals were detected at the end of every cycle). Baseline and threshold values were automatically set by the LightCycler® 96 Software. The number of PCR cycles required to reach the fluorescence threshold in each sample was defined as the Ct value. The $2^{-\Delta\Delta CT}$ method was used to normalize the fold change in gene expressions (Livak and Schmittgen 2001), using GAPDH as the housekeeping gene.

AChE activity

The AChE enzymatic assay was performed according to the method of Ellman et al. (1961), with some modifications, using acetylthiocholine (AcSCh) as substrate. Briefly, an aliquot of S1 is added to a medium containing 100 mM potassium phosphate buffer, pH 7.5. The enzymatic reaction is initiated by the addition of 0.5 mM 5,5-dithio-bis-(2-nitrobenzoic acid) (DTNB), and 0.8 mM AcSCh. The hydrolysis rate of the acetylcholin iodide was measured at 412 nm for 2 min. Results were expressed as µmol AcSCh/h/mg protein.

Protein determination

The protein concentration was measured by the method of Bradford (1976), using bovine serum albumin as the standard.

Statistical analysis

Data are expressed as means ± standard error of the mean (SEM). Data were analyzed by Graphpad Prism® 5. D'Agostino and Pearson omnibus normality tests evaluated data normality. Statistical analysis was performed using a one-way analysis of variance (ANOVA) followed by Tukey's test, when appropriate. Values less than 0.05 ($p < 0.05$) were considered statistically significant.

Results

4-PSQ attenuated ARS-induced depressive-like behavior without altering locomotor and exploratory activity

The data analysis of OFT showed no change in the number of crossings (ANOVA: $F_{(5,36)} = 0.9358$, $p = 0.4696$) and rearings (ANOVA: $F_{(5,36)} = 2.864$, $p = 0.0281$) after the different treatments in mice (Fig. 2a and b).

The effects of treatments on the grooming time of mice in the SPT are presented in Fig. 2c. The data analysis of SPT demonstrated that mice exposed to ARS decreased (around 59%) the grooming time when compared to the non-stressed control group. Treatment with 4-PSQ attenuated the grooming time of stressed mice. This effect was similar to the donepezil treatment but less than the administration of paroxetine in attenuating the grooming time in SPT. No changes in the grooming time in SPT were observed after per se treatment with 4-PSQ. (ANOVA: $F_{(5,36)} = 35.71$, $p < 0.0001$).

Figure 2d shows the effect of the treatments on the immobilization time in the TST. The one-way ANOVA followed by Tukey's post hoc test revealed that mice submitted to ARS showed an increase (around 34%) in the duration of immobility

Table 1 Primers used for quantitative real-time polymerase chain reaction. Listed are the forward and reverse primer sequences used to amplify each target gene as well as the GAPDH endogenous control

Primer name	Sequence	Reference
NF-κB forward	5′ AGAGAAGCACAGATACCACTAAG 3′	Li et al. (2017)
NF-κB reverse	5′ CAGCCTCATAGAAGCCATCC 3′	
IL-1β forward	5′ AGTTGACGGACCCCAAAAG 3′	Silverman et al. (2015)
IL-1β reverse	5′ AGCTG GATGCTCTCATCAGG 3′	
IL- 18 forward	5′ CAACTCAGGAGTCTTGCTCAACA 3′	Elhija et al. (2008)
IL-18 reverse	5′ CAGGCCTGACATCTTCTGCAA 3′	
IL-33 forward	5′ CTGCAAGTCAATCAGGCGAC 3′	Kurow et al. (2017)
IL-33 reverse	5′ TGCAGCCAGATGTCTGTGTC 3′	
PI3K forward	5′ CTCTCCTGTGCTGGCTACTGT 3′	Liu et al. (2014)
PI3K reverse	5′ GCTCTCGGTTGATTCCAAACT 3′	
AKT2 forward	5′ CAGCTGGGAGACCCAAGA 3′	Brand et al. (2015)
AKT2 reverse	5′ CACACGCTGTCACCTAGCTT 3′	
GAPDH forward	5′ TGCGACTTCAACAGCAACTC 3′	Bruckert et al. (2016)
GAPDH reverse	5′ ATGTAGGCAATGAGGTCCAC 3′	

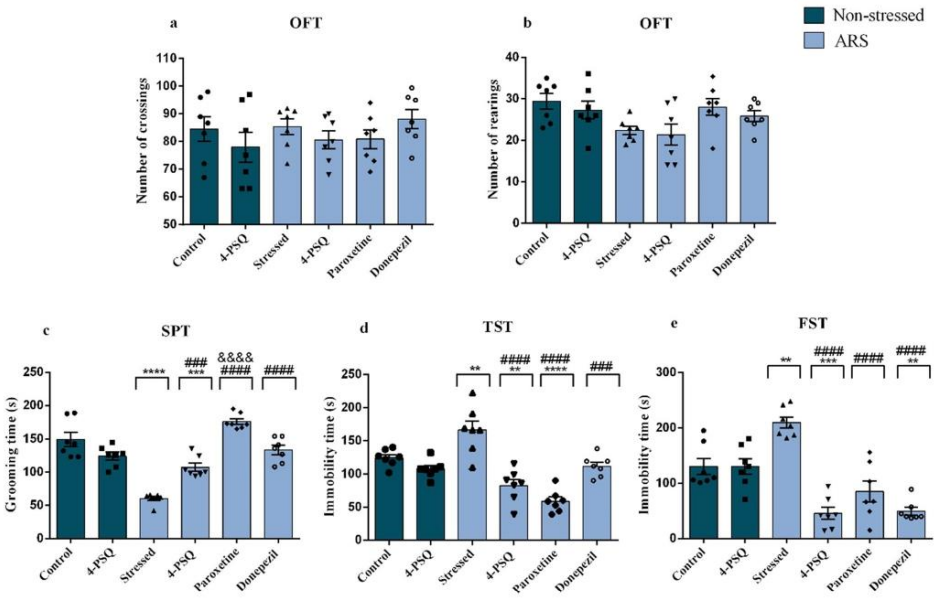

Fig. 2 Effect of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) or paroxetine or donepezil on the behaviors induced by acute restraint stress (ARS). **a** Crossings and **b** rearings in the open field test (OFT), **c** splash test (SPT), **d** tail suspension test (TST), and **e** forced swimming test (FST). Values are expressed as mean ± standard error of the mean (S.E.M.) ($n=7$). (**) denotes $p<0.01$, (***) denotes $p<0.001$, and (****) denotes $p<0.0001$ when compared to the non-stressed control group. (###) denotes $p<0.001$ and (####) denotes $p<0.0001$ when compared to the ARS-induced group. (&&&&) denotes $p<0.0001$ when compared to the ARS + 4-PSQ group (one-way ANOVA followed by Tukey's test)

time, when compared to the non-stressed control group. The immobility time was attenuated by the 4-PSQ similarly to the treatment with the positive controls (paroxetine and donepezil). 4-PSQ per se did not change the immobility time in TSTin mice (ANOVA: $F_{(5,36)}=20.55$, $p<0.0001$).

Figure 2e shows the effect of 4-PSQ, paroxetine, and donepezil on the immobilization time in the FST. The post hoc analysis demonstrated that mice exposed to ARS showed an increase (around 61%) in the duration of immobility time, when compared to the non-stressed control group. Treatment with 4-PSQ was able to attenuate the increase in immobility time induced by ARS similar to paroxetine and donepezil. Treatment with 4-PSQ per se did not change the behavior of mice in FST (ANOVA: $F_{(5,36)}=22.10$, $p<0.0001$).

4-PSQ attenuated the memory impairment caused by ARS-induction

The one-way ANOVA followed by Tukey's post hoc test revealed that ARS reduced (around 30%) the spontaneous alternation behavior in the Y-maze task, when compared to the non-stressed control group (Fig. 3a). This behavior was attenuated by treatment with 4-PSQ in a manner similar to donepezil. On the other hand, treatment with paroxetine did not alter the spontaneous alternation behavior in the Y-maze task. Treatment with 4-PSQ per se did not change the spontaneous alternation behavior of mice (ANOVA: $F_{(5,36)}=6.447$, $p=0.0002$). No change was observed in the number of arm entries after treatments in mice (ANOVA: $F_{(5,36)}=2.191$, $p=0.0767$) (data not shown).

Figures 3b and c illustrate the results obtained in the STM and LTM in the ORT, respectively. One-way ANOVA followed by Tukey's test revealed that there was no difference in exploratory preference for objects between groups in the training phase (ANOVA: $F_{(5,36)}=2.836$, $p=0.0293$) (data not shown). On the other hand, ARS reduced (around 34%) the exploratory preference for the new object in the STM, when compared to the non-stressed control group (Fig. 3b). This behavior was attenuated by treatment with 4-PSQ similar to donepezil, but it was superior to paroxetine

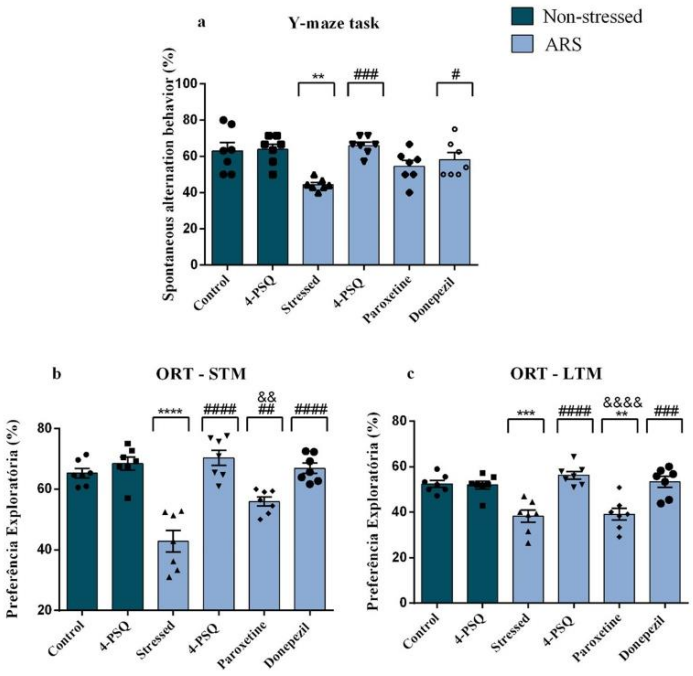

Fig. 3 Effect of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) or paroxetine or donepezil on the behaviors induced by acute restraint stress (ARS). **a** Y-maze task, **b** short-term memory (STM), and **c** long-term memory (LTM) in an object recognition test (ORT). Values are expressed as mean ± standard error of the mean (S.E.M.) ($n=7$). (**) denotes $p<0.01$, (***) denotes $p<0.001$, and (****) denotes $p<0.0001$ when compared to the non-stressed control group. (#) denotes $p<0.05$, (##) $p<0.01$, (###) denotes $p<0.001$, and (####) denotes $p<0.0001$ when compared to the ARS-induced group. (&&) denotes $p<0.01$ and (&&&&) denotes $p<0.0001$ when compared to the ARS + 4-PSQ group (one-way ANOVA followed by Tukey's test)

(ANOVA: $F_{(5,36)}=21.02$, $p<0.0001$). The results described in Fig. 3c demonstrate that ARS reduced (around 27%) the exploratory preference for the new object in the LTM, when compared to the non-stressed control group (Fig. 3c), assessed 24 h after the training phase. Treatment with 4-PSQ attenuated this behavior similarly to donepezil. On the other hand, treatment with paroxetine did not alter the exploratory preference for the new object in LTM (ANOVA: $F_{(5,36)}=13.21$, $p<0.0001$). Treatment with 4-PSQ per se did not change the exploratory preference for the new object in STM and LTM on ORT.

4-PSQ attenuated the HPA axis activation caused by ARS-induction

As illustrated in Fig. 4, ARS increased (around 256%) circulating corticosterone levels in stressed animals, when compared to the non-stressed control group. The effect of 4-PSQ in reducing corticosterone levels in stressed mice was similar to paroxetine, but it was superior to donepezil. 4-PSQ per se did not change the circulating corticosterone levels in mice (ANOVA: $F_{(5,36)}=40.40$, $p<0.0001$).

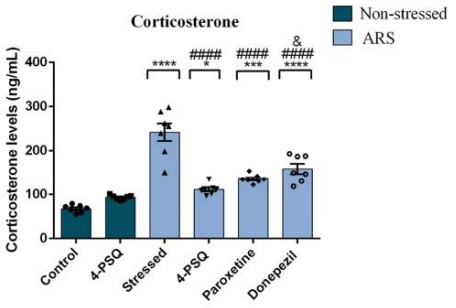

Fig. 4 Effect of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) or paroxetine or donepezil on corticosterone plasma levels in mice submitted to acute restraint stress (ARS). Values are expressed as mean ± standard error of the mean (S.E.M.) ($n=7$). (*) denotes $p<0.05$, (***) denotes $p<0.001$, and (****) denotes $p<0.0001$ when compared to the non-stressed control group. (####) denotes $p<0.0001$ when compared to the ARS-induced group. (&) denotes $p<0.05$ when compared to the ARS + 4-PSQ group (one-way ANOVA followed by Tukey's test)

4-PSQ modulated the MAO-A and MAO-B enzymes activity in the prefrontal cortex and hippocampus of stressed mice

The results described in Table 2 demonstrate that ARS increased MAO-A activity in the prefrontal cortex (around 216%) and in the hippocampus (around 129%) of mice, when compared to the non-stressed control group. MAO-A activity was normalized in the prefrontal cortex and hippocampus of stressed mice after treatment with 4-PSQ (Table 2). Paroxetine treatment normalized the activity of this enzyme only in the prefrontal cortex of stressed mice. On the other hand, donepezil was not able to normalize MAO-A activity in the evaluated brain structures. No changes in the activity of the MAO-A enzyme in the prefrontal cortex and hippocampus were observed after per se treatment with 4-PSQ (ANOVA: $F_{(5,36)}=22.77$, $p<0.0001$ for the prefrontal cortex; ANOVA: $F_{(5,36)}=9.47$, $p<0.0001$ for the hippocampus).

ARS increased MAO-B activity in the prefrontal cortex (around 88%) and hippocampus (around 62%), when compared to the non-stressed control group (Table 2). Treatment with 4-PSQ normalized MAO-B activity in the prefrontal cortex and hippocampus of stressed mice (Table 2). No changes were observed in MAO-B activity in cerebral structures of stressed mice after treatment with paroxetine or donepezil. 4-PSQ per se did not change MAO-B activity in the brain structures of mice (ANOVA: $F_{(5,36)}=6.15$, $p=0.0003$ for the prefrontal cortex; ANOVA: $F_{(5,36)}=4.70$, $p=0.0021$ for the hippocampus).

4-PSQ reduced oxidative damage in the prefrontal cortex and hippocampus of stressed mice

Figures 5a and b illustrate RS levels in the prefrontal cortex and hippocampus of mice. ARS increased RS levels in the prefrontal cortex (around 36%) (Fig. 5a) and in the hippocampus (around 124%) (Fig. 5b) of mice, when compared to the non-stressed control group. Treatment with 4-PSQ significantly reduced the production of RS caused by ARS in the cerebral structures (Fig. 5a and b). Treatment with donepezil reduced the formation of RS only in the hippocampus of stressed mice (Fig. 5b). However, the effect of 4-PSQ in reducing the RS levels in the hippocampus of stressed mice was superior to treatment with donepezil (Fig. 5b). The treatment with paroxetine noticeably reduced RS levels in the prefrontal cortex (Fig. 5a) and hippocampus (Fig. 5b) of stressed mice. No changes in the RS levels in the prefrontal cortex and hippocampus were observed after per se treatment with 4-PSQ (ANOVA: $F_{(5,36)}=8.15$, $p<0.0001$ for prefrontal cortex; ANOVA: $F_{(5,36)}=73.94$, $p=0.0001$ for hippocampus).

In the TBARS levels, ARS increased this parameter in the prefrontal cortex (around 32%) (Fig. 5c) and in the hippocampus (around 38%) (Fig. 5d), when compared to the non-stressed control group. 4-PSQ treatment reduced TBARS levels in the prefrontal cortex (Fig. 5c) and hippocampus (Fig. 5d) of stressed mice, while treatments with positive controls did not have any effect (Fig. 5c on the prefrontal cortex and Fig. 5d hippocampus). 4-PSQ per se did not change the TBARS levels in the prefrontal cortex and hippocampus of mice (ANOVA: $F_{(5,36)}=4.563$, $p=0.0025$ for prefrontal cortex; ANOVA: $F_{(5,36)}=5.998$, $p=0.0004$ for hippocampus).

4-PSQ modulated the antioxidant enzymes in the prefrontal cortex and hippocampus of stressed mice

Figures 6a and b summarize the results of SOD activity in the prefrontal cortex and hippocampus, respectively, of mice after the experimental protocol. ARS increased the SOD activity in the prefrontal cortex (around 96%) (Fig. 6a) and in the hippocampus (around 82%) (Fig. 6b), when compared to the non-stressed control group. Treatment with 4-PSQ normalized the SOD activity in the cerebral structures of stressed mice, while donepezil and paroxetine did not have

Table 2 Effect of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) or paroxetine or donepezil on the activity of monoamine oxidase (MAO)-A and B isoforms in the prefrontal cortex and hippocampus of mice subjected to acute restraint stress (ARS)

	Prefrontal cortex		Hippocampus	
Experimental groups	MAO A	MAO B	MAO A	MAO B
Non-stressed – control	0.54 ± 0.09	0.65 ± 0.06	0.99 ± 0.09	1.05 ± 0.09
Non-stressed – 4-PSQ	0.82 ± 0.05	0.55 ± 0.11	1.65 ± 0.30	1.60 ± 0.06
ARS—stressed	1.73 ± 0.12****	1.22 ± 0.09**	2.26 ± 0.14**	1.71 ± 0.11**
ARS – 4-PSQ	0.42 ± 0.04####	0.68 ± 0.08#	1.05 ± 0.12##	1.11 ± 0.16#
ARS—Paroxetine	1.46 ± 0.15 ****&	0.93 ± 0.14	1.28 ± 0.20#	1.28 ± 0.12
ARS—Donepezil	1.45 ± 0.15****&	1.16 ± 0.15*	2.70 ± 0.30****&	1.58 ± 0.17

Values are expressed as mean ± standard error of the mean (S.E.M.) ($n=7$). (**) denotes $p<0.01$ and (****) denotes $p<0.0001$ when compared to the non-stressed control group. (#) denotes $p<0.05$, (##) denotes $p<0.01$, and (####) denotes $p<0.0001$ when compared to the ARS-induced group. (&) denotes $p<0.05$ when compared to the ARS + 4-PSQ group (one-way ANOVA followed by Tukey's test)

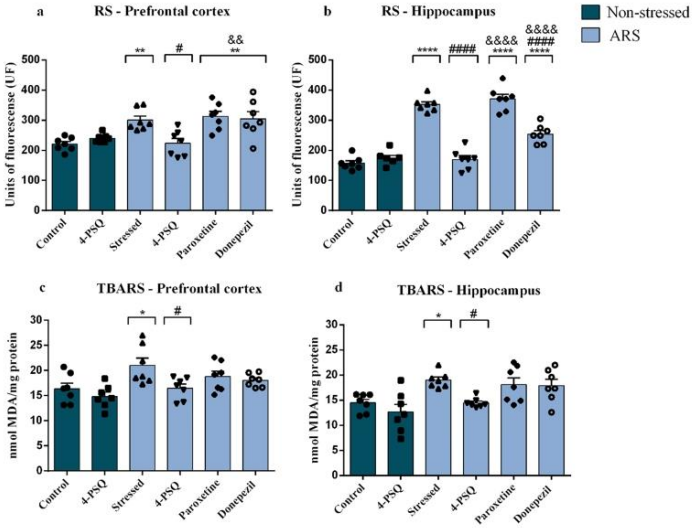

Fig. 5 Effect of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) or paroxetine or donepezil on markers of oxidative stress in mice submitted to acute restraint stress (ARS). Reactive species (RS) levels in **a** prefrontal cortex and **b** hippocampus; thiobarbituric acid reactive species (TBARS) levels in **c** prefrontal cortex and **d** hippocampus. Values are expressed as mean ± standard error of the mean (S.E.M.) ($n = 7$). (*) denotes $p < 0.05$, (**) denotes $p < 0.01$, and (****) denotes $p < 0.0001$ when compared to the non-stressed control group. (#) denotes $p < 0.05$ and (####) denotes $p < 0.0001$ when compared to the ARS-induced group. (&&) denotes $p < 0.01$ and (&&&&) denotes $p < 0.0001$ when compared to the ARS + 4-PSQ group (one-way ANOVA followed by Tukey's test)

any effect (Fig. 6a for prefrontal cortex and Fig. 6b for hippocampus). No changes were observed in the SOD activity in the prefrontal cortex and hippocampus after per se treatment with 4-PSQ (ANOVA: $F_{(5,36)} = 13.42$, $p < 0.0001$ for the prefrontal cortex; ANOVA: $F_{(5,36)} = 43.71$, $p < 0.001$ for the hippocampus).

For GPx activity, the one-way ANOVA followed by Tukey's post hoc test showed that ARS increased enzyme activity in the prefrontal cortex (around 64%) (Fig. 6c) and in the hippocampus (around 48%) (Fig. 6d) of mice, when compared to the non-stressed control group. Treatment with 4-PSQ normalized the GPx activity in cerebral structures of stressed mice, and this effect was similar to paroxetine. On the other hand, the effect of donepezil was superior to that of 4-PSQ in normalizing the enzyme activity in the prefrontal cortex. 4-PSQ per se did not change the GPx activity in the prefrontal cortex and hippocampus of mice (ANOVA: $F_{(5,36)} = 11.70$, $p < 0.0001$ for the prefrontal cortex; ANOVA: $F_{(5,36)} = 5.223$, $p = 0.0010$ for the hippocampus).

GR activity in the prefrontal cortex and hippocampus of mice is shown in Fig. 6e and f, respectively. ARS increased GR activity in the prefrontal cortex (around 34%) (Fig. 6e) and in the hippocampus (around 57%) (Fig. 6f) of mice, when compared to the non-stressed control group. Treatment with 4-PSQ normalized GR activity in the prefrontal cortex (Fig. 6e) and hippocampus (Fig. 6f) of stressed mice, while treatment with paroxetine normalized enzyme activity only in the hippocampus of stressed mice (Fig. 6f). Donepezil had no effect in normalizing GR activity in the brain structures of stressed mice (Fig. 6e for the prefrontal cortex and Fig. 6f for the hippocampus). No changes in GR activity were observed after per se treatment with 4-PSQ in the evaluated brain structures (ANOVA: $F_{(5,36)} = 6.326$, $p = 0.0003$ for the prefrontal cortex; ANOVA: $F_{(5,36)} = 6.292$, $p = 0.0001$ for the hippocampus).

4-PSQ modulated the NF-κB, IL-1β, IL-18, IL-33, PI3K, and AKT2 levels in the prefrontal cortex and hippocampus of stressed mice

Figure 7 illustrates the effects of treatment with 4-PSQ on NF-κB, IL-1β, IL-18, IL- 33, PI3K, and AKT2 mRNA expression levels in the prefrontal cortex and hippocampus of mice exposed to the ARS protocol. ARS increased the mRNA expression levels of NF-κB in the prefrontal cortex (around 34%) (Fig. 7a) and hippocampus (around 37%) (Fig. 7b), when compared to the non-stressed control. Treatment with 4-PSQ normalized this increase in the prefrontal cortex and hippocampus of stressed mice, similarly to paroxetine and donepezil. 4-PSQ per se did not change the mRNA expression levels of NF-κB in the prefrontal cortex and hippocampus of mice (ANOVA: $F_{(5,24)} = 9.894$, $p < 0.0001$ for the prefrontal cortex; ANOVA: $F_{(5,24)} = 15.45$, $p < 0.0001$ for the hippocampus).

ARS increased IL-1β expression in the prefrontal cortex (around 70%) (Fig. 7a), when compared to the non-stressed control group, but no changes were found in the hippocampus of stressed mice (Fig. 7b). Treatment with

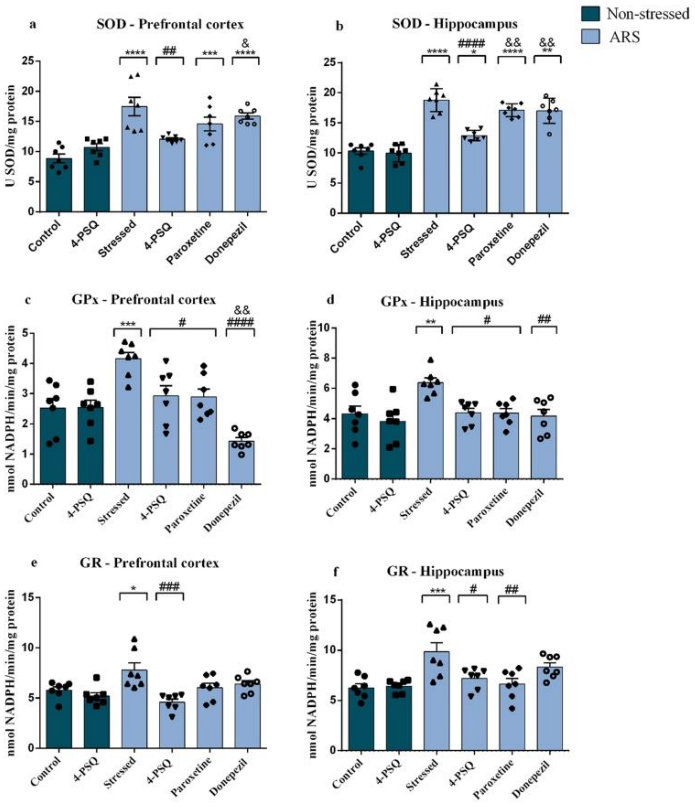

Fig. 6 Effect of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) or paroxetine or donepezil on the activity of antioxidant enzymes in mice submitted to acute restraint stress (ARS). Superoxide dismutase (SOD) activity in **a** prefrontal cortex and **b** hippocampus; Glutathione peroxidase (GPx) activity in **c** prefrontal cortex and **d** hippocampus; Glutathione reductase (GR) activity in **e** prefrontal cortex and **f** hippocampus. Values are expressed as mean ± standard error of the mean (S.E.M.) ($n = 7$). (*) denotes $p < 0.05$, (**) denotes $p < 0.01$, (***) denotes $p < 0.001$, and (****) denotes $p < 0.0001$ when compared to the non-stressed control group. (#) denotes $p < 0.05$, (##) denotes $p < 0.01$, (###) denotes $p < 0.001$, and (####) denotes $p < 0.0001$ when compared to the ARS-induced group. (&) denotes $p < 0.05$ and (&&) denotes $p < 0.01$ when compared to the ARS + 4-PSQ group (one-way ANOVA followed by Tukey's test)

4-PSQ significantly normalized the mRNA expression levels of IL-1β in the prefrontal cortex of stressed mice, similar to paroxetine. On the other hand, the effect of 4-PSQ was superior to that of donepezil. No changes in the mRNA expression levels of IL-1β were observed after per se treatment with 4-PSQ in the evaluated brain structures (ANOVA: $F_{(5,24)} = 19.82$, $p < 0.0001$ for the prefrontal cortex; ANOVA: $F_{(5,24)} = 19.16$, $p < 0.0001$ for the hippocampus).

IL-18 expression in the prefrontal cortex and hippocampus of mice is shown in Fig. 7a and b, respectively. ARS increased IL-18 expression in the prefrontal cortex (around 182%) (Fig. 7a), when compared to the non-stressed control group, but no changes were found in the hippocampus of stressed mice (Fig. 7b). Treatment with 4-PSQ normalized the mRNA expression levels of IL-18 in the prefrontal cortex of stressed mice, similarly to paroxetine and donepezil. No changes in the IL-18 expression were observed after per se treatment with 4-PSQ in the evaluated brain structures (ANOVA: $F_{(5,24)} = 24.32$, $p < 0.0001$ for the prefrontal cortex; ANOVA: $F_{(5,24)} = 4.45$, $p = 0.0052$ for the hippocampus).

In the IL-33 expression, ARS increased this parameter in the prefrontal cortex (around 185%) (Fig. 7a), when compared to the non-stressed control group, but no changes were found in the hippocampus of stressed mice (Fig. 7b). 4-PSQ treatment normalized the mRNA expression levels of IL-33 in the prefrontal cortex of stressed mice, similarly to paroxetine and donepezil. 4-PSQ per se did not change the IL-33 expression in the prefrontal cortex and hippocampus of mice (ANOVA: $F_{(5,24)} = 74.40$, $p < 0.0001$ for prefrontal cortex; ANOVA: $F_{(5,24)} = 1.245$, $p = 0.3196$ for hippocampus).

ARS decreased PI3K expression in the prefrontal cortex (around 45%) (Fig. 7a), when compared to the non-stressed control group, but no changes were found in the

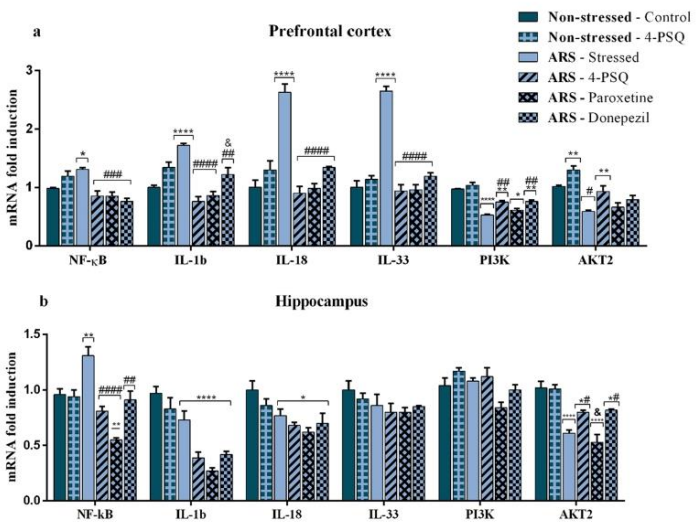

Fig. 7 Effect of 7-chloro-4- (phenylselanyl) quinoline (4-PSQ) or paroxetine or donepezil on the levels of mRNA expression of cell signaling molecules in mice subjected to acute restraint stress (ARS). Nuclear factor-kappa B (NF-κB) in **a** prefrontal cortex and **b** hippocampus; Interleukin (IL)-1β in **a** prefrontal cortex and **b** hippocampus; IL-18 in **a** prefrontal cortex and **b** hippocampus; IL-33 in **a** prefrontal cortex and **b** hippocampus; Phosphoinositide-3 kinase (PI3K) in **a** prefrontal cortex and **b** hippocampus; Protein kinase B -2 (AKT2) in **a** prefrontal cortex and **b** hippocampus. Values are expressed as mean ± standard error of the mean (S.E.M.) ($n = 5$). (*) denotes $p < 0.05$, (**) denotes $p < 0.01$ and (****) denotes $p < 0.0001$ when compared to the non-stressed control group. (#) denotes $p < 0.05$, (##) denotes $p < 0.01$, (###) denotes $p < 0.001$, and (####) denotes $p < 0.0001$ when compared to the ARS-induced group. (&) denotes $p < 0.05$ when compared to the ARS + 4-PSQ group (one-way ANOVA followed by Tukey's test)

hippocampus of stressed mice (Fig. 7b). Treatment with 4-PSQ significantly normalized the mRNA expression levels of PI3K in the prefrontal cortex of stressed mice, similarly to donepezil while paroxetine had no changes on mRNA expression levels of PI3K in the brain structures evaluated. No changes in the mRNA expression levels of PI3K were observed after per se treatment with 4-PSQ in the evaluated brain structures (ANOVA: $F_{(5,24)} = 30.99$, $p < 0.0001$ for the prefrontal cortex; ANOVA: $F_{(5,24)} = 4{,}220$, $p = 0.0068$ for the hippocampus).

ARS decreased the mRNA expression levels of AKT2 in the prefrontal cortex (around 42%) (Fig. 7a) and hippocampus (around 40%) (Fig. 7b), when compared to the non-stressed control group. Treatment with 4-PSQ significantly reversed the decrease in mRNA expression levels of AKT2 in the prefrontal cortex and hippocampus of stressed mice, while donepezil had an effect only on the hippocampus (Fig. 7a for prefrontal cortex and Fig. 7b for hippocampus). No changes were observed in the mRNA expression levels of AKT2 in cerebral structures of stressed mice after treatment with paroxetine (Fig. 7a for the prefrontal cortex and Fig. 7b for the hippocampus). 4-PSQ per se did not change the mRNA expression levels of AKT2 in the brain structures of mice (ANOVA: $F_{(5,24)} = 16.39$, $p < 0.0001$ for the prefrontal cortex; ANOVA: $F_{(5,24)} = 21.12$, $p < 0.0001$ for the hippocampus).

4-PSQ modulated the AChE activity in the prefrontal cortex and hippocampus of stressed mice

Results showed that ARS increased the AChE activity in the prefrontal cortex (around 120%) (Fig. 8a) and in the hippocampus (around 31%) (Fig. 8b) of mice, when compared to the non-stressed control group. Enzyme activity was completely normalized in the prefrontal cortex (Fig. 8a) and hippocampus (Fig. 8b) of stressed mice after treatment with 4-PSQ. Treatment with paroxetine or donepezil partially normalized AChE activity in the prefrontal cortex (Fig. 8a), but they completely normalized it in the hippocampus (Fig. 8b) of stressed mice. No changes were observed in the activity of the AChE enzyme in the prefrontal cortex and hippocampus after per se treatment with 4-PSQ (ANOVA: $F_{(5,36)} = 11.07$, $p < 0.0001$ for the prefrontal cortex; ANOVA: $F_{(5,36)} = 6.967$, $p < 0.0001$ for the hippocampus).

Discussion

The present study revealed, for the first time, the effect of the administration of 4-PSQ, a quinoline functionalized with the organoselenium group, in an ARS-induction protocol in mice. It was found that 4-PSQ attenuated depressive-like and self-care behaviors, as well as memory impairment induced by stress. Based on the evidence, we believe that the effects of 4-PSQ may be associated, at least in part, with the (I) attenuation of HPA axis activation; (II) attenuation of alterations in the monoaminergic system; (III) modulation of oxidative stress; (IV) modulation of the transcription factors involved in neuroinflammation and neuroplasticity; and (V) restoration of the cholinergic system (Fig. 9). These results suggest the pharmaceutical potential of 4-PSQ for the treatment of comorbidities, depression, and memory impairment.

In view of studies prospecting new drugs, the use of agents containing the quinoline nucleus for the treatment of diseases has been receiving attention in our research. In this context, several studies demonstrated that the quinoline fraction is the pharmacophore of several anti-AD drugs (Freeman and Dawson 1991; Mancino et al. 2009; Oz et al. 2009; Jiang et al. 2011). In parallel, selenium is an essential micronutrient for the body and plays a critical role in the proper functioning of several selenoproteins involved in the brain's antioxidant defenses (Sher 2000). Given its neuroprotective role in neuronal function, recent studies have investigated a relationship between selenium levels in preventing AD onset and progression and in depression (Wang et al. 2018; Varikasuvu et al. 2019). In addition, it is worth emphasizing that previous studies by our research group have shown that the phenylselanyl group present in the quinoline structure is critical for the antioxidant activity of 4-PSQ in a model of cerebral oxidative stress induced by sodium nitroprusside (Vogt et al. 2018). In this context, we have demonstrated the importance of selenium in positive biomedical effects of 4-PSQ.

Moreover, Couto et al. (2019) demonstrated that 4-PSQ was able to restore selenium levels in the head and body of *Drosophila melanogaster* flies in a Parkinson-like disease model, preventing the damage caused in this model. The authors suggested that 4-PSQ was able to penetrate the blood–brain barrier (BBB), since high levels of selenium in the heads of flies were observed after treatment with 4-PSQ (Couto et al. 2019). In addition, Barth et al. (2021) demonstrated that treatment with 4-PSQ restored plasma selenium levels in elderly rats, thus contributing to the restoration of damage caused by aging. Therefore, it is suggested that 4-PSQ is able to reach the bloodstream after its absorption (Barth et al. 2021). In this sense, considering that complex disorders, such as depression and memory impairment, are more likely to be attenuated through the simultaneous modulation of multiple targets, the versatility of 4-PSQ led us to consider its use as a possible therapeutic strategy for comorbidities depression and memory impairment.

Recent studies have shown that stress has a negative impact on the mental health of the population (Li et al. 2020; Huang and Zhao 2020). Among the main issues linked to stress is the current world situation, with the COVID-19 pandemic. Important data reveal that when the pandemic began, social isolation was proposed as one of the most effective measures to combat the spread of the new coronavirus SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) (Adalja et al. 2020). Despite this, social isolation has had a negative psychological influence, through increased psychosocial stress, anxiety, and neuropsychiatric symptoms, revealing significant psychiatric morbidities (Holmes et al. 2020; Xiang et al. 2020). Thus, society

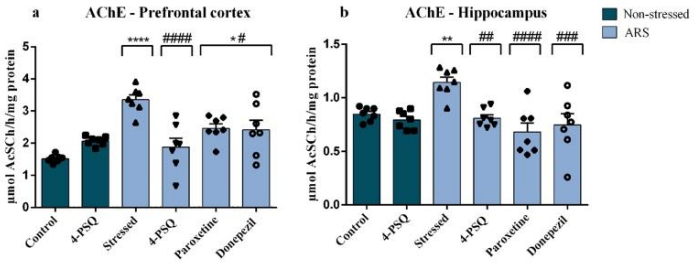

Fig. 8 Effects of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) or paroxetine or donepezil on the activity of acetylcholinesterase (AChE) in **a** prefrontal cortex and **b** hippocampus in mice submitted to acute restraint stress (ARS). Values are expressed as mean ± standard error of the mean (S.E.M.) ($n = 7$). (*) denotes $p < 0.05$, (**) denotes $p < 0.01$, and (****) denotes $p < 0.0001$ when compared to the non-stressed control group. (#) denotes $p < 0.05$, (##) denotes $p < 0.01$, (###) denotes $p < 0.001$, and (####) denotes $p < 0.0001$ when compared to the ARS-induced group (one-way ANOVA followed by Tukey's test)

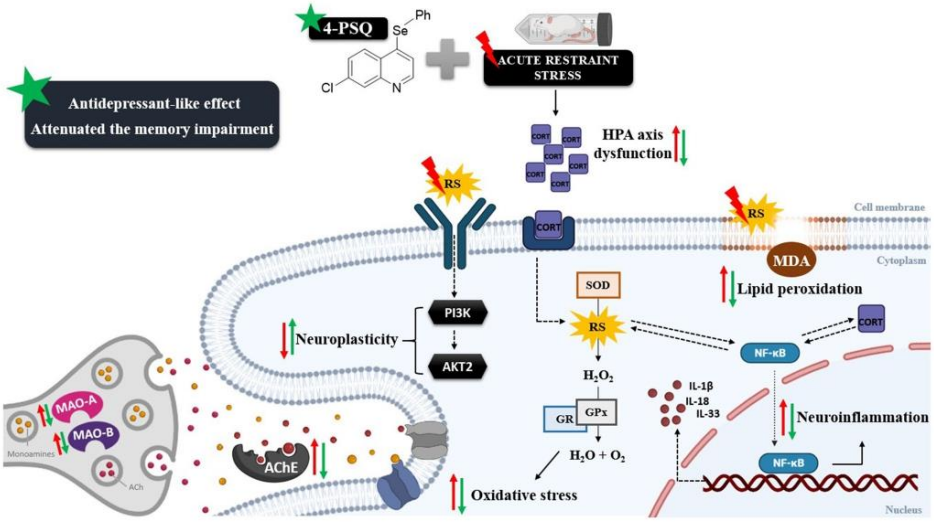

Fig. 9 Summary of the effects of acute containment stress (ARS) on the monoaminergic system, oxidative pathways, neuroplasticity, neuroinflammatory pathway, hypothalamic–pituitary–adrenal (HPA) axis, cholinergic system, and the possible targets of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ). Treatment with 4-PSQ HPA attenuates the activation of the HPA axis by reducing plasma corticosterone (CORT) levels, attenuates changes in the monoaminergic system, by reestablishing the activity of isoforms of the monoaminooxidase (MAO) (MAO-A and MAO-B) enzyme, modulating oxidative stress, reducing levels of reactive species (RS), thiobarbituric acid reactive species (TBARS) and activity of antioxidant enzymes (superoxide dismutase [SOD], glutathione peroxidase [GPx] and glutathione reductase [GR]), attenuates neuroinflammation by reducing the levels of nuclear factor B kappa (NF-κB), interleukin (IL)-1β, IL-18, and IL-33, positively modulated the transcription factors (phosphatidylinositol-3-kinase [PI3K] and protein kinase B-2 [AKT2]) involved in neuroplasticity and finally modulated the cholinergic system by reducing the activity of the acetylcholinesterase (AChE) enzyme. Based on the evidence, we believe that this set of results contributed to the reduction of depressive-like behavior and the memory impairment induced by ARS. Abbreviations: ACh, acetylcholine; MDA, malondialdehyde; H_2O_2, hydrogen peroxide; H_2O, molecule of water; O_2, molecular oxygen

urgently needs to achieve an understanding of psychosocial stress. According to this, understanding of mechanisms involved in the depression and memory impairment dyad, caused by stress is extremely important in order to study new therapeutic alternatives capable of reducing psychosocial stress and associated symptoms.

In view of this, ARS was proposed as an accepted stress-inducing experimental model to investigate depressive-like behavior, as well as its relationship with memory impairment in rodents (Baker and Kim 2002; Walesiuk et al. 2005; Li et al. 2012; Mosaffa et al. 2021; Niksiyar et al. 2021). In response to stress, the HPA axis is rapidly activated and results in the release of glucocorticoids (Del Giudice et al. 2011). ARS is a validated model for causing hyperactivation of the HPA axis, with a notable increase in plasma corticosterone levels (Joseph and Golden 2017). The action of these glucocorticoids occurs mainly through their binding to their receptors in the brain, the glucocorticoid receptor, and the mineralocorticoid receptor in order to lead complementarily to the regular activity of the HPA axis (Harris et al. 2013). Mineralocorticoid receptors are involved in basal conditions and at the beginning of activation of the HPA axis during stress and are therefore important for the development of stress resilience (Joels and de Kloet 2017). On the other hand, glucocorticoid receptors are activated after stress is established, thus leading to a reduction in the activity of the HPA axis (de Kloet et al. 2018). Accordingly, an imbalance between mineralocorticoid receptors and glucocorticoid receptor signaling may contribute to a dysregulation of the HPA axis in response to stress, thus contributing mainly to the development of mood disorder; however, little is known about the underlying molecular mechanisms between this relationship (Harris et al. 2013; Joels and de Kloet 2017; de Kloet et al. 2018).

In the present study, ARS-induced activation of the HPA axis was confirmed by the increase in plasma corticosterone

levels in stressed mice. Importantly, our results demonstrated that 4-PSQ treatment was able to attenuate the activation of the HPA axis by reducing the plasma corticosterone levels, with an effect similar and superior to positive controls used in this study (paroxetine and donepezil, respectively). It is known that deregulation of the HPA axis causes the binding of cortisol to its glucocorticoid receptors and mineralocorticoid receptors in the limbic system, mainly in the hippocampus and prefrontal cortex that have a high density of these receptors (Lowy et al. 1984). Consequently, neuronal atrophy occurs in these regions, resulting in emotional changes and memory dysfunction, symptoms that are found in patients with depression and AD (Lupien et al. 2009; Colciago et al. 2015). In this context, we confirmed that ARS caused depressive-like behavior, as demonstrated by increasing immobility time in TST and FST, and memory impairment, as demonstrated by reducing spontaneous alternations in the Y-maze task and reducing exploratory preference for the new object in the ORT. In accordance, other authors showed that the ARS protocol increased the immobility time in TST and FST (Mosaffa et al. 2021; Niksiyar et al. 2021). In addition, a study by Wolf (2008) reported that elevated cortisol responses in humans in the presence of psychosocial stressors triggered impairments in tasks that required participants to remember information previously learned. Another study found that sustained elevation of corticosterone had deleterious effects on the structure and function of important brain regions in animal models (Mcewen and Gianaros 2010). In this way, our data confirmed that the hyperactivation of the HPA axis contributed to the development of depressive-like behavior and memory impairment observed in animals submitted to ARS.

Moreover, patients with depression have reduced self-care behavior, a symptom that can be assessed in the SPT through grooming time (Freitas et al. 2014). However, the physiological mechanisms of a relationship between stress and self-care behavior are not fully understood. It is believed that a possible mechanism to explain this relationship is the neural reward circuits, since the self-cleansing behavior induces endocrine and neural responses even under stress-free conditions (Dunlop and Nemeroff 2007; Shiota et al. 2016). Accordingly, a study by Van Erp et al. (1994) demonstrated that self-cleaning time is correlated with plasma levels of the adrenocorticotropic hormone (responsible for stimulating the release of cortisol), suggesting that this behavior is linked to the neuroendocrine response to stress. In view of this, our results showed that ARS reduced self-care behavior, demonstrating that stress can induce depression with characteristics very similar to those found in humans, as already indicated previously (Vasconcelos et al. 2015; Gong et al. 2016).

It is important to highlight that in this study, 4-PSQ treatment attenuated depressive-like behavior (as demonstrated by reducing immobility time in TST and FST), work memory (as evidenced by increasing spontaneous alternation behavior in the Y-maze test), STM, and LTM (as demonstrated by the increase in exploratory preference for the new object in the ORT), and a symptom of self-care behavior (as shown by increasing grooming time in SPT). In this context, our results highlight that 4-PSQ has an antidepressant-like action and has a role in improving memory, supporting its pharmacological effect in the treatment of stress-induced comorbidities (depression and memory impairment). We can suggest that, at least in part, 4-PSQ mitigated behavioral changes by its ability to normalize corticosterone levels, attenuating the activation of the HPA axis. Importantly, our results demonstrated that the 4-PSQ treatment was able to attenuate the behavior changes similarly to positive controls used in this study (paroxetine and donepezil). Moreover, none of the treatments caused changes in the spontaneous locomotor and exploratory activities of mice, indicating, mainly, that the effect of 4-PSQ is not due to nonspecific changes, such as psychostimulant activity. Hence, this is an important result since psychostimulant drugs may give a false-positive result in animal models (Cryan et al. 2005).

As already mentioned, stress has a major impact on different biological systems in the brain, including the monoaminergic system (Higuchi et al. 2017), an important target that has been studied in depression and memory impairment/DA comorbidity, with reduced levels of monoamines in the CNS (Vermeiren et al. 2014; Otte et al. 2016; Behl et al. 2021). MAO is the enzyme of the monoaminergic system responsible for regulating the levels of monoaminergic neurotransmitters, such as serotonin (5-HT), norepinephrine, and/or dopamine, through their oxidative deamination, thus reducing the availability of these monoamines in synapses (Finberg and Rabey 2016). This enzyme exists in two enzymatic isoforms, MAO-A and MAO-B, which differ in their substrate and inhibitor specificity and in their tissue distribution (Shih et al. 1999). Selective MAO-A isoform inhibitors act as potent antidepressants, while MAO-B inhibitors are useful in neurodegenerative disorders such as in AD (Saura et al. 1994). Thus, the dual inhibition of MAO-A and MAO-B may be valuable for the therapy of depression and memory impairment/DA comorbidity. According to this, the evaluation of MAO isoforms became a target in this study. Our results show that ARS induced an increase in the activity of MAO-A and MAO-B isoforms in the prefrontal cortex and hippocampus of mice. Thus, an increase in MAO isoform activity is associated with an imbalance in the monoaminergic system, with a reduction in neurotransmitters; and based on the evidence, we believe that this has contributed to the depressive-like behavior and memory impairment observed in our study. On the other hand, treatment with 4-PSQ attenuated the changes in MAO-A and MAO-B activity in the prefrontal cortex and hippocampus of stressed

mice, being useful for the treatment of the comorbidity in question. According to this, studies demonstrate that modulation of the monoaminergic system with increased levels of monoamines at synapses reverses some of the impacts caused by stress (Holsboer 2000; Jiang et al. 2019).

There is evidence that stress can make the brain vulnerable to oxidative damage, through the activation of intracellular pathways involved in the formation of free radicals, and consequently, it produces behavioral changes related to neuropsychiatric disorders (Che et al. 2015). In this sense, studies report a relationship between HPA axis hyperactivation and an increase in oxidative parameters (Balmus et al. 2016; Kv et al. 2018). At the cellular level, glucocorticoids, such as corticosterone, favor the increase in the metabolic rate in conjunction with catecholamines, and in turn in this process, there is an increase in RS production (Balmus et al. 2016). In the present study, our results demonstrated that ARS increased RS production and favored lipid peroxidation, as evidenced by increasing TBARS levels in the prefrontal cortex and hippocampus of mice. Furthermore, we observed an increase in SOD, GPx, and GR activities in the cerebral structures of mice submitted to ARS. Indeed, an increase in RS levels induced by stress favors the process of lipid peroxidation and changes in antioxidant defenses in limbic regions (Kim et al. 2016), causing an impairment of the antioxidant state in the brain (Moretti et al. 2013; Sousa et al. 2018; Casaril et al. 2019). Moreover, SOD, GPx, and GR are the main antioxidant defenses responsible for maintaining the redox balance (Kulak et al. 2013) and an increase in these enzyme activities is an attempt to eliminate the RS generated by ARS in cerebral structures. In accordance, some reports showed results similar to those found in our study, in which ARS caused an increase in RS content, lipid peroxidation, and SOD, GPX, and GR activities in the prefrontal cortex and hippocampus of rodents (Moretti et al. 2013; Sousa et al. 2018; Casaril et al. 2019). Therefore, based on the evidence we believe that the neurotoxic effects of hyperactivation of the HPA axis induced by stress may have helped the oxidative stress process, contributing to the development of depressive-like behavior and memory impairment.

In the present study, oxidative changes induced by ARS were reversed by treatment with 4-PSQ, as evidenced by normalization of the RS content, lipid peroxidation, and activity of antioxidant enzymes, with an action similar or superior to positive controls (donepezil and paroxetine). These results demonstrate that 4-PSQ treatment is able to reverse neuronal oxidative damage and modulate the antioxidant defense system. Previously, 4-PSQ showed that it modulated oxidative stress in different experimental models (Pinz et al. 2016, 2018; Luchese et al. 2020; Paltian et al. 2020; Lemos et al. 2021; Rodrigues et al. 2021). These results are supported by a study by Vogt et al. (2018) in which he demonstrated the antioxidant action of 4-PSQ in a cerebral oxidative stress model in mice, and they evidenced that compound action is entirely attributed to the presence of the organoselenium group in the molecule. Indeed, we cannot say whether 4-PSQ acts directly in the redox state or whether it modulates other pathways/systems, reducing the process of cerebral oxidative stress. However, we can assume that 4-PSQ acts on different lines of antioxidant defense, by attenuating neuronal oxidative damage and reestablishing the antioxidant defense system, as evidenced by its beneficial effect against depressive-like behavior and impairment of memory caused by ARS.

In addition, hyperactivation of the HPA axis in response to stress progressively generates an inflammatory environment (de Baumont et al. 2019). In the current study, we found that ARS increased NF-κB mRNA expression levels in the prefrontal cortex and hippocampus of stressed animals. ARS also increased the expression levels of IL-1β, IL-18, and IL-33 mRNA in the prefrontal cortex of mice. In fact, neuroinflammatory events are orchestrated by transcription factors, such as NF-κB, which can be activated by different stimuli, such as stress, the presence of RS, and inflammatory responses (Siomek 2012; Balmus et al. 2016). NF-κB plays a key role in inflammation through its ability to modulate the transcription of numerous pro-inflammatory genes, such as IL-1β, IL-18, and IL-33 (Jope et al. 2017). This deregulation of pro-inflammatory cytokines ultimately favors oxidative imbalance and consequently causes an increase in RS production, which can synergistically induce tissue damage, neuroinflammation, neurodegeneration, and neuronal apoptosis (Bakunina et al. 2015). In parallel, another key component in these responses is the action of pro-inflammatory cytokines on the hypothalamus, promoting an exacerbated increase in the release of cortisol, and consequently triggering hyperactivity of the HPA axis (Iwata et al. 2013). In view of these mechanisms, previous reports have shown that NF-κB activation as well as the increase in pro-inflammatory cytokines is strongly related to the development of depressive-like behavior and memory impairment in rodents (O'Neill and Kaltschmidt 1997; Miller et al. 2009; Eyre and Baune 2012; Heppner et al. 2015). According to Koo et al. (2010) stress-induced anhedonia, one of the main symptoms of depression, depends on NF-κB activation. In parallel, previous studies describe that IL-1β and NF-κB signaling is activated by stressful events, indicating that this signaling pathway is necessary for the antineurogenic and behavioral effects of stress (Miller et al. 2009; Koo et al. 2010). Additionally, the overexpression of IL-18 and IL-33 is linked to neuroinflammatory processes, resulting in neuropsychiatric and cognitive changes (Alboni et al. 2010; Kudinova et al. 2016).

According to the above, our results are consistent with previous studies, as ARS induced the expression of NF-κB

and pro-inflammatory cytokines such as IL-1β, IL-18, and IL-33 in mouse brain structures. In this context, based on the evidence, we believe that HPA axis hyperactivation and increased oxidative stress were essential for NF-κB nuclear translocation, promoting the transcription of proinflammatory genes and thus initiating a neuroinflammatory response. In contrast, it is suggested that these neuroinflammatory responses contributed to the development of depressive-like behavior and memory impairment in stressed mice. Nonetheless, an important finding of our study was that 4-PSQ treatment reversed the increase in neuroinflammatory parameters (NF-κB, IL-1β, IL-18, and IL-33) in the prefrontal cortex and/or in the hippocampus of stressed mice, similar or superior to the positive controls (paroxetine and donepezil). We suggested that the effect of 4-PSQ in modulating neuroinflammation is associated with the attenuation of HPA axis activation and reduction of oxidative stress in the prefrontal cortex and hippocampus of stressed mice. Accordingly, previous studies have demonstrated that the attenuation of the HPA axis and oxidative stress is correlated with the reduction of neuroinflammatory processes (Casaril et al. 2019; Paltian et al. 2020). Thus, our findings demonstrated the importance of the therapeutic use of 4-PSQ, since it was able to attenuate neuroinflammation and oxidative stress, as well as the activation of the HPA axis, reducing depressive-like symptoms and memory impairment caused by stress.

Notably, in order to adapt to stressful events, intracellular signal transduction pathways are activated to promote neuronal survival and neuroplasticity (Begni et al. 2017). Outstanding among these signaling pathways is PI3K/AKT which is involved in synaptic plasticity, learning, memory, and depression (Yang et al. 2008; Qi et al. 2016), and can modulate neurotransmitter release, cell viability, apoptosis, and postsynaptic responses (Li et al. 2015b). The PI3K enzyme is activated in the presence of growth factors, hormones, cytokines, and/or neurotransmitters. Its lipid products act as second messengers, since they activate proteins such as AKT, being the effector of PI3K for cellular responses (Beaulieu 2012). Taking into account the significant role of PI3K/AKT signaling pathway modulation in the mechanisms underlying depression and memory impairment and in an attempt to advance our understanding of the mechanisms involved in the effect of 4-PSQ, it was found useful to assess this pathway. PI3K activates the three AKT isoforms: AKT1, AKT2, and AKT3 (Beaulieu 2012; Li et al. 2015b). In this study, we chose to evaluate the AKT2 mRNA expression, due to its participation in the regulation of neuron differentiation and survival, in addition to its inhibition being associated with psychiatric diseases, such as depression (Deng et al. 2019). We found that the mRNA expression of PI3K and AKT was reduced in the prefrontal cortex in stressed mice. Regarding the hippocampal response, only AKT2 mRNA expression levels were reduced after ARS in mice. A study by Cunha et al. (2015) reported that the activation of these kinases can affect sub-regions of the brain, making their detection difficult. In this way, based on the evidence we believe that this may have influenced our results, considering that we did not detect changes in the levels of PI3K mRNA expression in the hippocampus. Thus, other detection techniques, such as immunoblotting or even assessing other sub-regions, will be targets for future studies to better elucidate this pathway. According to this, our results suggested an involvement of the PI3K/AKT2 signaling pathway in the effect of 4-PSQ, considering that the compound was able to normalize PI3K and AKT2 mRNA expression levels in the prefrontal cortex, contributing to the reestablishment of neuroplasticity processes and behavioral changes. These effects of treatment with 4-PSQ were similar to the effect of the positive controls (paroxetine and donepezil).

We also investigated the activity of AChE in an attempt to expand the action mechanisms of 4-PSQ. AChE is responsible for maintaining the proper functioning of the cholinergic system by maintaining the levels of the neurotransmitter acetylcholine (ACh) (English and Webster 2012). Our results showed that ARS induced an increase in AChE activity in the prefrontal cortex and hippocampus of mice. Thus, an increase in AChE activity is associated with an imbalance in the cholinergic, system with loss of brain function, and based on the evidence, we believe that this contributed to the memory impairment observed in our study. The reduction in ACh levels is mainly responsible for causing memory impairment, an important mechanism that leads to neurodegeneration (Lu et al. 2018). Interestingly, treatment with 4-PSQ attenuated the memory impairment induced by ARS and normalized the stimulation of AChE activity in the prefrontal cortex and hippocampus of mice, similarly to paroxetine and donepezil. Previous studies have also pointed out that 4-PSQ exhibited a neuroprotective effect and an important improvement in parameters associated with cognition in animal models by modulating the cholinergic system (Rodrigues et al. 2021; Barth et al. 2019; Pinz et al. 2018). Thus, we can suggest that one of the possible justifications for the neuroprotector effect of 4-PSQ on memory impairment is the modulation of AChE activity, associated with the ability to modulate oxidative stress and synaptic plasticity regulation of the PI3K/AKT pathway, mechanisms that are associated with memory disorders and neuropsychiatric diseases, such as depression.

The findings of this study are highly important, since the treatments available for depression are not effective against the associated memory impairment (Galts et al. 2019). Thus, the adverse effects of antidepressants are added to those of drugs used for the treatment of AD (which mitigate memory impairment), and as a consequence, there is a reduction in the quality of life of the patient affected by this comorbidity

(Banerjee et al. 2013). Thus, one of the main advantages of 4-PSQ is the possibility of a single drug for the treatment of two diseases, depression and its comorbidity, memory impairment/AD.

Conclusion

In conclusion, 4-PSQ exhibited an antidepressant-like effect and attenuated the memory impairment induced by ARS in mice. Based on the evidence, we believe that these effects are associated, at least in part, with its ability to attenuate the activation of the HPA axis, attenuate changes in the monoaminergic system, modulate oxidative stress, restore neuroplasticity, modulate the cholinergic system, and attenuate neuroinflammation. Therefore, the findings of this study support the use of 4-PSQ as a possible promising alternative for the treatment of depression associated with stress-induced memory impairment. Thus, we are motivated to continue investigating the potential of this compound in search of new mechanisms linked to its effect.

Author contribution R.L.O. performed the experiments, the analysis of data, and wrote the manuscript. R.L.O., G.T.V., K.C.R., and M.P.P. performed the experiments. R.L.O., E.A.W., and C.L. designed the project. A.L. and D.A. synthesized the compound 4-PSQ E.B., W.B.D., and V.F.C. performed the genetic expression. E.A.W. and C.L. supervised the experiments. All authors critically reviewed the content and approved the final version for publication.

Funding We are grateful to Universidade Federal de Pelotas (UFPel), Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq) (UNIVERSAL 429859/2018–0), Fundação de Amparo à Pesquisa do Estado do Rio Grande do Sul (FAPERGS) (PRONEM 16/2551–0000240-1, PqG 17/2551–0001013-2 and 19/2551–0001745-6) for financial support. This study was also financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível superior – Brasil (CAPES)—Finance Code 001. R.L.O. is the recipient of a CAPES fellowship. V.F.C, D.A., E.A.W., and C.L. are recipients of CNPq fellowships.

Declarations

Conflict of interest The authors declare no competing interests.

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4.2 Manuscrito

7-chloro-4- (phenylselanyl) quinoline attenuates depressant-like behavior and memory impairment induced by β-Amyloid in mice

Manuscrito submetido para publicação na revista *Molecular Neurobiology*

7-chloro-4- (phenylselanyl) quinoline attenuates depressant-like behavior and memory impairment induced by β-Amyloid in mice

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Abstract

Although memory impairment is the main symptom of patients with Alzheimer's disease (AD), depression is the most common neuropsychiatric complication in this disease. Therefore, looking for a new therapeutic alternative capable of easing the comorbidity in question is extremely important. Thus, this study investigated the effect of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) on depressant-like behavior associated for memory impairment in a model of AD induced by the β-amyloid (Aβ) peptide (fragment 25-35) in male Swiss mice. The mice were intragastrically pretreated with canola oil (10 mL/kg) or 4-PSQ (1 mg/kg) or positive controls (1 mg/kg), for seven days. Thirty minutes after the treatment, the animals received a single intracerebroventricular injection of Aβ or saline (3 µl/per site). Mice were submitted to the behavioral tasks (tail suspension test, open-field test, forced swimming test and step-down inhibitory avoidance test) from the fifth day onwards of experimental protocol. On the seventh day, blood was collected to measure corticosterone levels, and the prefrontal cortices and hippocampus were removed to assess oxidative stress parameters, neuroinflammatory and activity of the enzyme acetylcholinesterase (AChE). 4-PSQ attenuated the depressant-like behavior and memory impairment caused by Aβ. We believe that these effects of 4-PSQ may be associated, with the restoration of the HPA axis, modulation of oxidative stress, attenuation of neuroinflammation and modulation of AChE enzyme activity. In conclusion, these results suggested that 4-PSQ had an antidepressant-like effect and attenuated memory impairment in an AD model induced by Aβ and could be a promising molecule for the treatment of these comorbidities.

Keywords: comorbidity, Alzheimer’s disease, depression, oxidative stress, selenium, corticosterone.

1. *Introduction*

Alzheimer's disease (AD) is a neurodegenerative disease, which is characterized by cognitive dysfunction and neuronal lost in brain areas involved in cognition and emotional behaviors [1]. The main neuropathological characteristics of AD include the deposition of β-amyloid (βA) peptide in brain regions, accompanied by the presence of intracellular neurofibrillary tangles, composed of hyperphosphorylated tau protein [2].

Although memory loss is the main symptom of patients with AD, depression is the most common neuropsychiatric complication in this disease, affecting about 50% of these individuals [3, 4]. Depression is extremely common in the early AD stages and can represent a prodrome for AD-related dementia in some individuals [5]. In line with this, the high incidence of depression in individuals with AD is associated with a high risk of morbidity and mortality [6]. Parallel to this, the neuroanatomical changes observed in depressive individuals are also observed in patients with AD, such as atrophy of the prefrontal cortices and hippocampus [7], thus reinforcing the relationship between depression and AD.

The molecular mechanisms related to the depression/AD dyad are not fully understood, however, there is evidence of the relationship of Aβ peptide neurotoxicity in the pathophysiology of these diseases [8]. The amyloid peptide Aβ (25-35) is widely used to examine comorbidity, depression and AD in rodents [9], as it is able to mimic the cognitive symptoms observed in patients with AD [10, 11] and induce depressive behavior, such as anhedonia and behavioral despair [12]. In view of this, several studies seek to clarify the mechanisms that cover this comorbidity. An overdrive of the hypothalamic-pituitary-adrenal (HPA) axis has been demonstrated, promoting an increase in the levels of glucocorticoids in patients with depression and AD [13]. In addition, other pathophysiological mechanisms are common in depression and AD, including oxidative stress, neurodegeneration, dysfunction of neuronal function and neuroinflammation [14, 15]. Still, it has been shown that impairment in cholinergic neurotransmission is one of the mechanisms that contributes to cognitive dysfunction in AD associated with depression [14].

Existing antidepressant drugs have reduced efficacy to treat depression in patients with AD [16]. In addition, the adverse effects of antidepressants are

added to those of drugs used for the treatment of AD, and therefore there is a reduction in the quality of life of the patient affected with this comorbidity [17]. Thus, there is a great need to find effective strategies to mitigate depression in AD. In view of this, 7-chloro-4- (phenylselanyl) quinoline (4-PSQ) appears as a promising agent for the treatment of disorders that affect the central nervous system, including AD and depression [11, 18]. There is an increasing number of studies demonstrating the neuroprotective effects of 4-PSQ in different animal models; and mechanisms related to neuroprotection include antioxidant, anti-inflammatory, anticholinesterase, anxiogenic and neurogenic effects [11, 18–25]. Recently, the anti-AD effect of 4-PSQ was evidenced, as well as its antidepressant-like and anxiolytic-like action in different experimental models in mice [11, 18]. Thus, important reasons persist to extend the study of the neuroprotective effect of 4-PSQ. In view of this, the objective of the following study was to evaluate the effect of 4-PSQ on comorbidity, depression and AD, as well as the mechanisms that mediate the neuroprotective effects of 4-PSQ in an AD model induced by Aβ (25-35) peptide in mice.

2. Materials and methods

2.1. Animals and ethical approval

The experiments were conducted using Swiss mice male (25-35g), from a local breeding colony were used. The animals were housed in cages with free access to food and water in a room maintained at 25 ± 1°C with a relative humidity of 50 ± 5%. They were kept on a 12 h light/12 h dark cycle. The experiments were performed according to the guidelines of the Committee on Care and Use of Experimental Animal Resources, Federal University of Pelotas, Brazil (CEEA 1974–2016), following the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978). All behavioral tests were conducted during the light period of the light/dark cycle. Every effort was made to minimize the number of animals used and their discomfort.

2.2. Chemicals

The 4-PSQ (Fig. 1) was prepared according to the literature [26]. The chemical purity of the compound (99.9%) was determined by gas chromatography - mass spectrometry (GC/MS). Drugs used as a positive control

(paroxetine and donepezil) were obtained commercially. 4-PSQ and positive controls was dissolved in canola oil (a non-polar and inert substance) and administered intragastrically (i.g.) at a dose of 1 mg/kg and at a constant volume of 10 mL/kg body weight. Aβ (fragment 25-35) was obtained from Sigma (St. Louis, MO). It was dissolved in sterile filtered water and added by incubation at 37°C for 4 days prior to use. All other chemicals were analytical grade and obtained from standard commercial suppliers.

2.3. Experimental protocol

Mice were randomly divided into 6 experimental groups (7 animals/group). On the first day of the experimental protocol, thirty minutes before initiating induction, the mice belonging to Sham and Aβ-induced groups received the canola oil (10 ml/kg), animals of 4-PSQ and Aβ + 4-PSQ groups received the compound (1 mg/kg, i.g.), and the mice belonging to Aβ + paroxetine and Aβ + donepezil received the paroxetine (1 mg/kg, i.g.) and donepezil (1 mg/kg, i.g.) via gavage, respectively. After treatments, mice belonging to Aβ-induced, Aβ + 4-PSQ, Aβ + paroxetine and Aβ + donepezil groups received Aβ (fragment 25–35) aggregated form (3 nmol/3 µl/per site, by intracerebroventricular (i.c.v.)) [27]. Sham and 4-PSQ groups received saline (3 µl/per site, i.c.v.). The i.c.v. injection of Aβ or vehicle (saline) was administered using a microsyringe with a 28-gauge stainless-steel needle 3.0 mm long (Hamilton) according to a previous report [28].

All animals were anesthetized with isoflurane before i.c.v. injection. Mice were treated with 4-PSQ, paroxetine, donepezil or canola oil every day, until the end of the experimental protocol. On the fifth-day of the experimental protocol, behavioral tests were initiated. On the seventh-day, after the behavioral assessment, the mice were anesthetized (isoflurane inhalation) before blood collection by cardiac puncture [29]. Subsequently, the animals were euthanized to remove brain structures, such as the prefrontal cortices and hippocampus for biochemical analysis. The experimental protocol is demonstrated in Figure 2.

2.4. Behavioral tests

All behavioral tests were scored by an observer-blinded, when the researcher does not know treatment that a mouse undergoes.

2.4.1. Open-field test (OFT)

The OFT evaluated the general locomotor and exploratory behavior of the mice in order to exclude any psychomotor alterations, after administration with Aβ, 4-PSQ, paroxetine or donepezil, on the sixth-day of the experimental protocol [30]. The open-field was made of plywood (30 cm in height x 45 cm in length x 45 cm in width) and divided by masking tape markers into 09 squares (3 rows of 3). Each animal was placed individually at the center of the apparatus and observed for 4-minutes period to record the locomotor (number of segments crossed with the four paws) and exploratory (expressed by the number of time rearing on the hind limbs) activities.

2.4.2. Tail suspension test (TST)

The TST was conducted as described by Steru et al. [31], being considered a behavioral parameter used to assess the antidepressant-like effect possible of 4-PSQ, paroxetine and donepezil. On the fifth-day of the experimental protocol, the mice were suspended 50 cm above the ground by an adhesive tape placed approximately 1 cm from the tip of the animals tail. Mice were considered immobile only when they hung passively and completely motionless. Immobility time was manually recorded during a 6-minutes period by an experienced observer. In this test, a decrease in the duration of immobility is an indicative of antidepressant-like effect.

2.4.3. Forced swimming test (FST)

The FST was conducted using the method described by Porsolt et al. [32], on the sixth-day of the experimental protocol. This test is performed to assess the antidepressant-like effect possible of 4-PSQ, paroxetine and donepezil. In this test, mice were individually forced to swim in an open cylindrical container (10 cm in diameter and 25 cm in height), containing 19 cm of water at 25 ± 1°C. The duration of immobility was scored during a 6-minutes period by an experienced observer. Each mouse was considered as immobile when floating motionless or making only those movements necessary to keep its head above water. A decrease in the duration of immobility is an indicative of antidepressant-like effect.

2.4.4. Step-down inhibitory avoidance (SDIAT)

The STDIAT was conducted using the method described by Sakaguchi et al. [33], with modifications of the intensity of electric shock. This test is performed to evaluate non-spatial long-term memory of animals treated with 4-PSQ, paroxetine or donepezil. On the sixth day of experimental protocol, the training session is held, where each mouse was placed on the platform. When it stepped down and placed its four paws on the grid floor, an electric shock (0.5 mA) was delivered for 2 s. The test was performed 24 h after training (seventh-day), where each mouse was placed again on the platform, and the transfer latency time (i.e., time it took to step down from the platform) (seconds) was measured as in the training session, but no electric shock was delivered. The maximum transfer latency time (seconds) was 300 s).

2.5. Biochemical analysis

2.5.1. Tissue processing

On the seventh day of the experimental protocol, mice were anesthetized with isoflurane and blood samples collected from the heart ventricle, using heparin as anticoagulant to obtain plasma. The plasma was obtained by centrifugation (900 × g) for 15 minutes and used to measure the levels of corticosterone. Then, prefrontal cortices and hippocampus were removed and immediately homogenized in cold 50 mM Tris-HCl, pH 7.4 (1/10, weight (w)/volume(v)). The homogenates were centrifuged at 900 *x*g at 4°C for 10 minutes and supernatant fractions (S1) were used to determine reactive species (RS) levels, thiobarbituric acid reactive species (TBARS) levels and activity of the enzymes superoxide dismutase (SOD), glutathione peroxidase (GPx) and Na^+, K^+-ATPase. For the determination of the activity of the enzyme acetylcholinesterase (AChE), brain structures of mice were homogenized in 0.25 M sucrose buffer (1/10, w/v) and centrifuged at 900 *x*g at 4°C for 10 minutes.

2.5.2. Plasma corticosterone level

The changes in the HPA axis were evaluated through the levels of plasma corticosterone, estimated by the fluorescence method previously described by Zenker and Bernstein [34]. Briefly, corticosterone in plasma aliquot was extracted with chloroform. The tubes were shaken for 15 s, centrifuged (5 minutes at 900

*x*g), and the aqueous layer was discarded. Then, 0.1 M NaOH was added to tubes and another round of agitation and centrifugation was performed. Lastly, after the addition of the fluorescence reagent (H_2SO_4 and 50% ethanol), samples were agitated and centrifuged (5 minutes at 900 *x*g) and incubated at room temperature for 2 h. After that, the fluorescence intensity emission was recorded at 540 nm (with 257 nm excitation) and corticosterone levels were expressed ng corticosterone/mL plasma.

2.5.3. Oxidative parameters

Samples of prefrontal cortices and hippocampus were collected to determine RS, and TBARS levels. These measurements were performed to evaluate the effect of 4-PSQ on the modulation of cerebral oxidative stress. RS levels were used as a marker of oxidative damage. The levels of RS formed in the brain structures were determined by spectrofluorimetric using the dichloro-dihydro-fluorescein diacetate (DCHF-DA) reagent [35]. In order to do it, DCHF-DA (1 mM) was incubated together with the S1 and Tris-HCl buffer (10 mM, pH 7.4). The oxidation of DCHF to fluorescent dichlorofluorescein (DCF) was measured for the intracellular RS detection. The fluorescence intensity was measured with emission at 520 nm and excitation at 488 nm in spectrofluorometer (Shimadzu RF-5301 PC fluorometer) and the results are expressed in units of fluorescence (UF).

TBARS content was used as a marker of lipid peroxidation. TBARS levels were determined as described by Ohkawa et al. [36]. An aliquot of S1 was added to the reaction mixture containing: thiobarbituric acid (0.8 %), sodium dodecyl sulfate (8.1 %), and acetic acid (pH 3.4) and incubated at 95°C for 2 h. The absorbance was measured at 532 nm in a spectrophotometer (Shimadzu RF-5301 PC). Results were reported as nmol malondialdehyde (MDA)/mg protein.

2.5.4. Antioxidant enzymes

Antioxidant enzymes have a high capacity to neutralize the formation of RS, being considered the primary cellular defense system [37], so this study evaluated these neurochemical targets. SOD activity was assayed spectrophotometrically as described by Misra and Fridovich [38]. This method is based on the capacity of SOD to inhibit the autoxidation of epinephrine. Briefly,

S1 was diluted 1:10 (v/v) to determine SOD activity. S1 aliquot was added to a 0.05 M Na_2CO_3 buffer, and the enzymatic reaction was started by adding the epinephrine. The color reaction was measured at 480 nm (Shimadzu RF-5301 PC spectrophotometer). One unit of enzyme was defined as the amount of enzyme required to inhibit the rate of epinephrine autoxidation by 50% at 37°C. Results were expressed as units U SOD/mg protein.

GPx activity was evaluated spectrophotometrically (Shimadzu RF-5301 PC) using the method described by Wendel [39], which involves monitoring the reduction of hydrogen peroxide (H_2O_2) in the presence of S1 at 340 nm. S1 was added in a system composed by reduced glutathione (GSH)/ nicotinamide adenine dinucleotide phosphate (NADPH)/GR (reduced glutathione), and the enzymatic reaction was initiated by the addition of H_2O_2. In this assay, the enzyme activity is indirectly measured by NADPH decay. H_2O_2 is reduced and generates oxidized glutathione (GSSG) from GSH. GSSG is regenerated back to GSH by the GR present in the analysis medium at the expense of NADPH. Enzymatic activity was expressed as nmol NADPH/min/mg protein.

2.5.5. RNA extraction and expression of tumor necrosis factor alpha (TNF-α) and interleukin-6 (IL-6) by real-time PCR

The total RNA of paw tissue was directly isolated after the completion of treatment using TRIZOL® (Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. Total RNA was treated with RNase-free DNase (Invitrogen, Carlsbad, CA). RNA was quantified using the NanoDrop Lite (Thermo Scientific) equipment. The primers used for the real-time PCRs were synthesized by Invitrogen (São Paulo, Brazil). The real-time PCR amplification reaction was carried out using SYBR® Green One- Step qRT-PCR with Rox (Invitrogen, Carlsbad, CA), performed according to the manufacturer's protocol. cDNA was synthesized from 0.5µg of total RNA, using Oligo(dT) Primer (Thermo Scientific) according to manufacturer's instructions. PCR reactions were run in a 7500 Real time Fast thermocycler (Applied Biosystems), under the following conditions: 50°C for 15 min, 95°C for 52 min, followed by 40 cycles at 95°C for 15 s and 60°C for 30 s, after the dissociation curve was performed at 95°C for 5 min with a final step of 4°C. The assay was accomplished for each gene and included cDNA of the samples treated and control without template.

Results were obtained as CT (threshold cycle) values. The software determines a threshold line at the base of the baseline fluorescent signal, and the data point that meets the threshold is given as CT, which is inversely proportional to the starting template copy number. The differences in CT values between the control group, treated group and endogenous control β-actin gene for each reaction (ΔCT) were analyzed using the 2−ΔΔCT method. All measurements were performed in duplicate in two independent experiments. The results were expressed as relative concentration calculated as described by Giongo et al. [40].

2.5.6. Immunofluorescence assay

Perfused and fixed brains were sectioned on a microtome (Leica, CM3050S), in the coronal plane, sequentially from the beginning of the hippocampus (14 µm) and collected in 6-well culture plates with 1x PBS buffer. Sections were incubated for free-floating for 2 hours in blocking buffer PBS containing 0.1% (v/v) Triton X-100 (PBS-Tx) and 10% (v/v) normal donkey serum at room temperature. After the blocking step, for the immunofluorescence reaction, the sections were incubated overnight at 4°C with the following primary antibodies: anti- glial fibrillary acidic protein (GFAP) (1:400; Sigma). Subsequently, they were washed 3 times for 10 min with 1x PBS buffer and incubated for 2 hours at room temperature with the appropriate secondary antibodies to the primary, including: Alexa fluor (1:1000). After incubation the slices were washed 3 times for 10 min with 1x PBS buffer and after were incubated with 5 µg/ml of DAPI (Invitrogen/1:1000) for 5 min. The slices were transferred to laminas and analyzed using a Nikon Ti2 fluorescence microscope (Nikon, Tokyo, Japan) which has an image capture system. Quantitative analysis of marked cells was made using the Image J software and results expressed as arbitrary units.

2.5.7. AChE activity

The AChE activity was measured by a modified method of Ellman et al. [44], using acetylthiocholine (AcSCh) as substrate. The method is based on the formation of the yellow anion, 5,50-dithio-bis-acid-nitrobenzoic, measured by

absorbance at 412 nm during. Results are expressed as µmol/AcSCh/h/mg protein.

2.5.8. Protein determination

The protein concentration was measured by the method of Bradford [41], using bovine serum albumin as the standard.

2.6. Statistical analysis

The normality of data was evaluated by the D'Agostino and Pearson omnibus normality test. The data were analyzed by one-way analysis of variance (ANOVA) followed by Tukey's test. All analyses were performed using the GraphPad software (GraphPad software, San Diego, CA, USA). Data were expressed as mean ± standard error of the mean (S.E.M.). Probability values less than ($p < 0.05$) were considered statistically significant.

3. Results

3.1. 4-PSQ protects against Aβ-induced memory impairment

The results of SDIAT can be seen in figure 3a and 3b. In the training phase, there was no difference in the transfer latency time among groups (Fig. 3a). On the other hand, in the test phase, Aβ decreased (around 85%) the transfer latency time, when compared to the Sham group. 4-PSQ attenuated the reduction in transfer latency time similarly to donepezil, (Fig. 3b). On the other hand, the pretreatment with paroxetine did not change of transfer latency time in SDIAT. Treatment with 4-PSQ *per se* did not change of transfer latency time of mice. (ANOVA: $F_{(5,36)} = 2.668$, $p < 0.0001$ for training phase and ANOVA: $F_{(5,36)} = 21.79$, $p < 0.0001$ for test phase).

3.2. 4-PSQ protects against Aβ-induced depressive-like behavior without altering locomotor and exploratory activity

The effect of pretreatments on immobility time in the TST and FST in mice can be seen in figure 3c and 3d. The one-way ANOVA followed by Tukey's post-hoc test revealed that mice induced with Aβ showed an increase (around 18% in TST and 52% in FST) in duration of immobility time, when compared to the Sham

group. The immobility time was attenuated by pretreatment with 4-PSQ, fluoxetine and donepezil in the TST (Fig. 3c) and FST (Fig. 3d). 4-PSQ effect was superior to donepezil and paroxetine in attenuating the depressant-like behavior caused by Aβ. Pretreatment with 4-PSQ decreased *per se* the immobility time in TST and FST, when compared with the sham group. (ANOVA: $F_{(5,36)} = 146.0$, $p < 0.0001$ for TST and ANOVA: $F_{(5,36)} = 115.6$, $p < 0.0001$ for FST).

The data analysis of OFT showed no change in the number of crossings (ANOVA: $F_{(5,36)} = 0.9207$, $p = 0.46798$) and rearings (ANOVA: $F_{(5,36)} = 2.466$, $p = 0.0508$) after the treatments in mice (Fig. 3e and 3f).

3.3.4-PSQ attenuated the HPA axis activation caused by Aβ-induction

The one-way ANOVA followed by Tukey's post-hoc test revealed that Aβ increased (around 137%) circulating corticosterone levels of mice, when compared with the Sham group. Pretreatment with 4-PSQ normalized these levels (Fig. 4). 4-PSQ *per se* did not change the circulating corticosterone levels in mice. (ANOVA: $F_{(3,24)} = 233.20$, $p < 0.0001$).

3.4.4-PSQ reduced oxidative damage in the prefrontal cortices and hippocampus of Aβ-induced mice

Aβ increased a RS level in prefrontal cortices (around 26%) (Fig. 5a) and in hippocampus (around 38%) (Fig. 5b) of mice, when compared with the Sham group. Pretreatment with 4-PSQ significantly reduced the production of RS caused by Aβ in the cerebral structures. No changes in the RS levels in the prefrontal cortices and hippocampus were observed after *per se* treatment with 4-PSQ. (ANOVA: $F_{(3,24)} = 5.06$, $p = 0.0074$ for the prefrontal cortices and ANOVA: $F_{(3,24)} = 6.44$, $p = 0.0023$ for the hippocampus).

Aβ caused an increase in TBARS levels in prefrontal cortices (around 149%) (Fig. 5c) and in hippocampus (around 37%) (Fig. 5d) of mice, when compared with the Sham group. Pretreatment with 4-PSQ protected against this increase in cerebral structures of mice. 4-PSQ *per se* did not change the TBARS levels in prefrontal cortices and hippocampus of mice. (ANOVA: $F_{(3,24)} = 53.82$, $p < 0.0001$ for the prefrontal cortices and ANOVA: $F_{(3,24)} = 18.85$, $p < 0.0001$ for the hippocampus).

3.5.4-PSQ modulated the antioxidant enzymes in the prefrontal cortices and hippocampus of Aβ-induced mice

SOD activity in prefrontal cortices and hippocampus of mice are shown in figures 6a and 6b, respectively. Aβ decreased the SOD activity in prefrontal cortices (around 19%) (Fig. 6a) and in hippocampus (around 24%) (Fig. 6b) of mice, when compared with the Sham group. Pretreatment with 4-PSQ normalized the SOD activity in the prefrontal cortices and hippocampus of mice. No changes in the SOD activity in the prefrontal cortices and hippocampus were observed after *per se* treatment with 4-PSQ. (ANOVA: $F_{(3,24)} = 6.003$, $p = 0.0033$ for the prefrontal cortices and ANOVA: $F_{(3,24)} = 8.14$, $p = 0.0007$ for the hippocampus).

The one-way ANOVA followed by Tukey's post-hoc test showed that Aβ caused an increase in the GPx activity in prefrontal cortices (around 24%) (Fig. 6c) and in hippocampus (around 104%) (Fig. 6d) of mice, when compared with the Sham group. Pretreatments with 4-PSQ normalized the activity of this enzyme in prefrontal cortices and hippocampus of the mice. 4-PSQ *per se* did not change the GPx activity in cerebral structures of mice. (ANOVA: $F_{(3,24)} = 41.18$, $p < 0.0001$ for the prefrontal cortices and ANOVA: $F_{(3,24)} = 33.23$, $p < 0.0001$ for the hippocampus).

3.6 4-PSQ reduced levels of TNF-α and IL-6 in the prefrontal cortices and hippocampus of Aβ-induced mice

Figure 7 demonstrated the changes of inflammatory genes in RNA sequencing analysis by the levels of pro-inflammatory cytokines in cerebral structures of mice. Aβ increased the levels of TNF-α and IL-6 in prefrontal cortices (around 340% and 246%, respectively) (Fig. 7a and 7c) and in hippocampus (around 340% and 385%, respectively) (Fig. 7b and 7d) of mice, when compared with the Sham group. Pretreatment with 4-PSQ protected against the increase caused by Aβ induction in mice cerebral structures. 4-PSQ reduced *per se* the levels of inflammatory cytokines in the evaluated structures, except in the prefrontal cortices (in the case of IL-6), when compared with the Sham group. (TNF-α - ANOVA: $F_{(3,8)} = 188.00$, $p < 0.0001$ for the prefrontal cortices and ANOVA: $F_{(3,8)} = 270.30$, $p < 0.0001$ for the hippocampus); (IL-6 - ANOVA: $F_{(3,8)} = 40.32$, $p < 0.0001$ for the prefrontal cortices and ANOVA: $F_{(3,8)} = 132.00$, $p < 0.0001$ for the hippocampus).

3.7 4-PSQ protected against the increase in GFAP levels Aβ-induced mice

The one-way ANOVA followed by Tukey's post-hoc test showed that Aβ caused an increase in the GFAP levels (around 41%) in the hippocampus of mice (Fig. 8a and 8b), when compared with the Sham group. Pretreatment with 4-PSQ were effective in protecting against this change. 4-PSQ *per se* did not change the GFAP levels. (ANOVA: $F_{(3,12)} = 7.32$, $p = 0.0048$).

3.84-PSQ modulated the AChE activity in the prefrontal cortices and hippocampus of Aβ-induced mice

The results showed that Aβ increased the AChE activity in prefrontal cortices (around 52%) (Fig. 9a) and in hippocampus (around 32%) (Fig. 9b) of mice, when compared with the Sham group. This increase was normalized through pretreatment with 4-PSQ in the cerebral structures of mice. No changes in the AChE activity in the prefrontal cortices and hippocampus were observed after *per se* treatment with 4-PSQ. (ANOVA: $F_{(3,24)} = 46.82$, $p < 0.0001$ for the prefrontal cortices and ANOVA: $F_{(3,24)} = 18.13$, $p < 0.0001$ for the hippocampus).

4. Discussion

The high prevalence rates between depression and AD, the multifactorial etiology of comorbidity, and yet, the adverse effects of using combined drugs motivated us to seek a new effective treatment with two or more complementary biological activities to represent an important advance for the treatment of these diseases. According to this, the present study showed, for the first time, the neuprotective effect of 4-PSQ, a quinoline functionalized with selenium, on the antidepressant-like effect and against memory impairment caused by Aβ (25–35) in mice. Based on the evidence, we believe that the effects of 4-PSQ may be associated, at least in part, with (I) restoration the changes in the HPA axis; (II) modulation of oxidative stress; (III) attenuation of neuroinflammatory markers; as well as (IIII) restoration of the cholinergic system. Accordingly, our findings demonstrated that 4-PSQ is a multi-target molecule, suggesting its clinical potential for the treatment of comorbidity depression and AD caused by Aβ.

It is worth mentioning that to validate our study and compare our findings with the drugs used for depression and AD, we used as positive controls

paroxetine (antidepressant, selective serotonin reuptake inhibitor) and donepezil (acetylcholinesterase inhibitor, used in the treatment of the DA). Accordingly, we revealed that unlike 4-PSQ, paroxetine did not attenuate the memory impairment caused by Aβ-induction and the effect of 4-PSQ was significantly superior to the antidepressant-like effect of positive controls.

The administration of Aβ (25-35) is known to mimic cognitive symptoms of AD, thus validating this model [10, 11, 42]. The neurotoxicity of this peptide results from the cleavage of the amyloid precursor protein (APP) by β and γ-secretases [43]. These Aβ peptides unite, forming amyloid plaques, which are deposited in brain regions causing several consequences, including neuronal death, resulting in impaired cognitive functions, such as memory impairment [44]. As expected, we showed that Aβ caused memory impairment, as evidenced by the reduction in transfer latency time in SDIAT in mice. 4-PSQ attenuated this memory impairment caused by Aβ. These findings extend the previous results made in our research group which demonstrated that 4-PSQ attenuated learning and memory impairment in different behavioral paradigms in an AD model induced by Aβ in mice [11].

In addition to memory impairment, a neuropsychiatric symptom commonly seen in patients with AD is depression [12]. Several behavioral tests can be done to investigate depression in animal models. TST and FST are tests with mechanistic validity to assess behavioral despair in rodents (which resembles symptoms of depression in humans) [45]. Both tests serve to screen for new molecules with antidepressant potential (which tend to reduce immobility time) [31, 46]. In our study, injection of Aβ increased the immobility time in the TST and FST, demonstrating that Aβ caused a depressant-like behavior in mice. These results are in accordance with a previous study, which demonstrated depression caused by Aβ injection in mice [12]. It is worth mentioning that 4-PSQ attenuated the depressant-like behavior induced by Aβ and decreased *per se* the immobility time in the TST and FST, suggesting an antidepressant-like effect of 4-PSQ. It is known that drugs that are psychostimulants or sedatives may be responsible for the effects observed in behavioral tests [47]. Therefore, it is important to note that treatment with 4-PSQ, paroxetine and donepezil did not alter the animal's locomotor and exploratory spontaneous activity in the OFT, ruling out the hypothesis that these drugs have psychostimulant or sedative properties.

To verify the mechanisms by which the 4-PSQ could be acting and exerting its beneficial effects in this study, some pathways and systems were evaluated. It is known that hyperactivity of the HPA axis results in an increase in circulating glucocorticoids (GC), such as cortisol (corticosterone in rodents) [48]. In our study, Aβ caused an increase in plasma levels of corticosterone and treatment with 4-PSQ was able to reestablish the dysfunction of the HPA axis by reducing the levels of this hormone. Indeed, clinical studies have found that this change is found both in depressed patients and those with AD [49]. Stressful stimuli to the organism, such as Aβ neurotoxicity are associated with hyperactivity of the HPA axis and an increase in the neuroinflammation process [50]. This hyperactivity can be transient due to a negative feedback system, which reduces the production of GC under physiological conditions to avoid pathological effects [51]. In contrast, in patients with depression and AD, it is believed that this negative feedback system is compromised. In view of this, the exacerbated increase in these GCs damage neuronal cells, especially the hippocampus, inducing memory impaired and emotional changes, symptoms found in patients with depression and AD [3, 52, 53]. In this sense, our data suggest that HPA axis hyperactivation contributed to the exacerbation of depressive behavior and cognitive impairment observed in animals submitted to Aβ induction and 4-PSQ was able to attenuate these behaviors by modulating the HPA axis.

Previous reports described a possible relationship between the hyperactivation of the HPA axis and the increase in oxidative parameters, in which GCs favor an increase in the metabolic rate, and in turn there is an increase in the production of RS [54]. In parallel, Aβ-induced neurotoxicity contributes to the exacerbated production of RS and lipid peroxidation products (such as MDA, evaluated in the TBARS test), inducing mitochondrial damage and consequently cell death [55]. In this way, the neurotoxicity of Aβ can be attenuated by antioxidants [56]. Here, we evaluated oxidative stress markers (RS and TBARS) to elucidate the neuroprotective/antioxidant effects of 4-PSQ. We found that Aβ increased the levels of RS and the lipid peroxidation process in the prefrontal cortices and in the hippocampus of the mice, indicating that Aβ caused oxidative damage in lipids. Importantly, 4-PSQ protected against these increases, indicating its possible antioxidant effect. In this sense, previous studies carried out in our laboratory have already demonstrated the antioxidant action of this

compound in different experimental models [11, 18, 19, 23, 24, 57]. Therefore, we suggested that the neurotoxic effects of the Aβ peptide and the consequent hyperactivation of the HPA axis may have cooperated in the oxidative stress process and, thus, contributed to the development of depressive behavior and cognitive deficit, in which they were mitigated by treatment with 4-PSQ.

In contrast, there are reports that patients with depression and AD have an impaired antioxidant system [54]. SOD and GPx are endogenous antioxidant enzymes which inhibit the formation of RS and/or remove free radicals and their precursors [58]. Thus, the deficiency of these antioxidant enzymes is implicated in the etiology of neuropsychiatric and neurodegenerative diseases [59]. In this study, we observed that the oxidative damage caused by exposure to Aβ peptide caused a reduction in the activity of the SOD enzyme and an increase in the activity of GPx in the prefrontal cortices and hippocampus of mice. We believe that the reduction in SOD activity is due to its ability to eliminate the excess RS generated, such as the superoxide anion radical ($O_2^{\cdot -}$) [54]. The SOD enzyme can dismute $O_2^{\cdot -}$, leading to the formation of H_2O_2 [60]. One of the enzymes responsible by antioxidant defense against peroxide toxicity is GPx [61]. Therefore, the increase in GPx activity found in this study could be justified as a compensatory action to reduce the concentration of H_2O_2 (toxic to cells), playing an important role in maintaining the redox environment of the cell. In this regard, 4-PSQ was able to normalize changes in the activity of these antioxidant enzymes in the prefrontal cortices and in the hippocampus of mice submitted to Aβ. In this way, we can suggest that the antidepressant-like action and improving memory effect of 4-PSQ may be, in part, through the oxidative stress modulation. It is worth noting that although the ability of 4-PSQ to modulate oxidative stress is not surprising, we cannot affirm that 4-PSQ directly modulates the redox state or other pathways/systems, reducing the process of cerebral oxidative stress.

Parallel to this, neuroinflammation is directly related to Aβ peptide neurotoxicity [50]. Previously, it was demonstrated that the accumulation and aggregation of Aβ peptide in brain regions (mainly the hippocampus) results in the activation of glial cells, mainly astrocytes, triggering a series of conformational, transcriptional, and functional changes [62]. Activation of astrocytes involves overexpression of glial fibrillary acidic protein (GFAP), as well as the release of oxidative mediators and inflammatory cytokines, such as IL-6

and TNF-α, initiating the neuroinflammatory process [62–64]. Furthermore, depression and AD cause changes in the blood-brain barrier, allowing the permeability of peripheral cytokines to the central nervous system, intensifying neuroinflammation [65]. Therefore, our results are consistent with previous studies, since the Aβ peptide induced the expression of pro-inflammatory cytokines, such as IL-6 and TNF-α in the prefrontal cortices and hippocampus and caused an increase in GFAP levels in the hippocampus of mice. Based on the previously demonstrated evidence, we believed that HPA axis hyperactivation and increased oxidative stress favored the neuroinflammatory response observed in this study. Moreover, an important finding was that treatment with 4-PSQ reversed the increase in neuroinflammatory parameters (IL-6, TNF-α and GFAP) in the evaluated brain structures. We suggested that the effect of 4-PSQ on neuroinflammation attenuation is associated with modulation of HPA axis activation and reduction of oxidative stress in prefrontal cortices and hippocampus of mice. In addition, previous studies have shown that attenuation of the HPA axis and oxidative stress is correlated with the reduction of neuroinflammatory processes [18, 24, 66]. In this context, our findings demonstrate the importance of the therapeutic use of 4-PSQ, since it was able to attenuate neuroinflammation and oxidative stress, as well as activate the HPA axis, reducing depressive-type symptoms and memory impairment caused by Aβ.

It was previously proposed that the neurotoxicity of the Aβ peptide is also capable of causing changes in the cholinergic system [67]. AChE is the enzyme of the cholinergic system responsible for hydrolyzing the acetylcholine (ACh) neurotransmitter, ending cholinergic transmission [68]. Therefore, the stimulation of AChE activity results in a reduction in the availability of ACh, being this an enzyme responsible for maintaining the processing of synapses and modulation of cognition and memory [69]. Therefore, the reduction in ACh levels is the main cause of cognitive impairment [70]. In addition, it has been shown that AChE also has non-cholinergic functions, through interaction with the Aβ peptide in its peripheral anionic site. With this, there is the formation of amyloid fibrils, which favor the increase of toxicity and deposition in neuronal cells of Aβ peptide [71, 72].

In view of this, to explain the mechanisms by which 4-PSQ attenuated the behavioral changes caused by the Aβ peptide, we also evaluated the activity of

the AChE enzyme. In the current study, induction with Aβ increased the activity of AChE in the prefrontal cortices and hippocampus of mice, indicating an association with memory impairment also observed in these animals. 4-PSQ normalized the stimulation of AChE activity caused by Aβ peptide in the evaluated brain structures. In addition, previous reports demonstrated that drugs which can inhibit AChE also act reducing neuropsychiatric symptoms, such as apathy, a common feeling in depressed patients [73]. Therefore, our findings indicate that the anticholinesterase effect of 4-PSQ may be related with attenuation of memory impairment and antidepressant-like behavior in the AD model. Thus, we believed that the neuroprotective effects of 4-PSQ can be partially explained by its ability to modulate AChE activity, as previously reported [11, 74]**.**

In conclusion, 4-PSQ attenuated depressant-like behavior and cognitive impairment induced by Aβ. We associated the effect of this compound with the restoration of the HPA axis, modulation of oxidative stress, attenuation of neuroinflammatory markers and modulation of the cholinergic system thought AChE enzyme. Our findings are extremely important, considering that these comorbidities share similar neurochemical changes. Therefore, the results of this study support the use of 4-PSQ as a possible promising alternative for the treatment of depression associated with AD. Hence, further studies are required to elucidate the other mechanisms involved in this pharmacological action of 4-PSQ.

Declarations

Ethics Approval

The study was approved by the Committee on Care and Use of Experimental Animal Resources, Federal University of Pelotas, Brazil (CEEA 1974–2016), following the National Institutes of Health guide for the care and use of laboratory animals (NIH Publications No. 8023, revised 1978).

Consent to Participate

Informed consent was obtained from all individual participants included in the study.

Consent for Publication

Not applicable.

Availability of data and materials

The datasets analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Competing Interests

The authors declare no competing interests.

Funding

We are grateful to UFPel, CNPq (429859/2018-0), FAPERGS (PqG 17/2551-0001013-2 and 19/2551-0001745-6) for financial support. This study was also financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível superior – Brasil (CAPES) - Finance Code 001. R.L.O. is the recipient of a CAPES fellowship. S.P., D.A., E.A.W., and C.L. are recipients of CNPq fellowship.

Author contribution

R.L.O performed the experiments, the analysis of data and wrote the manuscript. R.L.O., G.T.V., K.C.R. A.G.V. and M.P.P. performed the experiments. R.L.O., E.A.W. and C.L. designed the project. A.S.L. and D.A. synthesized the compound 4-PSQ. V.C.B, R.A.V., J.L.G, C.B.Q, E.M.F and S.P performed additional experiments. E.A.W. and C.L. supervised the experiments. All authors critically reviewed the content and approved the final version for publication.

Acknowledgements

Not applicable.

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Legend of figures

Fig. 1 Chemical structure of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ)

Fig. 2 Scheme of experimental protocol. Thirty minutes before starting intragastric (i.g.) treatments, mice received amyloid β-peptide (Aβ, fragment 25–35) in aggregated form or vehicle (saline), both intracerebroventricularly (i.c.v.). Treatments were performed every day, until the end of the experimental protocol. Between the fifth and seventh day of the protocol, the animals were submitted to behavioral tests: tail suspension test (TST), open field test (OFT), forced swimming test (FST) and step-down inhibitory avoidance tests (SDIAT). On the seventh- day, after the behavioral assessment, the animals were sacrificed for biochemical analyzes (ex vivo)

Fig. 3 Effect of 7-chloro-4-(phenylselanyl)quinoline (4-PSQ) or paroxetine (Parox.) or donepezil (Done) in the behavioral changes induced by amyloid β (Aβ) peptide. (a) Training and (b) test at the Step-down inhibitory avoidance (SDIAT), (c) tail suspension test (TST), (d) forced swimming test (FST), (e) crossings and (f) rearings at the open field test (OFT). Values are expressed as mean ± standard error of the mean (S.E.M.) (n = 7). (*) denotes $p < 0.05$, (***) denotes $p < 0.001$ and (****) denotes $p < 0.0001$ when compared to the sham group. (#) denotes $p < 0.05$, (###) denotes $p < 0.001$ and (####) denotes $p < 0.0001$ when compared with the Aβ-induced group. (&) denotes denotes $p < 0.05$ when compared with the Aβ + 4-PSQ group (One-way ANOVA followed by the Tukey's test)

Fig. 4 Effect of 7-chloro-4- (phenylselanyl) quinoline (4-PSQ) on corticosterone plasma levels in mice submitted to induction with amyloid β-peptide (Aβ). Values are expressed as mean ± standard error of the mean (S.E.M.) (n = 7). (****) denotes $p < 0.0001$ when compared to the sham group. (####) denotes $p < 0.0001$ when compared with the Aβ-induced group (One-way ANOVA followed by the Tukey's test)

Fig. 5 Effect of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) on markers of oxidative stress in mice submitted to induction with amyloid β-peptide (Aβ).

Reactive species (RS) levels in (a) prefrontal cortex and (b) hippocampus; Thiobarbituric acid reactive species (TBARS) levels in (c) prefrontal cortex and (d) hippocampus. Values are expressed as mean ± standard error of the mean (S.E.M.) (n = 7). (**) denotes $p < 0.01$, (***) denotes $p < 0.001$ and (****) denotes $p < 0.0001$ when compared to the sham group. (#) denotes $p < 0.05$ and (####) denotes $p < 0.0001$ when compared with the Aβ-induced group (One-way ANOVA followed by the Tukey's test)

Fig. 6 Effect of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) in the activity of antioxidant enzymes in mice submitted to induction with amyloid β-peptide (Aβ). Superoxide dismutase (SOD) activity in (a) prefrontal cortex and (b) hippocampus; Glutathione peroxidase (GPx) activity in (c) prefrontal cortex and (d) hippocampus. Values are expressed as mean ± standard error of the mean (S.E.M.) (n = 7). (**) denotes $p < 0.01$, (***) denotes $p < 0.001$ and (****) denotes $p < 0.0001$ when compared to the sham group. (##) denotes $p < 0.01$ and (####) denotes $p < 0.0001$ when compared with the Aβ-induced group (One-way ANOVA followed by the Tukey's test)

Fig. 7 Effect of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) on the levels of pro-inflammatory cytokines in the brain regions of mice submitted to induction with amyloid β-peptide (Aβ). Tumor necrosis factor alpha (TNF-α) expression in (a) prefrontal cortex and (b) hippocampus; Interleukin-6 (IL-6) expression in (c) prefrontal cortex and (d) hippocampus. Values are expressed as mean ± standard error of the mean (S.E.M.) (n = 3). (*) denotes $p < 0.05$, (**) denotes $p < 0.01$, (***) denotes $p < 0.001$ and (****) denotes $p < 0.0001$ when compared to the sham group. (#) denotes $p < 0.05$ and (####) denotes $p < 0.0001$ when compared with the Aβ-induced group (One-way ANOVA followed by the Tukey's test)

Fig. 8 Effect of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) treatment aganist alterations induced by amyloid β-peptide (Aβ) in (a) Glial Fibrillary Acidic Protein (GFAP) levels in the hippocampus of animals and (b) representative images of immunofluorescence assay (GFAP-positive astrocytes appear as red signals

generated by Alexa 594. The nuclei were stained blue by DAPI. The GFAP and DAPI content was evaluated by immunofluorescence microscopy. Scale bar: 25 μm). Values are expressed as mean ± standard error of the mean (S.E.M.) (n = 4). (*) denotes $p < 0.05$ when compared to the sham group. (##) denotes $p < 0.01$ when compared with the Aβ-induced group (One-way ANOVA followed by the Tukey's test)

Fig. 9 Effects of 7-chloro-4-(phenylselanyl) quinoline (4-PSQ) in the activity of acetylcholinesterase (AChE) in (a) prefrontal cortex and (b) hippocampus in mice submitted to induction with amyloid β-peptide (Aβ). Values are expressed as mean ± standard error of the mean (S.E.M.) (n = 7). (*) denotes $p < 0.05$, (**) denotes $p < 0.01$ and (****) denotes $p < 0.0001$ when compared to the sham group. (##) denotes $p < 0.01$ and (####) denotes $p < 0.0001$ when compared with the Aβ-induced group (One-way ANOVA followed by the Tukey's test)

Figures

Fig. 1

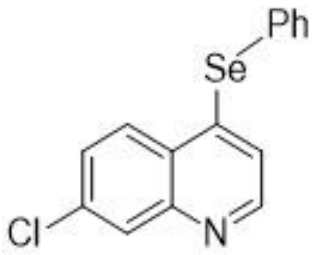

Fig. 2

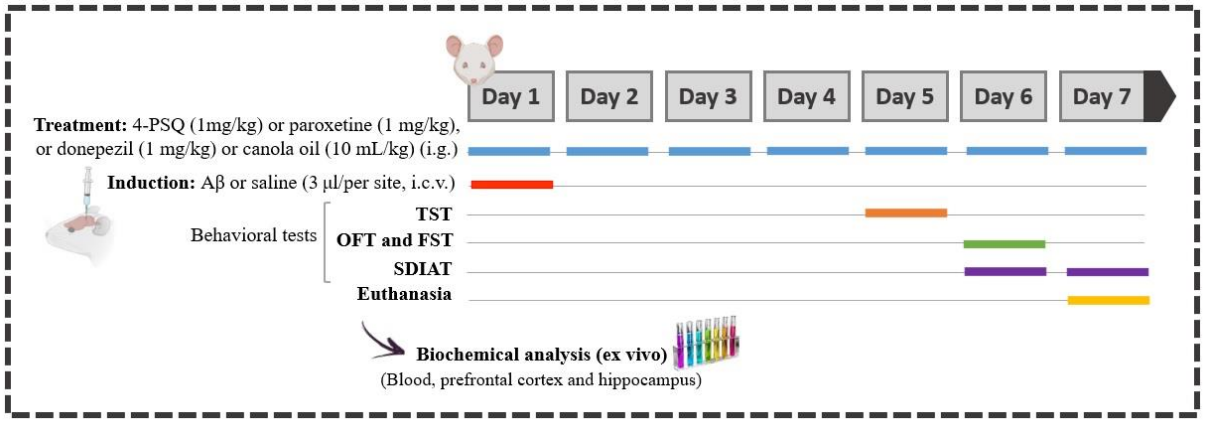

Fig. 3

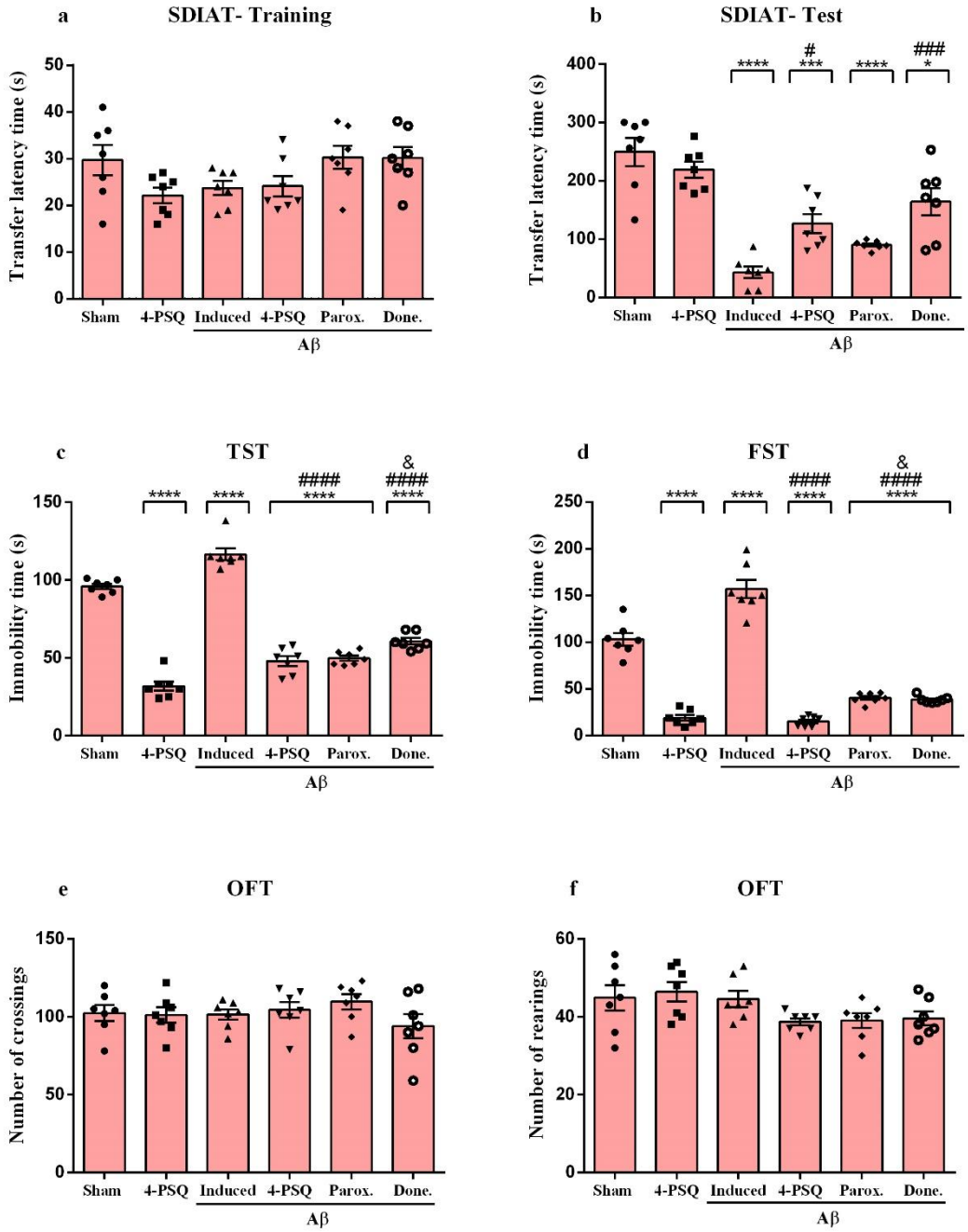
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Fig. 4

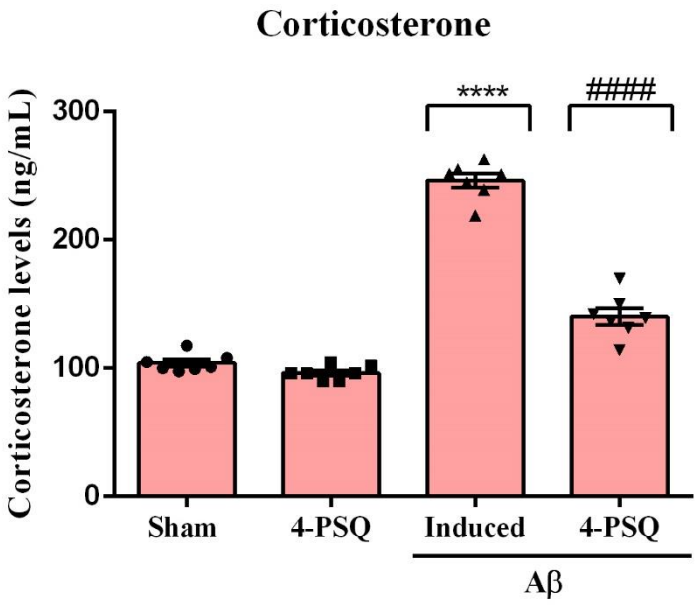
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Fig. 5

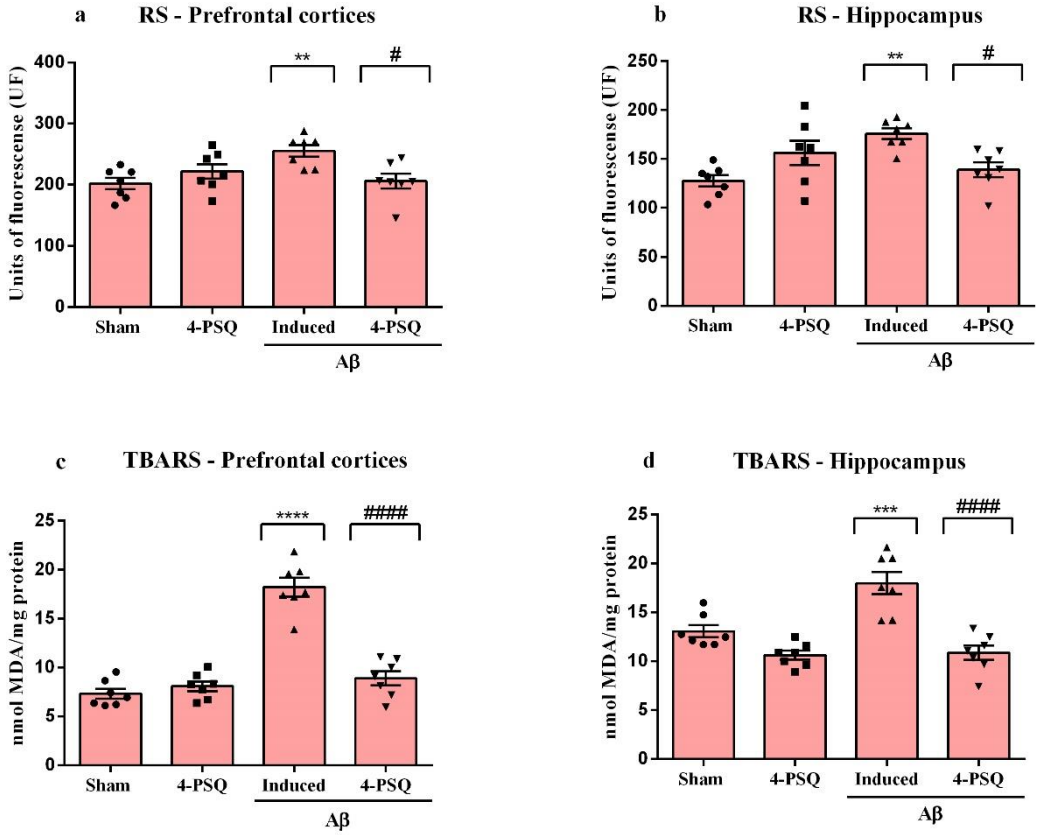
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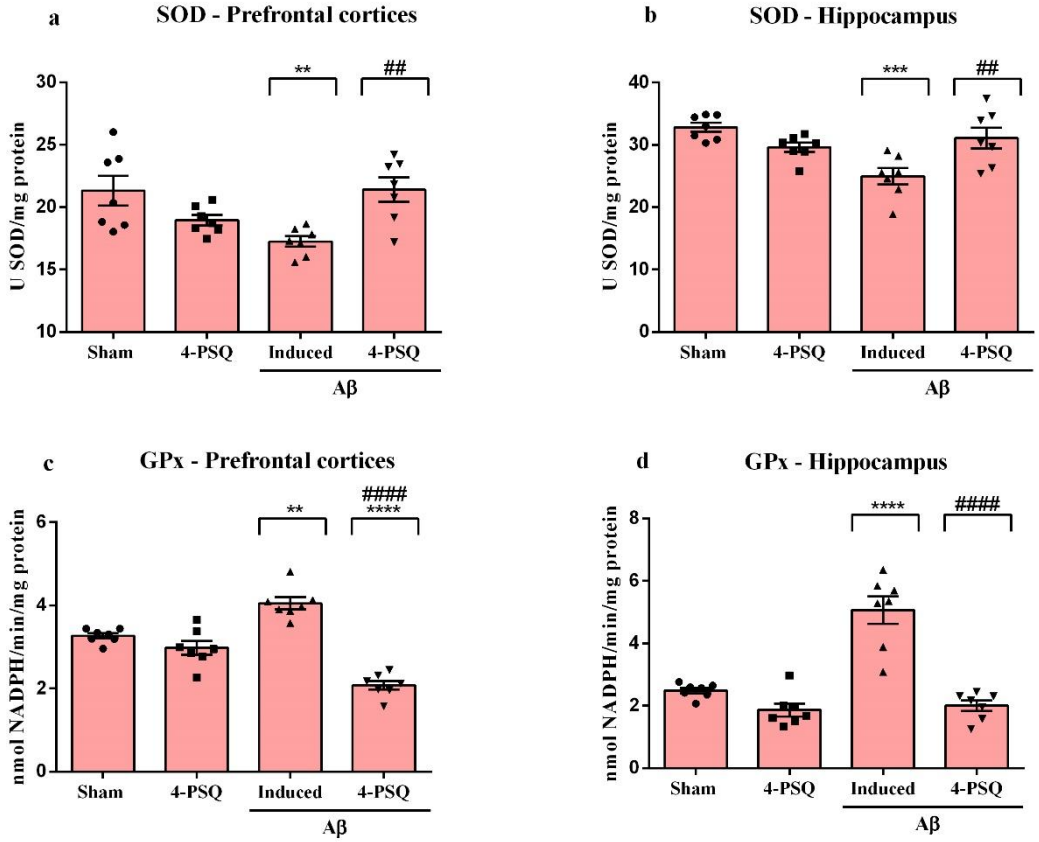
Fig. 6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****
Sham
4-PSQ
Induced
4-PSQ
Aβ
d
GPx - Hippocampus
nmol NADPH/min/mg protein
8
6
4
2
0
****
####
Sham
4-PSQ
Induced
4-PSQ
Aβ

Fig. 7

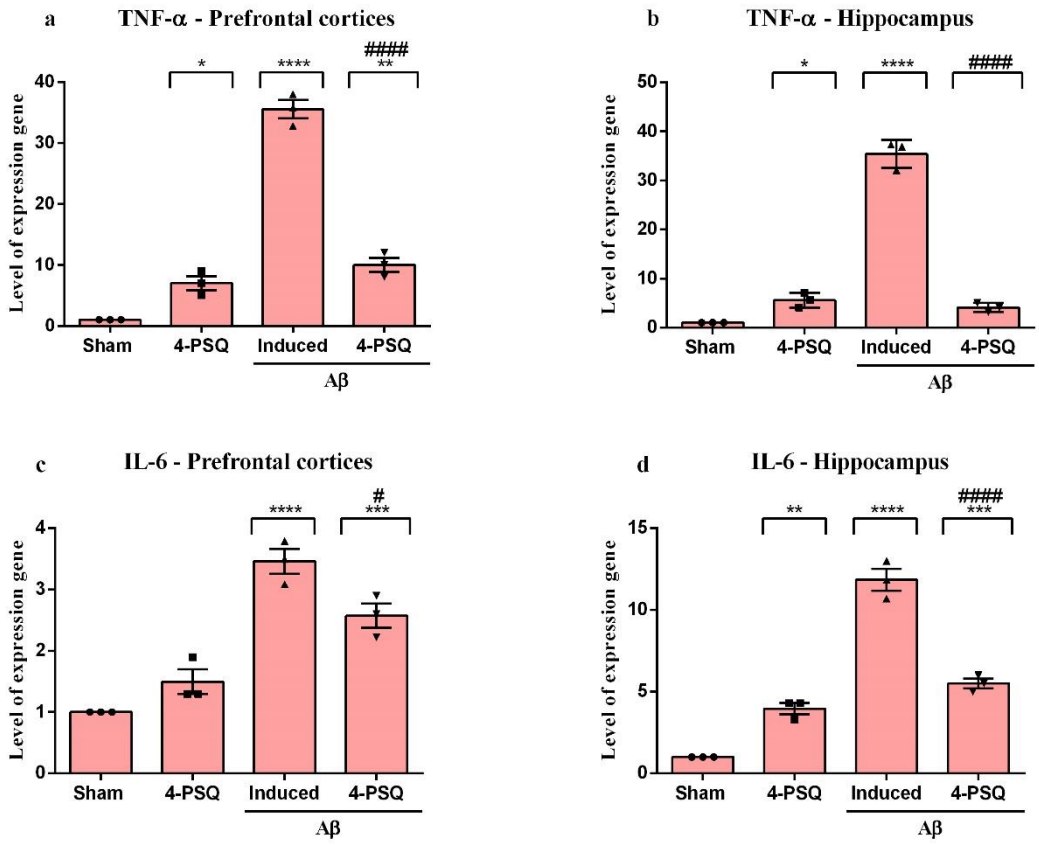
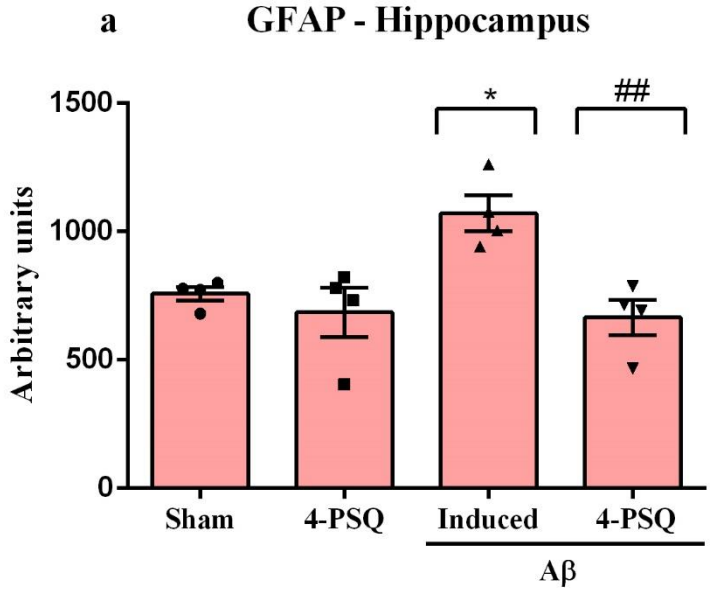

a
TNF-α - Prefrontal cortices
Level of expression gene
Sham
4-PSQ
Induced
4-PSQ
Aβ
b
TNF-α - Hippocampus
c
IL-6 - Prefrontal cortices
d
IL-6 - Hippocampus

Fig. 8


a
GFAP - Hippocampus
Arbitrary units
Sham
4-PSQ
Induced
4-PSQ
Aβ

b

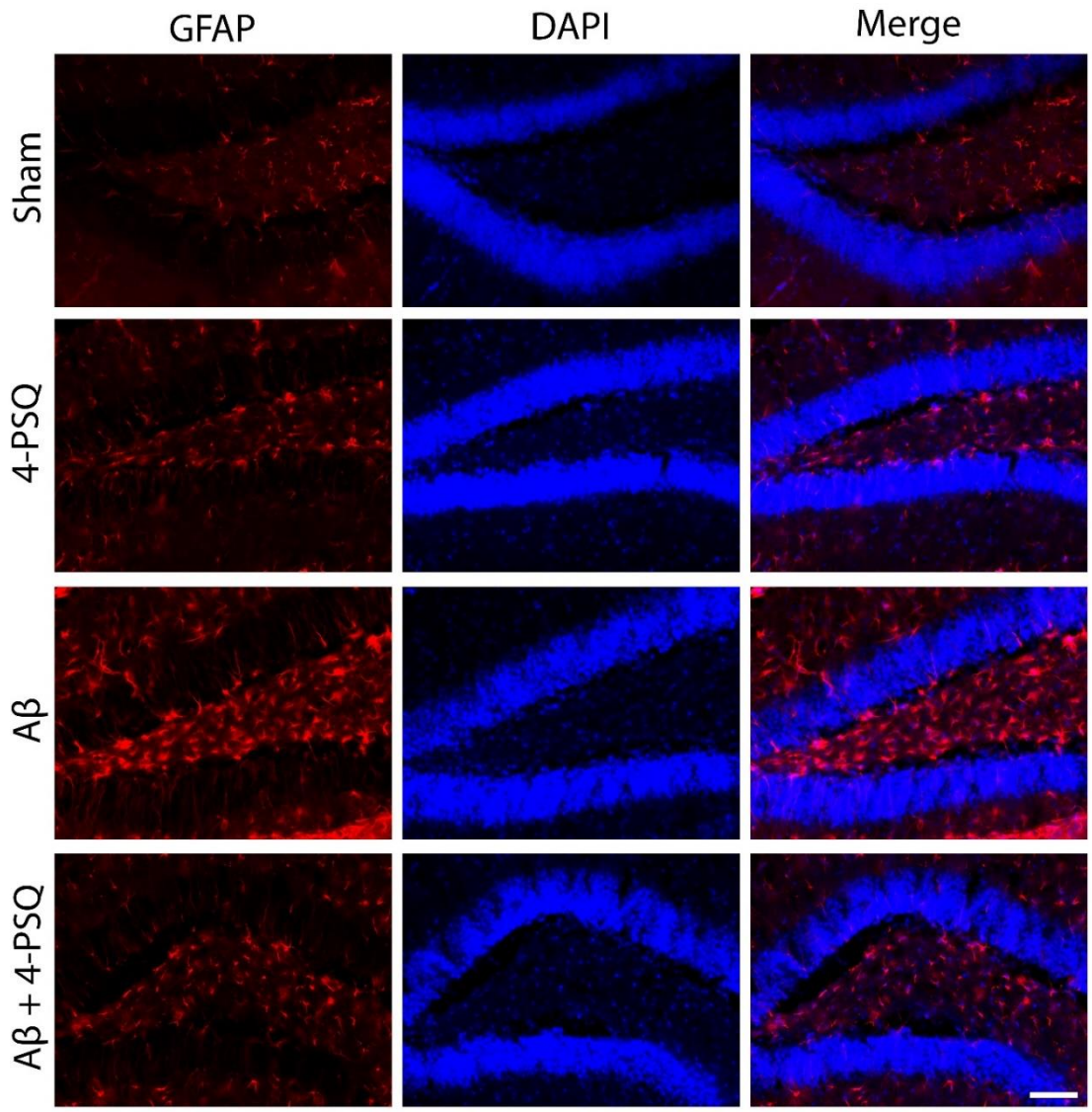
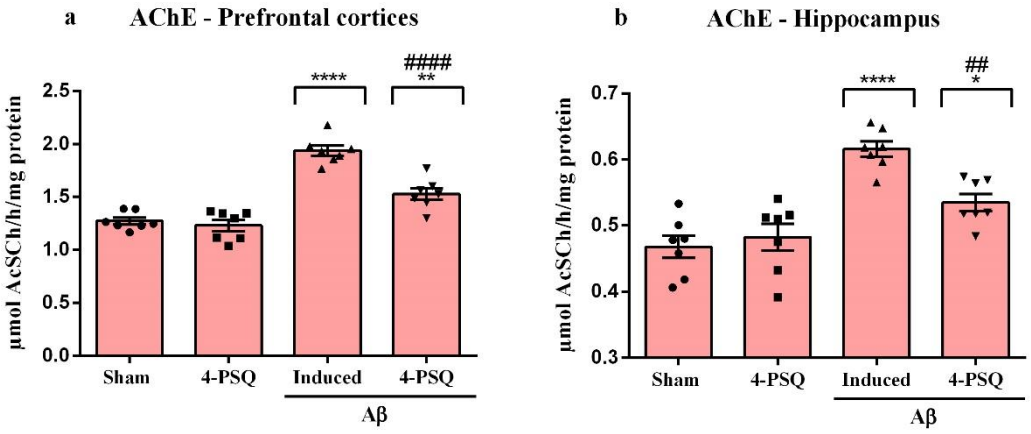

GFAP
DAPI
Merge
Sham
4-PSQ
Aβ
Aβ + 4-PSQ

Fig. 9


a
AChE - Prefrontal cortices
µmol AcSCh/h/mg protein
2.5
2.0
1.5
1.0
0.5
0.0
****
**
####
Sham
4-PSQ
Induced
4-PSQ
Aβ
b
AChE - Hippocampus
µmol AcSCh/h/mg protein
0.7
0.6
0.5
0.4
0.3
****
*
##
Sham
4-PSQ
Induced
4-PSQ
Aβ

5. DISCUSSÃO

Na presente tese demonstrou-se pela primeira vez, o efeito da 4-PSQ em atenuar o comportamento do tipo-depressivo associado ao comprometimento de memória/DA, em dois diferentes modelos experimentais em camundongos. De acordo com os resultados obtidos em ambos os estudos, acredita-se que os efeitos benéficos da 4-PSQ na comorbidade depressão e comprometimento de memória/DA, possam estar relacionados com a sua capacidade de restaurar o eixo HPA, reduzir o estresse oxidativo cerebral, modular o sistema monoaminérgico, restabelecer as alterações no sistema colinérgico, bem como modular fatores de transcrição envolvidos na neuroinflamação e neuroplasticidade. Nesse sentido, nossos achados demonstraram que a 4-PSQ é uma molécula multialvo, sugerindo seu potencial clínico para o tratamento da comorbidade, depressão e comprometimento de memória/DA.

No **primeiro estudo (Artigo)**, utilizou-se o modelo de ARS que é responsável por induzir alterações comportamentais e neuroquímicas semelhantes as encontradas em pacientes depressivos, além de causar comprometimento de memória em roedores, e por isso foi útil em avaliar a comorbidade depressão e comprometimento de memória (AMIN et al., 2020; WADA et al., 2020). Tendo em vista que obteve-se resultados positivos no primeiro estudo, optou-se por aprofundar os conhecimentos sobre os efeitos da 4-PSQ na comorbidade depressão e DA, e para isto, no **segundo estudo (Manuscrito)** utilizou-se o modelo de DA induzido pelo peptídeo βa (fragmento 25-35), tendo em vista que este modelo é conhecido por mimetizar os sintomas cognitivos da DA, como o comprometimento de memória (IANISKI et al., 2012; PINZ et a., 2018). Ademais, diversos estudos demostram que a indução com o peptídeo βa também é capaz de causar sintomas do tipo-depressivo em roedores (CHENG et al., 2009; FIDELIS et al., 2019; SOULTANOV et al., 2017).

Para avaliar a depressão em modelos animais, são utilizados diferentes testes comportamentais, como o teste da suspensão da cauda (TST, do inglês *tail suspension test*) e o teste do nado forçado (FST, do inglês *forced swimming test*) que avaliam sintomas do tipo-depressivos em roedores. Estes testes apresentam validade mecanista para avaliar o desespero comportamental em roedores, no qual se assemelha aos sintomas de depressão em humanos (MCGONIGLE, 2014). Ainda, esses testes são considerados testes preditivos

utilizados para avaliar o rastreamento de compostos com propriedades do tipo-antidepressivas em modelos animais (PLANCHEZ; SURGET; BELZUNG, 2019). De acordo com isto, em **ambos os estudos**, as induções (ARS (**Artigo**) e βa (**Manuscrito**)) causaram um comportamento do tipo-depressivo nos camundongos, demonstrados pelo aumento no tempo de imobilidade no TST e FST. O tratamento com a 4-PSQ atenuou o comportamento do tipo-depressivo induzido por ARS e βa, sugerindo um efeito do tipo-antidepressivo da 4-PSQ nestes modelos. Vale ressaltar, que no **segundo estudo (Manuscrito)** o tratamento *per se* com 4-PSQ apresentou efeito do tipo-antidepressivo, sendo que este efeito não foi observado no **primeiro estudo (Artigo)**. De fato, pode-se sugerir que as diferentes doses utilizadas e os períodos de tratamento foram os responsáveis pelas diferenças deste efeito, uma vez que no **primeiro estudo (Artigo)** a 4-PSQ foi administrado em uma única dose de 10 mg/kg e no **segundo estudo (Manuscrito)** o tratamento com a 4-PSQ se deu durante sete dias na dose de 1 mg/kg.

Além disso, outra característica de pacientes com depressão é a redução do comportamento de autocuidado (comparado a anedonia), sintoma que pode ser avaliado no teste da borrifagem de sacarose (SPT, do inglês *splash test*), por meio do tempo de limpeza (*grooming)* (FREITAS et al., 2014). Os resultados encontrados no **primeiro estudo (Artigo)** mostraram que o ARS reduziu o comportamento de autocuidado, o que demonstra que o estresse é capaz de induzir sintomas do tipo-depressivos com características semelhantes àquelas encontradas em pacientes com depressão (GONG et al., 2016). Por outro lado, o tratamento com 4-PSQ atenuou o comportamento de autocuidado nos camundongos estressados, conforme demonstrado pelo aumento do tempo de limpeza *(grooming)* no SPT.

O ARS é um modelo de depressão validado por meio da hiperativação do eixo HPA, no qual resulta no aumento nos níveis de GC circulantes, como a corticosterona em roedores (JOSEPH; GOLDEN, 2017). Esta alteração pode ser observada tanto em pacientes depressivos quanto naqueles com DA (CARACI et al., 2010; MARQUES; SILVERMAN; STERNBERG, 2009; POPP et al., 2015). Estímulos estressantes para o organismo, como o estresse e a neurotoxicidade do peptídeo βa, estão associados à hiperatividade do eixo HPA (JAYASINGH CHELLAMMAL et al., 2019). De acordo com isto, em **ambos os estudos (Artigo**

e Manuscrito), a 4-PSQ foi capaz de reestabelecer a disfunção do eixo HPA causada pelo ARS e pela neurotoxicidade do peptídeo βa, por meio da redução nos níveis de corticosterona plasmática. Nesse sentido, os dados do presente estudo sugerem que a hiperativação do eixo HPA contribuiu para o desenvolvimento do comportamento do tipo-depressivo e o comprometimento de memória observado em animais submetidos ao ARS e ao βa, e que a 4-PSQ foi capaz de atenuar esses comportamentos modulando o eixo HPA. Neste contexto, um estudo de WOLF (2008) demonstrou que pacientes com níveis elevados de cortisol frente a estressores psicossociais, apresentavam deficiência em tarefas que exigiam recordação de informações aprendidas previamente. Adicionalmente, outro estudo relatou que a elevação sustentada da corticosterona causa efeitos deletérios na estrutura e função de importantes regiões do cérebro em modelos animais, como córtex pré-frontal e hipocampo (MCEWEN; GIANAROS, 2010). De acordo com isto, os nossos resultados estão de acordo com o que está descrito na literatura levando em consideração que o aumento nos níveis de GCs circulantes prejudicam a funcionalidade neuronal e desencadeiam alterações cognitivas e comportamentais.

É importante destacar que, os resultados apresentados nesta tese demonstram que a 4-PSQ é capaz de atenuar o comprometimento da memória de trabalho (como evidenciado pelo aumento comportamento alternâncias espontâneas no teste de labirinto Y) e a memória de curto e longo prazo (conforme demonstrado pelo aumento na preferência exploratória pelo novo objeto no teste do reconhecimento do objeto), no modelo de ARS (**primeiro estudo – Artigo)** e a memória não espacial de longo prazo (evidenciado pelo aumento no tempo de latência de transferência no teste da esquiva inibitória, no modelo de βa (**segundo estudo – Manuscrito II**). Essas descobertas estendem os achados anteriores do nosso grupo de pesquisa que demonstram que a 4-PSQ atenuou o aprendizado e o comprometimento da memória em diferentes parâmetros comportamentais em um modelo de DA induzido por βa em camundongos (PINZ et al., 2018). Neste sentido, os resultados deste estudo evidenciam que a 4-PSQ apresenta efeito do tipo-antidepressivo e reverte o comprometimento de memória, suportando o seu efeito farmacológico no tratamento da comorbidade depressão e comprometimento de memória/DA em diferentes modelos experimentais. Além disso, nenhum dos tratamentos causou

alterações nas atividades locomotora e exploratória espontânea dos camundongos, indicando, principalmente, que o efeito da 4-PSQ não se deve a alterações inespecíficas, como a atividade psicoestimulante. Desta forma, este é um resultado importante, visto que drogas psicoestimulantes podem dar um resultado falso positivo em modelos animais (CRYAN; PAGE; LUCKI, 2005).

Relatos anteriores descrevem uma possível relação entre a hiperativação do eixo HPA e o aumento dos parâmetros oxidativos, no qual os GCs favorecem um aumento na taxa metabólica, e por sua vez há um aumento na produção de espécies reativas (RS, do inglês *reactive species*) (BALMUS et al., 2016). Além disso, o estresse pode tornar o cérebro vulnerável aos danos oxidativos, através da ativação de vias de sinalização intracelular envolvidos na formação de radicais livres (CHE et al., 2015). Em paralelo, sabe-se que a exposição a neurotoxicidade do peptídeo βa contribui para a produção exacerbada de RS e produtos da peroxidação lipídica, indução de dano mitocondrial e, consequentemente, a morte celular (CHAUHAN; CHAUHAN, 2006). Portanto, em ambos os estudos se avaliou os marcadores de estresse oxidativo (RS e TBARS) e a atividade das enzimas antioxidantes, na tentativa de elucidar os possíveis efeitos modulatórios/antioxidantes da 4-PSQ. De acordo com os resultados obtidos em **ambos os estudos (Artigo e Manuscrito)**, descobriu-se que tanto o ARS como o peptídeo βa, aumentaram os níveis de RS e o processo de peroxidação lipídica no córtex pré-frontal e no hipocampo dos camundongos, indicando que ambos os modelos de indução ocasionaram um dano oxidativo cerebral. Além disso, observou-se alterações na atividade das enzimas SOD, GPx e GR nas estruturas cerebrais dos camundongos submetidos ao ARS e ao βa. De fato, um aumento nos níveis de RS favorece o processo de peroxidação lipídica e altera as defesas antioxidantes em regiões límbicas (KIM et al., 2016), causando uma redução no estado antioxidante no cérebro (CASARIL et al., 2019; MORETTI et al., 2013; SOUSA et al., 2018). De acordo com isto, na tentativa de eliminar as RS, ocorre um desequilíbrio na atividade das enzimas antioxidantes com o intuito de manter o equilíbrio redox das células neuronais (KULAK et al., 2013).

Em contraste, a 4-PSQ modulou o estresse oxidativo em **ambos os estudos (Artigo e Manuscrito)**, reduzindo os níveis de RS e o processo de peroxidação lipídica e reestabeleceu as alterações na atividade das enzimas

antioxidantes nas estruturas cerebrais avaliadas, indicando seu possível efeito antioxidante. De acordo, estudos anteriores realizados em nosso laboratório já foi demonstrado o efeito antioxidante desse composto em diferentes modelos experimentais (DUARTE et al., 2017; LEMOS et al., 2021; LUCHESE et al., 2020; PINZ et al. 2016, 2018, VOGT et al., 2018). Portanto, sugerimos que os efeitos neurotóxicos do peptídeo βa, o ARS e a consequente hiperativação do eixo HPA podem ter cooperado no processo de estresse oxidativo e, assim, contribuído para o desenvolvimento de comportamento do tipo depressivo e comprometimento de memória, que foram mitigados pelo tratamento com 4-PSQ.

A redução dos níveis de neurotransmissores monoaminérgicos no SNC é considerado um importante alvo que tem sido estudado na comorbidade depressão e comprometimento da memória/DA (BEHL et al., 2021; OTTE et al., 2016; VERMEIREN et al., 2014), no qual a enzima MAO é a enzima responsável por regular estes níveis (FINBERG; RABEY, 2016). Essa enzima existe em duas isoformas enzimáticas, MAO-A (ao inibir se tem efeito do tipo-antidepressivos) e MAO-B (é modulada em distúrbios neurodegenerativos, como na DA) (SAURA et al., 1994; SHIH; CHEN; RIDD, 1999). Nossos resultados do **primeiro estudo (Artigo)** mostram que a ARS induziu um aumento na atividade das isoformas MAO-A e MAO-B no córtex pré-frontal e hipocampo de camundongos. Assim, um aumento na atividade das isoformas da MAO está associado a um desequilíbrio no sistema monoaminérgico, com redução de neurotransmissores, e com base nas evidências, acreditamos que isso tenha contribuído para o comportamento do tipo-depressivo e comprometimento da memória observados em nosso estudo. Por outro lado, o tratamento com 4-PSQ atenuou as alterações na atividade da MAO-A e MAO-B no córtex pré-frontal e hipocampo de camundongos estressados. Assim, a inibição dupla de MAO-A e MAO-B pode ser valiosa para a terapia de depressão e comorbidade comprometimento da memória/DA.

Além dos mecanismos acima relatados, a neuroinflamação está frequentemente associada à depressão e à DA (AMANI et al., 2019; MAES et al., 2011). De acordo com isto, os eventos neuroinflamatórios são orquestrados por fatores de transcrição, como NF-κB, que podem ser ativados por diferentes estímulos, como estresse, presença de RS e respostas inflamatórias (BALMUS

et al., 2016; SIOMEK, 2012). No **primeiro estudo (Artigo)** verificou-se que o ARS aumentou a expressão de RNAm do NF-κB no córtex pré-frontal e hipocampo dos animais estressados. O NF- κB desempenha um papel fundamental na inflamação por meio de sua capacidade de modular a transcrição de citocinas pró-inflamatórias e assim favorecer o desequilíbrio oxidativo (JOPE et al., 2017). Ademais, outra componente chave nessas respostas é na ativação de células gliais, principalmente astrócitos, devido ao acúmulo e agregação do peptídeo βa em regiões do cérebro (principalmente o hipocampo) (UDDIN et al., 2020). Esta ativação de envolve a superexpressão da proteína glial fibrilar ácida (GFAP, do inglês *glial fibrillary acidic protein*), bem como a ativação e liberação de mediadores inflamatórias, como NF-κB e ILs (MUCKE, 2009; UDDIN et al., 2020; VETRIVEL; THINAKARAN, 2010). Neste contexto, um achado importante do presente estudo foi que o tratamento com a 4-PSQ reverteu o aumento do NF-κB, IL-1β, IL-18, IL-33 no **primeiro estudo (Artigo)** e os níveis de IL-6, TNF-α e GFPA no **segundo estudo (Manuscrito)** nas estruturas cerebrais dos camundongos. Assim, os achados da presente tese sugerem que o efeito da 4-PSQ em modular a neuroinflamação e o estresse oxidativo, bem como restabelecer o eixo HPA, está associado à redução dos sintomas do tipo-depressivos e do comprometimento de memória causados pelo estresse.

Para expandir a avaliação dos possíveis mecanismos de ação da 4-PSQ na comorbidade depressão e comprometimento de memória/DA, investigou-se o sistema colinérgico, por meio da avaliação da atividade da enzima AChE. Levando em consideração que a AChE é responsável por reduzir a transmissão colinérgica através da hidrólise do neurotransmissor ACh, uma estimulação na atividade da AChE está diretamente relacionada com o desenvolvimento do comprometimento cognitivo e de memória (MOKRANI et al., 2019). Diante disso, em **ambos os estudos (Artigo e Manuscrito)**, tanto o ARS como o βa causaram um aumento na atividade da AChE no córtex pré-frontal e hipocampo dos camundongos. Assim, um aumento na atividade da AChE está associado com um desequilíbrio no sistema colinérgico, com perda da função cerebral, e acredita-se que isso tenha contribuído para o comprometimento da memória observado neste estudo. Além disso, relatos anteriores demonstram que medicamentos que inibem a AChE também atuam na redução dos sintomas neuropsiquiátricos, como apatia, um sentimento comum em pacientes

deprimidos (MCCLOSKEY; YOUNG; ANDERSON, 2017). Nesse sentido, a 4-PSQ atenuou as mudanças comportamentais e normalizou a atividade da AChE causada pela exposição ao ARS e ao peptídeo βa nas estruturas cerebrais avaliadas. Portanto, estes resultados indicam que o efeito anticolinesterásico da 4-PSQ pode estar relacionado à atenuação do comprometimento da memória e comportamento do tipo-antidepressivo nos modelos experimentais avaliados. Assim, acredita-se que os efeitos da 4-PSQ podem ser parcialmente explicados por sua capacidade de modular a atividade da AChE, conforme relatado anteriormente pelo nosso grupo de pesquisa (BARTH et al., 2019; DUARTE et al., 2017; PINZ et al., 2018; RODRIGUES et al., 2021).

Notavelmente, algumas moléculas de sinalização celular estão envolvidas na progressão da depressão associada a DA (BEGNI; RIVA; CATTANEO, 2017). Entre estas vias de sinalização destaca-se a via PI3K/AKT, no qual a sua ativação é responsável por regular a sobrevivência neuronal e a neuroplasticidade, além de estar envolvida nos processos de aprendizagem, memória e depressão (QI et al., 2016). A PI3K ativa as três isoformas de AKT, porém neste estudo avaliou-se os níveis de expressão de RNAm de AKT2, devido à sua participação na regulação, diferenciação e sobrevivência neuronal. Além disso, a redução na expressão da AKT2 está associada com doenças psiquiátricas, como a depressão (DENG et al., 2019). De acordo com os resultados obtidos no **primeiro estudo (Artigo)**, verificou-se que a expressão de RNAm de PI3K e AKT2 foi reduzida no córtex pré-frontal de camundongos estressados. Em contrapartida, no hipocampo apenas os níveis de expressão do RNAm de AKT2 foram reduzidos após a exposição ao ARS em camundongos. Uma possível explicação para estas diferenças baseia-se no estudo de CUNHA e colaboradores (2015), que relatam que a ativação dessas quinases pode acontecer em sub-regiões do cérebro, dificultando sua detecção. Dessa forma, acredita-se que isso possa ter influenciado os resultados deste estudo, visto que não detectamos alterações nos níveis de expressão do RNAm de PI3K no hipocampo. De acordo com isso, os resultados sugeriram um envolvimento da via de sinalização PI3K/AKT2 no efeito da 4-PSQ, considerando que o composto foi capaz de normalizar os níveis de expressão destas moléculas

de sinalização a nível cortical, contribuindo para o restabelecimento dos processos de neuroplasticidade, neurogênese e mudanças comportamentais.

As descobertas desta tese são extremamente importantes, uma vez que os tratamentos disponíveis para depressão não são totalmente eficazes contra o comprometimento da memória associado (GALTS et al., 2019). Assim, os efeitos adversos dos antidepressivos somam-se aos das drogas utilizadas para o tratamento da DA (que atenuam o comprometimento da memória), e como consequência há uma redução na qualidade de vida do paciente acometido por essa comorbidade (BANERJEE et al., 2013). Assim, uma das principais vantagens da 4-PSQ é a possibilidade de um único fármaco para o tratamento de duas doenças, depressão e sua comorbidade, comprometimento da memória/DA.

6. CONCLUSÃO

Com base nos resultados apresentados nesta tese pode-se concluir que a 4-PSQ foi capaz de atenuar o comportamento do tipo-depressivo e o comprometimento de memória/DA induzido por diferentes protocolos experimentais (ARS e βa). A 4-PSQ orquestrou sua resposta multialvo atuando por diferentes mecanismos (Figura 7), como no eixo HPA, estresse oxidativo, neuroinflamação, disfunsão monoaminérgica, alterações em fatores de transcrição envolvidos na neuroplasticidade, bem como disfunção colinérgica.

De acordo com isto, os achados desta tese são de grande importância, considerando que a depressão e o comprometimento de memória/DA compartilham mecanismos neuroquímicos semelhantes e a 4-PSQ é capaz de normalizar estas alterações. Portanto, os resultados desta tese apoiam o uso da 4-PSQ como uma possível alternativa promissora para o tratamento da depressão associada a DA. Contudo, a fim de complementar os achados, estudos adicionais devem ser conduzidos para evidenciar outros mecanismos envolvidos no efeito da 4-PSQ.

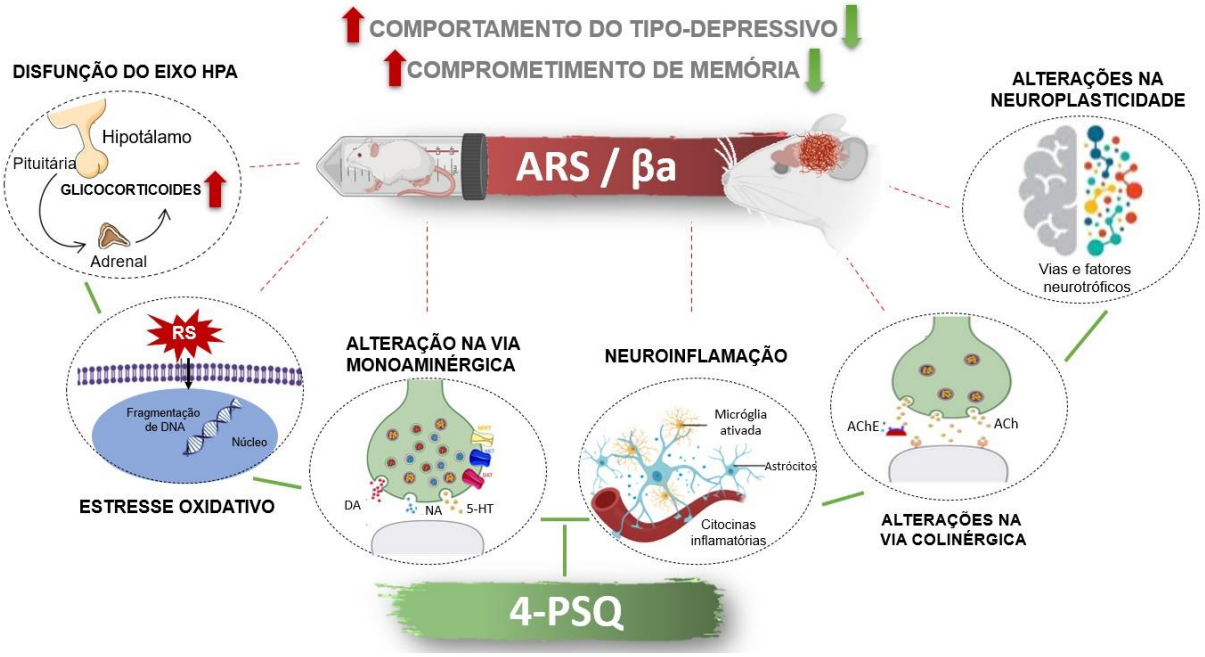


Figura 7. Resumo das ações do estresse agudo de contenção (ARS) e da indução com peptídeo beta amiloide (βa), bem como os efeitos da 7-cloro-4-(fenilselanil)quinolina (4-PSQ). O tratamento com 4-PSQ atenuou a ativação do

eixo hipotálamo-hipófise-adrenal (HPA) reduzindo os níveis plasmáticos de corticosterona; atenuou as alterações no sistema monoaminérgico, restabelecendo a atividade de isoformas da enzima monoaminooxidase (MAO) (MAO-A e MAO-B); modulou o estresse oxidativo, reduzindo os níveis de espécies reativas (RS), espécies reativas ao ácido tiobarbitúrico (TBARS) e atividade de enzimas antioxidantes; atenuou a neuroinflamação, modulou positivamente os fatores de transcrição (fosfatidilinositol-3-quinase [PI3K] e proteína quinase B-2 [AKT2]) envolvido na neuroplasticidade, bem como modulou o sistema colinérgico reduzindo a atividade da enzima acetilcolinesterase (AChE). Com base nas evidências, acreditamos que esse conjunto de resultados contribuiu para a redução do comportamento do tipo-depressivo e do comprometimento de memória induzido pelo ARS e por meio da indução com peptídeo βa.

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ANEXO 1

Carta de aprovação dos protocolos experimentais pela Comissão de Ética em Experimentação Animal da Universidade Federal de Pelotas

UNIVERSIDADE FEDERAL DE PELOTAS

PARECER Nº **44/2019/CEEA/REITORIA**
PROCESSO Nº 23110.028008/2019-15

Certificado

Certificamos que a proposta intitulada **"Prospecção de uma nova estratégia terapêutica para a comorbidade depressão e déficit cognitivo/doença de Alzheimer utilizando um derivado de quinolina: envolvimento de moléculas de sinalização celular e dos sistemas colinérgico e monoaminérgico "**, registrada com o nº 23110.028008/2019-15, sob a responsabilidade de **Cristiane Luchese** - que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica (ou ensino) – encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e recebeu parecer **FAVORÁVEL** a sua execução pela Comissão de Ética em Experimentação Animal, em reunião de **16/07/2019.**

É necessário que o fornecedor dos animais assine o TCLE.

Finalidade	(x) Pesquisa ( ) Ensino
Vigência da autorização	01/12/2019 a 01/12/2022
Espécie/linhagem/raça	*Mus musculus*/Swiss
N° de animais	2608
Idade	2283 animais com 60 dias e 325 animais com 20 meses
Sexo	Machos
Origem	Biotério Central - UFPel

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