UNIVERSIDADE FEDERAL DE PELOTAS

Programa de Pós-Graduação em Biotecnologia

Dissertação

Estudos pré-clínicos da ação anti-Alzheimer do octilseleno-xilofuranosídeo

Rodolfo da Silva Mazzarini Baldinotti

Pelotas, 28 de Fevereiro de 2018

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Estudos pré-clínicos da ação anti-Alzheimer do octilseleno-xilofuranosídeo

Dissertação apresentada ao Programa de Pós-Graduação em Biotecnologia da Universidade Federal de Pelotas, como requisito parcial à obtenção do título de Mestre em Ciências.

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Coorientadora: Lucielli Savegnago

Pelotas, 28 de Fevereiro de 2018

Universidade Federal de Pelotas / Sistema de Bibliotecas
Catalogação na Publicação

B177e Baldinotti, Rodolfo da Silva Mazzarini

Estudos pré-clínicos da ação anti-alzheimer do octilseleno-xilofuranosídeo / Rodolfo da Silva Mazzarini Baldinotti ; Diego da Silva Alves, orientador ; Lucielli Savegnago, coorientadora. — Pelotas, 2019.

86 f. : il.

Dissertação (Mestrado) — Programa de Pós-Graduação em Biotecnologia, Centro de Desenvolvimento Tecnológico, Universidade Federal de Pelotas, 2019.

1. Anti-alzheimer. 2. Selenocarbohidratos. 3. Via amiloidogênica. 4. Inibidor multi-alvo. I. Alves, Diego da Silva, orient. II. Savegnago, Lucielli, coorient. III. Título.

CDD : 616.858

Elaborada por Ubirajara Buddin Cruz CRB: 10/901

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Dedico este trabalho a minha família, que sempre está comigo me apoiando e com quem sempre posso contar.

Agradecimentos

Gostaria de agradecer inicialmente a minha família, em especial meus pais e minha irmã. Aos meus pais, por todo ensinamento, dedicação, suporte aos meus sonhos e ao meu futuro, sem mencionar todo o carinho e dedicação. À minha irmã agradeço também por todo o carinho, companheirismo e por ser aquela pessoa que sei que posso contar para resto da vida, embora seja minha “irmãzinha” ainda. Enfim, de um modo geral, devo a minha família muito do que alcancei até o momento e que ainda busco alcançar, sempre presentes como uma base e porto seguro, modelo e inspiração, além de um carinho e amor inesgotável.

Além da família, claro, cabe agradecer a todos amigos e amigas que se fizeram presentes em todo este processo, durante os últimos dois anos. Dentre eles os que fazem parte do meu dia-a-dia no laboratório e também a todos os que passaram ou estiveram comigo nesse período, em diferentes situações, atém mesmo em situações inusitadas, como em um pub. Como são várias as pessoas, cito aqui algumas das quais fizerem parte, como a Denise, descoberta de uma amizade inesperada e muito rápida e divertida, com seus altos e baixos, mas muito grande acima disso, algo como uma amizade pra vida e que sempre me aconselhou, sempre me apoiando e acreditando em mim. Também agradeço aos amigos, embora mais a nível virtual, como a Camena principalmente, Pedro, Rachel e Bruna, que ainda mantive contato e me auxiliaram com problemas e dúvidas, além de sempre me incentivarem e apoiarem meus sonhos. Além destes, agradeço a Viviane, dona do pensionato onde morei novamente, mais uma vez estando presente e me dando suporte, além do Heron e Amanda, antigos moradores.

Além destes gostaria de agradecer a todos do GPN, desde os estagiários à alunos da pós, por sempre me ajudarem de todas as maneiras possíveis, e sem eles seria impossível finalizar e alcançar os objetivos deste trabalho. Agradeço a professora Lucielli e professor Diego por toda orientação e dedicação. Em especial, agradeço a Mariana (Cajamate), que além de colega de trabalho e parte da dupla dinâmica, é uma amiga quase irmã que vou levar comigo, mesmo que não esteja em Pelotas, e tenho felicidade de ter conhecido e espero sempre manter contato. Também a agradeço todos os funcionários da UFPEL e Biotério da mesma por tornarem possível a execução do presente trabalho. Enfim, sou grato a todos, por mais que minimamente, contribuíram nesse processo de alguma forma. Muito obrigado.

Resumo

BALDINOTTI, Rodolfo. **Estudos pré-clínicos da ação anti-Alzheimer do octilseleno-xilofuranosídeo.** 2019. 86f. Disssertação (Mestrado) – Programa de Pós-Graduação em Biotecnologia. Universidade Federal de Pelotas, Pelotas.

A doença de Alzheimer é uma doença de caráter multifatorial, caracterizada por uma neurodegeneração progressiva, com uma patofisiologia ainda não completamente elucidada. Neste sentido, diferentes teorias foram desenvolvidas buscando explicar como esse processo patológico funciona. No presente estudo, alguns desses aspectos foram avaliados, como a via amiloidogênica e colinérgica, neurotransmissão monoaminérgica, estresse oxidativo e ainda alterações comportamentais referentes a cognição e formação da memória, visando avaliar a ação protetora da molécula octilseleno-xilofuranosídeo (OSX). Para atingir tais objetivos, o protocolo experimental consistiu na administração do composto OSX via intragástrica, na dose de 0,1 mg/kg, durante os primeiros 20 dias do delineamento experimental. Após o tratamento prévio com o composto, os camundongos foram submetidos a injeção intracerebroventricular de estreptozotocina (duas doses de 1,5 mg/kg, no 21º e 23º dias), para a indução da doença. Decorridos 15 dias após a ultima injeção, testes comportamentais avaliando a formação da memória e cognição foram realizados. Após os testes comportamentais, os animais passaram pela eutanásia para remoção do cérebro para avaliação de marcadores do estresse oxidativo, atividade cinética das enzimas acetilcolinesterase e monoamina oxidase e avaliação da via amiloidogênica por qRT-PCR. Os resultados obtidos mostraram que o OSX protegeu os camundongos de alterações causadas no padrão comportamental, em diferentes tipos de memória. Além disso, o OSX protegeu frente ao aumento dos níveis de peroxidação lipídica, inibiu a atividade das enzimas acetilcolinesterase e monoamino oxidase, além de reduzir a expressão relativa dos genes APP, β e γ-secretase, pontos cruciais na via amiloidogênica. Assim, o OSX apresentou efeito anti-Alzheimer, protegendo os camundongos de alterações induzidas pela estreptozotocina, em diferentes parâmetros da doença.

Palavras-chave: anti-Alzheimer, selenocarbohidratos, via amiloidogênica, inibidor multi-alvo

Abstract

BALDINOTTI, Rodolfo. **Pre-clinical studies of anti-Alzheimer action of octylseleno-xylofuranoside.** 2019. 86p. Dissertation (Master's Degree) - Graduate Program in Biotechnology. Federal University of Pelotas, Pelotas.

Alzheimer's disease is a multifactorial disease, characterized by progressive neurodegeneration, with a pathophysiology not yet fully elucidated. In this sense, different theories have been developed aiming to explain how this pathological process works. In the present study, some of these aspects were evaluated, such as the amyloidogenic and cholinergic pathways, monoaminergic neurotransmission, oxidative stress and behavioral alterations related to cognition and memory formation, aiming to evaluate the protective action of the octylselenoxylofuranoside (OSX). To achieve these objectives, OSX was administered orally at a dose of 0.1 mg/kg during the first 20 days of the experimental design. After pretreatment with the compound, mice underwent intracerebroventricular injection of streptozotocin (two doses of 1.5 mg/kg, in the 21st and 23rd days), as an Alzheimer-like model. 15 days after the last injection, behavioral tests evaluating memory formation and cognition were performed. After behavioral tests, animals underwent euthanasia to collect the brain for evaluation of markers of oxidative stress, kinetic activity of the enzymes acetylcholinesterase and monoamine oxidase, and evaluation of the amyloidogenic pathway by qRT-PCR. The results showed that OSX protected mice from abnormalities caused by the behavioral pattern in different types of memory. In addition, OSX protected against increased levels of lipid peroxidation, inhibited acetylcholinesterase and monoamine oxidase enzymes, and reduced the relative expression of APP, β and γ-secretase, main points of amiloidogenic pathway. Overall, OSX presented anti-Alzheimer's abilities, protecting the mice from changes induced by streptozotocin, in different parameters of the disease.

Key words: anti-Alzheimer, selenocarbohydrates, amiloidogenic pathway, multimodal inhibitor

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Lista de Abreviaturas e Siglas

Aβ - Beta amiloide

ACh - Acetilcolina

AChE - Acetilcolinesterase

ApoE - Apolipoproteína

APP - Proteína precursora amiloide

BDNF - Fator neurotrófico derivado do cérebro

CAMKII - Proteína quinase II dependente de Ca^{+2}/calmodulina

DA - Doença de Alzheimer

DM - Depressão maior

EO - Estresse oxidativo

ER - Espécies reativas

ERK - Proteína quinase regulada extracelular

FDA - Food and drug admnistration

GPN - Grupo de pesquisa em Neurobiotecnologia

GSK-3β - Glicogênio quinase sintase 3 beta

iAChE - Inibidores da enzima acetilcolinesterase

ICV - Intracerebroventricular

LPS - Lipopolissacarídeo

NMDA - N-methyl-D-aspartate

OSX - Octilseleno-xilofuranosídeo

PKA - Proteína quinase A

PKC - Proteína quinase C

PS - Presinilina

SNC - Sistema nervoso central

STZ - Estreptozotocina

Sumário

1 INTRODUÇÃO GERAL

A Doença de Alzheimer (DA) é uma doença neurodegenerativa e progressiva, que afeta principalmente idosos, representando a causa mais comum de demência no mundo. Embora a DA seja um problema socioeconômico global, atingindo cerca de 47 milhões de pessoas no mundo, a fisiopatologia da doença não está totalmente elucidada (Alzheimer's Disease International, 2015; Cervellati et al., 2016; Alzheimer's Association (USA), 2018), devido a suas características multifatoriais e complexas.

Devido a tais caracteristicas, teorias sobre a etiologia da DA foram desenvolvidas, visando explicar possíveis causas e a patofisiologia da doença. Uma delas é a teoria amiloidogênica, que trata do processamento da proteína precursora amiloide (APP), sendo clivada pelas enzimas γ e β-secretases, gerando os peptídeos β-amilóide (Aβ), levando a um processo neurodegenerativo do sistema nervoso central (SNC) (Thinakaran & Koo, 2008; Vandersteen et al., 2012). Outra teoria é a colinérgica, que está relacionada a baixos níveis de acetilcolina (ACh) no SNC (Francis et al., 1999; Hampel et al., 2018), correlacionado com o déficit cognitivo e da memória característicos da DA (Hasselmo, 2006; Wang et al., 2016). Essa redução dos níveis de ACh pode ser explicada pelo aumento da atividade da enzima acetilcolinesterase (AChE), caracterizando um importante alvo na DA (Wilcock et al., 1982; Chigurupati et al., 2016).

Além das teorias apresentadas, a teoria do estresse oxidativo (EO) vem sendo amplamente ligada a doenças neurodegenerativas e desordens neuropsiquiátricas, (Liu et al., 2017). Essa teoria trata do desequilíbrio entre moléculas pró e antioxidantes, levando a danos as biomoléculas, causando a degradação celular (Blagosklonny, 2008; Van Raamsdonk et al., 2017). Além disso, o EO está relacionado aos processos de envelhecimento, que estão intimamente relacionados a DA, visto que a mesma afeta principalmente idosos (Mecocci et al., 2018). Adicionado a isso, os agregados de Aβ podem induzir processo inflamatórios e alterações nas sinalizações intracelulares, que culminam no EO, danificando as células (Allan Butterfield & Boyd-Kimball, 2018).

Embora a DA seja multifatorial, os tratamentos atualmente disponíveis focam somente em um dos fatores da mesma, como inibidores da AChE (iAChE), reduzindo somente os sintomas, sem interromper o processo neurodegenerativo (Cummings et

al., 2014; Weller & Budson, 2018). Levando isso em consideração, novas possibilidades de tratamento visando o processo neurodegenerativo, são interessantes como alvos de estudo.

Além de seu caráter multifatorial, a DA está associada a sintomas neuropsiquiátricos, presentes em aproximadamente 80% dos pacientes acometidos, sendo a depressão maior (DM) o mais comum (Andrade et al., 2017). Embora essas doenças estejam relacionadas, não está claro ainda se a DM é um fator de predisposição para o desenvolvimento da DA, ou se é uma característica inical da DA (Chi et al., 2015). Além da comorbidade entre as doenças, a DM apresenta alterações patológicas semelhantes à DA, como o EO, disfunção na memória e redução de neurotransmissores e neurotrofinas (Rodrigues et al., 2014; Ismail et al., 2017; Morgese et al., 2017). Ainda, pacientes afetados pela DA e depressivos, apresentam um aumento nos marcadores da doença (Rapp et al., 2006, 2008).

Nesse sentido, o octilseleno-xilofuranosídeo (OSX), é um composto orgânico de selênio, que já foi avaliado por nosso grupo de pesquisa (Grupo de pesquisa em Neurobiotecnologia), quanto a sua capacidade antioxidante e antidepressiva. Nos estudos apontados, a molécula apresentou efeito tipo-antidepressivo, modulando vias intracelulares e o sistema monoaminérgico (Pinto Brod et al., 2016a, 2017a). Interessantemente, o OSX foi capaz de interagir com proteínas de vias intracelulares, as quais ativam neurotrofinas e suas vias de neuroplasticidade, como a do fator neurotrófico derivado do cérebro (BDNF) (Manosso et al., 2015). Nas doenças apresentadas, DA e DM, o BDNF apresenta níveis reduzidos, sendo associados a redução da neuroplasticidade (Budni et al., 2015; Bus et al., 2015). Além disso, compostos contendo selênio vem demonstrando efeitos terapêuticos interessantes, como efeito anti-inflamatório, antioxidante e antidepressivo (Pinto Brod et al., 2017b; Sousa et al., 2018; Xie et al., 2018; Casaril et al., 2019).

Tendo em vista o que foi exposto, em relação aos tratamentos atualmente disponíveis e suas limitações, a busca por novas moléculas bioativas que possam agir sobre um ou mais marcadores da DA, é essencial. Ainda, considerando a atividade terapêutica demonstrada pelo OSX, o objetivo do presente estudo foi avaliar a atividade anti-Alzheimer dessa molécula frente a indução por estreptozotocina (STZ), em camundongos, visto que a STZ é um modelo bem estabelecido para estudo da DA.

2 REVISÃO BIBLIOGRÁFICA

2.1 Doença de Alzheimer (DA)

A DA é caracterizada por um processo neurodegenerativo e progressivo, além de apresentar um caráter multifatorial e complexo (Tramutola et al., 2016a; dos Santos Picanco et al., 2018). Esta doença afeta principalmente a população de idosos, além de ser a principal causa de demência a nível mundial, afetando mais de 47 milhões de pacientes, com tendência a um aumento significativo (Alzheimer's Disease International, 2015). Somado a isso, a DA é caracterizada por um déficit cognitivo e na formação de memória progressivos, levando a um grande impacto socioeconômico global (Prince et al., 2015; Karlawish et al., 2017).

Além disso, a DA pode ser dividida nas formas genética ou esporádica. A forma genética está relacionada ao aumento da expressão de genes, afetando principalmente jovens e adultos, representando a forma menos prevalente da doença (Bettens et al., 2013). Tais genes são: APP, presenilina 1 e 2 (PS), e apolipoproteína (ApoE), aumentando a produção do peptídeo Aβ e sua cascata neurodegenerativa, como consequência (Ye et al., 2012; Karch & Goate, 2015). A forma esporádica é a mais prevalente, afetando a população de idosos, correspondendo a mais de 90% dos casos da DA (Bekris et al., 2010). Isto ocorre pois, a DA esporádica está associada ao processo de envelhecimento, que é caracterizado pelo estresse oxidativo (EO), depleção do mecanismo de reparo celular e envelhecimento celular (Rehman et al., 2017). Essa forma da doença não possui somente uma teoria que explique o processo patológico como um todo, devido a seu caráter multifatorial e complexo, resultando em uma patofisiologia ainda não completamente elucidada.

Devido à ausência de uma única sobre a patologia da doença, diferentes teorias foram desenvolvidas ao longo dos anos de estudo da DA, buscando possíveis causas que desencadeiam o processo neurodegenerativo e os sintomas da doença.

2.2 Teorias da DA esporádica

2.2.1 Teoria colinérgica

A teoria colinérgica está relacionada a redução dos níveis do neurotransmissor ACh na DA, sendo uma das teorias mais antigas que visam explicar os sintomas da doença (Wilcock et al., 1982; Francis et al., 1999). Nessa teoria, a redução dos níveis

de ACh são relacionados ao déficit cognitivo e disfunção na formação da memória, visto que esse neurotransmissor está envolvido nestes processos cognitivos, além de outras funções essenciais no SNC, como a resposta ao estresse (H. Ferreira-Vieira et al., 2016).

Uma das explicações para essa redução é o aumento da atividade da enzima AChE, visto que, a mesma é responsável pela hidrólise da ACh, reduzindo sua permanência na fenda sináptica e sua ligação aos seus receptores (Chigurupati et al., 2016). Outra explicação para a redução presente na DA de ACh, é a morte dos neurônios colinérgicos no SNC, desse modo, também reduzindo os níveis desse neurotransmissor essencial, devido a parada de sua produção pela morte neuronal (Whitehouse et al., 1981, 1982). De ambas as formas, esse neurotransmissor chave para os processos de cognição e formação da memória, tem seus níveis reduzidos e como consequência a surgimento de alguns dos sintomas clássicos da DA.

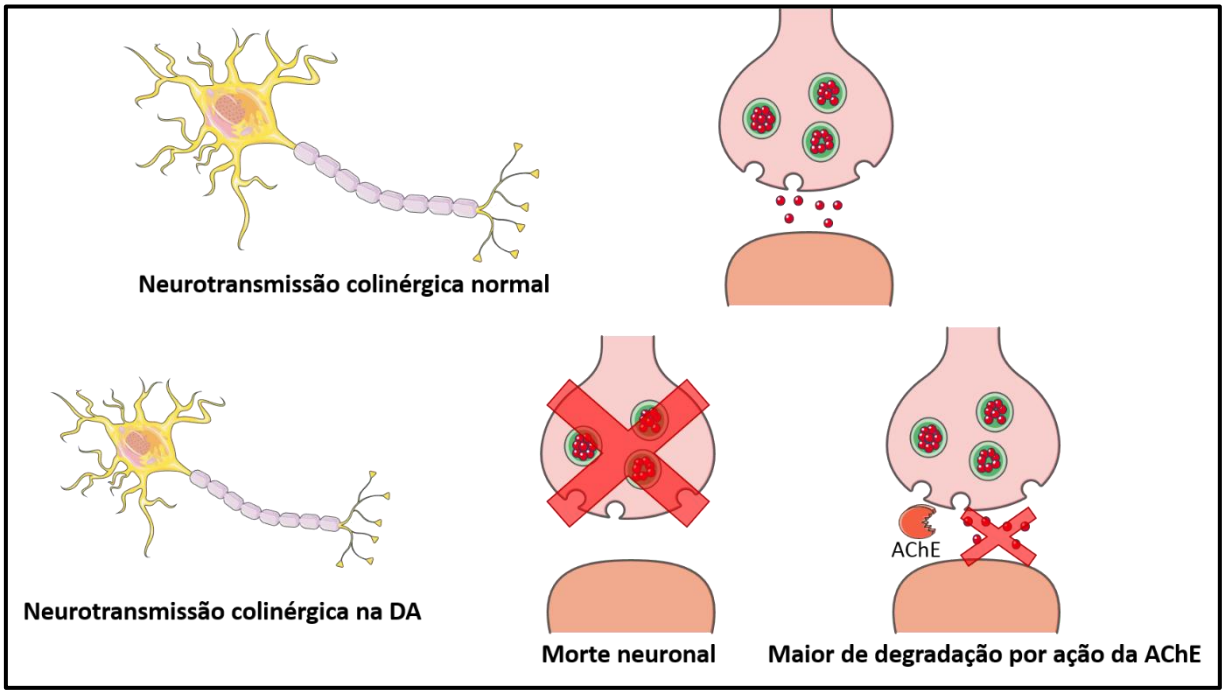

Figura 1. Ilustração da teoria colinérgica, representado a redução da acetilcolina (ACh) e aumento da atividade da acetilcolinesterase (AChE), características da doença de Alzheimer (DA).

2.2.2 Teoria amiloidogênica

Outra importante linha de pesquisa no estudo da DA visa o processamento da APP. Na fisiologia normal do SNC a APP pode ser processada por duas diferentes vias, a via amilodogênica e não amiloidogênica (O'Brien & Wong, 2011). Na via não

amiloidogênica, a APP é clivada pela enzima α-secretase, gerando produtos não tóxicos e com função neuroprotetora (Kojro & Fahrenholz, 2005). Na via amiloidogênica, as enzimas β-secretase e γ-secretase clivam a APP, gerando os peptídeos Aβ característicos da DA (LaFerla et al., 2007). No quadro patológico da DA a via amiloidogênica encontra-se mais ativa, produzindo Aβ em excesso, formando agregados extracelulares nocivos SNC, que levam a respostas fisiológicas no mesmo (Thinakaran & Koo, 2008; Vandersteen et al., 2012).

Uma das explicações para o aumento da atividade amiloidogênica na DA é o aumento da expressão das enzimas chave nessa rota, a β-secretase e γ-secretase, assim tornando mais ativo o processamento da APP e formação de seu produto em excesso (Liang et al., 2008). Além do aumento na expressão dessas enzimas, a sua atividade encontra-se aumentada, clivando mais APP em Aβ (Fang et al., 2018), levando a formação dos agregados proteicos e lavando a respostas como a neuroinflamação, ativando a micróglia e astrócitos, além da indução da formação do EO (Heneka et al., 2015a).

Assim, além das próprias respostas danosas ao SNC, a formação de peptídeos Aβ acarreta outras respostas e cascatas, como a neuroinflamação e EO, levando ao processo neurodegenerativo (Dá Mesquita et al., 2016; Cheignon et al., 2018).

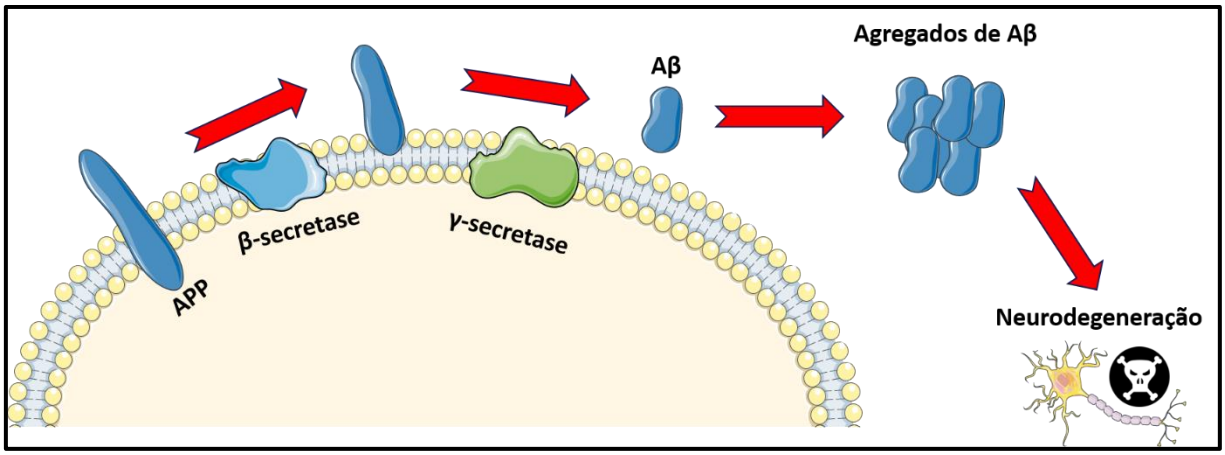

Figura 2. Ilustração da teoria amiloidogênica e o processamento da proteína precursora amiloide (APP) à peptídeos β-amilóides (Aβ).

2.2.3 Teoria neurofibrilar

Outro dos *hallmarks* da DA são os agregados neurofibrilares compostos pela proteína TAU hiperfosforilada, além dos agregados extracelulares do peptídeo Aβ

(Guedes et al., 2018). A proteína TAU em seu estado nativo auxilia na estabilização dos microtúbulos neuronais, responsáveis pelo transporte e estabilidade estrutural no neurônio (Mandelkow & Mandelkow, 2011). Na DA devido a alterações na expressão gênica ou atividade anormal de quinases, como a glicogênio sintase quinase 3β (GSK-3β), são responsáveis por hiperfosforilar a proteína TAU, desestabilizando sua estrutura, impedindo que a mesma execute sua função (Gong & Iqbal, 2008; Gandini et al., 2018). Além disso, quando hiperfosforilada, essa proteína forma agregados neurofibrilares intracelulares, que por sua vez desencadeiam uma resposta que culmina na morte neuronal e colabora para o processo neurodegenerativo (Buée et al., 2010).

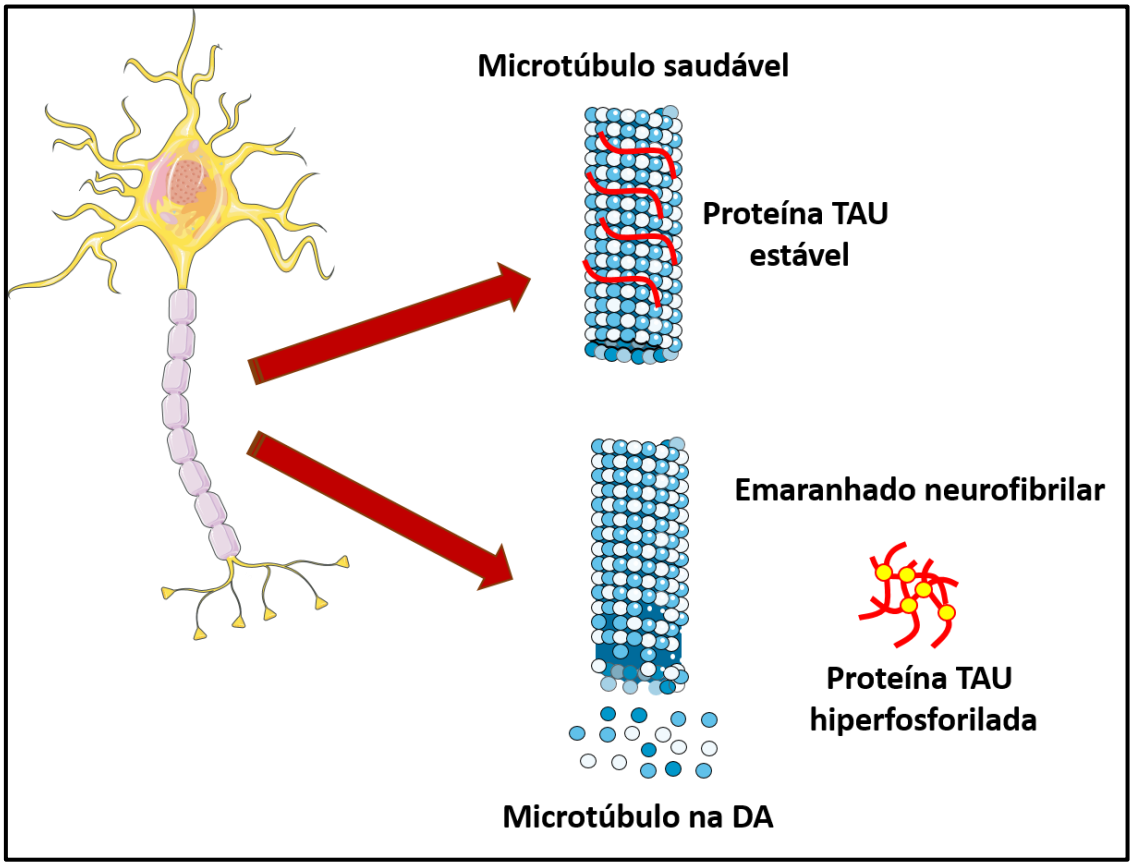

Figura 3. Ilustração da teoria neurofibrilar, demonstrando a formação dos agregados neurofibrilares de proteína TAU, na doença de Alzheimer (DA).

2.2.4 Teoria do estresse oxidativo (EO)

O EO é caracterizado pelo desequilíbrio entre moléculas pró e antioxidantes, onde há um aumento das pró oxidantes ou redução das defesas antioxidantes no organismo humano (Blagosklonny, 2008; Van Raamsdonk et al., 2017). Em condições normais fisiológicas, existe a produção de espécies reativas (ER), porém essas são

neutralizadas por moléculas e defesas antioxidantes presentes no organismo (Nimse & Pal, 2015). No entanto, quando há o desequilíbrio e organismo não consegue reestabelecer o equilíbrio novamente, ocorre o EO, danificando biomoléculas, como os lipídeos, proteínas e ácidos nucleicos (Valavanidis et al., 2006).

Visto que o EO está ligado ao dano as biomoléculas essenciais para o funcionamento celular, o mesmo está amplamente relacionado a diversas patologias, como por exemplo as neurodegenerativas, como a DA (Cervellati et al., 2016; Paloczi et al., 2017). Esse fato pode ser explicado pela correlação existente entre a DA e do EO com o envelhecimento, sendo um fator de risco determinante para o estabelecimento dessa doença (Mecocci et al., 2018). O envelhecimento é caracterizado pelo EO, envelhecimento celular e mitocondrial, depleção do mecanismo de reparo celular, corroborando com o processo neurodegenerativo e sintomas como déficit cognitivo e perda da memória (Rehman et al., 2017).

Somado a outras teorias e marcadores da DA, o EO pode ser relacionado com os agregados de Aβ, havendo um aumento no dano oxidativo, além da indução da neuroinflamação, levando a um aumento da morte de células neurais (Allan Butterfield & Boyd-Kimball, 2018). Isso ocorre, pois, embora didaticamente separadas, as teorias ocorrem simultaneamente no quadro patológico da doença, agindo como um todo no processo neurodegenerativo e na fisiopatologia da doença (Halliwell, 2018).

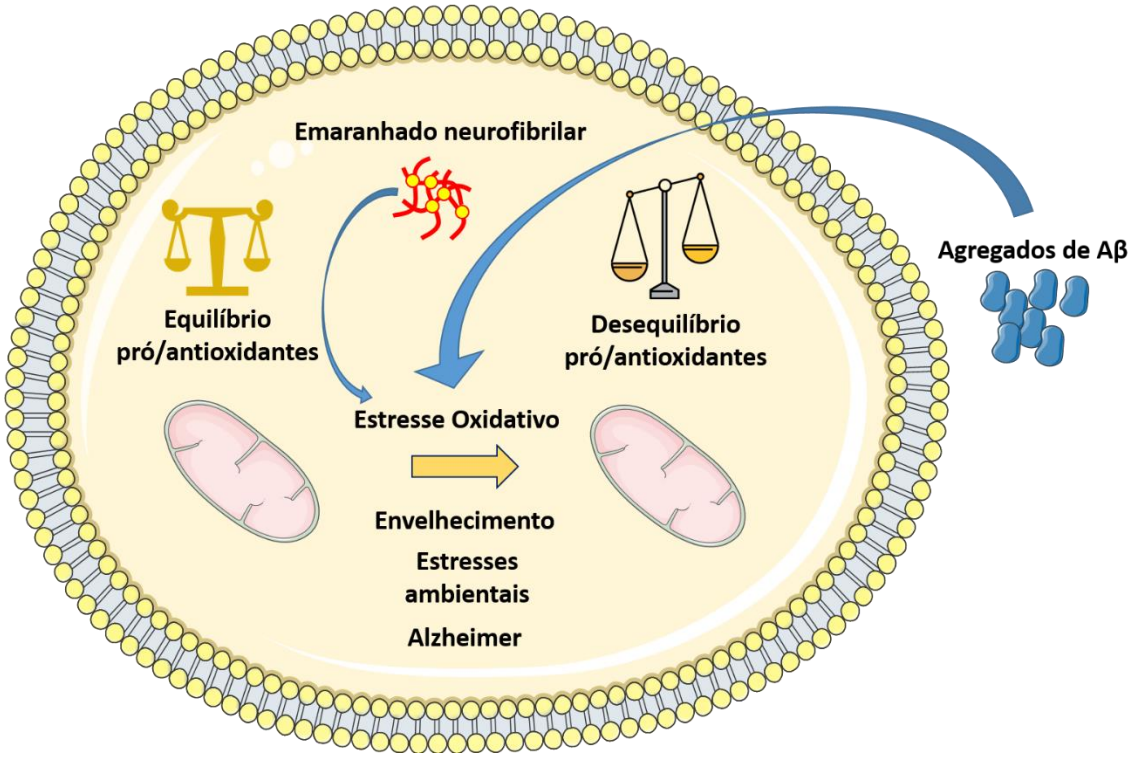

Figura 4. Ilustração da teoria do estresse oxidativo e correlação com as outras teorias da doença de Alzheimer (DA), onde o aumento dos agregados extracelulares de Aβ e intracelulares de emaranhados neurofibrilares da proteína TAU hipefosforilada, corroboram com o aumento do estresse oxidativo (EO).

2.3 Depressão maior (DM) na DA

Além de seu caráter complexo, a DA, pode ser associada a outras patologias, como as desordens neuropsiquiátricas, presentes em 80% dos pacientes acometidos pela DA, sendo a DM a forma mais representativa (Andrade et al., 2017). Porém, embora tal relação exista, ainda não está claro se a DM é um fator do risco para o desenvolvimento da DA ou se faz parte das consequências e sintomatologia da mesma (Chi et al., 2015).

Além dessa relação de comorbidade entre essas doenças, existem algumas características fisiopatológicas e sintomáticas similares entre as mesmas. Quanto a patofisiologia, o EO está presente em ambas as doenças, causando danos a nível celular e prejudicando o processo de neurotransmissão, colaborando com o processo neurodegenerativo da DA (Rodrigues et al., 2014). Além do EO, a redução dos níveis de neurotransmissores e neurotrofinas pode ser evidenciado em ambos os quadros patológicos, levando a um desequilíbrio neuroquímico e redução da neuroplasticidade sináptica (Ismail et al., 2017; Morgese et al., 2017).

Outra semelhança entre a DM e DA é a disfunção na memória e na cognição. Essa característica sintomatológica está relacionada ao circuito cerebral de neurônios colinérgicos, os quais estão relacionados a processos da cognição e formação da memória, além de resposta ao estresse (Janowsky & Risch, 1984; Newman et al., 2001). Assim, a disfunção da neurotransmissão colinérgica representa outra similaridade sintomatológica entre as doenças descritas (Rosenberg et al., 2015).

Somado ao que foi exposto, evidências indicam um aumento em marcadores conhecidos, como os agregados de Aβ e emaranhados neurofibrilares, em pacientes acometidos pela DA com DM diagnosticada (Rapp et al., 2006, 2008).

Desse modo, estudos de novas moléculas com potencial bioativo, capazes de inibir ou melhorar os sintomas depressivos, podem vir a ser interessantes em tratamentos e estudos aplicados à DA.

2.4 Tratamentos atualmente disponíveis para a DA

Embora a DA provoque um grande impacto global e apresente uma tendência no aumento de sua ocorrência, o cenário global atual referente a seu tratamento é limitado e necessita de tratamentos mais efetivos (Yiannopoulou & Papageorgiou, 2013). Os tratamentos atualmente disponíveis são inibidores da AChE, como Donepezila, Galantamina e Rivastigmina, aprovados pela FDA (Food and Drug Administration) e o antagonista de receptor N-methyl-D-aspartate (NMDA) glutamatérgico. Embora tais medicamentos possam aumentar a disponibilidade da ACh e retardar a excitotoxicidade presente na DA, respectivamente, os tratamentos muitas vezes aliviam os sintomas e possuem curta duração de efeito, além de não interromperem o processo neurogenerativo (Doody, 2003; Solanki et al., 2016).

Desse modo, novos tratamentos que visem uma melhor eficácia e duração de efeito, são necessários. Levando em conta o que foi exposto sobre a DA, vale ressaltar a investigação de novas moléculas capazes de agir sobre mais de um dos aspectos relacionados a DA, como por exemplo associar a inibição da enzima AChE com o efeito antioxidante (Tundis et al., 2017; Zhang et al., 2018). Nesse sentido, vale destacar os compostos orgânicos de selênio, que vem demonstrando atividades biológicas e um potencial farmacológico interessante (Kamal et al., 2018).

2.5 Compostos orgânicos de selênio

Um dos micronutrientes essenciais para o funcionamento do organismo humano é o selênio, sendo esse adquirido através da dieta (Huawei, 2009). Tal nutriente é de grande importância para o organismo humano visto que compõe diferentes moléculas, como proteínas e faz parte do aminoácido selenocisteína. Dentre as funções desempenhadas por essas moléculas contendo o selênio pode-se citar a atividade antioxidante, além do envolvimento com a ativação da proliferação e diferenciação celular, além de agir sobre o sistema imune inato e adaptativo (Brown & Arthur, 2001). A deficiência desse nutriente está associada a efeitos deletérios no organismo, uma vez que apresenta importante papel sobre o equilíbrio redox e outras vias de ativação e sinalização (Levander, 1982; Medeiros, 2016; Wrobel et al., 2016). Além disso, a deficiência do selênio pode ser associada a doenças que atingem o SNC, visto que o selênio também possui papel sobre esse sistema e seu desenvolvimento (Cardoso et al., 2015; Fradejas-Villar, 2018; Hall, 2018).

Um dos exemplos de moléculas contendo selênio são as selenoproteínas O, K e P, as quais atuam em diferentes vias de sinalização celular ou mesmo no transporte do selênio pelo organismo (Labunskyy et al., 2014). Outro exemplo são proteínas do sistema endógeno de defesa antioxidante, como as enzimas glutationa peroxidase e tioredoxina redutase. Dentre essas, a enzima glutationa peroxidase é uma das mais estudadas no sistema endógeno de defesa antioxidante, atuando na detoxificação de ER, como o peróxido de hidrogênio e hidroperóxidos fosfolipídicos (Gaur & Agnihotri, 2017).

Desse modo, tendo em vista a teoria do estresse oxidativo e os danos decorrentes do desequilíbrio redox característicos das doenças neurodegenerativas e neuropsiquiátricas (Sbodio et al., 2018), novas moléculas com potencial antioxidante são interessantes como alvo de estudo. Ainda, visto que o selênio faz parte do sítio catalítico de enzimas componentes do sistema de defesa antioxidante do organismo humano (Brigelius-Flohé, 2015), a síntese de moléculas contendo selênio em sua estrutura, são de grande interesse de estudo em doenças como a DA.

Nesse sentido, os compostos orgânicos contendo selênio vem demonstrando diversas atividades biológicas, como por exemplo a atividade anti-inflamatória, antinociceptiva, antidepressiva, antioxidante, anticancerígena, neuroprotetora e anti-Alzheimer (Meotti et al., 2004; Savegnago et al., 2008; Gill et al., 2010; De Freitas & Rocha, 2011; Kelany et al., 2016; Casaril et al., 2017; Fronza et al., 2017; Sousa et al., 2018; Rodrigues et al., 2018).

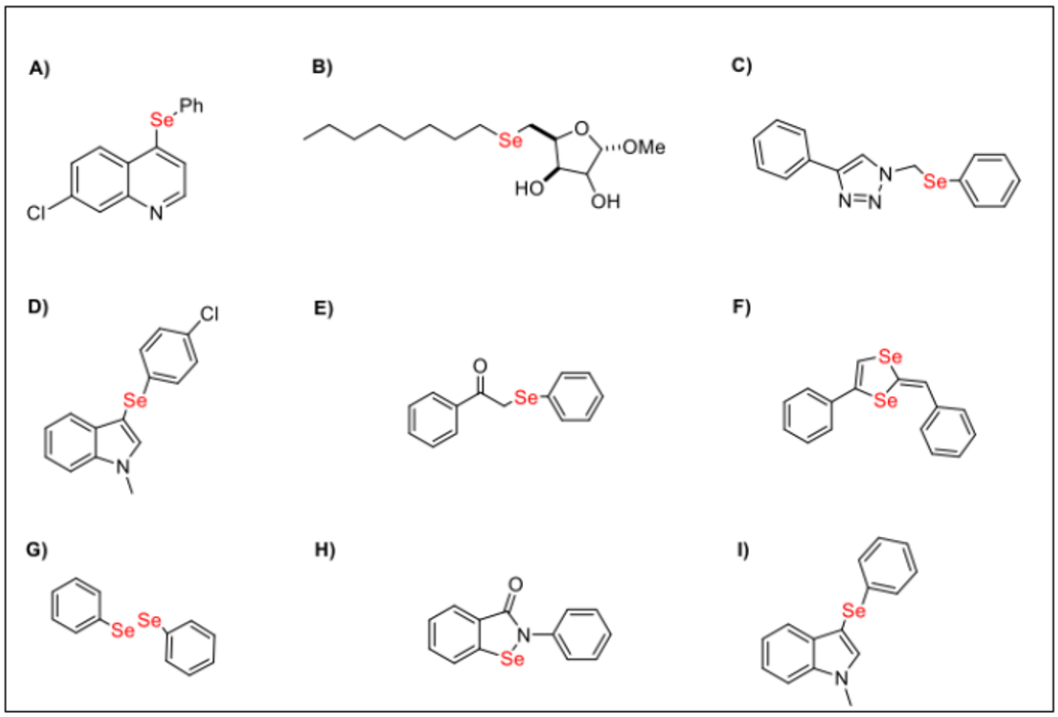

Figura 5. Exemplos de compostos orgânicos: (A) 4-fenilselenil-7-cloroquinolina; (B) octilseleno-xilofuranosídeo; (C) 4-fenil-1-(fenilselenilmetil)-1,2,3-triazol; (D) 3-((4-clorofenil)selenil)-1-metil-1*H*-indol; (E) α-(fenilselanil) acetofenona; (F) 2-benzilideno-4-fenil-1,3-disselenol; (G) disseleneto de difenila; (H) ebselen; (I) 1-metil-3-(fenilselenil)-1*H*-indol. Fonte: Bampi, 2018.

Parte das atividade citadas foram avaliadas e descobertas, ao longo dos anos de estudo por nosso Grupo de Pesquisa em Neurobiotecnologia (GPN). Dentres esse anos de estudo, tais compostos demonstraram efeitos benéficos, frente ao estudo de doenças que afetam o SNC, como a depressão, dados que corroboram com outros estudos na literatura, que também apontam efeitos benéficos desses compostos (Xie et al., 2017; Martini et al., 2019). Dentre esses compostos, a molécula octilseleno xilofuranosídeo (OSX) pode ser destacada, por demonstrar a atividade tipo antidepressiva.

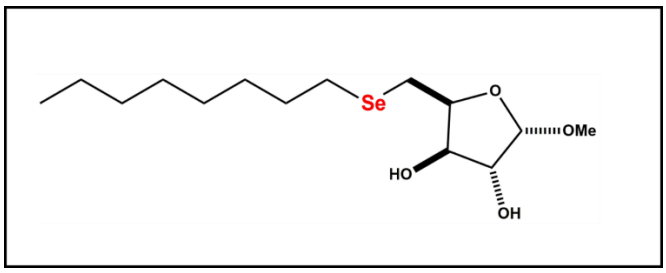

Figura 6. Estrutura química do octilseleno-xilofuranosídeo (OSX).

A atividade do tipo antidepressiva do OSX advém de sua habilidade de interagir com o sistema monoaminérgico, além de modular vias intracelulares de sinalização, envolvendo proteínas como a proteína quinase A (PKA) e C (PKC), proteína quinase II dependente de Ca^{+2}/calmodulina (CAMKII) e proteína quinase regulada extracelular (ERK) (Pinto Brod et al., 2016b, 2017a). Estas proteínas estão relacionadas a ativação de neurotrofinas e suas vias de neuroplasticidade, como a do BDNF (Manosso et al., 2015). Em doenças que afetam o SNC, como DA e DM, o BDNF apresenta níveis reduzidos, sendo associados a redução da neuroplasticidade característica dessas doenças (Budni et al., 2015; Bus et al., 2015).

Tendo em visto o que foi exposto sobre a relação da DM e a DA, além dos efeitos farmacológicos demonstrados pelo composto, OSX, o mesmo se torna um potencial candidato para novas análises, visando uma ação anti-Alzheimer, devido a correlação existente entre o Alzheimer e a DM.

2.6 Modelos animais para o estudo da DA

Atualmente, para o estudo de doenças neurodegenerativas, existem modelos animais que visam mimetizar o máximo possível as características do quadro patológico da doença, como na DA (Dawson et al., 2018). Alguns dos exemplos que podem ser citados são o uso de animais transgênicos, uso de lipopolissacarídeo (LPS) ou uso da STZ.

O uso de animais transgênicos permitiu o estudo e novas descobertas relacionadas a DA, como os fatores fisiopatológicos, como os agregados de Aβ ou emaranhados de TAU hiperfosforilada (Götz et al., 2004). Isso se deve ao fato, que ao longo dos anos de estudo e desenvolvimento desse modelo, genes envolvidos na cascata amiloidogênica foram utilizados para induzir um quadro semelhante ao da doença (Spires & Hyman, 2005). Tais genes são os das proteínas APP, PS1 e 2, por exemplo, induzindo assim a formação de marcadores clássicos da DA, como nos triplos transgênicos atualmente utilizados (Oddo et al., 2003; Belfiore et al., 2018). Porém, uma das limitações de modelos transgênicos, é o fato de serem mais semelhantes a DA genética, sendo esse o tipo menos prevalente da doença.

Visando a forma esporádica da doença, outros modelos foram desenvolvidos, como por exemplo o modelo neuroinflamatório induzido por LPS. Esse modelo visa o estudo da faze inicial da doença, fase essa, que é caracterizada por um caráter

neuroinflamatório, semelhante ao que se tem na DA, além de fatores, como o EO (Rocha & Amorim, 2011; Heneka et al., 2015b).

Ainda, outro modelo amplamente utilizado é o da injeção ICV de STZ. Esse modelo visa também a forma esporádica da doença, porém em sua fase mais avançada, onde pacientes já estão com um quadro neurodegenerativo avançado, visto que ainda o diagnóstico da DA é tardio (Alzheimer's Association, 2018). Nesse modelo, acredita-se que o mecanismo pelo qual a STZ age a nível de SNC é através da entrada na célula, interagindo com receptores de glicose do tipo 2 (GLUT2), levando a um estado de hipometabolismo, além de induzir um aumento na produção de ER e do EO (Salkovic-Petrisic, 2008; Ishrat et al., 2009). Além disso, a injeção de STZ, provoca um aumento na expressão de genes relacionados a produção e processamento de Aβ, culminando no processo neurodegenerativo. Essas alterações, por sua vez, levam a alterações comportamentais nos animais modelo, além de modificações neuroquímicas, bioquímicas, morfológicas e histológicas semelhantes ao que ocorre na DA (Mehan et al., 2012; Grieb, 2016).

3 HIPÓTESE E OBJETIVOS

3.1 Hipótese

A partir do que foi exposto, a hipótese do presente trabalho é que o composto OSX possui potencial anti-Alzheimer, devido a suas propriedade apresentadas e a existência da comorbidade entre a DA e a DM.

3.2 Objetivo geral

O objetivo do presente estudo, foi avaliar a ação protetora do OSX, frente a indução do tipo Alzheimer, induzida por STZ em camundongos, segundo parâmetros comportamentais e biológicos ligados a DA.

3.3 Objetivos específicos

- Avaliar o efeito protetor do OSX nos parâmetros comportamentais ligados a formação da memória e cognição frente aos danos induzidos por STZ em camundongos.

- Estudar o efeito protetor do OSX frente a marcadores do estresse oxidativo induzido por STZ em camundongos.

- Investigar o efeito inibitório do OSX nas enzimas acetilcolinesterase e monoamino oxidase frente a indução por STZ em camundongos.

- Verificar o efeito do OSX na via amiloidogênica, característica da DA, em camundongos induzidos por STZ.

4 CAPÍTULOS

4.1 Manuscrito – Anti-Alzheimer properties of octylseleno-xylofuranoside (OSX) in a streptozotocin mice model.

Anti-Alzheimer properties of octylseleno-xylofuranoside (OSX) in a streptozotocin mice model

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Abstract

Octylseleno-xylofuranoside (OSX), an organic selenium compound, has been studied and presented few interesting results in the literature in the last years. OSX demonstrated its antidepressive-like activity, modulating intracellular pathways and monoaminergic system. Recently, new studies have been showing different correlations between major depression and Alzheimer's disease (AD). In that sense, in the present study we evaluated OSX in different aspects of AD, such as amiloidogenic pathway, cholinergic and monoaminergic neurotransmission, oxidative stress and behavioural abnormalities in memory formation and cognition. To achieve these goals, OSX was administered by oral gavage, in a 0.1 mg/kg dose, during the first 20 days of the experimental design. After the pre-treatment, the compound was administration was followed by intracerebroventricular injections of streptozotocin (two times 1.5 mg/kg dose), as an Alzheimer-like model. After 15 days from the last streptozotocin injection, behavioural tests including object and social recognition tests, Y maze, passive avoidance and open field tests were performed, followed by evaluation of markers of oxidative stress, acetylcholinesterase, monoamine oxidase activities and amiloidogenic pathway via qRT-PCR. The results showed that OSX protected mice from anomalies caused in behavioural pattern in memory tests presented here, in different types of memory. Furthermore, OSX protected against lipid peroxidation increased levels, inhibited acetylcholinesterase and monoamine oxidase activities, as well, reduced relative expression of main protein related to amiloidogenic pathway in Alzheimer's disease: amyloid precursor protein (APP), γ-secretase and β-secretase. Overall, OSX presented anti-Alzheimer activity, protecting mice from streptozotocin abnormalities in behavioural and different parameters of the disease.

Key words: anti-Alzheimer; selenocarbohydrates; amiloidogenic pathway;

1. Introduction

Octilseleno-xylofuranoside, a selenocarbohydrate molecule, has been studied and presented few interesting results in the literature in the last years. OSX demonstrated its antidepressive-like activity, modulating intracellular pathways and monoaminergic system (Pinto Brod et al., 2016a, 2017b). Moreover, organoselenium compounds have been showing interesting biological activities in the literature, such as anti-inflammatory, antioxidant and antidepressive (Casaril et al., 2017; Fronza et al., 2017; Sousa et al., 2018; Xie et al., 2018).

The proteins of intracellular pathways that OSX was able to interact with were protein kinase A (PKA), protein kinase C (PKC), Ca^{+2}/calmodulin-dependent protein kinase II (CAMKII) and extracellular-regulated protein kinase (ERK). Those proteins can be associated with neutrophins and its neural plasticity pathway, such as brain-derived neurotrophic factor (BDNF) (Manosso et al., 2015). Furthermore, BDNF reduced levels can be associated with diseases that affects central nervous system (CNS), such as major depression (MD) and Alzheimer's disease (AD), related with a reduction in neuroplasticity and stablishment of pathological conditions in these diseases (Budni et al., 2015; Bus et al., 2015).

Recently, a relation between MD and AD have noticed. AD has been associated to neuropsychiatric symptoms, present in approximately 80% of the affected patients and the most frequent is major depression (MD), representing 50% of those cases (Andrade et al., 2017). Although these diseases seem to be related, it is not clear yet, if depression is a risk factor of developing AD or if it is a characteristic early event of the disease (Chi et al., 2015). Other than the comorbid relating these two pathologies, MD presents some of alterations presented in AD, such as OE, memory dysfunction, neurotransmitters and neurotrophic factors impairment (Ismail et al., 2017; Morgese et al., 2017). Furthermore, depressive AD patients show increased levels of hallmarks of the disease when compared with AD patients that are not depressive (Rapp et al., 2008).

Alzheimer's Disease (AD) is a progressive complex chronic neurodegenerative disease and is the most common cause of dementia in the world (Tramutola et al., 2016b). Although AD presents itself as a major socioeconomic global problem, the

pathophysiology of the disease is not completely understood (Alzheimer's Disease International, 2015; Cervellati et al., 2016).

Due to these very complex's aspects, few theories have been developed to explain possible causes and pathological pathways related to the disease. These theories relate different aspects of the disease, such as the amyloidogenic pathway (Vandersteen et al., 2012), lower levels of acetylcholine (ACh) (Francis et al., 1999; Hampel et al., 2018), reduced neuroplasticity in AD (Wang et al., 2016) and increased oxidative stress (OS) levels, that has been widely connected to neurodegenerative and neuropsychiatric diseases (Liu et al., 2017).

Although AD represents an important global impact, available tretments, such as the AChE inhibitors (iAChE) and glutamatergic antagonist, are only able to reduce symptoms, but not to stop the neurodegenerative process (Cummings et al., 2014; Weller & Budson, 2018). Taking that into account, new possibilities for AD treatment might have activities in the different targets, aiming for a more effective treatment.

Since none of the mentioned diseases present any fully functioning treatment, the search for new molecules presenting positive effects in neurological diseases are important.

Taking into consideration the available treatment for AD and its limitations, the search for new bioactive molecules, that might act in more than one factor of the disease is essential. Added to that, considering the previous anti-depressive activity of OSX, modulating monoaminergic system and intracellular signalling, the objective of the present study was to evaluate the anti-Alzheimer activity of OSX against STZ Alzheimer-like mice model.

2. Materials and methods:

2.1. Chemicals

Octylseleno-xylofuranoside (OSX) (Fig. 1) was synthetized in the Chemical Institute of Universidade Federal do Rio Grande do Sul, Brazil and characterized as a previously described method (Vargas et al., 2015). Streptozotocin (STZ) was purchased from Sigma-Aldrich (Sigma, St. Louis, MO).

All other chemicals of analytical grade were obtained from standard commercial suppliers.

Insert figure 1 here

2.2. Animals

The experiments were performed using Swiss mice (60 days old) obtained from Federal University of Pelotas (UFPEL) breeding colony. The animals were maintained on 12h light/dark cycle, at a temperature of 22 ± 2 ºC, with free access to water and food. All experimental procedures were performed according to the guidelines of Ethics and Animal Experimentation Committee (CEEA 8316-2017), and efforts were made to minimize animals suffering and to reduce the number of animals used in the experiments.

2.3. Experimental design

The experimental design of this is study is presented in Fig. 2. Mice were randomly divided in four different groups (n=10 animals/group, except in molecular analyses and toxicity, were utilized n=6 animals/group). During the first 20 days of the experiment, group I and III were treated with OSX's vehicle (canola oil 0,1 ml/kg body weight dose). Group II and IV were treated with OSX (0,1 mg/kg body weight dose). After the pre-treatment, groups I and II were exposed to a intracerebroventricular (ICV) injection with STZ's vehicle (0.9% saline solution in a 1 ml/kg body weight) on day 21 and repeated on day 23. On the same days, group III and IV, were exposed to a intracerebroventricular (ICV) injection with STZ (1,5 mg/kg body weight). After 15 days past the last ICV injection, mice performed behavioural tests and afterwards, were submitted to euthanasia to remove brain for *ex vivo* analysis.

Insert figure 2 here

2.4. Surgical procedure

Animals were anesthetized with isoflurane (Iso Fo Abbott Laboratories) via inhalation before the beginning of the surgery. A hypodermic needle coupled to a Hamilton syringe of 25 µl was used to perform free-hand ICV, using the bregma as a reference point, according to a previously described method (HALEY & MCCORMICK, 1957; Laursen & Belknap, 1986). More specifically 2 mm behind the ears line. 1.0 mm lateral to sagittal suture, and 3.0 mm beneath the surface of the brain.

2.5. Behavioural tests

2.5.1. Open Field Test (OFT)

OFT was performed in order to exclude possible effects of the compound in the locomotor and exploratory behaviour of the mice (Walsh & Cummins, 1976). Briefly, the animals were placed in a wooden box (30 cm x 30 cm x 15 cm), marked with lines dividing the box into 9 squares of equal area. During five minutes, mice were evaluated about their exploratory and locomotor activity. The exploratory activity is corresponding to the number of rearings the animals performed, and the locomotor activity to the number of crossings with the 4 paws between squares. Moreover, the animals were left in the box an extra 5 minutes for setting them for the next behavioural test, after 24 hours, Object Recognition Test (ORT).

2.5.2. Object recognition task (ORT)

ORT was performed to evaluate memory formation and learning capability of the mice in short-term memory (STM) and long-term memory (LTM) (Rosa et al., 2003). In order to realize the test, animals were placed in the same apparatus from the OFT, 24 hours after setting in the first day, with two identical objects, for the first interaction (FI). 1 and onde and half hour later (STM), one of the objects was replaced for another one (familiar and novel object), of different colour and shape. Next day, 24 hours later (LTM), the novel object was replaced for a new one. During each phase, mice interaction time with the objects were accounted for 5 minutes. The interaction was considered as touching, stepping or sniffing the objects. To determine memory and learning, a recognition index was calculated, according to the following formula: [time spent in the novel object/ (time spent in the familiar object + time spent in the novel object)].

2.5.3. Y maze test

In order to evaluate spatial and work memory, Y maze test was performed (Jackson, 1943). Mice were placed in a Y shaped apparatus, 35 cm long, 5 cm wide and 10 cm high, and were allowed to explore the maze. The test is based in the willingness of mice to explore new environments, in that case, the arms of the maze, rather than a previously visited one. The sequence of entries in the arms (A, B or C arms) and spontaneous alternations (alternation between 3 different arms consecutively = 1 spontaneous alternation) were noted during 6 minutes, and expressed as spontaneous alternation (%): [(nº of spontaneous alternation x 3) / (nº of

entries in the arms – 2)] x 100. The number of entries were considered as parameter to check mice mobility, in order to validate the previous obtained result.

2.5.4. Social recognition task (SRT)

One crucial point of social behaviour for humans and mice is social recognition memory, important in the process of discriminating between humans and animals. Taking that into account, the present experiment aims to analyse the memory formation and learning of mice, during a social recognition task (Tanimizu et al., 2017). Animals were placed in the same wooden box from OFT, with a juvenile mouse from the same species, in order to evaluate its interaction, during 5 minutes (experiment animal with the juvenile mouse). After one hour, the animal evaluated and the same juvenile were placed again together, during 5 minutes, and had its interaction time accounted. Juvenile animals were used in order to avoid fight between mice, and the box was cleaned between each mouse evaluated. The results were expressed in the form of a ratio between the second and the first investigation, by the time spent interacting with the juvenile animal.

2.5.5. Passive avoidance task – step-down

The passive avoidance test works with aversive conditioning pattern, based on a negative stimulus against mice primary instinct, like stepping into a dark compartment, or going down in a lower level from a high platform. Moreover, the test combines both fear conditioning and a training context (Roesler et al., 1999). Thereby, the test is divided in two phases, the first one is the training phase. In that part, mice were placed in an apparatus, in a higher platform and allowed to explore the lower grid floor. Once it goes down, mice received a shock (2 seconds of 0,5 mA), as the aversive stimulus, leading it back to the higher safe platform. 24 hours after the training, mice were placed into the apparatus again and had the time to go down in the lower grid floor (latency time) accounted (cut-off of 5 minutes). Results were expressed in seconds of latency.

2.6. Ex vivo assays

2.6.1. Tissue preparation

Mice were euthanized and had their brain tissue rapidly removed and placed on ice. The brains of the animals were dissected to separate total cortex and

hippocampus, kept chilled and homogenized in different buffer according to test performed. Both of the homogenates was centrifuged for 10 min at 2500 rpm to yield a pellet that was discarded and a low speed supernatant (S_1) collected for each tissue.

2.6.2. Thiobarbituric acid reactive species (TBARS) assay

Lipid peroxidation was evaluated by TBARS assay, in total cortex and hippocampus. In an acid and high temperature reaction, TBARS were formed and its product was analysed in a spectrophotometer (Ohkawa et al., 1979). Total cortex S_1 and an aliquot of hippocampus S_1 were incubated with 8.1 % SDS, TBA (0.8%) and acetic acid/HCl (pH 3.4) at 95 ºC for 2 h. MDA was used as a biomarker of fatty-acid peroxidation, detected by a spectrophotometer (532 nm).

2.6.3. Reactive species (RS) formation assay

In order to further scope our knowledge about the antioxidant activity of the compound, RS formation assay was performed (Loetchutinat et al., 2005). RS formation was evaluated in total cortex and hippocampus. S_1 of both tissues were incubated with dichlorofluorescein at room temperature for 30 minutes, in the dark. Dichlorofluorescein was used as a detector of the RS, emitting fluorescence, detected by a spectrofluorimeter.

2.6.4. Lipid extraction and hippocampal and cortical cholesterol measurement

Briefly, tissue from total cortex and hippocampus of mice were weighted homogenized with chloroform and methanol, and washed with water. After homogenizing the samples, they were centrifuged and processed, in order to get the cholesterol rich phase, letting it dry (Santos et al., 2015). The dry pellet was suspended in a 0.1 M HCl isotonic NaCl solution. Afterwards, the solution obtained was used to quantify the cholesterol content in the tissues, using an enzymatic colorimetric method, according to the manufacturer's protocol (LABTEST, Diagnostica S.A., Brazil). The results were expressed in µg of cholesterol/mg tissue.

2.6.5. Acetylcholinesterase inhibitory activity

Acetylcholinesterase (AChE) activity was evaluated according to Ellman's method (1961) (ELLMAN et al., 1961), in total cortex and hippocampus. This assay consists in a rapid colorimetric determination of AChE activity, based on the reaction of dithiobisnitrobenzoate (DTNB) acid with the product of the enzyme's action

(acetylthiocoline cleaved into thiocoline). The interaction of DTNB and thiocoline generates a yellow colour, detected by a spectrophotometer (412 nM).

2.6.6. Monoamine oxidase (MAO) B activity

MAO activity was determined according to previously described method, with some alterations (Matsumoto et al., 1984). The cerebral total cortex and hippocampus extracted from mice were processed, in a few steps of centrifuging, until obtain the mitochondrial fraction for the analysis. The last supernatant obtained was incubated with MAO substrate, kynuramine dihydrobromide, in order to evaluate its consumption, to determine MAO activity. Clorgiline (MAO A inhibitor) was used to evaluate MAO B activity. The fluorescence generated after kynuramine's consumption was detected spectrofluorimetrically with excitation at 315 nm and emission at 380 nm.

2.7. RNA extraction and Gene Expression evaluation by qRT-PCR

Seeking to evaluate another parameter on AD, amiloidogenic pathway proteins, such as APP, γ-secretase and β-secretase expression, were analysed by qRT-PCR. A different group of mice were used to the gene expression evaluation. Total RNA was extracted and purified from mice hippocampus using Trizol reagent (Invitrogen™, Carlsbad, CA, United States). cDNA was obtained with 1 µg RNA using the High Capacity cDNA Reverse Transcription kit (Applied Biosystems™, United Kingdom) according to the manufacturer's instructions. Relative gene expression was normalized using GAPDH gene and the reaction's conditions included 95ºC for 15 s, 60ºC for 60 s, and 72ºC for 30 s. The Delta-Delta Comparative Threshold method was used to normalize the fold change in gene expressions. The used primer sequences and full name of the genes are provided in Table 1.

Insert table 1 here

2.8. Toxicological assays

A sub chronic toxicological evaluation of OSX (0,1 mg/kg) was accomplished performing 20 continuous days of intragastric treatment with the compound, according to the Figure 3., with a different group of animals (n=6).

Insert figure 3 here

2.8.1. Plasmatic AST and ALT activities

Aiming to investigate the endpoints of hepatotoxicity, plasmatic aspartate (AST) and alanine aminotransferase (ALT) were quantified with biochemical commercial kits (LABTEST, Diagnostica S.A., Brazil), according to Reitman and Frankel (1957) (Hospital, 1957).

2.8.2. Plasmatic urea and creatinine

Renal function was evaluated determining plasmatic levels of urea (Mackay & Mackay, 1927) and creatinine (Peake & Whiting, 2006), in order to check any kidney damage, using commercial kits (LABTEST, Diagnostica S.A., Brazil).

2.8.3. Plasmatic glucose levels

Glucose levels were quantified using commercial kits (LABTEST, Diagnostica S.A., Brazil), in order to check if there were any changes in glucose levels, and to validate STZ ICV injection, proving that STZ was not able to reach the systemic level inducing diabetes.

2.9. Statistical analysis

Statistical analysis was performed using two-way analysis of variance (ANOVA), except for toxicological assays, where one-way ANOVA was applied, both followed by Newman-Keuls *post-hoc* test when appropriate. Experimental results were expressed as mean ± standard deviation (SD) to show variation among groups. The results were considered different for a $p<0,05$. All data was analysed by GraphPad Prism 7.0.

3. Results

3.1. Effect of OSX on locomotor and exploratory activity on Open Field Test (OFT)

With the purpose of checking if OSX or STZ injections might cause any psychomotor changes, OFT was performed. Neither OSX or STZ injected mice presented any changes. Two-way ANOVA showed no significant STZ X OSX interaction in the number of crossings ($F(1,36) = 1.266$; $P = 0.2681$) and rearings ($F(1,36) = 1.347$; $P = 0.2528$). Those results show that any of the groups had any influence in their psychomotor parameters and that other behavioural test would not be influenced by these parameters.

3.2. Effect of OSX on object recognition in STZ injected mice on Object Recognition Task (ORT)

STZ induced a decrease in recognition index on ORT, indicated by the reduction of time spent in the novel object, both in short-term and long-term memory. OSX treatment prevented the memory impairment caused by STZ injection, exhibited by an increase in recognition index. Two-way ANOVA indicated an STZ X OSX interaction in short-term ($F(1,36) = 7.07$; $P = 0.01$) and long-term memory ($F(1,36) = 31.3$; $P < 0.001$), presented in Figure 4a and 4b respectively.

Insert figure 4 here

3.3. Effect of OSX on spontaneous alternation in STZ injected mice on Y maze

In order to evaluate spatial memory, Y maze test was performed. STZ decreased the spontaneous alternation, while this reduction was prevented by OSX treatment. Two-way ANOVA indicated an STZ X OSX interaction ($F(1,36) = 13.2$; $P < 0.001$) (Fig. 5). These results indicate that OSX prevented cognitive deficit and memory impairment caused by STZ injection.

Insert figure 5 here

3.4. Effect of OSX on social recognition in STZ injected mice on Social Recognition Task (SRT)

Aiming to evaluate social behaviour, relating to the capability of one animal to discriminate other individual, SRT was performed. STZ injection caused an increase in the interaction time with the juvenile mice, representing that these mice did not remember the first contact with the strange animal, increasing recognition index. OSX treatment was able to reduce recognition index levels, preventing social behaviour abnormality caused by STZ. Two-way ANOVA showed an STZ X OSX interaction ($F(1,36) = 14.9$; $P < 0.001$) (Fig. 6).

Insert figure 6 here

3.5. Effect of OSX on passive avoidance task in STZ injected mice

Looking forward to investigating another memory performance of mice, we applied passive avoidance test (step-down type), evaluating aversive conditioning pattern. STZ injection reduced latency time (s) for mice to go down to the grid floor. On

other hand, OSX treatment protected against STZ memory damage, increasing the latency time. Two-way ANOVA presented an STZ X OSX interaction ($F(1,36) = 17.5$; $P < 0.001$) (Fig. 7).

Insert figure 7 here

3.6. Effect of OSX on oxidative stress (OS) related parameters in STZ injected mice

The results are presented in Figures 8-10, on lipid peroxidation, RS formation and cholesterol levels, respectively, in total cortex (a) and hippocampus (b). STZ injected mice presented an elevated lipid peroxidation in total cortex and hippocampus, although it was not able to decrease cholesterol levels. Two-way ANOVA showed no significant STZ X OSX interaction in cholesterol levels in total cortex ($F(1,36) = 0.0248$; $P = 0.88$) or hippocampus ($F(1,36) = 2.87$; $P = 0.10$). The same happened on RS formation, presenting no significant changes. Two-way ANOVA revealed no significant STZ X OSX interaction in total cortex ($F(1,36) = 0.164$; $P = 0.69$) or hippocampus ($F(1,36) = 0.715$; $P = 0.40$). From this, OSX reduced the lipid peroxidation in STZ injected mice group. Two-way ANOVA presented a main effect of OSX in total cortex ($F(1,36) = 4.55$; $P = 0.04$) and STZ X OSX interaction in hippocampus ($F(1,36) = 11.8$; $P = 0.002$). Furthermore, OSX did not change the other parameters, revealed by Two-way ANOVA ($P > 0.05$). We believe that the dosage of STZ and the induction time of the presented protocol, was not enough to create a damage in the cholesterol mostly present in the myelin sheath, even though the lipid peroxidation production was increased.

Insert figures 8-10 here

3.7. Effect of OSX on AChE activity in STZ injected mice

STZ injection led to an increase in AChE activity in both structures, while OSX protected against this cholinergic dysfunction, showing its potential as a cholinesterase inhibitor. Two-way ANOVA noticed an STZ X OSX interaction in total cortex ($F(1,36) = 8.57$; $P = 0.006$) and hippocampus ($F(1,36) = 7.72$; $P = 0.009$) (Fig. 11).

Insert figure 11 here

3.8. Effect of OSX on MAO B activity in STZ injected mice

In the present study, STZ ICV injections were not able to produce any increased response on MAO B activity. However, OSX was able to reduce MAO B activity in total cortex. Two-way ANOVA presented a main effect of OSX in total cortex ($F(1,36) = 7.69$; $P = 0.009$), although did not show STZ X OSX interaction ($F(1,36) = 0.0001$; $P = 0.78$), neither have main effect in hippocampus ($F(1,36) = 0.0801$; $P = 0.78$) (Fig. 12).

Insert figure 12 here

3.9. Effect of OSX on mRNA levels in STZ injected mice on qRT-PCR

The amiloidogenic pathway was evaluated by qRT-PCR in the present study. Based on two-way ANOVA, STZ group was able to induce an increase in all the evaluated genes from the amiloidogenic pathway (APP, β-secretase and γ-secretase), both in total cortex and hippocampus (Fig. 13-14). Furthermore, OSX was able to reduce the increased mRNA levels, in both tissues, protecting from STZ injections. Two-way ANOVA showed an STZ X OSX interaction in total cortex ($F(1,20) = 8.44$; $P = 0.009$), ($F(1,20) = 17.3$; $P < 0.001$) ($F(1,20) = 6.69$; $P = 0.02$) (APP, β-secretase, γ-secretase, respectively). Moreover, Two-way ANOVA presented an STZ X OSX interaction in hippocampus in APP and β-secretase mRNA levels($F(1,20) = 17.7$; $P < 0.001$), ($F(1,20) = 13.1$; $P = 0.002$) and a main effect of OSX in γ-secretase gene ($F(1,20) = 13.4$; $P = 0.002$).

Insert figure 13-14 here

3.10. Effect of OSX on toxicological parameters

Some of common markers of toxicity were evaluated in the present study, checking if OSX present any sub-chronic toxicological effects. Plasmatic urea and creatinine were quantified aiming to evaluate kidney's integrity, whereas, AST and ALT were measured to verify if there was liver damage. The 20 days oral OSX treatment in the 0.1 mg/kg dose, does not show any changes in the evaluated toxicological markers, indicating no toxicity in the present study. Moreover, glucose levels were not changed, validating STZ Alzheimer-like model, proving that there was not a systemic diabetes induction.

4. Discussion

The present study provided new evidence concerning OSX pharmacological properties, showing its anti-Alzheimer effects in mice, adding to its antidepressant-like

effect already exhibited in the literature, modulating intracellular cascades and monoaminergic pathways (Pinto Brod et al., 2016a, 2017a). This new evidence presented OSX inhibiting AChE and MAO B activities, reducing lipid peroxidation and relative expression of genes related to amiloidogenic pathway and protected mice against cognitive and memory impairment. Taken all together, the results presented in this study showed initial data on anti-Alzheimer effect of OSX, in different factors of the disease, in behavioural and physiological levels of AD markers.

With regard behavioural experiments, mice memory test presented here are already well stablished in the literature and can be related to AD characteristics, such as memory impairment and cognitive deficit in humans (Różyk-Myrta, 2014). The behavioural tests performed in the present study targeted different aspects of memory in each of them. ORT is a memory test capable of evaluate the ability of mice in differentiate between novel and familiar objects, relating with working memory in AD patients for example (Ennaceur & Delacour, 1988). Y maze evaluate animals willingness to explore a new environment and normally it prefers to explore a new arm in the maze rather then a previously visited one, indicating spontaneous alternation level and visuospatial memory (Darcet et al., 2014). Furthermore, social behaviours are formed by different aspects and components, such as social interaction and recognition. In that sense, SRT was performed, evaluating animals memory associated with its social interaction and recognition (Kogan et al., 2000; Gabor et al., 2012). Moreover, passive avoidance test look at the tendency of mice to explore new environment, such as going doing from the safe platform to the grid floor, and its associative memory, relating the mild foot-shock with a harmful stimulus, conditioning its response (Erden, 2014).

STZ injections reduced mice ability to recognize and differentiate familiar from novel objects in ORT and familiar mice in SRT, impairing working memory, indicated by a decreased recognition index in ORT and increased in SRT. Besides that, STZ induced visuospatial memory deficit in Y maze test and reduced conditioning pattern in passive avoidance test, indicated by a reduced spontaneous alternation (%) and latency, respectively. These results are in accordance with other studies in the literature involving memory in STZ Alzheimer models, showing memory impairment and cognitive deficit (Li et al., 2016; Ravelli et al., 2017; Bassani et al., 2018). On other hand, OSX treatment was able to prevent these behavioural changes, protecting mice,

proved by restored recognition indexes, higher spontaneous alternation and latency. These results can be related with MD and AD carachteristics, such as cognitive deficit, whereas, OSX, that already presented an antidepressant-like activity, showed an ability to improve the cognitive performance in the applied tests.

Besides behavioural alterations, STZ has been proved as a good oxidative stress inductor in AD model, increasing damages in biomolecules, such as lipid peroxidation and production of reactive species (Islam et al., 2015). CNS is a susceptible tissue to oxidative damage, due to its characteristics, like high oxygen use, lower antioxidant defences and higher contents of polyunsaturated fatty acids (Halliwell, 2001; Moreira et al., 2005). This higher susceptibility can be related to the damages caused in AD, due to OE, such as lipid peroxidation in the myelin sheath, because of its high levels of cholesterol, degenerating its structure, stopping or slowing neural transmission and proper functioning (Cai & Xiao, 2016; Wu et al., 2017). Moreover, OS is present in MD, due to the same susceptibility of CNS. Thereby, we evaluated RS formation, lipid peroxidation and indirect myelin integrity (cholesterol levels), aiming to evaluate part of OS involvement in this STZ Alzheimer model, taking in account the already presented antidepressant activity of OSX.

STZ injections were able to increase lipid peroxidation, but without any increment in RS formation or low cholesterol, as expected. These results might be explained by the fact that the STZ dose injected might not have been high enough to cause drastic changes in myelin integrity, when compared to other studies in the literature (Santos et al., 2015; Reeta et al., 2017; Pacheco et al., 2018). Other possibility, is that RS did not increase because the animals were left for 15 days to induce the neurodegeneration processes by STZ and treated during the next 20 days, leaving time enough for their organism to restore part of RS and antioxidant balance, but did not restore the damage caused, such lipid peroxidation. Although some of the parameters evaluated has not changed, OSX reduced lipid peroxidation, both in total cortex and hippocampus, probably related to its ability in reduce oxidative stress.

One other factor related to AD are the abnormalities in some enzymatic activities, changing neurotransmitters availability. One of these enzymes is AChE, responsible for regulating ACh levels in the synaptic cleft by degrading it. In AD, AChE activity is elevated and the same happens in STZ model, reducing ACh availability, causing behavioural irregularities, such as memory and cognitive impairment (Biswas

et al., 2016; Chigurupati et al., 2016). Furthermore, ACh levels can be related to neuroplasticity and neurogenesis, since iAChE treatments are capable of increasing hippocampal neurogenesis and modulate neurotrophins levels (Craig et al., 2011). Moreover, reduced levels of neurotrophins, such as BDNF, are related to cholinergic signalling malfunctioning (Barage & Sonawane, 2015).

Taking that into account, the available AD treatments are mostly iAChE, acting in the cholinergic signalling in the disease, reducing behavioural symptoms (Doody, 2003). STZ injections caused an increase in AChE activity as expected, that can be related to the observed results in mice behavioural tests (Berté et al., 2018), like cognitive deficit and memory impairment. Protecting from AChE increased activity, OSX increased ACh avilabillity, ameliorating mice behavioural performances, acting similarly to a iAChE. Also, OSX already presented in previous studies, modulation of intracellular signalling proteins, like PKC, CAMK and ERK, that might be related to BDNF-TRKB (tropomyosin receptor kinase B) pathway of synaptic plasticity (Minichiello, 2009) and its involvement with AD and MD (Zhang et al., 2012). Those results complement the previous results obtained from OSX action, proving itself not only as an antidepressant, but as molecule able to improve memory and cognitive impairments, whats can be related to AChE inhibition.

Other affected enzyme is MAO of B type, with an increased activity in AD, presenting itself as a promising new associated target for the treatment of the disease (Borroni et al., 2017). From MAO B activity test, the results showed no alterations in MAO B activity in STZ-injected mice, representing a new data, since there is no data relating ICV STZ injections with MAO B activity abnormalities in the literature. Although, STZ was not able to produce changes on this enzyme activity, OSX presented an inhibitory activity, significantly reducing enzymatic activity, acting as a multimodal inhibitor in both AChE and MAO B. A recent study presented a relation between and increased MAO B activity and a raise in γ-secretase expression, that is a limiting step to Aβ peptide production in amiloidogenic pathway (Schedin-Weiss et al., 2017).

In order to further scope our knowledge about different aspects of AD, qRT-PCR was performed to quantify mRNA levels of the main genes present in amiloidogenic pathway. The genes APP, β and γ-secretases correspond to proteins that are considered biomarkers of AD (Bagyinszky et al., 2014). With an increased relative expression in the disease, more APP is available to be processed by β and γ-

secretases into Aβ peptides, that aggregates and provoke a neurodegenerative response in CNS (Thinakaran & Koo, 2008; Heneka et al., 2015a). ICV STZ injections has also been related to increased quantitative expression of APP-pathway genes in recent studies (Park et al., 2015; Rajasekar et al., 2017). Our results showed that STZ injection, agreeing with results found in the literature, increased APP, β and γ-secretases mRNA's levels, validating our model in the amiloidogenic pathway. OSX previous treatment reduced APP-pathway genes relative expression, inhibiting the amiloidogenic pathway. Additionally, γ-secretase reduced relative expression can be related to OSX MAO B inhibitory activity, given that an increased MAO B activity is connected to γ-secretase expression, inducing a higher APP-pathway activity, collaborating with Aβ formation.

5. Conclusion

Based on what was exposed, it is possible to conclude that OSX presented anti-Alzheimer effect in the present study. OSX can act on different aspects, here presented, of AD, for instance, reducing lipid peroxidation, as a marker of oxidative stress, avoiding damage in cellular membranes. In addition, OSX presented inhibitory properties over AChE and MAO B, two important targets of the disease, increasing ACh availability and reducing γ-secretase expression. Also related to APP-pathway, OSX reduced main proteins responsible for Aβ peptide aggregates, reducing the chance of the presence of neurodegenerative process in CNS. All those factors contributed for an improvement in all the behavioural tests performed in mice and OSX protective action. Nonetheless, more studies are necessary to further evaluate more about OSX's protective action and its mechanisms associated with its anti-Alzheimer activity.

Aknowledgements

The authors of the present study are grateful to UFPel, especially to the Programa de Pós Graduação em Biotecnologia, and its facilities for providing the material and environment to carry out this work. Also, we would like to thank CNPq, CAPES and FAPERGS (PRONEM 16/2551-0000240-1, PqG 17/2551-00011046-9 and FAPERGS/CAPES 04/2018 - DOCFIX 18/2551-0000511-8) for the financial support. L.S., D.A, T.C. and F.K.S., are recipients of CNPq fellowship. All authors revised this manuscript and approved its final version and none declaration or conflict

of interest. This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

References

Allan Butterfield, D. & Boyd-Kimball, D. (2018). Oxidative Stress, Amyloid-β Peptide, and Altered Key Molecular Pathways in the Pathogenesis and Progression of Alzheimer's Disease. *Journal of Alzheimer's Disease*.

Alzheimer's Association (2018). *Alzheimer's Facts and Figures Report | Alzheimer's Association*. 2018. alz.org.

Alzheimer's Association (USA) (2018). 2018 Alzheimer's disease facts and figures includes a special report on the financial and personal benefits of early diagnosis. *Alzheimers Dement*.

Alzheimer's Disease International (2015). *The Global Impact of Dementia: An analysis of prevalence, incidenc, cost and treads World Alzhiemer Report 2015*.

Andrade, V., Cortes, N., Guzman-Martinez, L. & Maccioni, R. (2017). *An Overview of the Links between Behavioral Disorders and Alzheimer's Disease*. [Online]. 4. p.pp. 1–5. Available from: https://pdfs.semanticscholar.org/c416/2bd55e79de9416b553afacb1babe1e95b08d.pdf.

Bagyinszky, E., Youn, Y.C., An, S. & Kim, S. (2014). The genetics of Alzheimer's disease. *Clinical Interventions in Aging*. [Online]. p.p. 535. Available from: http://www.dovepress.com/the-genetics-of-alzheimer39s-disease-peer-reviewed-article-CIA.

Barage, S.H. & Sonawane, K.D. (2015). Amyloid cascade hypothesis: Pathogenesis and therapeutic strategies in Alzheimer's disease. *Neuropeptides*.

Bassani, T.B., Bonato, J.M., Machado, M.M.F., Cóppola-Segovia, V., Moura, E.L.R., Zanata, S.M., Oliveira, R.M.M.W. & Vital, M.A.B.F. (2018). Decrease in Adult Neurogenesis and Neuroinflammation Are Involved in Spatial Memory Impairment in the Streptozotocin-Induced Model of Sporadic Alzheimer's Disease in Rats. *Molecular Neurobiology*.

Bekris, L.M., Yu, C.E., Bird, T.D. & Tsuang, D.W. (2010). Review article: Genetics of Alzheimer disease. *Journal of Geriatric Psychiatry and Neurology*.

Belfiore, R., Rodin, A., Ferreira, E., Velazquez, R., Branca, C., Caccamo, A. & Oddo, S. (2018). Temporal and regional progression of Alzheimer's disease-like pathology in 3xTg-AD mice. *Aging Cell*. [Online]. (September). p.p. e12873. Available from: http://doi.wiley.com/10.1111/acel.12873.

Berté, T.E., Dalmagro, A.P., Zimath, P.L., Gonçalves, A.E., Meyre-Silva, C., Bürger, C., Weber, C.J., dos Santos, D.A., Cechinel-Filho, V. & de Souza, M.M. (2018). Taraxerol as a possible therapeutic agent on memory impairments and Alzheimer's disease: Effects against scopolamine and streptozotocin-induced cognitive dysfunctions. *Steroids*.

Bettens, K., Sleegers, K. & Van Broeckhoven, C. (2013). Genetic insights in Alzheimer's disease. *The Lancet Neurology*.

Biswas, J., Goswami, P., Gupta, S., Joshi, N., Nath, C. & Singh, S. (2016). Streptozotocin Induced Neurotoxicity Involves Alzheimer's Related Pathological Markers: a Study on N2A Cells. *Molecular Neurobiology*.

Blagosklonny, M. V. (2008). Aging: ROS or TOR. *Cell Cycle*. 7 (21) p.pp. 3344–3354.

Borroni, E., Bohrmann, B., Grueninger, F., Prinssen, E., Nave, S., Loetscher, H., Chinta, S.J., Rajagopalan, S., Rane, A., Siddiqui, A., Ellenbroek, B., Messer, J., Pahler, A., Anderson, J.K., Wyler, R. & Cesura, A.M. (2017). Sembragiline: a novel, selective monoamine oxidase type B inhibitor for the treatment of Alzheimer's disease. *Journal of Pharmacology and Experimental Therapeutics*.

Brigelius-Flohé, R. (2015). The Evolving Versatility of Selenium in Biology. *Antioxidants & Redox Signaling*.

Brown, K. & Arthur, J. (2001). Selenium, selenoproteins and human health: a review. *Public Health Nutrition*.

Budni, J., Bellettini-Santos, T., Mina, F., Lima Garcez, M. & Ioppi Zugno, A. (2015). The involvement of BDNF, NGF and GDNF in aging and Alzheimer's disease. *Aging and Disease*.

Buée, L., Troquier, L., Burnouf, S., Belarbi, K., Van der Jeugd, A., Ahmed, T.,

Fernandez-Gomez, F., Caillierez, R., Grosjean, M.-E., Begard, S., Barbot, B., Demeyer, D., Obriot, H., Brion, I., Buée-Scherrer, V., Maurage, C.-A., Balschun, D., D'hooge, R., Hamdane, M., Blum, D. & Sergeant, N. (2010). From tau phosphorylation to tau aggregation: what about neuronal death? *Biochemical Society transactions*. 38 (4). p.pp. 967–72.

Bus, B.A.A., Molendijk, M.L., Tendolkar, I., Penninx, B.W.J.H., Prickaerts, J., Elzinga, B.M. & Voshaar, R.C.O. (2015). Chronic depression is associated with a pronounced decrease in serum brain-derived neurotrophic factor over time. *Molecular Psychiatry*.

Cai, Z. & Xiao, M. (2016). Oligodendrocytes and Alzheimer's disease. *International Journal of Neuroscience*.

Cardoso, B.R., Roberts, B.R., Bush, A.I. & Hare, D.J. (2015). Selenium, selenoproteins and neurodegenerative diseases. *Metallomics*.

Casaril, A.M., Domingues, M., Bampi, S.R., de Andrade Lourenço, D., Padilha, N.B., Lenardão, E.J., Sonego, M., Seixas, F.K., Collares, T., Nogueira, C.W., Dantzer, R. & Savegnago, L. (2019). The selenium-containing compound 3-((4-chlorophenyl)selanyl)-1-methyl-1H-indole reverses depressive-like behavior induced by acute restraint stress in mice: modulation of oxido-nitrosative stress and inflammatory pathway. *Psychopharmacology*. [Online]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30610349%0Ahttp://link.springer.com/10.1007/s00213-018-5151-x.

Casaril, A.M., Domingues, M., Fronza, M., Vieira, B., Begnini, K., Lenardão, E.J., Seixas, F.K., Collares, T., Nogueira, C.W. & Savegnago, L. (2017). Antidepressant-like effect of a new selenium-containing compound is accompanied by a reduction of neuroinflammation and oxidative stress in lipopolysaccharide-challenged mice. *Journal of Psychopharmacology*.

Cervellati, C., Wood, P.L., Romani, A., Valacchi, G., Squerzanti, M., Sanz, J.M., Ortolani, B. & Zuliani, G. (2016). Oxidative challenge in Alzheimer's disease: state of knowledge and future needs. *J Investig Med*. [Online]. 64 (1). p.pp. 21–32. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26755810.

Cheignon, C., Tomas, M., Bonnefont-Rousselot, D., Faller, P., Hureau, C. & Collin, F.

(2018). Oxidative stress and the amyloid beta peptide in Alzheimer's disease. *Redox Biology*.

Chi, S., Yu, J.T., Tan, M.S. & Tan, L. (2015). Depression in alzheimer's disease: Epidemiology, mechanisms, and management. In: *Handbook of Depression in Alzheimer's Disease*.

Chigurupati, S., Selvaraj, M., Mani, V., Selvarajan, K.K., Mohammad, J.I., Kaveti, B., Bera, H., Palanimuthu, V.R., Teh, L.K. & Salleh, M.Z. (2016). Identification of novel acetylcholinesterase inhibitors: Indolopyrazoline derivatives and molecular docking studies. *Bioorganic Chemistry*. 67. p.pp. 9–17.

Craig, L.A., Hong, N.S. & McDonald, R.J. (2011). Revisiting the cholinergic hypothesis in the development of Alzheimer's disease. *Neuroscience and Biobehavioral Reviews*.

Cummings, J.L., Morstorf, T. & Zhong, K. (2014). Alzheimer's disease drug-development pipeline: Few candidates, frequent failures. *Alzheimer's Research and Therapy*.

Dá Mesquita, S., Ferreira, A.C., Sousa, J.C., Correia-Neves, M., Sousa, N. & Marques, F. (2016). Insights on the pathophysiology of Alzheimer's disease: The crosstalk between amyloid pathology, neuroinflammation and the peripheral immune system. *Neuroscience and Biobehavioral Reviews*.

Darcet, F., Mendez-David, I., Tritschler, L., Gardier, A.M., Guilloux, J.-P. & David, D.J. (2014). Learning and memory impairments in a neuroendocrine mouse model of anxiety/depression. *Frontiers in Behavioral Neuroscience*.

Dawson, T.M., Golde, T.E. & Lagier-Tourenne, C. (2018). Animal models of neurodegenerative diseases. *Nature Neuroscience*.

Doody, R.S. (2003). Current treatments for Alzheimer's disease: cholinesterase inhibitors. *The Journal of clinical psychiatry*. [Online]. 64 Suppl 9. p.pp. 11–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12934969.

ELLMAN, G.L., COURTNEY, K.D., ANDRES, V. & FEATHER-STONE, R.M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical pharmacology*. [Online]. 7. p.pp. 88–95. Available from:

http://www.ncbi.nlm.nih.gov/pubmed/13726518.

Ennaceur, A. & Delacour, J. (1988). A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behavioural Brain Research*.

Erden, F. (2014). Effects of 7-NI and ODQ on memory in the passive avoidance, novel object recognition, and social transmission of food preference tests in mice. *Medical Science Monitor Basic Research*.

Fang, F., Yu, Q., Arancio, O., Chen, D., Gore, S.S., Yan, S.S. Du & Yan, S.F. (2018). RAGE mediates Aβ accumulation in a mouse model of Alzheimers disease via modulation of β- and γ-secretase activity. *Human Molecular Genetics*.

Fradejas-Villar, N. (2018). Consequences of mutations and inborn errors of selenoprotein biosynthesis and functions. *Free Radical Biology and Medicine*.

Francis, P.T., Palmer, A.M., Snape, M. & Wilcock, G.K. (1999). The cholinergic hypothesis of Alzheimer's disease: a review of progress. *Journal of Neurology, Neurosurgery & Psychiatry*. [Online]. 66 (2). p.pp. 137–147. Available from: http://jnnp.bmj.com/content/66/2/137%5Cnhttp://jnnp.bmj.com/content/66/2/137.full#xref-ref-2-1%5Cnhttp://jnnp.bmj.com/content/66/2/137.full.pdf%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/10071091.

De Freitas, A.S. & Rocha, J.B.T. (2011). Diphenyl diselenide and analogs are substrates of cerebral rat thioredoxin reductase: A pathway for their neuroprotective effects. *Neuroscience Letters*.

Fronza, M.G., Pinto Brod, L.M., Casaril, A.M., Sacramento, M., Alves, D. & Savegnago, L. (2017). Computational and biological evidences on the serotonergic involvement of SeTACN antidepressant-like effect in mice. *PLoS ONE*.

Gabor, C.S., Phan, A., Clipperton-Allen, A.E., Kavaliers, M. & Choleris, E. (2012). Interplay of oxytocin, vasopressin, and sex hormones in the regulation of social recognition. *Behavioral Neuroscience*.

Gandini, A., Bartolini, M., Tedesco, D., Martinez-Gonzalez, L., Roca, C., Campillo, N.E., Zaldivar-Diez, J., Perez, C., Zuccheri, G., Miti, A., Feoli, A., Castellano, S., Petralla, S., Monti, B., Rossi, M., Moda, F., Legname, G., Martinez, A. &

Bolognesi, M.L. (2018). Tau-Centric Multitarget Approach for Alzheimer's Disease: Development of First-in-Class Dual Glycogen Synthase Kinase 3β and Tau-Aggregation Inhibitors. *Journal of Medicinal Chemistry*.

Gaur, S. & Agnihotri, R. (2017). Trace Mineral Micronutrients and Chronic Periodontitis—a Review. *Biological Trace Element Research*.

Gill, R., Tsung, A. & Billiar, T. (2010). Linking oxidative stress to inflammation: Toll-like receptors. *Free Radical Biology and Medicine*.

Gong, C.-X. & Iqbal, K. (2008). Hyperphosphorylation of microtubule-associated protein tau: a promising therapeutic target for Alzheimer disease. *Current medicinal chemistry*. [Online]. 15 (23). p.pp. 2321–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18855662%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC2656563.

Götz, J., Streffer, J.R., David, D., Schild, A., Hoerndli, F., Pennanen, L., Kurosinski, P. & Chen, F. (2004). Transgenic animal models of Alzheimer's disease and related disorders: Histopathology, behavior and therapy. *Molecular Psychiatry*.

Grieb, P. (2016). Intracerebroventricular Streptozotocin Injections as a Model of Alzheimer's Disease: in Search of a Relevant Mechanism. *Molecular Neurobiology*.

Guedes, J.R., Lao, T., Cardoso, A.L. & El Khoury, J. (2018). Roles of microglial and monocyte chemokines and their receptors in regulating Alzheimer's disease-associated amyloid-β and tau pathologies. *Frontiers in Neurology*.

H. Ferreira-Vieira, T., M. Guimaraes, I., R. Silva, F. & M. Ribeiro, F. (2016). Alzheimer's disease: Targeting the Cholinergic System. *Current Neuropharmacology*.

HALEY, T.J. & MCCORMICK, W.G. (1957). Pharmacological effects produced by intracerebral injection of drugs in the conscious mouse. *British journal of pharmacology and chemotherapy*.

Hall, J.O. (2018). Selenium. *Veterinary Toxicology: Basic and Clinical Principles: Third Edition*. p.pp. 469–477.

Halliwell, B. (2018). Making sense of neurodegeneration: A unifying hypothesis. In: *Learning To Live Together: Promoting Social Harmony*.

Halliwell, B. (2001). Role of Free Radicals in the Neurodegenerative Diseases. *Drugs & Aging*.

Hampel, H., Mesulam, M.M., Cuello, A.C., Farlow, M.R., Giacobini, E., Grossberg, G.T., Khachaturian, A.S., Vergallo, A., Cavedo, E., Snyder, P.J. & Khachaturian, Z.S. (2018). The cholinergic system in the pathophysiology and treatment of Alzheimer's disease. *Brain*.

Hasselmo, M.E. (2006). The role of acetylcholine in learning and memory. *Current Opinion in Neurobiology*. 16 (6) p.pp. 710–715.

Heneka, M.T., Carson, M.J., Khoury, J. El, Landreth, G.E., Brosseron, F., Feinstein, D.L., Jacobs, A.H., Wyss-Coray, T., Vitorica, J., Ransohoff, R.M., Herrup, K., Frautschy, S.A., Finsen, B., Brown, G.C., Verkhratsky, A., Yamanaka, K., Koistinaho, J., Latz, E., Halle, A., Petzold, G.C., Town, T., Morgan, D., Shinohara, M.L., Perry, V.H., Holmes, C., Bazan, N.G., Brooks, D.J., Hunot, S., Joseph, B., Deigendesch, N., Garaschuk, O., Boddeke, E., Dinarello, C.A., Breitner, J.C., Cole, G.M., Golenbock, D.T. & Kummer, M.P. (2015a). Neuroinflammation in Alzheimer's disease. *The Lancet Neurology*. 14 (4) p.pp. 388–405.

Heneka, M.T., Carson, M.J., Khoury, J. El, Landreth, G.E., Brosseron, F., Feinstein, D.L., Jacobs, A.H., Wyss-Coray, T., Vitorica, J., Ransohoff, R.M., Herrup, K., Frautschy, S.A., Finsen, B., Brown, G.C., Verkhratsky, A., Yamanaka, K., Koistinaho, J., Latz, E., Halle, A., Petzold, G.C., Town, T., Morgan, D., Shinohara, M.L., Perry, V.H., Holmes, C., Bazan, N.G., Brooks, D.J., Hunot, S., Joseph, B., Deigendesch, N., Garaschuk, O., Boddeke, E., Dinarello, C.A., Breitner, J.C., Cole, G.M., Golenbock, D.T. & Kummer, M.P. (2015b). Neuroinflammation in Alzheimer's disease. *The Lancet Neurology*. 14 (4). p.pp. 388–405.

Hospital, T.J. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology*. p.pp. 56–63.

Huawei, Z. (2009). Selenium as an essential micronutrient: Roles in cell cycle and apoptosis. *Molecules*.

Ishrat, T., Hoda, M.N., Khan, M.B., Yousuf, S., Ahmad, M., Khan, M.M., Ahmad, A. & Islam, F. (2009). Amelioration of cognitive deficits and neurodegeneration by

curcumin in rat model of sporadic dementia of Alzheimer's type (SDAT). *European Neuropsychopharmacology*.

Islam, F., Javed, H., Vaibhav, K., Ahmed, M.E., Khan, A., Tabassum, R., Islam, F. & Safhi, M.M. (2015). Effect of hesperidin on neurobehavioral, neuroinflammation, oxidative stress and lipid alteration in intracerebroventricular streptozotocin induced cognitive impairment in mice. *Journal of the Neurological Sciences*.

Ismail, Z., Elbayoumi, H., Fischer, C.E., Hogan, D.B., Millikin, C.P., Schweizer, T., Mortby, M.E., Smith, E.E., Patten, S.B. & Fiest, K.M. (2017). Prevalence of depression in patients with mild cognitive impairment: A systematic review and meta-analysis. *JAMA Psychiatry*.

Jackson, L.L. (1943). VTE on an elevated T-maze. *Journal of Comparative Psychology*.

Janowsky, D.S. & Risch, S.C. (1984). Cholinomimetic and Anticholinomimetic Drugs Used To Investigate an Acetylcholine Hypothesis of Affective Disorders and Stress. *Drug Development Research*.

Kamal, A., Iqbal, M.A. & Bhatti, H.N. (2018). Therapeutic applications of selenium-derived compounds. *Reviews in Inorganic Chemistry*.

Karch, C.M. & Goate, A.M. (2015). Alzheimer's disease risk genes and mechanisms of disease pathogenesis. *Biological Psychiatry*.

Karlawish, J., Jack, C.R., Rocca, W.A., Snyder, H.M. & Carrillo, M.C. (2017). 2017 Alzheimer's disease facts and figures. *Alzheimer's and Dementia*.

Kelany, M.E., Hakami, T.M., Omar, A.H. & Abdallah, M.A. (2016). Combination of Sitagliptin and Insulin against Type 2 Diabetes Mellitus with Neuropathy in Rats: Neuroprotection and Role of Oxidative and Inflammation Stress. *Pharmacology*. 98 (5–6). p.pp. 242–250.

Kogan, J.H., Frankland, P.W. & Silva, A.J. (2000). Long-term memory underlying hippocampus-dependent social recognition in mice. *Hippocampus*.

Kojro, E. & Fahrenholz, F. (2005). The non-amyloidogenic pathway: structure and function of alpha-secretases. *Sub-cellular biochemistry*.

Labunskyy, V.M., Hatfield, D.L. & Gladyshev, V.N. (2014). Selenoproteins: Molecular Pathways and Physiological Roles. *Physiological Reviews*.

LaFerla, F.M., Green, K.N. & Oddo, S. (2007). Intracellular amyloid-beta in Alzheimer's disease. *Nature Reviews Neuroscience*.

Laursen, S.E. & Belknap, J.K. (1986). Intracerebroventricular injections in mice. Some methodological refinements. *Journal of Pharmacological Methods*.

Levander, O.A. (1982). CLINICAL CONSEQUENCES OF LOW SELENIUM INTAKE AND ITS RELATIONSHIP TO VITAMIN E. *Annals of the New York Academy of Sciences*.

Li, D., Huang, Y., Cheng, B., Su, J., Zhou, W.X. & Zhang, Y.X. (2016). Streptozotocin induces mild cognitive impairment at appropriate doses in mice as determined by long-term potentiation and the morris water maze. *Journal of Alzheimer's Disease*.

Liang, W.S., Dunckley, T., Beach, T.G., Grover, A., Mastroeni, D., Ramsey, K., Caselli, R.J., Kukull, W.A., McKeel, D., Morris, J.C., Hulette, C.M., Schmechel, D., Reiman, E.M., Rogers, J. & Stephan, D.A. (2008). Altered neuronal gene expression in brain regions differentially affected by Alzheimer's disease: a reference data set. *Physiological Genomics*.

Liu, Z., Zhou, T., Ziegler, A.C., Dimitrion, P. & Zuo, L. (2017). Oxidative Stress in Neurodegenerative Diseases: From Molecular Mechanisms to Clinical Applications. *Oxidative Medicine and Cellular Longevity*.

Loetchutinat, C., Kothan, S., Dechsupa, S., Meesungnoen, J., Jay-Gerin, J.P. & Mankhetkorn, S. (2005). Spectrofluorometric determination of intracellular levels of reactive oxygen species in drug-sensitive and drug-resistant cancer cells using the 2???,7???-dichlorofluorescein diacetate assay. *Radiation Physics and Chemistry*. 72 (2–3). p.pp. 323–331.

Mackay, E.M. & Mackay, L.L. (1927). The concentration of urea in the blood of normal individuals. *J Clin Invest*. [Online]. 4 (2). p.pp. 295–306. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=434672&tool=pmcentrez&rendertype=abstract.

Mandelkow, E.M. & Mandelkow, E. (2011). Biochemistry and cell biology of Tau protein

in neurofibrillary degeneration. *Cold Spring Harbor Perspectives in Biology*. 3 (10) p.pp. 1–25.

Manosso, L.M., Moretti, M., Ribeiro, C.M., Gonçalves, F.M., Leal, R.B. & Rodrigues, A.L.S. (2015). Antidepressant-like effect of zinc is dependent on signaling pathways implicated in BDNF modulation. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*.

Martini, F., Rosa, S.G., Klann, I.P., Fulco, B.C.W., Carvalho, F.B., Rahmeier, F.L., Fernandes, M.C. & Nogueira, C.W. (2019). A multifunctional compound ebselen reverses memory impairment, apoptosis and oxidative stress in a mouse model of sporadic Alzheimer's disease. *Journal of Psychiatric Research*. [Online]. 109. p.pp. 107–117. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0022395618308951.

Matsumoto, T., Furuta, T., Nimura, Y. & Suzuki, O. (1984). 3-(p-Hydroxyphenyl)propionic acid as a new fluorogenic reagent for amine oxidase assays. *Analytical Biochemistry*.

Mecocci, P., Boccardi, V., Cecchetti, R., Bastiani, P., Scamosci, M., Ruggiero, C. & Baroni, M. (2018). A Long Journey into Aging, Brain Aging, and Alzheimer's Disease Following the Oxidative Stress Tracks. *Journal of Alzheimer's Disease*.

Medeiros, D.M. (2016). Copper, iron, and selenium dietary deficiencies negatively impact skeletal integrity: A review. *Experimental Biology and Medicine*.

Mehan, S., Arora, R., Sehgal, V., Sharma, D. & Sharm, G. (2012). Dementia – A Complete Literature Review on Various Mechanisms Involves in Pathogenesis and an Intracerebroventricular Streptozotocin Induced Alzheimer's Disease. *Inflammatory Diseases - Immunopathology, Clinical and Pharmacological Bases*. [Online]. (February). Available from: http://www.intechopen.com/books/inflammatory-diseases-immunopathology-clinical-and-pharmacological-bases/alzheimer-s-disease-an-updated-review-on-pathogenesis-and-intracerebroventricular-streptozotocin-ind.

Meotti, F.C., Stangherlin, E.C., Zeni, G., Nogueira, C.W. & Rocha, J.B.T. (2004). Protective role of aryl and alkyl diselenides on lipid peroxidation. *Environmental Research*.

Minichiello, L. (2009). TrkB signalling pathways in LTP and learning. *Nature Reviews Neuroscience*.

Moreira, P.I., Siedlak, S.L., Aliev, G., Zhu, X., Cash, A.D., Smith, M.A. & Perry, G. (2005). Oxidative stress mechanisms and potential therapeutics in Alzheimer disease. *Journal of Neural Transmission*.

Morgese, M.G., Schiavone, S. & Trabace, L. (2017). Emerging role of amyloid beta in stress response: Implication for depression and diabetes. *European Journal of Pharmacology*.

Newman, M.B., Nazian, S.J., Sanberg, P.R., Diamond, D.M. & Shytle, R.D. (2001). Corticosterone-attenuating and anxiolytic properties of mecamylamine in the rat. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*.

Nimse, S.B. & Pal, D. (2015). Free radicals, natural antioxidants, and their reaction mechanisms. *RSC Adv.* [Online]. 5 (35). p.pp. 27986–28006. Available from: http://xlink.rsc.org/?DOI=C4RA13315C.

O'Brien, R.J. & Wong, P.C. (2011). Amyloid Precursor Protein Processing and Alzheimer's Disease. *Annual Review of Neuroscience*. [Online]. 34 (1). p.pp. 185–204. Available from: http://www.annualreviews.org/doi/10.1146/annurev-neuro-061010-113613.

Oddo, S., Caccamo, A., Shepherd, J.D., Murphy, M.P., Golde, T.E., Kayed, R., Metherate, R., Mattson, M.P., Akbari, Y. & LaFerla, F.M. (2003). Triple-transgenic model of Alzheimer's Disease with plaques and tangles: Intracellular Aβ and synaptic dysfunction. *Neuron*.

Ohkawa, H., Ohishi, N. & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*.

Pacheco, S.M., Soares, M.S.P., Gutierres, J.M., Gerzson, M.F.B., Carvalho, F.B., Azambuja, J.H., Schetinger, M.R.C., Stefanello, F.M. & Spanevello, R.M. (2018). Anthocyanins as a potential pharmacological agent to manage memory deficit, oxidative stress and alterations in ion pump activity induced by experimental sporadic dementia of Alzheimer's type. *Journal of Nutritional Biochemistry*.

Paloczi, J., Varga, Z. V., Hasko, G. & Pacher, P. (2017). Neuroprotection in Oxidative

Stress-Related Neurodegenerative Diseases: Role of Endocannabinoid System Modulation. *Antioxidants & Redox Signaling*.

Park, S.J., Kim, Y.H., Nam, G.H., Choe, S.H., Lee, S.R., Kim, S.U., Kim, J.S., Sim, B.W., Song, B.S., Jeong, K.J., Lee, Y., Park, Y. Il, Lee, K.M., Huh, J.W. & Chang, K.T. (2015). Quantitative expression analysis of APP pathway and tau phosphorylation-related genes in the ICV STZ-induced non-human primate model of sporadic alzheimer's disease. *International Journal of Molecular Sciences*.

Peake, M. & Whiting, M. (2006). Measurement of Serum Creatinine – Current Status and Future Goals. *Clinical Biochemist Reviews*.

Pinto Brod, L.M., Fronza, M.G., Vargas, J.P., L??dtke, D.S., Luchese, C., Wilhelm, E.A. & Savegnago, L. (2016a). Involvement of monoaminergic system in the antidepressant-like effect of (octylseleno)-xylofuranoside in the mouse tail suspension test. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*. [Online]. 65. p.pp. 201–207. Available from: http://dx.doi.org/10.1016/j.pnpbp.2015.10.008.

Pinto Brod, L.M., Fronza, M.G., Vargas, J.P., Lüdtke, D.S., Brüning, C.A. & Savegnago, L. (2017a). Modulation of PKA, PKC, CAMKII, ERK 1/2 pathways is involved in the acute antidepressant-like effect of (octylseleno)-xylofuranoside (OSX) in mice. *Psychopharmacology*.

Pinto Brod, L.M., Fronza, M.G., Vargas, J.P., Lüdtke, D.S., Brüning, C.A. & Savegnago, L. (2017b). Modulation of PKA, PKC, CAMKII, ERK 1/2 pathways is involved in the acute antidepressant-like effect of (octylseleno)-xylofuranoside (OSX) in mice. *Psychopharmacology*. [Online]. 234 (4). p.pp. 717–725. Available from: http://link.springer.com/10.1007/s00213-016-4505-5.

Pinto Brod, L.M., Fronza, M.G., Vargas, J.P., Lüdtke, D.S., Luchese, C., Wilhelm, E.A. & Savegnago, L. (2016b). Involvement of monoaminergic system in the antidepressant-like effect of (octylseleno)-xylofuranoside in the mouse tail suspension test. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*.

Prince, M., Wimo, A., Guerchet, M., Gemma-Claire, A., Wu, Y.-T. & Prina, M. (2015). *World Alzheimer Report 2015*.

Van Raamsdonk, J.M., Vega, I.E. & Brundin, P. (2017). Oxidative stress in neurodegenerative disease: causation or association? *Oncotarget*.

Rajasekar, N., Nath, C., Hanif, K. & Shukla, R. (2017). Intranasal Insulin Administration Ameliorates Streptozotocin (ICV)-Induced Insulin Receptor Dysfunction, Neuroinflammation, Amyloidogenesis, and Memory Impairment in Rats. *Molecular Neurobiology*. 54 (8). p.pp. 6507–6522.

Rapp, M.A., Schnaider-Beeri, M., Grossman, H.T., Sano, M., Perl, D.P., Purohit, D.P., Gorman, J.M. & Haroutunian, V. (2006). Increased hippocampal plaques and tangles in patients with Alzheimer disease with a lifetime history of major depression. *Archives of General Psychiatry*.

Rapp, M.A., Schnaider-Beeri, M., Purohit, D.P., Perl, D.P., Haroutunian, V. & Sano, M. (2008). Increased neurofibrillary tangles in patients with alzheimer disease with comorbid depression. *American Journal of Geriatric Psychiatry*.

Ravelli, K.G., Rosário, B. dos A., Camarini, R., Hernandes, M.S. & Britto, L.R. (2017). Intracerebroventricular Streptozotocin as a Model of Alzheimer's Disease: Neurochemical and Behavioral Characterization in Mice. *Neurotoxicity Research*.

Reeta, K.H., Singh, D. & Gupta, Y.K. (2017). Chronic treatment with taurine after intracerebroventricular streptozotocin injection improves cognitive dysfunction in rats by modulating oxidative stress, cholinergic functions and neuroinflammation. *Neurochemistry International*.

Rehman, S.U., Shah, S.A., Ali, T., Chung, J. Il & Kim, M.O. (2017). Anthocyanins Reversed D-Galactose-Induced Oxidative Stress and Neuroinflammation Mediated Cognitive Impairment in Adult Rats. *Molecular Neurobiology*. 54 (1). p.pp. 255–271.

Rocha, N.P. & Amorim, C. (2011). *Processo Inflamatório e Neuroimunomodulação na Doença de Alzheimer : Revisão de Literatura*. 19 (2). p.pp. 300–313.

Rodrigues, J., Saba, S., Joussef, A.C., Rafique, J. & Braga, A.L. (2018). KIO3-Catalyzed C(sp2)-H Bond Selenylation/Sulfenylation of (Hetero)arenes: Synthesis of Chalcogenated (Hetero)arenes and their Evaluation for Anti-Alzheimer Activity. *Asian Journal of Organic Chemistry*.

Rodrigues, R., Petersen, R.B. & Perry, G. (2014). Parallels Between Major Depressive Disorder and Alzheimer's Disease: Role of Oxidative Stress and Genetic Vulnerability. *Cellular and Molecular Neurobiology*.

Roesler, R., Walz, R., Quevedo, J., De-Paris, F., Zanata, S.M., Graner, E., Izquierdo, I., Martins, V.R. & Brentani, R.R. (1999). Normal inhibitory avoidance learning and anxiety, but increased locomotor activity in mice devoid of PrP(C). *Molecular Brain Research*.

Rosa, R.M., Flores, D.G., Appelt, H.R., Braga, A.L., Henriques, J.A.P. & Roesler, R. (2003). Facilitation of long-term object recognition memory by pretraining administration of diphenyl diselenide in mice. *Neuroscience Letters*. 341 (3). p.pp. 217–220.

Rosenberg, P.B., Nowrangi, M.A. & Lyketsos, C.G. (2015). Neuropsychiatric symptoms in Alzheimer's disease: What might be associated brain circuits? *Molecular Aspects of Medicine*.

Różyk-Myrta, A. (2014). In Search of Memory Tests Equivalent for Experiments on Animals and Humans. *Medical Science Monitor*.

Salkovic-Petrisic, M. (2008). Amyloid cascade hypothesis: Is it true for sporadic Alzheimer's disease. *Periodicum Biologorum*.

Santos, D.B., Colle, D., Moreira, E.L.G., Peres, K.C., Ribeiro, R.P., dos Santos, A.A., de Oliveira, J., Hort, M.A., de Bem, A.F. & Farina, M. (2015). Probucol mitigates streptozotocin-induced cognitive and biochemical changes in mice. *Neuroscience*.

dos Santos Picanco, L.C., Ozela, P.F., de Fatima de Brito Brito, M., Pinheiro, A.A., Padilha, E.C., Braga, F.S., de Paula da Silva, C.H.T., dos Santos, C.B.R., Rosa, J.M.C. & da Silva Hage-Melim, L.I. (2018). Alzheimer's Disease: A Review from the Pathophysiology to Diagnosis, New Perspectives for Pharmacological Treatment. *Current Medicinal Chemistry*.

Savegnago, L., Jesse, C.R., Pinto, L.G., Rocha, J.B.T., Barancelli, D.A., Nogueira, C.W. & Zeni, G. (2008). Diphenyl diselenide exerts antidepressant-like and anxiolytic-like effects in mice: Involvement of l-arginine-nitric oxide-soluble guanylate cyclase pathway in its antidepressant-like action. *Pharmacology Biochemistry and Behavior*.

Sbodio, J.I., Snyder, S.H. & Paul, B.D. (2018). Redox Mechanisms in Neurodegeneration: From Disease Outcomes to Therapeutic Opportunities. *Antioxidants & Redox Signaling*. [Online]. p.p. ars.2017.7321. Available from: http://www.liebertpub.com/doi/10.1089/ars.2017.7321.

Schedin-Weiss, S., Inoue, M., Hromadkova, L., Teranishi, Y., Yamamoto, N.G., Wiehager, B., Bogdanovic, N., Winblad, B., Sandebring-Matton, A., Frykman, S. & Tjernberg, L.O. (2017). Monoamine oxidase B is elevated in Alzheimer disease neurons, is associated with γ-secretase and regulates neuronal amyloid β-peptide levels. *Alzheimer's Research and Therapy*.

Solanki, I., Parihar, P. & Parihar, M.S. (2016). Neurodegenerative diseases: From available treatments to prospective herbal therapy. *Neurochemistry International*.

Sousa, F.S.S., Birmann, P.T., Baldinotti, R., Fronza, M.G., Balaguez, R., Alves, D., Brüning, C.A. & Savegnago, L. (2018). α- (phenylselanyl) acetophenone mitigates reserpine-induced pain–depression dyad: Behavioral, biochemical and molecular docking evidences. *Brain Research Bulletin*.

Spires, T.L. & Hyman, B.T. (2005). Transgenic models of Alzheimer's disease: Learning from animals. *NeuroRx*.

Tanimizu, T., Kenney, J.W., Okano, E., Kadoma, K., Frankland, P.W. & Kida, S. (2017). Functional Connectivity of Multiple Brain Regions Required for the Consolidation of Social Recognition Memory. *The Journal of Neuroscience*.

Thinakaran, G. & Koo, E.H. (2008). Amyloid precursor protein trafficking, processing, and function. *The Journal of biological chemistry*. [Online]. 283 (44). p.pp. 29615–9. Available from: http://www.jbc.org/cgi/doi/10.1074/jbc.R800019200%0Ahttp://www.ncbi.nlm.nih.gov/pubmed/18650430%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC2573065.

Tramutola, A., Lanzillotta, C., Perluigi, M. & Butterfield, D.A. (2016a). Oxidative stress, protein modification and Alzheimer disease. *Brain Research Bulletin*.

Tramutola, A., Lanzillotta, C., Perluigi, M. & Butterfield, D.A. (2016b). Oxidative stress, protein modification and Alzheimer disease. *Brain Research Bulletin*. [Online]. Available from: http://dx.doi.org/10.1016/j.brainresbull.2016.06.005.

Tundis, R., Loizzo, M.R., Nabavi, S.M., Orhan, I.E., Skalicka-Wozniak, K., D'Onofrio, G. & Aiello, F. (2017). Natural Compounds and Their Derivatives as Multifunctional Agents for the Treatment of Alzheimer Disease. In: *Discovery and Development of Neuroprotective Agents from Natural Products: Natural Product Drug Discovery*.

Valavanidis, A., Vlahogianni, T., Dassenakis, M. & Scoullos, M. (2006). Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. *Ecotoxicology and Environmental Safety*. 64. p.pp. 178–189.

Vandersteen, A., Masman, M.F., De Baets, G., Jonckheere, W., Van Der Werf, K., Marrink, S.J., Rozenski, J., Benilova, I., De Strooper, B., Subramaniam, V., Schymkowitz, J., Rousseau, F. & Broersen, K. (2012). Molecular plasticity regulates oligomerization and cytotoxicity of the multipeptide-length amyloid-β peptide pool. *Journal of Biological Chemistry*. 287 (44). p.pp. 36732–36743.

Vargas, J.P., Pinto, L.M., Savegnago, L. & Lüdtke, D.S. (2015). Synthesis of alkylseleno-carbohydrates and evaluation of their antioxidant properties. *Journal of the Brazilian Chemical Society*.

Walsh, R.N. & Cummins, R. a (1976). The Open-Field Test: a critical review. *Psychological bulletin*. 83 (3). p.pp. 482–504.

Wang, Z.M., Cai, P., Liu, Q.H., Xu, D.Q., Yang, X.L., Wu, J.J., Kong, L.Y. & Wang, X.B. (2016). Rational modification of donepezil as multifunctional acetylcholinesterase inhibitors for the treatment of Alzheimer's disease. *European Journal of Medicinal Chemistry*. [Online]. 123. p.pp. 282–297. Available from: http://dx.doi.org/10.1016/j.ejmech.2016.07.052.

Weller, J. & Budson, A. (2018). Current understanding of Alzheimer's disease diagnosis and treatment. *F1000Research*.

Whitehouse, P.J., Price, D.L., Clark, A.W., Coyle, J.T. & DeLong, M.R. (1981). Alzheimer disease: Evidence for selective loss of cholinergic neurons in the nucleus basalis. *Annals of Neurology*.

Whitehouse, P.J., Price, D.L., Struble, R.G., Clark, A.W., Coyle, J.T. & DeLong, M.R. (1982). Alzheimer's disease and senile dementia: Loss of neurons in the basal

forebrain. *Science*.

Wilcock, G.K., Esiri, M.M., Bowen, D.M. & Smith, C.C.T. (1982). Alzheimer's disease. Correlation of cortical choline acetyltransferase activity with the severity of dementia and histological abnormalities. *Journal of the Neurological Sciences*.

Wrobel, J.K., Power, R. & Toborek, M. (2016). Biological activity of selenium: Revisited. *IUBMB Life*.

Wu, Y., Ma, Y., Liu, Z., Geng, Q., Chen, Z. & Zhang, Y. (2017). Alterations of myelin morphology and oligodendrocyte development in early stage of Alzheimer's disease mouse model. *Neuroscience Letters*.

Xie, Y., Liu, Q., Zheng, L., Wang, B.T., Qu, X., Ni, J., Zhang, Y. & Du, X. (2018). Se-Methylselenocysteine Ameliorates Neuropathology and Cognitive Deficits by Attenuating Oxidative Stress and Metal Dyshomeostasis in Alzheimer Model Mice. *Molecular Nutrition and Food Research*.

Xie, Y., Tan, Y., Zheng, Y., Du, X. & Liu, Q. (2017). Ebselen ameliorates β-amyloid pathology, tau pathology, and cognitive impairment in triple-transgenic Alzheimer's disease mice. *Journal of Biological Inorganic Chemistry*.

Ye, X., Tai, W. & Zhang, D. (2012). The early events of Alzheimer's disease pathology: From mitochondrial dysfunction to BDNF axonal transport deficits. *Neurobiology of Aging*. 33 (6).

Yiannopoulou, K.G. & Papageorgiou, S.G. (2013). Current and future treatments for Alzheimer's disease. *Therapeutic Advances in Neurological Disorders*.

Zhang, F., Kang, Z., Li, W., Xiao, Z. & Zhou, X. (2012). Roles of brain-derived neurotrophic factor/tropomyosin-related kinase B (BDNF/TrkB) signalling in Alzheimer's disease. *Journal of Clinical Neuroscience*.

Zhang, X., He, X., Chen, Q., Lu, J., Rapposelli, S. & Pi, R. (2018). A review on the hybrids of hydroxycinnamic acid as multi-target-directed ligands against Alzheimer's disease. *Bioorganic and Medicinal Chemistry*.

Tables

Table 1 – Primer's sequences.

Gene	Sequence 5'-3'
GAPDH	S-AGGTCGGTGTGAACGGATTTG A-TGTAGACCATGTAGTTGAGGTCA
APP	S-CGGGACCCAAATGTCAAACT A-TCCGAGCATGTGGAGGGATA
BACE-1	S-CAGTGGAAGGTCCGTTTGTT A-GCAGAGTGGCAACATGAAGA
γ-SECRETASE	TATGGCCTCCTGATTTTTGG GATGCTAAGCCCTCATCTGC

Figures

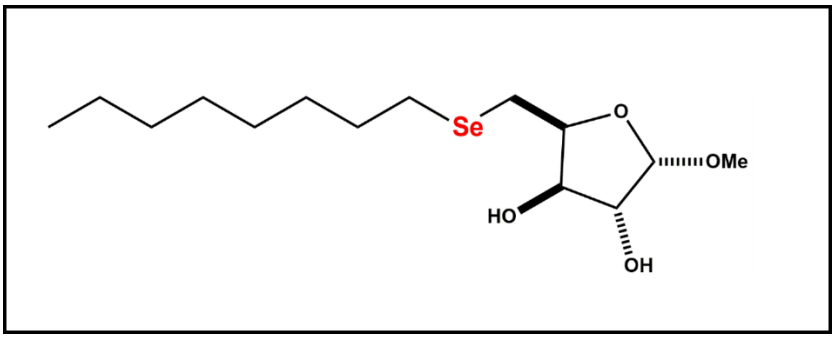

Figure 1. Chemical structure of octylseleno-xylofuranoside (OSX).

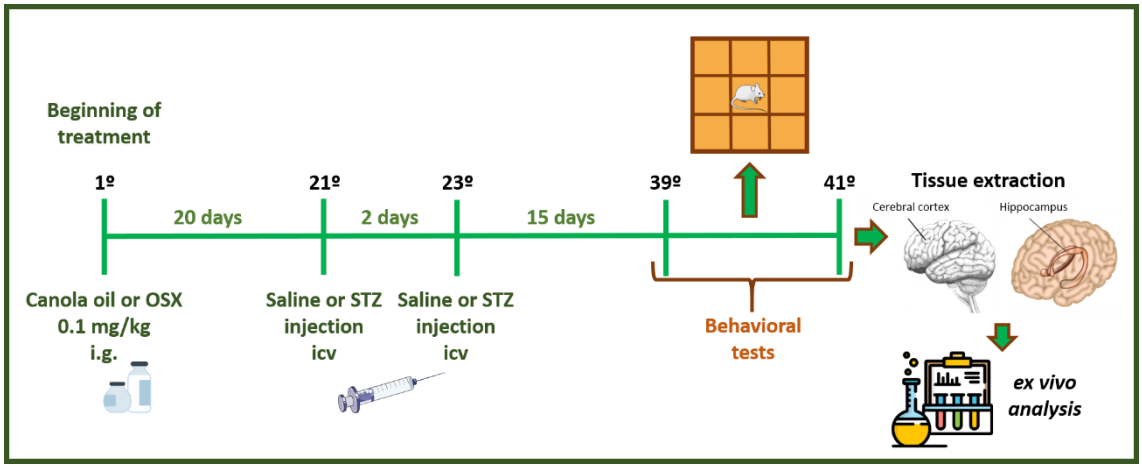

Figure 2. Experimental design of the protection evaluation of OSX in STZ injected mice.

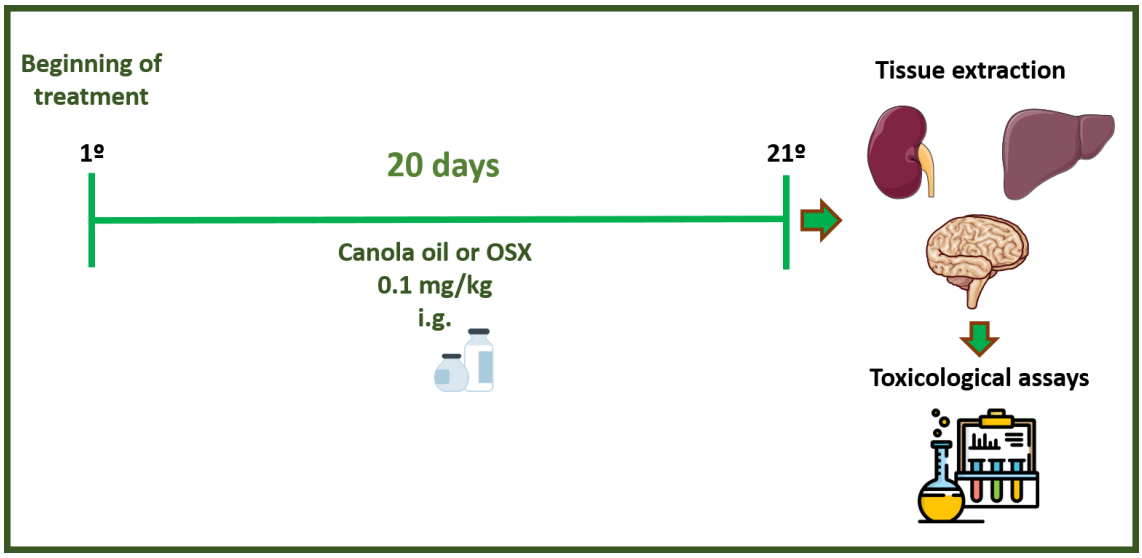

Figure 3. Experimental design of the toxicological evaluation of OSX.

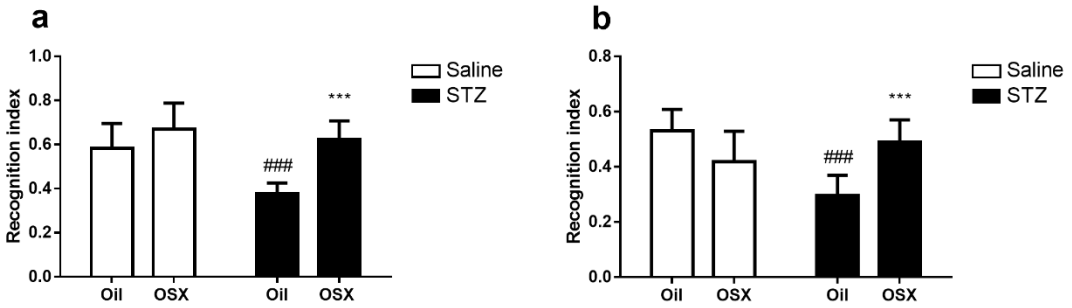

Figure 4. Evaluation of OSX on cognition and memory formation in mice injected with STZ on ORT. Values were expressed in the form of recognition index, both in short-term (a) and long-term (b) memory. Data are presented as mean ± SD (n=10). ***p<0.001 when compared to STZ Oil group. ###p<0.001 when compared to control group.

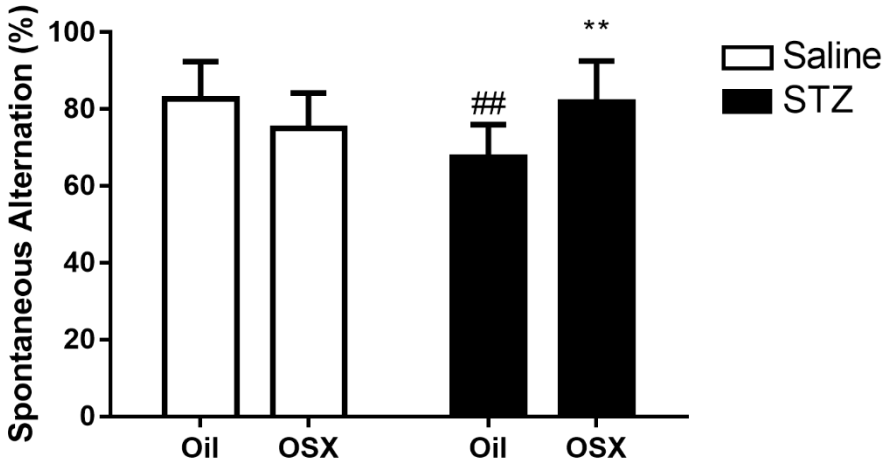

Figure 5. Evaluation of OSX on spontaneous alternation in mice injected with STZ on Y maze test. Values were expressed in percentage (%) of spontaneous alternation. Data are presented as mean ± SD (n=10). **p<0.01 when compared to STZ Oil group. ##p<0.01 when compared to control group.

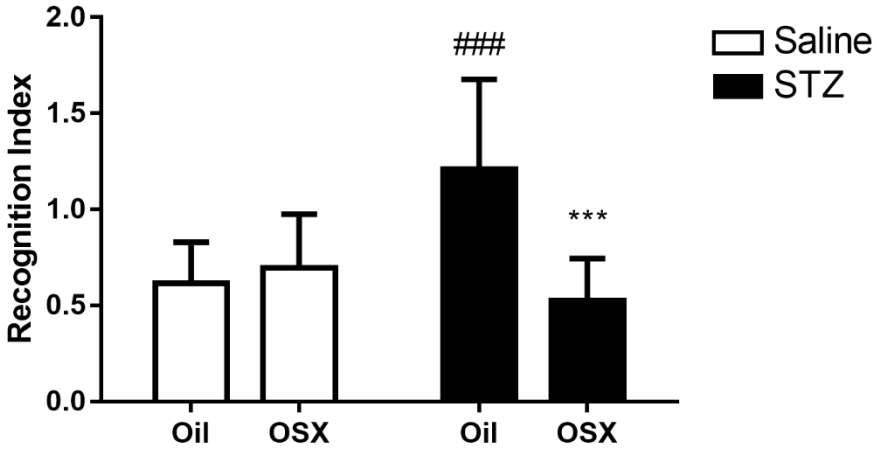

Figure 6. Evaluation of OSX on cognition and memory formation in mice injected with STZ on social recognition task. Values were expressed in the form of recognition index. Data are presented as mean ± SD (n=10). ***p<0.001 when compared to STZ Oil group. ###p<0.001 when compared to control group.

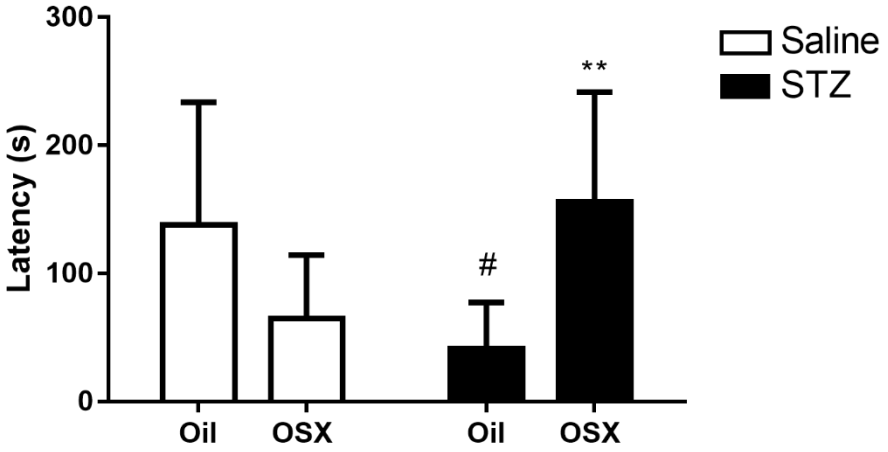

Figure 7. Evaluation of OSX on cognition and memory formation in mice injected with STZ on passive avoidance test. Values were expressed in seconds (latency time). Data are presented as mean ± SD (n=10). **p<0.01 when compared to STZ Oil group. #p<0.05 when compared to control group.

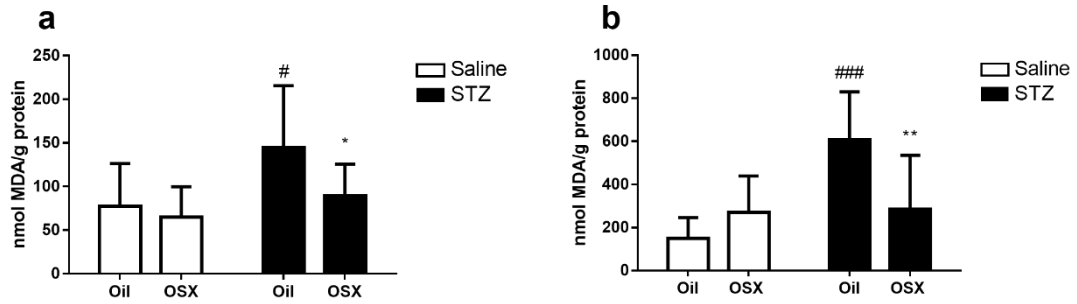

Figure 8. Evaluation of OSX on lipid peroxidation levels in mice injected with STZ, in total cortex (a) and hippocampus (b). Values were expressed in nM of MDA. Data are presented as mean ± SD (n=9-10). *p<0.05; **p<0,01 when compared to STZ Oil group. #p<0.05; ###p<0.001 when compared to control group.

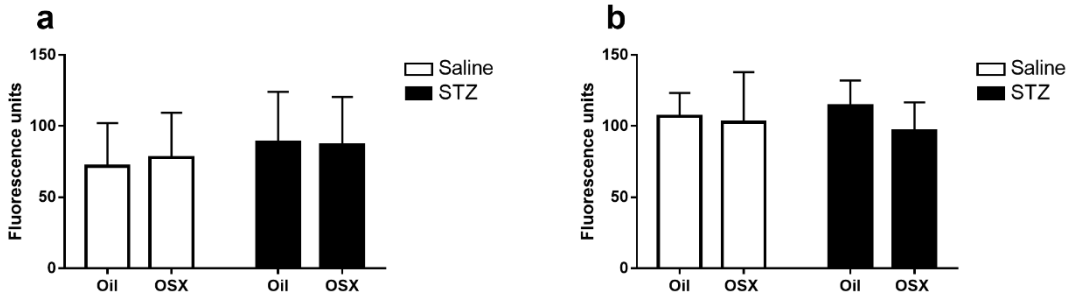

Figure 9. Evaluation of OSX on RS formation in mice injected with STZ, in total cortex (a) and hippocampus (b). Values were expressed in fluorescence units. Data are presented as mean ± SD (n=9-10).

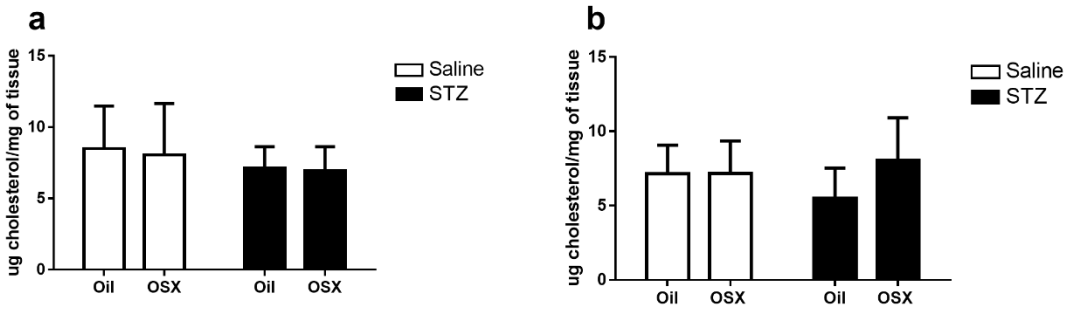

Figure 10. Evaluation of OSX on cholesterol levels in mice injected with STZ, in total cortex (a) and hippocampus (b). Values were expressed cholesterol/mg of tissue. Data are presented as mean ± SD (n=9-10).

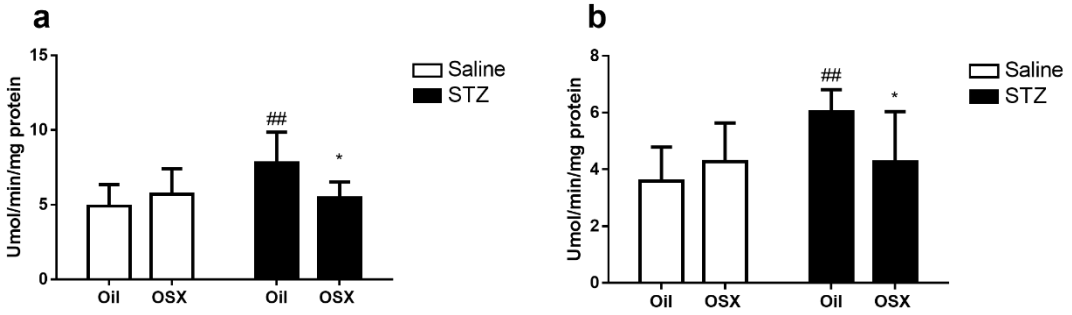

Figure 11. Evaluation of OSX inhibitory activity on AChE in mice injected with STZ, in total cortex (a) and hippocampus (b). Values were expressed in the form AChE activity. Data are presented as mean ± SD (n=9-10). *p<0.05 when compared to STZ Oil group. ##p<0.01 when compared to control group.

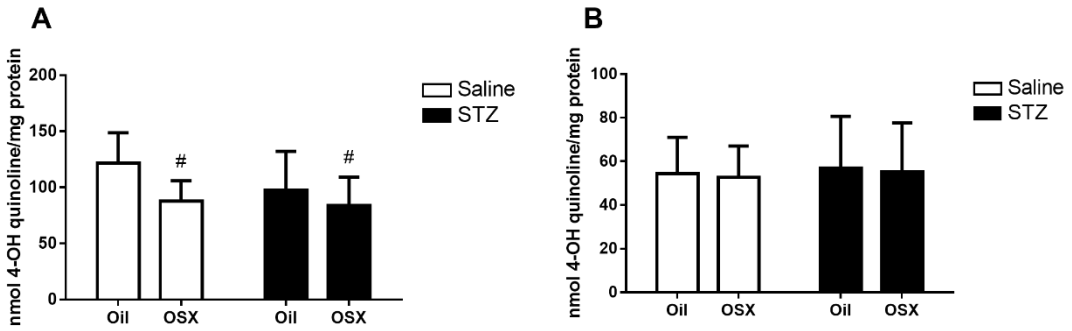

Figure 12. Evaluation of OSX inhibitory activity on MAO B in mice injected with STZ, in total cortex (a) and hippocampus (b). Values were expressed in the form MAO B activity. Data are presented as mean ± SD (n=9-10). #p<0.05 when compared to STZ Oil group.

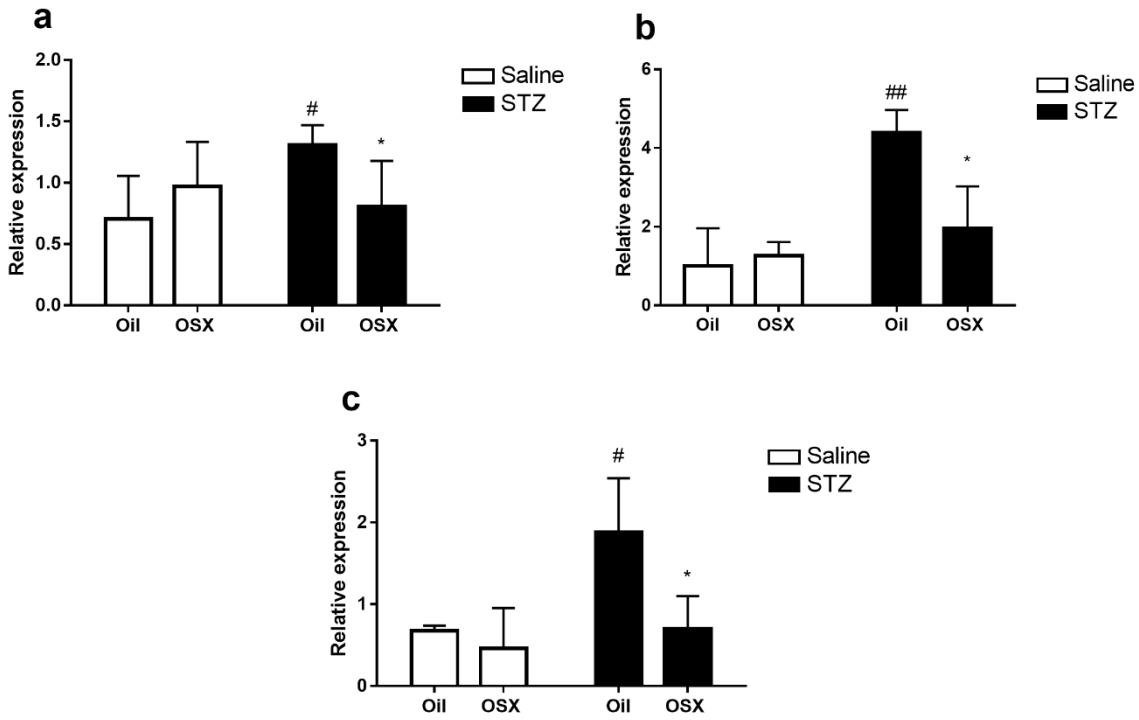

Figure 13. Levels of mRNA relative expression of APP, β-secretase and γ-secretase in total cortex, under OSX treatment in mice injected with STZ. Values were expressed in the form of relative expression. Data are presented as mean ± SD (n=5). $^{*}p<0.05$ when compared to STZ Oil group. $^{\#}p<0.05$; $^{\#\#}p<0.01$ when compared to control group.

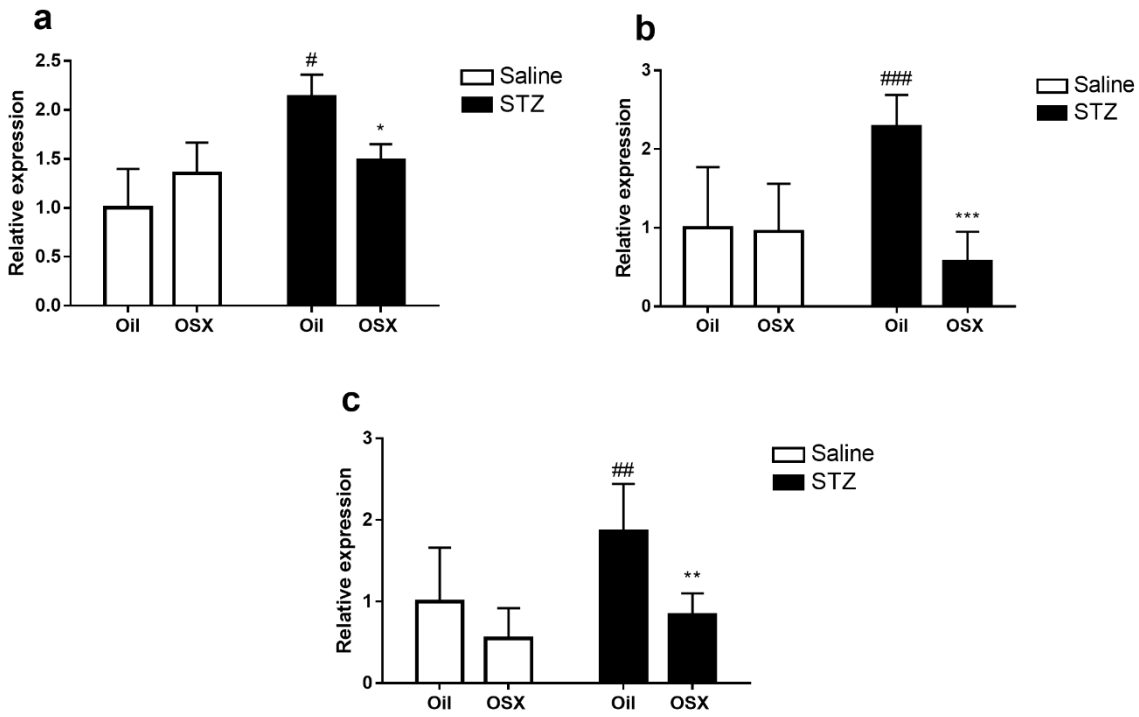

Figure 14. Levels of mRNA relative expression of APP, β-secretase and γ-secretase in hippocampus, under OSX treatment in mice injected with STZ. Values were expressed in the form of relative

expression. Data are presented as mean ± SD (n=5). $^{*}p<0.05$; $^{**}p<0.01$; $^{***}p<0.001$ when compared to STZ Oil group. $^{\#}p<0.05$; $^{\#\#}p<0.01$; $^{\#\#\#}p<0.001$ when compared to control group.

5 CONCLUSÃO GERAL

Baseado no que foi exposto, podemos concluir que o OSX demonstrou um efeito anti-Alzheimer em diferentes parâmetros avaliados, no modelo de demência induzido por STZ em camundongos. Dentre esses efeitos, pode-se citar a redução da peroxidação lipídica, referente ao EO e o dano causado nas membranas e lipídeos celulares. Além disso, o OSX demonstrou capacidade inibitória sobre as enzimas AChE e MAO B, possivelmente aumentando a disponibilidade da ACh e das monoaminas. Ainda, o OSX foi capaz de modular a expressão gênica relativa dos genes chave da via amiloidogênica, protegendo frente ao aumento provocado pela STZ. Tais ações, podem explicar a proteção frente ao dano cognitivo nos testes comportamentais apresentada pelo OSX, frente a STZ. Entretanto, embora tenha apresentado ação em diferentes parâmetros da DA, mais estudos são necessários para aprofundar o conhecimento sobre a ação biológica do OSX, nos parâmetros aqui avaliados, além de outros marcadores da doença.

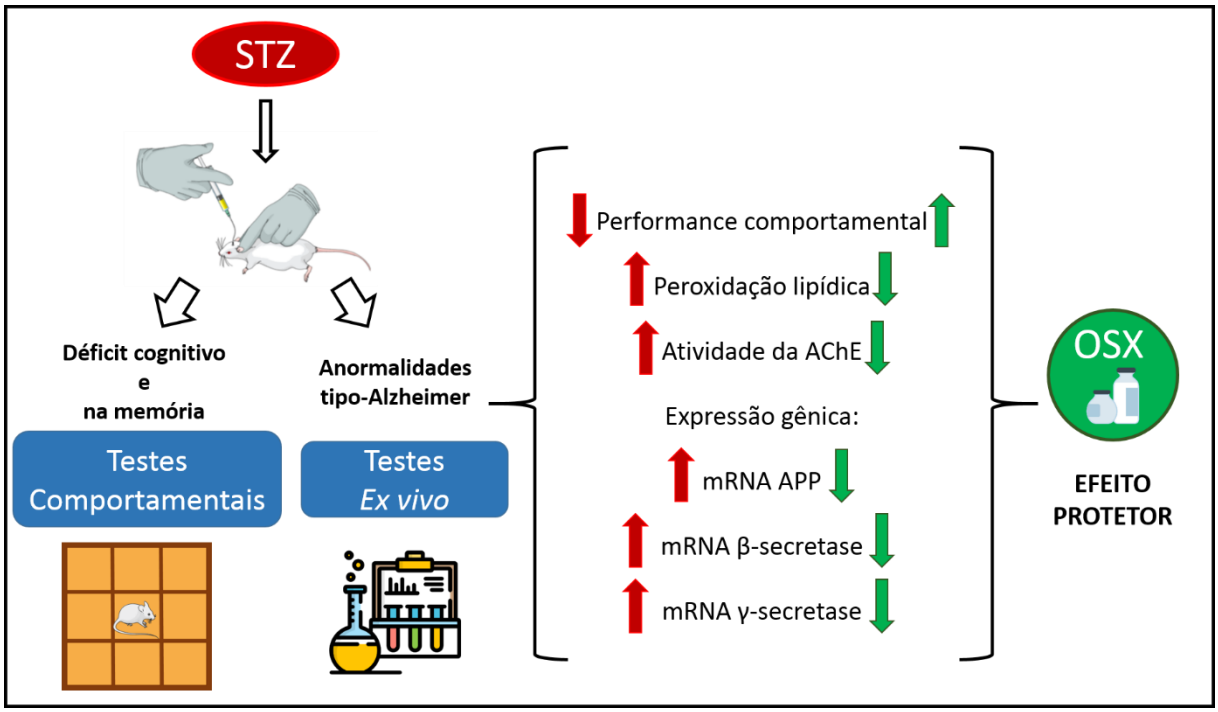

Figura 6. Resumo da ação protetora do octilseleno-xilofuranosídeo (OSX), frente a indução por estreptozotocina (STZ) do modelo da doença de Alzheimer (DA) esporádica, em camundongos, quanto aos parâmetros comportamentais e marcadores fisiológicos e moleculares da DA.

7 REFERÊNCIAS

Allan Butterfield, D. & Boyd-Kimball, D. (2018). Oxidative Stress, Amyloid-β Peptide, and Altered Key Molecular Pathways in the Pathogenesis and Progression of Alzheimer's Disease. *Journal of Alzheimer's Disease*.

Alzheimer's Association (2018). *Alzheimer's Facts and Figures Report | Alzheimer's Association*. 2018. alz.org.

Alzheimer's Association (USA) (2018). 2018 Alzheimer's disease facts and figures includes a special report on the financial and personal benefits of early diagnosis. *Alzheimers Dement*.

Alzheimer's Disease International (2015). *The Global Impact of Dementia: An analysis of prevalence, incidenc, cost and treads World Alzhiemer Report 2015*.

Andrade, V., Cortes, N., Guzman-Martinez, L. & Maccioni, R. (2017). *An Overview of the Links between Behavioral Disorders and Alzheimer's Disease*. [Online]. 4. p.pp. 1–5. Available from: https://pdfs.semanticscholar.org/c416/2bd55e79de9416b553afacb1babe1e95b08d.pdf.

Bagyinszky, E., Youn, Y.C., An, S. & Kim, S. (2014). The genetics of Alzheimer's disease. *Clinical Interventions in Aging*. [Online]. p.p. 535. Available from: http://www.dovepress.com/the-genetics-of-alzheimer39s-disease-peer-reviewed-article-CIA.

Barage, S.H. & Sonawane, K.D. (2015). Amyloid cascade hypothesis: Pathogenesis and therapeutic strategies in Alzheimer's disease. *Neuropeptides*.

Bassani, T.B., Bonato, J.M., Machado, M.M.F., Cóppola-Segovia, V., Moura, E.L.R., Zanata, S.M., Oliveira, R.M.M.W. & Vital, M.A.B.F. (2018). Decrease in Adult Neurogenesis and Neuroinflammation Are Involved in Spatial Memory Impairment in the Streptozotocin-Induced Model of Sporadic Alzheimer's Disease in Rats. *Molecular Neurobiology*.

Bekris, L.M., Yu, C.E., Bird, T.D. & Tsuang, D.W. (2010). Review article: Genetics of Alzheimer disease. *Journal of Geriatric Psychiatry and Neurology*.

Belfiore, R., Rodin, A., Ferreira, E., Velazquez, R., Branca, C., Caccamo, A. & Oddo,

S. (2018). Temporal and regional progression of Alzheimer's disease-like pathology in 3xTg-AD mice. *Aging Cell*. [Online]. (September). p.p. e12873. Available from: http://doi.wiley.com/10.1111/acel.12873.

Berté, T.E., Dalmagro, A.P., Zimath, P.L., Gonçalves, A.E., Meyre-Silva, C., Bürger, C., Weber, C.J., dos Santos, D.A., Cechinel-Filho, V. & de Souza, M.M. (2018). Taraxerol as a possible therapeutic agent on memory impairments and Alzheimer's disease: Effects against scopolamine and streptozotocin-induced cognitive dysfunctions. *Steroids*.

Bettens, K., Sleegers, K. & Van Broeckhoven, C. (2013). Genetic insights in Alzheimer's disease. *The Lancet Neurology*.

Biswas, J., Goswami, P., Gupta, S., Joshi, N., Nath, C. & Singh, S. (2016). Streptozotocin Induced Neurotoxicity Involves Alzheimer's Related Pathological Markers: a Study on N2A Cells. *Molecular Neurobiology*.

Blagosklonny, M. V. (2008). Aging: ROS or TOR. *Cell Cycle*. 7 (21) p.pp. 3344–3354.

Borroni, E., Bohrmann, B., Grueninger, F., Prinssen, E., Nave, S., Loetscher, H., Chinta, S.J., Rajagopalan, S., Rane, A., Siddiqui, A., Ellenbroek, B., Messer, J., Pahler, A., Anderson, J.K., Wyler, R. & Cesura, A.M. (2017). Sembragiline: a novel, selective monoamine oxidase type B inhibitor for the treatment of Alzheimer's disease. *Journal of Pharmacology and Experimental Therapeutics*.

Brigelius-Flohé, R. (2015). The Evolving Versatility of Selenium in Biology. *Antioxidants & Redox Signaling*.

Brown, K. & Arthur, J. (2001). Selenium, selenoproteins and human health: a review. *Public Health Nutrition*.

Budni, J., Bellettini-Santos, T., Mina, F., Lima Garcez, M. & Ioppi Zugno, A. (2015). The involvement of BDNF, NGF and GDNF in aging and Alzheimer's disease. *Aging and Disease*.

Buée, L., Troquier, L., Burnouf, S., Belarbi, K., Van der Jeugd, A., Ahmed, T., Fernandez-Gomez, F., Caillierez, R., Grosjean, M.-E., Begard, S., Barbot, B., Demeyer, D., Obriot, H., Brion, I., Buée-Scherrer, V., Maurage, C.-A., Balschun, D., D'hooge, R., Hamdane, M., Blum, D. & Sergeant, N. (2010). From tau

phosphorylation to tau aggregation: what about neuronal death? *Biochemical Society transactions*. 38 (4). p.pp. 967–72.

Bus, B.A.A., Molendijk, M.L., Tendolkar, I., Penninx, B.W.J.H., Prickaerts, J., Elzinga, B.M. & Voshaar, R.C.O. (2015). Chronic depression is associated with a pronounced decrease in serum brain-derived neurotrophic factor over time. *Molecular Psychiatry*.

Cai, Z. & Xiao, M. (2016). Oligodendrocytes and Alzheimer's disease. *International Journal of Neuroscience*.

Cardoso, B.R., Roberts, B.R., Bush, A.I. & Hare, D.J. (2015). Selenium, selenoproteins and neurodegenerative diseases. *Metallomics*.

Casaril, A.M., Domingues, M., Bampi, S.R., de Andrade Lourenço, D., Padilha, N.B., Lenardão, E.J., Sonego, M., Seixas, F.K., Collares, T., Nogueira, C.W., Dantzer, R. & Savegnago, L. (2019). The selenium-containing compound 3-((4-chlorophenyl)selanyl)-1-methyl-1H-indole reverses depressive-like behavior induced by acute restraint stress in mice: modulation of oxido-nitrosative stress and inflammatory pathway. *Psychopharmacology*. [Online]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/30610349%0Ahttp://link.springer.com/10.1007/s00213-018-5151-x.

Casaril, A.M., Domingues, M., Fronza, M., Vieira, B., Begnini, K., Lenardão, E.J., Seixas, F.K., Collares, T., Nogueira, C.W. & Savegnago, L. (2017). Antidepressant-like effect of a new selenium-containing compound is accompanied by a reduction of neuroinflammation and oxidative stress in lipopolysaccharide-challenged mice. *Journal of Psychopharmacology*.

Cervellati, C., Wood, P.L., Romani, A., Valacchi, G., Squerzanti, M., Sanz, J.M., Ortolani, B. & Zuliani, G. (2016). Oxidative challenge in Alzheimer's disease: state of knowledge and future needs. *J Investig Med*. [Online]. 64 (1). p.pp. 21–32. Available from: http://www.ncbi.nlm.nih.gov/pubmed/26755810.

Cheignon, C., Tomas, M., Bonnefont-Rousselot, D., Faller, P., Hureau, C. & Collin, F. (2018). Oxidative stress and the amyloid beta peptide in Alzheimer's disease. *Redox Biology*.

Chi, S., Yu, J.T., Tan, M.S. & Tan, L. (2015). Depression in alzheimer's disease:

Epidemiology, mechanisms, and management. In: *Handbook of Depression in Alzheimer's Disease*.

Chigurupati, S., Selvaraj, M., Mani, V., Selvarajan, K.K., Mohammad, J.I., Kaveti, B., Bera, H., Palanimuthu, V.R., Teh, L.K. & Salleh, M.Z. (2016). Identification of novel acetylcholinesterase inhibitors: Indolopyrazoline derivatives and molecular docking studies. *Bioorganic Chemistry*. 67. p.pp. 9–17.

Craig, L.A., Hong, N.S. & McDonald, R.J. (2011). Revisiting the cholinergic hypothesis in the development of Alzheimer's disease. *Neuroscience and Biobehavioral Reviews*.

Cummings, J.L., Morstorf, T. & Zhong, K. (2014). Alzheimer's disease drug-development pipeline: Few candidates, frequent failures. *Alzheimer's Research and Therapy*.

Dá Mesquita, S., Ferreira, A.C., Sousa, J.C., Correia-Neves, M., Sousa, N. & Marques, F. (2016). Insights on the pathophysiology of Alzheimer's disease: The crosstalk between amyloid pathology, neuroinflammation and the peripheral immune system. *Neuroscience and Biobehavioral Reviews*.

Darcet, F., Mendez-David, I., Tritschler, L., Gardier, A.M., Guilloux, J.-P. & David, D.J. (2014). Learning and memory impairments in a neuroendocrine mouse model of anxiety/depression. *Frontiers in Behavioral Neuroscience*.

Dawson, T.M., Golde, T.E. & Lagier-Tourenne, C. (2018). Animal models of neurodegenerative diseases. *Nature Neuroscience*.

Doody, R.S. (2003). Current treatments for Alzheimer's disease: cholinesterase inhibitors. *The Journal of clinical psychiatry*. [Online]. 64 Suppl 9. p.pp. 11–7. Available from: http://www.ncbi.nlm.nih.gov/pubmed/12934969.

ELLMAN, G.L., COURTNEY, K.D., ANDRES, V. & FEATHER-STONE, R.M. (1961). A new and rapid colorimetric determination of acetylcholinesterase activity. *Biochemical pharmacology*. [Online]. 7. p.pp. 88–95. Available from: http://www.ncbi.nlm.nih.gov/pubmed/13726518.

Ennaceur, A. & Delacour, J. (1988). A new one-trial test for neurobiological studies of memory in rats. 1: Behavioral data. *Behavioural Brain Research*.

Erden, F. (2014). Effects of 7-NI and ODQ on memory in the passive avoidance, novel object recognition, and social transmission of food preference tests in mice. *Medical Science Monitor Basic Research*.

Fang, F., Yu, Q., Arancio, O., Chen, D., Gore, S.S., Yan, S.S. Du & Yan, S.F. (2018). RAGE mediates Aβ accumulation in a mouse model of Alzheimers disease via modulation of β- and γ-secretase activity. *Human Molecular Genetics*.

Fradejas-Villar, N. (2018). Consequences of mutations and inborn errors of selenoprotein biosynthesis and functions. *Free Radical Biology and Medicine*.

Francis, P.T., Palmer, A.M., Snape, M. & Wilcock, G.K. (1999). The cholinergic hypothesis of Alzheimer's disease: a review of progress. *Journal of Neurology, Neurosurgery & Psychiatry*. [Online]. 66 (2). p.pp. 137–147. Available from: http://jnnp.bmj.com/content/66/2/137%5Cnhttp://jnnp.bmj.com/content/66/2/137.full#xref-ref-2-1%5Cnhttp://jnnp.bmj.com/content/66/2/137.full.pdf%5Cnhttp://www.ncbi.nlm.nih.gov/pubmed/10071091.

De Freitas, A.S. & Rocha, J.B.T. (2011). Diphenyl diselenide and analogs are substrates of cerebral rat thioredoxin reductase: A pathway for their neuroprotective effects. *Neuroscience Letters*.

Fronza, M.G., Pinto Brod, L.M., Casaril, A.M., Sacramento, M., Alves, D. & Savegnago, L. (2017). Computational and biological evidences on the serotonergic involvement of SeTACN antidepressant-like effect in mice. *PLoS ONE*.

Gabor, C.S., Phan, A., Clipperton-Allen, A.E., Kavaliers, M. & Choleris, E. (2012). Interplay of oxytocin, vasopressin, and sex hormones in the regulation of social recognition. *Behavioral Neuroscience*.

Gandini, A., Bartolini, M., Tedesco, D., Martinez-Gonzalez, L., Roca, C., Campillo, N.E., Zaldivar-Diez, J., Perez, C., Zuccheri, G., Miti, A., Feoli, A., Castellano, S., Petralla, S., Monti, B., Rossi, M., Moda, F., Legname, G., Martinez, A. & Bolognesi, M.L. (2018). Tau-Centric Multitarget Approach for Alzheimer's Disease: Development of First-in-Class Dual Glycogen Synthase Kinase 3β and Tau-Aggregation Inhibitors. *Journal of Medicinal Chemistry*.

Gaur, S. & Agnihotri, R. (2017). Trace Mineral Micronutrients and Chronic

Periodontitis—a Review. *Biological Trace Element Research*.

Gill, R., Tsung, A. & Billiar, T. (2010). Linking oxidative stress to inflammation: Toll-like receptors. *Free Radical Biology and Medicine*.

Gong, C.-X. & Iqbal, K. (2008). Hyperphosphorylation of microtubule-associated protein tau: a promising therapeutic target for Alzheimer disease. *Current medicinal chemistry*. [Online]. 15 (23). p.pp. 2321–8. Available from: http://www.ncbi.nlm.nih.gov/pubmed/18855662%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC2656563.

Götz, J., Streffer, J.R., David, D., Schild, A., Hoerndli, F., Pennanen, L., Kurosinski, P. & Chen, F. (2004). Transgenic animal models of Alzheimer's disease and related disorders: Histopathology, behavior and therapy. *Molecular Psychiatry*.

Grieb, P. (2016). Intracerebroventricular Streptozotocin Injections as a Model of Alzheimer's Disease: in Search of a Relevant Mechanism. *Molecular Neurobiology*.

Guedes, J.R., Lao, T., Cardoso, A.L. & El Khoury, J. (2018). Roles of microglial and monocyte chemokines and their receptors in regulating Alzheimer's disease-associated amyloid-β and tau pathologies. *Frontiers in Neurology*.

H. Ferreira-Vieira, T., M. Guimaraes, I., R. Silva, F. & M. Ribeiro, F. (2016). Alzheimer's disease: Targeting the Cholinergic System. *Current Neuropharmacology*.

HALEY, T.J. & MCCORMICK, W.G. (1957). Pharmacological effects produced by intracerebral injection of drugs in the conscious mouse. *British journal of pharmacology and chemotherapy*.

Hall, J.O. (2018). Selenium. *Veterinary Toxicology: Basic and Clinical Principles: Third Edition*. p.pp. 469–477.

Halliwell, B. (2018). Making sense of neurodegeneration: A unifying hypothesis. In: *Learning To Live Together: Promoting Social Harmony*.

Halliwell, B. (2001). Role of Free Radicals in the Neurodegenerative Diseases. *Drugs & Aging*.

Hampel, H., Mesulam, M.M., Cuello, A.C., Farlow, M.R., Giacobini, E., Grossberg,

G.T., Khachaturian, A.S., Vergallo, A., Cavedo, E., Snyder, P.J. & Khachaturian, Z.S. (2018). The cholinergic system in the pathophysiology and treatment of Alzheimer's disease. *Brain*.

Hasselmo, M.E. (2006). The role of acetylcholine in learning and memory. *Current Opinion in Neurobiology*. 16 (6) p.pp. 710–715.

Heneka, M.T., Carson, M.J., Khoury, J. El, Landreth, G.E., Brosseron, F., Feinstein, D.L., Jacobs, A.H., Wyss-Coray, T., Vitorica, J., Ransohoff, R.M., Herrup, K., Frautschy, S.A., Finsen, B., Brown, G.C., Verkhratsky, A., Yamanaka, K., Koistinaho, J., Latz, E., Halle, A., Petzold, G.C., Town, T., Morgan, D., Shinohara, M.L., Perry, V.H., Holmes, C., Bazan, N.G., Brooks, D.J., Hunot, S., Joseph, B., Deigendesch, N., Garaschuk, O., Boddeke, E., Dinarello, C.A., Breitner, J.C., Cole, G.M., Golenbock, D.T. & Kummer, M.P. (2015a). Neuroinflammation in Alzheimer's disease. *The Lancet Neurology*. 14 (4) p.pp. 388–405.

Heneka, M.T., Carson, M.J., Khoury, J. El, Landreth, G.E., Brosseron, F., Feinstein, D.L., Jacobs, A.H., Wyss-Coray, T., Vitorica, J., Ransohoff, R.M., Herrup, K., Frautschy, S.A., Finsen, B., Brown, G.C., Verkhratsky, A., Yamanaka, K., Koistinaho, J., Latz, E., Halle, A., Petzold, G.C., Town, T., Morgan, D., Shinohara, M.L., Perry, V.H., Holmes, C., Bazan, N.G., Brooks, D.J., Hunot, S., Joseph, B., Deigendesch, N., Garaschuk, O., Boddeke, E., Dinarello, C.A., Breitner, J.C., Cole, G.M., Golenbock, D.T. & Kummer, M.P. (2015b). Neuroinflammation in Alzheimer's disease. *The Lancet Neurology*. 14 (4). p.pp. 388–405.

Hospital, T.J. (1957). A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. *American Journal of Clinical Pathology*. p.pp. 56–63.

Huawei, Z. (2009). Selenium as an essential micronutrient: Roles in cell cycle and apoptosis. *Molecules*.

Ishrat, T., Hoda, M.N., Khan, M.B., Yousuf, S., Ahmad, M., Khan, M.M., Ahmad, A. & Islam, F. (2009). Amelioration of cognitive deficits and neurodegeneration by curcumin in rat model of sporadic dementia of Alzheimer's type (SDAT). *European Neuropsychopharmacology*.

Islam, F., Javed, H., Vaibhav, K., Ahmed, M.E., Khan, A., Tabassum, R., Islam, F. &

Safhi, M.M. (2015). Effect of hesperidin on neurobehavioral, neuroinflammation, oxidative stress and lipid alteration in intracerebroventricular streptozotocin induced cognitive impairment in mice. *Journal of the Neurological Sciences*.

Ismail, Z., Elbayoumi, H., Fischer, C.E., Hogan, D.B., Millikin, C.P., Schweizer, T., Mortby, M.E., Smith, E.E., Patten, S.B. & Fiest, K.M. (2017). Prevalence of depression in patients with mild cognitive impairment: A systematic review and meta-analysis. *JAMA Psychiatry*.

Jackson, L.L. (1943). VTE on an elevated T-maze. *Journal of Comparative Psychology*.

Janowsky, D.S. & Risch, S.C. (1984). Cholinomimetic and Anticholinomimetic Drugs Used To Investigate an Acetylcholine Hypothesis of Affective Disorders and Stress. *Drug Development Research*.

Kamal, A., Iqbal, M.A. & Bhatti, H.N. (2018). Therapeutic applications of selenium-derived compounds. *Reviews in Inorganic Chemistry*.

Karch, C.M. & Goate, A.M. (2015). Alzheimer's disease risk genes and mechanisms of disease pathogenesis. *Biological Psychiatry*.

Karlawish, J., Jack, C.R., Rocca, W.A., Snyder, H.M. & Carrillo, M.C. (2017). 2017 Alzheimer's disease facts and figures. *Alzheimer's and Dementia*.

Kelany, M.E., Hakami, T.M., Omar, A.H. & Abdallah, M.A. (2016). Combination of Sitagliptin and Insulin against Type 2 Diabetes Mellitus with Neuropathy in Rats: Neuroprotection and Role of Oxidative and Inflammation Stress. *Pharmacology*. 98 (5–6). p.pp. 242–250.

Kogan, J.H., Frankland, P.W. & Silva, A.J. (2000). Long-term memory underlying hippocampus-dependent social recognition in mice. *Hippocampus*.

Kojro, E. & Fahrenholz, F. (2005). The non-amyloidogenic pathway: structure and function of alpha-secretases. *Sub-cellular biochemistry*.

Labunskyy, V.M., Hatfield, D.L. & Gladyshev, V.N. (2014). Selenoproteins: Molecular Pathways and Physiological Roles. *Physiological Reviews*.

LaFerla, F.M., Green, K.N. & Oddo, S. (2007). Intracellular amyloid-beta in Alzheimer's

disease. *Nature Reviews Neuroscience*.

Laursen, S.E. & Belknap, J.K. (1986). Intracerebroventricular injections in mice. Some methodological refinements. *Journal of Pharmacological Methods*.

Levander, O.A. (1982). CLINICAL CONSEQUENCES OF LOW SELENIUM INTAKE AND ITS RELATIONSHIP TO VITAMIN E. *Annals of the New York Academy of Sciences*.

Li, D., Huang, Y., Cheng, B., Su, J., Zhou, W.X. & Zhang, Y.X. (2016). Streptozotocin induces mild cognitive impairment at appropriate doses in mice as determined by long-term potentiation and the morris water maze. *Journal of Alzheimer's Disease*.

Liang, W.S., Dunckley, T., Beach, T.G., Grover, A., Mastroeni, D., Ramsey, K., Caselli, R.J., Kukull, W.A., McKeel, D., Morris, J.C., Hulette, C.M., Schmechel, D., Reiman, E.M., Rogers, J. & Stephan, D.A. (2008). Altered neuronal gene expression in brain regions differentially affected by Alzheimer's disease: a reference data set. *Physiological Genomics*.

Liu, Z., Zhou, T., Ziegler, A.C., Dimitrion, P. & Zuo, L. (2017). Oxidative Stress in Neurodegenerative Diseases: From Molecular Mechanisms to Clinical Applications. *Oxidative Medicine and Cellular Longevity*.

Loetchutinat, C., Kothan, S., Dechsupa, S., Meesungnoen, J., Jay-Gerin, J.P. & Mankhetkorn, S. (2005). Spectrofluorometric determination of intracellular levels of reactive oxygen species in drug-sensitive and drug-resistant cancer cells using the 2???,7???-dichlorofluorescein diacetate assay. *Radiation Physics and Chemistry*. 72 (2–3). p.pp. 323–331.

Mackay, E.M. & Mackay, L.L. (1927). The concentration of urea in the blood of normal individuals. *J Clin Invest*. [Online]. 4 (2). p.pp. 295–306. Available from: http://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=434672&tool=pmcentrez&rendertype=abstract.

Mandelkow, E.M. & Mandelkow, E. (2011). Biochemistry and cell biology of Tau protein in neurofibrillary degeneration. *Cold Spring Harbor Perspectives in Biology*. 3 (10) p.pp. 1–25.

Manosso, L.M., Moretti, M., Ribeiro, C.M., Gonçalves, F.M., Leal, R.B. & Rodrigues,

A.L.S. (2015). Antidepressant-like effect of zinc is dependent on signaling pathways implicated in BDNF modulation. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*.

Martini, F., Rosa, S.G., Klann, I.P., Fulco, B.C.W., Carvalho, F.B., Rahmeier, F.L., Fernandes, M.C. & Nogueira, C.W. (2019). A multifunctional compound ebselen reverses memory impairment, apoptosis and oxidative stress in a mouse model of sporadic Alzheimer's disease. *Journal of Psychiatric Research*. [Online]. 109. p.pp. 107–117. Available from: https://linkinghub.elsevier.com/retrieve/pii/S0022395618308951.

Matsumoto, T., Furuta, T., Nimura, Y. & Suzuki, O. (1984). 3-(p-Hydroxyphenyl)propionic acid as a new fluorogenic reagent for amine oxidase assays. *Analytical Biochemistry*.

Mecocci, P., Boccardi, V., Cecchetti, R., Bastiani, P., Scamosci, M., Ruggiero, C. & Baroni, M. (2018). A Long Journey into Aging, Brain Aging, and Alzheimer's Disease Following the Oxidative Stress Tracks. *Journal of Alzheimer's Disease*.

Medeiros, D.M. (2016). Copper, iron, and selenium dietary deficiencies negatively impact skeletal integrity: A review. *Experimental Biology and Medicine*.

Mehan, S., Arora, R., Sehgal, V., Sharma, D. & Sharm, G. (2012). Dementia – A Complete Literature Review on Various Mechanisms Involves in Pathogenesis and an Intracerebroventricular Streptozotocin Induced Alzheimer's Disease. *Inflammatory Diseases - Immunopathology, Clinical and Pharmacological Bases*. [Online]. (February). Available from: http://www.intechopen.com/books/inflammatory-diseases-immunopathology-clinical-and-pharmacological-bases/alzheimer-s-disease-an-updated-review-on-pathogenesis-and-intracerebroventricular-streptozotocin-ind.

Meotti, F.C., Stangherlin, E.C., Zeni, G., Nogueira, C.W. & Rocha, J.B.T. (2004). Protective role of aryl and alkyl diselenides on lipid peroxidation. *Environmental Research*.

Minichiello, L. (2009). TrkB signalling pathways in LTP and learning. *Nature Reviews Neuroscience*.

Moreira, P.I., Siedlak, S.L., Aliev, G., Zhu, X., Cash, A.D., Smith, M.A. & Perry, G.

(2005). Oxidative stress mechanisms and potential therapeutics in Alzheimer disease. *Journal of Neural Transmission*.

Morgese, M.G., Schiavone, S. & Trabace, L. (2017). Emerging role of amyloid beta in stress response: Implication for depression and diabetes. *European Journal of Pharmacology*.

Newman, M.B., Nazian, S.J., Sanberg, P.R., Diamond, D.M. & Shytle, R.D. (2001). Corticosterone-attenuating and anxiolytic properties of mecamylamine in the rat. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*.

Nimse, S.B. & Pal, D. (2015). Free radicals, natural antioxidants, and their reaction mechanisms. *RSC Adv.* [Online]. 5 (35). p.pp. 27986–28006. Available from: http://xlink.rsc.org/?DOI=C4RA13315C.

O'Brien, R.J. & Wong, P.C. (2011). Amyloid Precursor Protein Processing and Alzheimer's Disease. *Annual Review of Neuroscience*. [Online]. 34 (1). p.pp. 185–204. Available from: http://www.annualreviews.org/doi/10.1146/annurev-neuro-061010-113613.

Oddo, S., Caccamo, A., Shepherd, J.D., Murphy, M.P., Golde, T.E., Kayed, R., Metherate, R., Mattson, M.P., Akbari, Y. & LaFerla, F.M. (2003). Triple-transgenic model of Alzheimer's Disease with plaques and tangles: Intracellular Aβ and synaptic dysfunction. *Neuron*.

Ohkawa, H., Ohishi, N. & Yagi, K. (1979). Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. *Analytical Biochemistry*.

Pacheco, S.M., Soares, M.S.P., Gutierres, J.M., Gerzson, M.F.B., Carvalho, F.B., Azambuja, J.H., Schetinger, M.R.C., Stefanello, F.M. & Spanevello, R.M. (2018). Anthocyanins as a potential pharmacological agent to manage memory deficit, oxidative stress and alterations in ion pump activity induced by experimental sporadic dementia of Alzheimer's type. *Journal of Nutritional Biochemistry*.

Paloczi, J., Varga, Z. V., Hasko, G. & Pacher, P. (2017). Neuroprotection in Oxidative Stress-Related Neurodegenerative Diseases: Role of Endocannabinoid System Modulation. *Antioxidants & Redox Signaling*.

Park, S.J., Kim, Y.H., Nam, G.H., Choe, S.H., Lee, S.R., Kim, S.U., Kim, J.S., Sim,

B.W., Song, B.S., Jeong, K.J., Lee, Y., Park, Y. Il, Lee, K.M., Huh, J.W. & Chang, K.T. (2015). Quantitative expression analysis of APP pathway and tau phosphorylation-related genes in the ICV STZ-induced non-human primate model of sporadic alzheimer's disease. *International Journal of Molecular Sciences*.

Peake, M. & Whiting, M. (2006). Measurement of Serum Creatinine – Current Status and Future Goals. *Clinical Biochemist Reviews*.

Pinto Brod, L.M., Fronza, M.G., Vargas, J.P., L??dtke, D.S., Luchese, C., Wilhelm, E.A. & Savegnago, L. (2016a). Involvement of monoaminergic system in the antidepressant-like effect of (octylseleno)-xylofuranoside in the mouse tail suspension test. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*. [Online]. 65. p.pp. 201–207. Available from: http://dx.doi.org/10.1016/j.pnpbp.2015.10.008.

Pinto Brod, L.M., Fronza, M.G., Vargas, J.P., Lüdtke, D.S., Brüning, C.A. & Savegnago, L. (2017a). Modulation of PKA, PKC, CAMKII, ERK 1/2 pathways is involved in the acute antidepressant-like effect of (octylseleno)-xylofuranoside (OSX) in mice. *Psychopharmacology*.

Pinto Brod, L.M., Fronza, M.G., Vargas, J.P., Lüdtke, D.S., Brüning, C.A. & Savegnago, L. (2017b). Modulation of PKA, PKC, CAMKII, ERK 1/2 pathways is involved in the acute antidepressant-like effect of (octylseleno)-xylofuranoside (OSX) in mice. *Psychopharmacology*. [Online]. 234 (4). p.pp. 717–725. Available from: http://link.springer.com/10.1007/s00213-016-4505-5.

Pinto Brod, L.M., Fronza, M.G., Vargas, J.P., Lüdtke, D.S., Luchese, C., Wilhelm, E.A. & Savegnago, L. (2016b). Involvement of monoaminergic system in the antidepressant-like effect of (octylseleno)-xylofuranoside in the mouse tail suspension test. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*.

Prince, M., Wimo, A., Guerchet, M., Gemma-Claire, A., Wu, Y.-T. & Prina, M. (2015). *World Alzheimer Report 2015*.

Van Raamsdonk, J.M., Vega, I.E. & Brundin, P. (2017). Oxidative stress in neurodegenerative disease: causation or association? *Oncotarget*.

Rajasekar, N., Nath, C., Hanif, K. & Shukla, R. (2017). Intranasal Insulin Administration

Ameliorates Streptozotocin (ICV)-Induced Insulin Receptor Dysfunction, Neuroinflammation, Amyloidogenesis, and Memory Impairment in Rats. *Molecular Neurobiology*. 54 (8). p.pp. 6507–6522.

Rapp, M.A., Schnaider-Beeri, M., Grossman, H.T., Sano, M., Perl, D.P., Purohit, D.P., Gorman, J.M. & Haroutunian, V. (2006). Increased hippocampal plaques and tangles in patients with Alzheimer disease with a lifetime history of major depression. *Archives of General Psychiatry*.

Rapp, M.A., Schnaider-Beeri, M., Purohit, D.P., Perl, D.P., Haroutunian, V. & Sano, M. (2008). Increased neurofibrillary tangles in patients with alzheimer disease with comorbid depression. *American Journal of Geriatric Psychiatry*.

Ravelli, K.G., Rosário, B. dos A., Camarini, R., Hernandes, M.S. & Britto, L.R. (2017). Intracerebroventricular Streptozotocin as a Model of Alzheimer's Disease: Neurochemical and Behavioral Characterization in Mice. *Neurotoxicity Research*.

Reeta, K.H., Singh, D. & Gupta, Y.K. (2017). Chronic treatment with taurine after intracerebroventricular streptozotocin injection improves cognitive dysfunction in rats by modulating oxidative stress, cholinergic functions and neuroinflammation. *Neurochemistry International*.

Rehman, S.U., Shah, S.A., Ali, T., Chung, J. Il & Kim, M.O. (2017). Anthocyanins Reversed D-Galactose-Induced Oxidative Stress and Neuroinflammation Mediated Cognitive Impairment in Adult Rats. *Molecular Neurobiology*. 54 (1). p.pp. 255–271.

Rocha, N.P. & Amorim, C. (2011). *Processo Inflamatório e Neuroimunomodulação na Doença de Alzheimer : Revisão de Literatura*. 19 (2). p.pp. 300–313.

Rodrigues, J., Saba, S., Joussef, A.C., Rafique, J. & Braga, A.L. (2018). KIO3-Catalyzed C(sp2)-H Bond Selenylation/Sulfenylation of (Hetero)arenes: Synthesis of Chalcogenated (Hetero)arenes and their Evaluation for Anti-Alzheimer Activity. *Asian Journal of Organic Chemistry*.

Rodrigues, R., Petersen, R.B. & Perry, G. (2014). Parallels Between Major Depressive Disorder and Alzheimer's Disease: Role of Oxidative Stress and Genetic Vulnerability. *Cellular and Molecular Neurobiology*.

Roesler, R., Walz, R., Quevedo, J., De-Paris, F., Zanata, S.M., Graner, E., Izquierdo, I., Martins, V.R. & Brentani, R.R. (1999). Normal inhibitory avoidance learning and anxiety, but increased locomotor activity in mice devoid of PrP(C). *Molecular Brain Research*.

Rosa, R.M., Flores, D.G., Appelt, H.R., Braga, A.L., Henriques, J.A.P. & Roesler, R. (2003). Facilitation of long-term object recognition memory by pretraining administration of diphenyl diselenide in mice. *Neuroscience Letters*. 341 (3). p.pp. 217–220.

Rosenberg, P.B., Nowrangi, M.A. & Lyketsos, C.G. (2015). Neuropsychiatric symptoms in Alzheimer's disease: What might be associated brain circuits? *Molecular Aspects of Medicine*.

Różyk-Myrta, A. (2014). In Search of Memory Tests Equivalent for Experiments on Animals and Humans. *Medical Science Monitor*.

Salkovic-Petrisic, M. (2008). Amyloid cascade hypothesis: Is it true for sporadic Alzheimer's disease. *Periodicum Biologorum*.

Santos, D.B., Colle, D., Moreira, E.L.G., Peres, K.C., Ribeiro, R.P., dos Santos, A.A., de Oliveira, J., Hort, M.A., de Bem, A.F. & Farina, M. (2015). Probucol mitigates streptozotocin-induced cognitive and biochemical changes in mice. *Neuroscience*.

dos Santos Picanco, L.C., Ozela, P.F., de Fatima de Brito Brito, M., Pinheiro, A.A., Padilha, E.C., Braga, F.S., de Paula da Silva, C.H.T., dos Santos, C.B.R., Rosa, J.M.C. & da Silva Hage-Melim, L.I. (2018). Alzheimer's Disease: A Review from the Pathophysiology to Diagnosis, New Perspectives for Pharmacological Treatment. *Current Medicinal Chemistry*.

Savegnago, L., Jesse, C.R., Pinto, L.G., Rocha, J.B.T., Barancelli, D.A., Nogueira, C.W. & Zeni, G. (2008). Diphenyl diselenide exerts antidepressant-like and anxiolytic-like effects in mice: Involvement of l-arginine-nitric oxide-soluble guanylate cyclase pathway in its antidepressant-like action. *Pharmacology Biochemistry and Behavior*.

Sbodio, J.I., Snyder, S.H. & Paul, B.D. (2018). Redox Mechanisms in Neurodegeneration: From Disease Outcomes to Therapeutic Opportunities. *Antioxidants & Redox Signaling*. [Online]. p.p. ars.2017.7321. Available from:

http://www.liebertpub.com/doi/10.1089/ars.2017.7321.

Schedin-Weiss, S., Inoue, M., Hromadkova, L., Teranishi, Y., Yamamoto, N.G., Wiehager, B., Bogdanovic, N., Winblad, B., Sandebring-Matton, A., Frykman, S. & Tjernberg, L.O. (2017). Monoamine oxidase B is elevated in Alzheimer disease neurons, is associated with γ-secretase and regulates neuronal amyloid β-peptide levels. *Alzheimer's Research and Therapy*.

Solanki, I., Parihar, P. & Parihar, M.S. (2016). Neurodegenerative diseases: From available treatments to prospective herbal therapy. *Neurochemistry International*.

Sousa, F.S.S., Birmann, P.T., Baldinotti, R., Fronza, M.G., Balaguez, R., Alves, D., Brüning, C.A. & Savegnago, L. (2018). α- (phenylselanyl) acetophenone mitigates reserpine-induced pain–depression dyad: Behavioral, biochemical and molecular docking evidences. *Brain Research Bulletin*.

Spires, T.L. & Hyman, B.T. (2005). Transgenic models of Alzheimer's disease: Learning from animals. *NeuroRx*.

Tanimizu, T., Kenney, J.W., Okano, E., Kadoma, K., Frankland, P.W. & Kida, S. (2017). Functional Connectivity of Multiple Brain Regions Required for the Consolidation of Social Recognition Memory. *The Journal of Neuroscience*.

Thinakaran, G. & Koo, E.H. (2008). Amyloid precursor protein trafficking, processing, and function. *The Journal of biological chemistry*. [Online]. 283 (44). p.pp. 29615–9. Available from: http://www.jbc.org/cgi/doi/10.1074/jbc.R800019200%0Ahttp://www.ncbi.nlm.nih.gov/pubmed/18650430%0Ahttp://www.pubmedcentral.nih.gov/articlerender.fcgi?artid=PMC2573065.

Tramutola, A., Lanzillotta, C., Perluigi, M. & Butterfield, D.A. (2016a). Oxidative stress, protein modification and Alzheimer disease. *Brain Research Bulletin*.

Tramutola, A., Lanzillotta, C., Perluigi, M. & Butterfield, D.A. (2016b). Oxidative stress, protein modification and Alzheimer disease. *Brain Research Bulletin*. [Online]. Available from: http://dx.doi.org/10.1016/j.brainresbull.2016.06.005.

Tundis, R., Loizzo, M.R., Nabavi, S.M., Orhan, I.E., Skalicka-Wozniak, K., D'Onofrio, G. & Aiello, F. (2017). Natural Compounds and Their Derivatives as Multifunctional

Agents for the Treatment of Alzheimer Disease. In: *Discovery and Development of Neuroprotective Agents from Natural Products: Natural Product Drug Discovery*.

Valavanidis, A., Vlahogianni, T., Dassenakis, M. & Scoullos, M. (2006). Molecular biomarkers of oxidative stress in aquatic organisms in relation to toxic environmental pollutants. *Ecotoxicology and Environmental Safety*. 64. p.pp. 178–189.

Vandersteen, A., Masman, M.F., De Baets, G., Jonckheere, W., Van Der Werf, K., Marrink, S.J., Rozenski, J., Benilova, I., De Strooper, B., Subramaniam, V., Schymkowitz, J., Rousseau, F. & Broersen, K. (2012). Molecular plasticity regulates oligomerization and cytotoxicity of the multipeptide-length amyloid-β peptide pool. *Journal of Biological Chemistry*. 287 (44). p.pp. 36732–36743.

Vargas, J.P., Pinto, L.M., Savegnago, L. & Lüdtke, D.S. (2015). Synthesis of alkylseleno-carbohydrates and evaluation of their antioxidant properties. *Journal of the Brazilian Chemical Society*.

Walsh, R.N. & Cummins, R. a (1976). The Open-Field Test: a critical review. *Psychological bulletin*. 83 (3). p.pp. 482–504.

Wang, Z.M., Cai, P., Liu, Q.H., Xu, D.Q., Yang, X.L., Wu, J.J., Kong, L.Y. & Wang, X.B. (2016). Rational modification of donepezil as multifunctional acetylcholinesterase inhibitors for the treatment of Alzheimer's disease. *European Journal of Medicinal Chemistry*. [Online]. 123. p.pp. 282–297. Available from: http://dx.doi.org/10.1016/j.ejmech.2016.07.052.

Weller, J. & Budson, A. (2018). Current understanding of Alzheimer's disease diagnosis and treatment. *F1000Research*.

Whitehouse, P.J., Price, D.L., Clark, A.W., Coyle, J.T. & DeLong, M.R. (1981). Alzheimer disease: Evidence for selective loss of cholinergic neurons in the nucleus basalis. *Annals of Neurology*.

Whitehouse, P.J., Price, D.L., Struble, R.G., Clark, A.W., Coyle, J.T. & DeLong, M.R. (1982). Alzheimer's disease and senile dementia: Loss of neurons in the basal forebrain. *Science*.

Wilcock, G.K., Esiri, M.M., Bowen, D.M. & Smith, C.C.T. (1982). Alzheimer's disease. Correlation of cortical choline acetyltransferase activity with the severity of dementia and histological abnormalities. *Journal of the Neurological Sciences*.

Wrobel, J.K., Power, R. & Toborek, M. (2016). Biological activity of selenium: Revisited. *IUBMB Life*.

Wu, Y., Ma, Y., Liu, Z., Geng, Q., Chen, Z. & Zhang, Y. (2017). Alterations of myelin morphology and oligodendrocyte development in early stage of Alzheimer's disease mouse model. *Neuroscience Letters*.

Xie, Y., Liu, Q., Zheng, L., Wang, B.T., Qu, X., Ni, J., Zhang, Y. & Du, X. (2018). Se-Methylselenocysteine Ameliorates Neuropathology and Cognitive Deficits by Attenuating Oxidative Stress and Metal Dyshomeostasis in Alzheimer Model Mice. *Molecular Nutrition and Food Research*.

Xie, Y., Tan, Y., Zheng, Y., Du, X. & Liu, Q. (2017). Ebselen ameliorates β-amyloid pathology, tau pathology, and cognitive impairment in triple-transgenic Alzheimer's disease mice. *Journal of Biological Inorganic Chemistry*.

Ye, X., Tai, W. & Zhang, D. (2012). The early events of Alzheimer's disease pathology: From mitochondrial dysfunction to BDNF axonal transport deficits. *Neurobiology of Aging*. 33 (6).

Yiannopoulou, K.G. & Papageorgiou, S.G. (2013). Current and future treatments for Alzheimer's disease. *Therapeutic Advances in Neurological Disorders*.

Zhang, F., Kang, Z., Li, W., Xiao, Z. & Zhou, X. (2012). Roles of brain-derived neurotrophic factor/tropomyosin-related kinase B (BDNF/TrkB) signalling in Alzheimer's disease. *Journal of Clinical Neuroscience*.

Zhang, X., He, X., Chen, Q., Lu, J., Rapposelli, S. & Pi, R. (2018). A review on the hybrids of hydroxycinnamic acid as multi-target-directed ligands against Alzheimer's disease. *Bioorganic and Medicinal Chemistry*.