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VICTOR HUGO BITENCOURT DE ANDRADE

A GRANDISINA E SEU ANÁLOGO ISOXAZÓLICO PREVINEM O DÉFICIT DE MEMÓRIA ATRAVÉS DE EFEITOS ANTIOXIDANTES EM ANIMAIS SUBMETIDOS A UM MODELO DA DOENÇA DE ALZHEIMER

CAMPO GRANDE-MS
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Orientador: Prof. Dr. Davi Campos La Gatta

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RESUMO

A doença de Alzheimer (DA) causa declínio progressivo das funções cognitivas e está relacionada ao acúmulo e deposição da proteína β-amilóide (βA) em áreas cerebrais, como o córtex (CTX) e hipocampo (HPC), resultando na ativação de vias neurotóxicas, oxidativas e inflamatórias. A grandisina (GRA), bem como seu derivado isoxazólico (AIG) são neolignanas com propriedades antiinflamatórias e antioxidantes, possuindo assim potencial neuroprotetor. Outras neolignanas exibiram propriedades neuroprotetores contra βA *in vitro*. Além disso, um bioisóstero triazólico da grandisina preveniu o déficit cognitivo de animais que receberam βA através da redução de citocinas e lipoperoxidação no CTX e HPC. Entretanto, o potencial neuroprotetor da GRA e o AIG ainda não foi avaliado em modelos *in vivo* da DA. Sendo assim, foram investigados os efeitos da GRA e do AIG sobre a memória e perfil oxidativo em um modelo animal da DA. Para isso, camundongos C57/Bl6 machos (protocolo CEUA: 1.272/2023) foram submetidos a cirurgia para injeção intracerebroventricular (i.c.v.) de βA ou veículo. Após 24 horas, iniciou-se o tratamento oral ou intraperitoneal (i.p.) diário com GRA (1 mg/kg), AIG (1 mg/kg) ou veículo, por 7 dias. Assim, os grupos experimentais foram divididos em Controle i.c.v./ Veículo i.p. ou oral, Aβ i.c.v./ Veículo i.p. ou oral, Aβ i.c.v./ GRA i.p. ou oral, e Aβ i.c.v./ AIG i.p. ou oral. Após o final do tratamento foi realizado o Teste de Reconhecimento de Objeto (TRO). Por fim, foram coletados o HPC e CTX para análise dos níveis de lipoperoxidação, mieloperoxidase (MPO) e glutationa reduzida (GSH). O tratamento por via i.p. com GRA, porém não com o AIG, evitou o déficit de memória dos animais no TRO, além de reduzir os níveis de marcadores oxidativos no HPC. Da mesma forma, o tratamento por via oral com GRA ou AIG demonstrou ser eficaz na prevenção do prejuízo à memória no TRO, bem como na melhora do perfil oxidativo. Estes resultados sugerem que a GRA e seu análogo isoxazólico possuem efeitos neuroprotetores *in vivo* frente aos danos induzidos pelos oligômeros de βA.

Palavras-chave: Doença de Alzheimer; Drogas multi-alvo; Grandisina; Neuroproteção

SUMÁRIO

Sumário

1. REVISÃO BIBLIOGRÁFICA

1.1. Descoberta da Doença de Alzheimer

Em novembro de 1906, aconteceu o 37º Encontro de Psiquiatras do Sudoeste da Alemanha na universidade Tubingen, no qual o médico alemão Alois Alzheimer apresentou um relato de caso de uma paciente que estava aos seus cuidados no Hospital Psiquiátrico de Frankfurt, Alemanha. Em seu relato continham informações sobre Auguste Deter, 51 anos de idade, a qual apresentava grave prejuízo na memória, além de confusão mental, dificuldades na leitura, pronúncia e escrita. Após a autópsia da paciente, foi realizada uma análise histológica de amostras de tecido cerebral da paciente, sendo encontrados emaranhados neurofibrilares e placas senis espalhadas por todo o córtex cerebral. Esses seriam os primeiros achados histopatológicos do que seria futuramente conhecida como Doença de Alzheimer (DA). Adiante, o caso de Auguste D. foi observado e documentado desde o início da sua admissão no hospital até sua morte em abril de 1906. O registro clínico e a autópsia do cérebro da paciente durante esses 4 anos foram a base para o relato do médico alemão no encontro em Tubingen. Entretanto, os achados de Alois Alzheimer não foram encarados com grande entusiasmo pela comunidade científica da época.

Dando continuidade aos trabalhos, em 1909 colaboradores de Alzheimer publicaram mais quatro casos com detalhes clínicos, incluindo o caso do paciente Johan F., de 56 anos de idade, do sexo masculino, que também apresentava sintomas de deterioração cognitiva e outras alterações comportamentais muito semelhantes ao de Auguste Deter. Entre 1906 e 1909 Emil Kraepelin publicou tais achados no lançamento da 8ª edição do livro “Psychiatrie”, propondo pela primeira vez o termo “Doença de Alzheimer”. No entanto, a morte do doutor Alois Alzheimer em 1915 acabou por inviabilizar a continuação dos estudos acerca desta patologia.

Apenas em 1976, novos estudos epidemiológicos e histopatológicos do grupo de pesquisa do Dr. Robert Katzman confirmaram as pesquisas realizadas por Alzheimer e Kraeplin sobre a doença, sendo identificada uma nova entidade clínica denominada a partir de então como Doença de Alzheimer. Em 1984, George Glenner e Cai’ne W. Wong determinaram que as então chamadas “placas senis” eram compostas por aglomerados de proteína β-amiloide (βA). Dois anos mais tarde, identificou-se que a proteína *tau* era o componente predominante nos

emaranhados neurofibrilares. Em 1993, a tacrina foi aprovada como primeiro tratamento da DA [44; 45].

1.2. Proteína beta-amiloide: produção e mecanismos envolvidos na hipersecreção

A βA é formada a partir de processos de clivagem e expressão da proteína precursora amilóide (APP), que é uma proteína integral de membrana presente em neurônios do Sistema Nervoso Central (SNC) [46]. A clivagem dessa proteína pode ser realizada por duas vias diferentes. A primeira é a via amiloidogênica na qual a clivagem é regulada pela enzima β-secretase e tem como produto uma proteína com cadeia mais curta denominada β-amilóide (βA). A segunda via é chamada de não-amiloidogênica, visto que a quebra da APP resulta em um segmento proteico diferente da βA verdadeira, denominado P83. Esta via é regulada pela enzima α-secretase [47]. De forma suplementar, a atividade dessas duas vias também é controlada por enzimas γ-secretases, que tem como co-fatores as presenilinas que são codificadas pelos genes PSEN1 e PSEN2 [48]. Não se sabe ao certo o papel fisiológico do segmento P83, porém é sugerido que a via não-amiloidogênica funcionaria para desviar a fragmentação das APP, evitando sua clivagem excessiva pela via amiloidogênica, e mantendo níveis fisiológicos de βA ao redor dos neurônios [49]. Adicionalmente, o receptor de apolipoproteína E (APOEr), participa da remoção da βA das sinapses através da barreira hematoencefálica, contribuindo para a manutenção de níveis normais desta proteína no espaço extracelular [50].

Nestas condições, as vias de produção e remoção de βA estão em equilíbrio, de forma que uma quantidade moderada da mesma facilita processos de plasticidade e transmissão sináptica, tais como formação de brotos dentríticos durante o remodelamento e reparo das sinapses [51, 52]. Entretanto, formas polimórficas nos genes que regulam a expressão da APP, bem como nos loci das enzimas secretases e das presenilinas, como também no gene que codifica o APOEr resultam em acúmulo de monômeros de βA, facilitando a sua polimerização e posterior agregação de modo a formar oligômeros de βA. Estes oligômeros se depositam ao redor das sinapses neurais e geram processo de desagregação das sinapses [53, 54].

1.3. Neurobiologia da doença de Alzheimer

No córtex pré-frontal (CTX) e Hipocampo (HPC) acontecem importantes processos neuroplásticos mediados pelos receptores glutamatérgicos ionotrópicos (NMDA) localizados nas sinapses (S-NMDA). A ativação desses receptores, culmina na liberação de neurotrofinas como o fator neurotrófico derivado do cérebro (brain derived neurotrophic factor - BDNF) que é responsável por eventos celulares com efeitos neuroprotetores, como aumento do número de conexões entre neurônios, formação de espinhas dendríticas e neurogênese [55, 56]. No CTX e HPC tais processos neuroplásticos estão relacionados à formação de memória. O glutamato é liberado pelo neurônio pré-sináptico e pelos astrócitos, enquanto que a recaptação do glutamato da fenda sináptica é realizada por transportadores presentes nos astrócitos. A deposição de βA no CTX e HPC alteram a cinética de liberação e recaptação do glutamato, de modo que, ocorre um aumento na liberação pelo neurônio pré-sináptico e astrócitos, através da estimulação de receptores colinérgicos nicotínicos mediada pela βA [30]. Além disso, a recaptação de glutamato é inibida pela βA, por meio da interação com os transportadores presentes nos astrócitos. Dessa forma, ocorre um acúmulo de glutamato nas sinapses e extravasamento do mesmo para o espaço extrasináptico, atingindo assim os receptores glutamatérgicos ionotrópicos extrasinápticos (E-NMDA) [30]. Estes possuem ação antagônica aos S-NMDA, uma vez que eles são capazes de inibir o estímulo ao BDNF, reduzindo processos de sobrevivência neural, como também estimulando a liberação de fatores de apoptose, como caspase-3 e substâncias oxidantes (ex: óxido nítrico e ânion superóxido). Os E-NMDA ainda conseguem ativar quinases como a glicogênio sintase quinase (GSK) que auxiliam na hiperfosforilação da proteína tau, presente no citoesqueleto de neurônios, resultando na desagregação da mesma e formação dos “emaranhados neurofibrilares” no espaço intracelular favorecendo ainda mais a disfunção sináptica e morte neuronal [57]. Ademais, a βA em astrócitos consegue estimular a expressão de genes que codificam a enzima ciclooxigenase-2 (COX-2), responsável pela produção de prostaglandinas inflamatórias [58]. Além disso, o acúmulo de βA também pode induzir neuroinflamação através do estímulo a liberação citocinas pró-inflamatórias, tais como as interleucinas (IL-6), fator de necrose tumoral (TNF) e interferón (IFN) pela micróglia ativada, Tais eventos inflamatórios potencializam a morte neuronal [59].

Uma outra possível via inflamatória envolvida na patogênses da doença, inclui a enzima mieloperoxidase (MPO). Esta enzima está presente em granulócitos presentes no sangue e também na micróglia, sendo um marcador de infiltração celular. Estudos prévios demonstram que a aplicação intracerebroventricular de βA aumentou a atividade da mieloperoxidase no CTX e HPC de camundongos, demonstrando que os oligômeros são capazes de atrair e ativar células de defesa [36].

Além disso, a MPO é um marcador de estresse oxidativo, visto que esta enzima produz ácido hipocloroso, uma substância altamente oxidante. Deste modo a micróglia ativada pela βA pode participar também do dano oxidativo durante a evolução da doença [37]. O ácido hipocloroso produzido pela MPO leva à produção de ânions superóxido que por sua vez reagem com os lipídios presentes na bainha de mielina e na membrana dos neurônios, contribuindo com a lesão oxidativa através da lipoperoxidação [60]. Por outro lado, grupos carbonil também podem ser formados durante estados de elevada oxidação, contribuindo com a lesão proteica nos neurônios. O produto desta lesão são as proteínas carboniladas que também sugerem dano oxidativo [36]. Estudos *in vivo* mostram que a βA foi capaz de aumentar a lipoperoxidação e o conteúdo de proteína carbonilada no CTX e HPC, demonstrando que a patogênese da doença também envolve estresse oxidativo [61]. Este ambiente inflamatório e oxidativo leva à perda de sinapses e prejuízo da neurogênese, levando às manifestações características da DA, tais como o intenso prejuízo cognitivo, alterações comportamentais e transtornos de humor [44].

1.4. Tratamento farmacológico atual

Há tratamentos que visam retardar o declínio cognitivo e funcional presente na doença e também existem os tratamentos focados em reduzir os sintomas da demência causada na DA [62].

Dentre as substâncias aprovadas que visam retardar o declínio cognitivo causado pela doença estão o Lecanemab e o Donanemab. Ambos são terapias intravenosas de anticorpos anti-amilóide que visam facilitar a remoção das placas de beta-amilóide do SNC, retardando o declínio cognitivo e funcional em pacientes com DA precoce. Alguns dos efeitos colaterais dos tratamentos anti-amiloides são reações alérgicas graves, eventos decorrentes da infusão e também as anormalidades de imagens relacionadas ao amiloide (ARIA), indicando possíveis

microhemorragias (ARIA-H) ou então edemas cerebrais (ARIA-E) [62-64]. Além disso, os medicamentos que contêm tais fármacos são de alto custo, o que dificulta o acesso da população em geral. No ano de 2024, o primeiro anti-corpo desta classe, o aducanumabe (Aduhelm®), que obteve aprovação acelerada para uso em 2021, teve seu uso descontinuado nos EUA pela fabricante [29].

Para tratar os sintomas da DA, existem apenas duas classes de medicamentos que são clinicamente usadas para este manejo, sendo elas os inibidores da acetilcolinesterase (iAChE) e um antagonista de receptores NMDA. Deste modo, existem apenas 4 medicamentos estabelecidos e aprovados para realizar o tratamento, sendo eles, a memantina que é um antagonista não competitivo de receptores NMDA e outros três iAChE, sendo eles, o donepezil, a galantamina e a rivastigmina [62].

Considerando que o efeito deletério do excesso de βA sobre a memória e processos neuroplásticos é uma consequência direta da excitotoxicidade glutamatérgica, foi demonstrado que o bloqueio não competitivo de receptores NMDA, consegue atenuar a morte de neurônios em cultura induzida pela aplicação da βA [31]. Na clínica a memantina é capaz de retardar a progressão dos sintomas e conferir uma maior qualidade de vida ao indivíduo, apresentando boa tolerabilidade, sendo os efeitos adversos mais comuns tontura, diarreia e confusão mental [65].

Adicionalmente, os processos neurotóxicos relacionados à DA, afetam também neurônios produtores de acetilcolina (Ach), resultando na diminuição da transmissão colinérgica. Nas sinapses colinérgicas a enzima acetilcolinesterase (AchE) atua na degradação da Ach endógena, mantendo níveis fisiológicos deste neurotransmissor no meio extracelular. No contexto da DA, os iAChE reduzem a degradação de Ach e consequentemente aumentam a sua disponibilidade na fenda sináptica [66]. A tolerabilidade dos iAChE hoje em dia é boa, porém apresenta efeitos adversos pelo aumento da atividade colinérgica, principalmente relacionados ao sistema gastrointestinal (TGI), tais como, náusea, diarreia, cólicas abdominais e vômito. Também pode ocorrer, insônia e câimbras [26].

É importante enfatizar que todos os medicamentos estabelecidos e aprovados para o tratamento da DA são utilizados em estratégias terapêuticas que visam retardar sintomas e progressão da doença. Portanto, nenhum deles possuí efeito preventivo ou curativo, bem como, sua maior efetividade terapêutica está relacionada principalmente aos casos leves e moderados da doença. Mesmo assim,

eles têm se mostrado ineficazes à medida que a doença avança, pois são incapazes de interromper a morte neuronal [26, 67].

Assim sendo, é urgente o desenvolvimento de novas substâncias com capacidade de neuroproteção, visto que as opções terapêuticas são limitadas por questões de eficácia, segurança e custo.

1.6. Grandisina e seu análogo isoxazólico

Compreendendo a natureza multifatorial e frente as limitações dos tratamentos medicamentosos disponíveis para a DA, uma corrente crescente de pesquisadores voltou-se para a investigação de drogas com perfil de ação multialvo, dado que apresentam o potencial de modular simultaneamente diferentes vias biológicas, oferecendo uma abordagem mais abrangente para mitigar o déficit cognitivo e os danos neuronais induzidos pela βA [10, 11].

Os produtos naturais têm despertado crescente interesse por suas notáveis propriedades antioxidantes e anti-inflamatórias, duas propriedades essenciais no combate aos danos neuronais e ao estresse oxidativo que caracterizam a DA. Diversos estudos destacam que substâncias naturais, como flavonoides, gingeróis, taninos, antocianinas, triterpenos e alcaloides, podem reduzir a formação de placas beta-amiloides, bem como atenuar processos inflamatórios e oxidativos cerebrais, fatores críticos na progressão da doença. Esse tipo de abordagem, pode oferecer uma alternativa ou um tratamento adjuvante aos medicamentos sintéticos, visando não apenas retardar o avanço da DA, mas também melhorar a qualidade de vida dos pacientes [68-70].

Nesse contexto, estudos anteriores demonstraram que a Grandisina (GRA), uma neolignana isolada da planta *Virola surinamensis*, apresenta atividade anti-inflamatória *in vivo*, evidenciada por sua capacidade de inibir a contorção induzida por ácido acético, redução do tempo de lambida no teste de dor induzido por formalina e redução edema de orelha induzido por óleo de cróton [14]. Além disso, também foi demonstrado efeito antioxidante *in vivo* devido ao metabólito 4-O-desmetilgrandisina da grandisina apresentar efeito antioxidante [15].

Além disso, Zhai e colaboradores (2005) demonstraram que neolignanas tetrahidrofurânicas estruturalmente relacionadas apresentam efeitos neuroprotetores contra peptídeos Aβ em modelos *in vitro* [20]. No mesmo trabalho, foi observado que a incubação de neurônios com estas neolignanas resultou em aumento dos neuritos,

sugerindo que tais moléculas possuem efeito neurogênico. De forma complementar, uma pesquisa realizada pelo nosso grupo evidenciou que um derivado triazólico da grandisina foi capaz de prevenir déficits cognitivos em animais submetidos à injeção intracerebral de Aβ, mediados por mecanismos antioxidantes e anti-inflamatórios. [41].

De forma análoga, pesquisas com neolignanas cujos núcleos foram substituídos por grupos isoxazólicos revelaram um vasto espectro de atividades biológicas, abrangendo efeitos antitripomastigota, antileishmania e anti-inflamatórios, observados tanto em ensaios *in vitro* quanto *in vivo* [16-19].

1.7. Justificativa

Baseado no que foi exposto, a DA apresenta mecanismos inflamatórios e oxidativos bem estabelecidos, o que contribui com a morte neural progressiva. Considerando que neolignanas apresentam efeitos anti-inflamatórios, antioxidantes e neuroprotetores é possível supor que tanto a GRA quanto o seu análogo isoxazólico (AIG) possam contrapor os efeitos deletérios causados pela βA. Neste sentido, tais moléculas nunca foram testadas num modelo *in vivo* da DA. Sendo assim este estudo investigou os efeitos da GRA e AIG em um modelo animal da DA, avaliando os efeitos sobre a memória e as alterações no perfil oxidativo.

2. OBJETIVOS

2.1. Objetivo geral

Avaliar o efeito do tratamento repetido com grandisina (GRA) e do seu análogo isoxazólico (AIG), sobre a memória de reconhecimento em camundongos com submetidos ao modelo animal da doença de Alzheimer.

2.2. Objetivos específicos

3.2.1 - Mensurar o efeito do tratamento repetido com GRA e AIG por via intraperiotonial (i.p.) e oral, sobre a memória de reconhecimento em camundongos com deposição de βA.

3.2.2 - Avaliar os possíveis efeitos antioxidantes do tratamento repetido com GRA ou AIG sobre os níveis de peroxidação lipídica (TBARS), glutationa reduzida (GSH) e mieloperoxidase (MPO) no CTX e HPC.

ARTIGO

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GRANDISIN AND ITS ISOXAZOLE ANALOG PREVENT MEMORY DEFICITS THROUGH ANTIOXIDANT EFFECTS IN ANIMALS SUBJECTED TO AN ALZHEIMER'S DISEASE MODEL

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ABSTRACT

Alzheimer's disease (AD) is associated with the deposition of β-amyloid (βA) protein in brain regions such as the cortex (CTX) and hippocampus (HPC), leading to the activation of neurotoxic, oxidative, and inflammatory pathways. Grandisin (GRA) and its isoxazole derivative (IGA) are neolignans with that shows neuroprotective potential, since structurally related compounds have shown to protect neurons against βA *in vitro*. Additionally, a triazole bioisoster of grandisin prevented cognitive deficits in animals exposed to Aβ by reducing cytokine levels in HPC and lipid peroxidation in CTX and HPC. However, GRA and AIG neuroprotective effects remain unexplored *in vivo*. This study evaluated GRA and AIG effects on memory and oxidative profiles in an AD mouse model. Male C57/Bl6 mice (CEUA protocol: 1.272/2023) received intracerebroventricular (i.c.v.) Aβ or vehicle injection. After 24 hours, mice underwent daily oral or intraperitoneal (i.p.) treatments with GRA (1 mg/kg), IGA (1 mg/kg), or vehicle for 7 days. Groups included Control i.c.v. /Vehicle (oral or i.p)., βA i.c.v./Vehicle (oral or i.p.), βA i.c.v./GRA (oral or i.p.), and βA i.c.v./IGA (oral or i.p.). At the end of the treatment, the Object Recognition Test (ORT) was performed. Immediately after ORT animals were euthanized for HPC and CTX collection for lipid peroxidation, myeloperoxidase (MPO), and reduced glutathione (GSH) measurement. GRA (i.p.) but not IGA (i.p.) prevented memory deficits and reduced oxidative markers in HPC. Despite GRA (oral) protected memory and improved oxidative profiles, IGA (oral) demonstrated to be superior in memory improvement. These findings show that GRA and its derivative have *in vivo* neuroprotective effects against βA oligomer-induced damage and suggest that AIG may act as a pro-drug.

Keywords: Alzheimer's Disease; Grandisin; Multi-targeted drugs; Neuroprotection

3. INTRODUCTION

Dementia is characterized by a progressive and significant decline in cognitive and functional abilities, reducing autonomy in daily activities, social interactions, and economic participation. Currently, dementia is the seventh leading cause of death worldwide, affecting over 55 million people, with approximately 10 million new cases each year [1]. Today, Alzheimer's Disease (AD) accounts for 60–70% of global dementia cases [2]. Several demographic factors, such as age, sex, race, and social class, are risk factors for the development of AD, with age being the most significant [3].

The overproduction of the β-amyloid peptide in AD results in the formation of β-amyloid oligomers (AβO), which accumulate in various regions of the central nervous system (CNS) involved in memory formation and other cognitive processes, such as the cortex (CTX) and hippocampus (HPC). This leads to the activation of oxidative and neuroinflammatory pathways [4]. For example, AβO deposition stimulates the secretion of glutamate by presynaptic neurons and inhibits its reuptake by astrocytes, resulting in an excess of glutamate in the synaptic cleft. This excess of glutamate diffuses into the spiny neck and activates extrasynaptic glutamatergic receptors (E-NMDA), triggering oxidative damage by reactive oxygen species (ROS) and mediating tau protein hyperphosphorylation, impairing synaptic plasticity and neural survival [5].

It is also believed that AβO themselves act as damage-associated molecular patterns (DAMPs), which can be recognized and phagocytosed by microglia. However, when phagocytosis fails, microglia remain chronically activated, releasing reactive oxygen species and pro-inflammatory cytokines. This prolonged activation triggers a continuous inflammatory response, promoting the migration of additional immune cells, thereby intensifying oxidative damage and neuroinflammation [6]. Studies show that intracerebroventricular administration of AβO increases the activity of microgial myeloperoxidase (MPO) in the CTX and HPC of mice, demonstrating that oligomers attract and activate immune cells [36]. MPO, in turn, produces hypochlorous acid, a highly reactive substance that contributes to the oxidative damage characteristic of AD [37]. The hypochlorous acid released by MPO generates superoxide anions, which attack the proteins and lipids in neuronal membranes, triggering a lipid peroxidation process that compromises cellular integrity, resulting in dysfunction and neuronal death [38].

During neurodegenerative processes, levels of reduced glutathione (GSH), a potent antioxidant, are often decreased. This decrease indicates a deficiency in the ability to protect against oxidative stress, suggesting that GSH is not sufficiently available to combat the cellular damage associated with these processes [39, 40].

Therapeutic strategies for AD aim to reduce symptom intensity and slow disease progression to extend patient survival. Currently approved medications are effective in mild to moderate cases but are ineffective in more advanced stages. Furthermore, current therapy cannot halt cognitive decline in AD and often presents severe side effects that may preclude their use [7, 8, 9].

Considering the multifactorial nature of AD, one of the most current pharmacological approaches involves drugs with multitarget profiles, which could act on various sites, contributing to more effective control of cognitive impairment and the deleterious effects of β-amyloid on neurons [10, 11, 12]. In this context, natural products can serve as a source of new drugs due to the variety of compounds found in plants with anti-inflammatory and antioxidant properties [13].

Previous studies have reported the anti-inflammatory and antioxidant effects *in vivo* of grandisin (GRA), a neolignan found in the plant *Virola surinamensis* [14, 15]. Similarly, studies using neolignans modified with isoxazole groups have demonstrated a wide range of biological activities, such as antitrypanosomal, antileishmanial, and anti-inflammatory effects, both *in vitro* and *in vivo* [16, 17, 18, 19]. Additionally, the study by Zhai et al. (2005) demonstrated that several structurally related neolignans have neuroprotective effects against Aβ peptides *in vitro* [20]. Furthermore, a study by our group showed that a triazole derivative of grandisin prevented cognitive deficits in animals injected intracerebrally with Aβ through antioxidant and anti-inflammatory effects [41]. However, neither GRA nor its isoxazole analog (IGA) has been tested in an *in vivo* AD model. Therefore, this study investigated the potential effects of both substances in an animal model of AD, evaluating their effects on memory and oxidative profile changes in the HPC and CTX.

4. MATERIALS AND METHODS

4.1. Animals

Male C57/Bl6 mice, 3 months-old, were used in this study. The animals were housed with *ad libitum* access to food and water in a temperature-controlled room. The project was approved by the Ethics Committee of the Federal University of Mato Grosso do Sul (protocol number: 1.272/2023).

4.2. Preparation of fractionated Aβ protein oligomers

The preparation of amyloid-beta oligomers (AβO) was conducted under the supervision of Dr. Adriano Sebollela, in the Department of Biochemistry, School of Medicine of Ribeirão Preto. The oligomers were prepared from the synthetic βA 1-42 peptide (California Peptides). The peptide was solubilized in hexafluoroisopropanol, and the solvent was evaporated to produce dry films, which were then dissolved in sterile, anhydrous dimethyl sulfoxide to create a 5 mM solution. This solution was further diluted to 100 µM in PBS and incubated for 16 hours at 4°C. The preparation was then centrifuged at 14,000 rpm for 10 minutes at 4°C to remove protofibrils and insoluble fibrils. The supernatant containing soluble AβO) was stored at 4°C. The concentration of AβO was measured by ELISA [21].

4.3. Intracerebroventricular injection of βA oligomers

For intracerebroventricular (i.c.v.) injection of βA, the animals were anesthetized via intraperitoneal (i.p.) injection with ketamine (70 mg/kg) and xylazine (10 mg/kg) at a volume of 1 mg/kg. The stereotaxic coordinates for the unilateral implantation of the guide cannula into the left ventricle are described in the work by Diniz et al. (2017) (LANE RM et al., 2006). The final injection volume was 2 µL, using a Hamilton syringe with a 10 µL capacity [22].

4.4. Pharmacological treatment

The extraction and purification of GRA (Figure 1-A) were carried out under the supervision of Professor Nídia Cristiane Yoshida from the Institute of Chemistry (INQUI-UFMS). IGA (Figure 1-B) was synthesized and handled in the Pharmaceutical Chemistry Laboratory together with the Pharmaceutical Technology Laboratory at the Federal University of Mato Grosso do Sul (UFMS), supervised by Dr. Adriano César de Morais Baroni and Dr. Éverton do Nascimento Alencar. The animals were treated

once per day, for 7 days via intraperitoneal (i.p.) or oral administration with GRA (1 mg/kg), IGA (1 mg/kg), or vehicle (nanoemulsion). The animals were divided into the following experimental groups: Control (i.c.v.) / Vehicle (i.p. or oral), βA (i.c.v.) / Vehicle (i.p. or oral), βA (i.c.v.) / GRA (i.p. or oral), and βA (i.c.v.) / IGA (i.p. or oral).

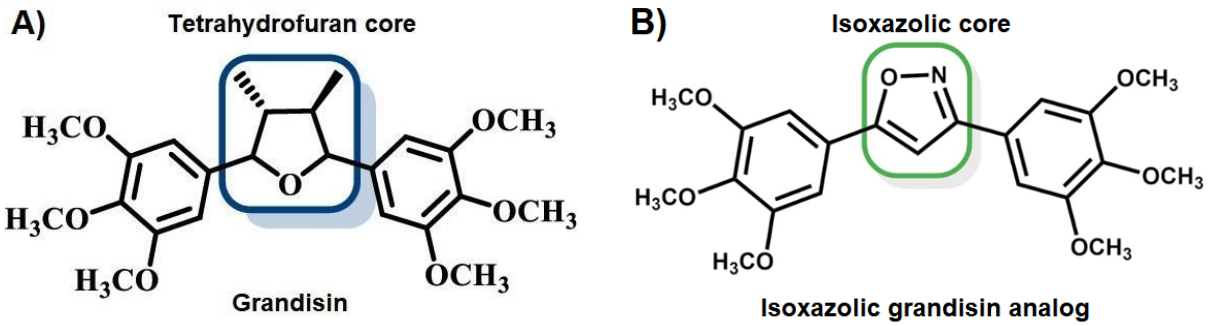

Fig. 1 Chemical structure of grandisin (A) and the isoxazole grandisin analog (B)

4.5. Object recognition test

The Object Recognition Test (ORT) was conducted according to the methodology described by Figueiredo et al., 2013 [22]. Initially, the animals were placed individually in a circular acrylic arena for the habituation session (t0). One day later, the animals were reintroduced to the same arena with two identical objects placed symmetrically, and the time spent exploring each object was measured (acquisition session – t1). In the next day, mice were placed back in the arena, with one of the objects from t1 replaced by a new object (retention session – t2). The total time for each session was 5 minutes. The discrimination index (d2) was used to analyze the exploration time between the new and familiar objects. This index is calculated as the difference between the exploration of the familiar object (FO) and the new object (NO), divided by the total exploration time of the new and familiar objects: d2 = (NO – FO) / (NO + FO).

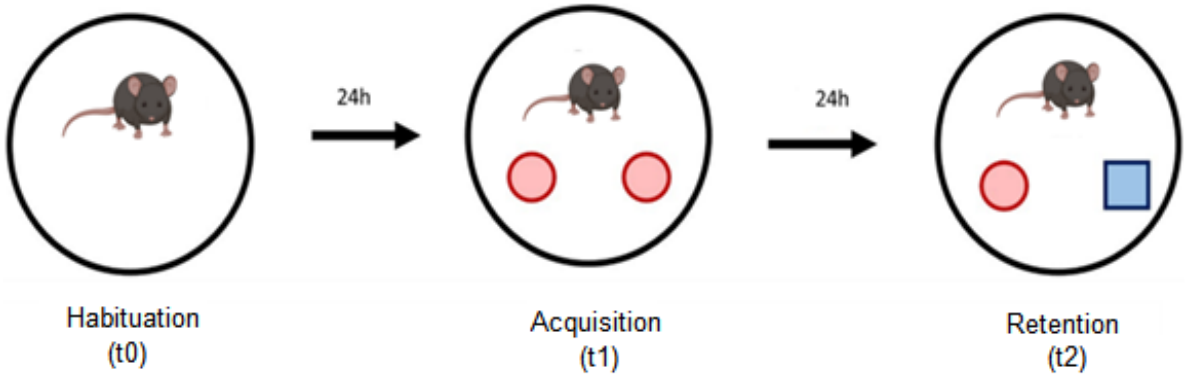

Fig. 2 Representative scheme of the experimental protocol for the 24-hour object recognition test (ORT). Figure created using BioRend software

4.6. Determination of myeloperoxidase activity

For the oxidative profile measurements, myeloperoxidase (MPO) activity was determined. For this purpose,20 µL of the samples were transferred into tubes containing 150 µL of a pre-prepared solution (165 µL of o-dianisidine hydrochloride, 50 µL of 30% H2O2, distilled water, and 50 nM monosodium phosphate). The tubes were then incubated for 15 minutes at 37°C. To stop the reaction, 15 µL of 1% sodium azide was added, and the tubes were incubated again for 10 minutes at 37°C. Absorbance was measured at 450 nm using a spectrophotometer, and the results were compared to a standard curve. MPO activity is expressed as mU/mL [23].

4.7. Evaluation of lipoperoxidation

The evaluation of lipid oxidation was performed by measuring the levels of thiobarbituric acid reactive species (TBARS), particularly malondialdehyde (MDA). The lysate solution was precipitated with 800 µL of 12% (v/v) trichloroacetic acid (TCA) and then incubated with 0.73% TBA for 60 minutes at 100°C in Tris-HCl buffer (60 nM) + 0.1 nM DTPA (diethylenetriaminepentaacetic acid), pH 7.4. The solutions were then centrifuged for 5 minutes at 1500 RCF. Absorbance was measured at 535 nm using a spectrophotometer, in duplicate. The formula used was ε = 1.56 × 10^5 M^-1cm^-1, with results expressed in nmol/mL. The results were normalized by calculating the protein content in each sample [24].

4.8. Evaluation of reduced glutathione

The concentration of GSH was determined through non-protein thiols. Trichloroacetic acid (TCA 12%, 1:4, v/v) was used to precipitate the thiols. Then, 0.2 mL of 2.5 mM 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) was added to cuvettes containing 1.9 mL of 0.2 M phosphate buffer (pH 8.0) and 0.1 mL of the sample. After incubation and agitation, the result was the formation of the yellow-colored thiolate anion (TNB), which was read at 412 nm [25].

4.9. Evaluation of carbonylated protein

For analysis of protein carbonyl concentrations, 100 µL of sample was added to 600 µL of 2,4-dinitrophenylhydrazine (DNPH, Sigma-Aldrich) for derivatization, with a later addition of ethanol/ethyl acetate (1:1 v/v) to remove DNPH. Afterward, 600 µL of guanidine chloride was added, and the optical density was read at 370 nm in the ELISA reader (Thermo Fischer Scientific Oy, Vantaa, Finland) [71, 72].

4.10. Statistical analysis

The statistical analysis of the data was performed using ANOVA for paired group comparison against the control, with a minimum significance level of $p<0.05$. The software used was GraphPad Prism version 8.0.1.

5. RESULTS

5.1. GRA, but not IGA prevents recognition memory impairment induced by AβO in mice treated intraperitoneally

In this protocol, we observed that the animals in the Aβ i.c.v. / Vehicle i.p. and Aβ i.c.v. / IGA i.p. groups showed a reduction in the discrimination index (d2) in the ORT test compared to the Control i.c.v. / Vehicle i.p. group (Cont.-Veh: 0.42 ± 0.07; Aβ-Veh: -0.16 ± 0.06; Aβ-IGA: -0.25 ± 0.08; p< 0.05* and <0.01**). On the other hand, AβO animals treated with GRA i.p. did not show a significant difference in the d2 index compared to the Control i.c.v. / Vehicle i.p. group (Cont.-Veh: 0.42 ± 0.07; Aβ-GRA: 0.43 ± 0.04; p> 0.05#) (Figure 3).

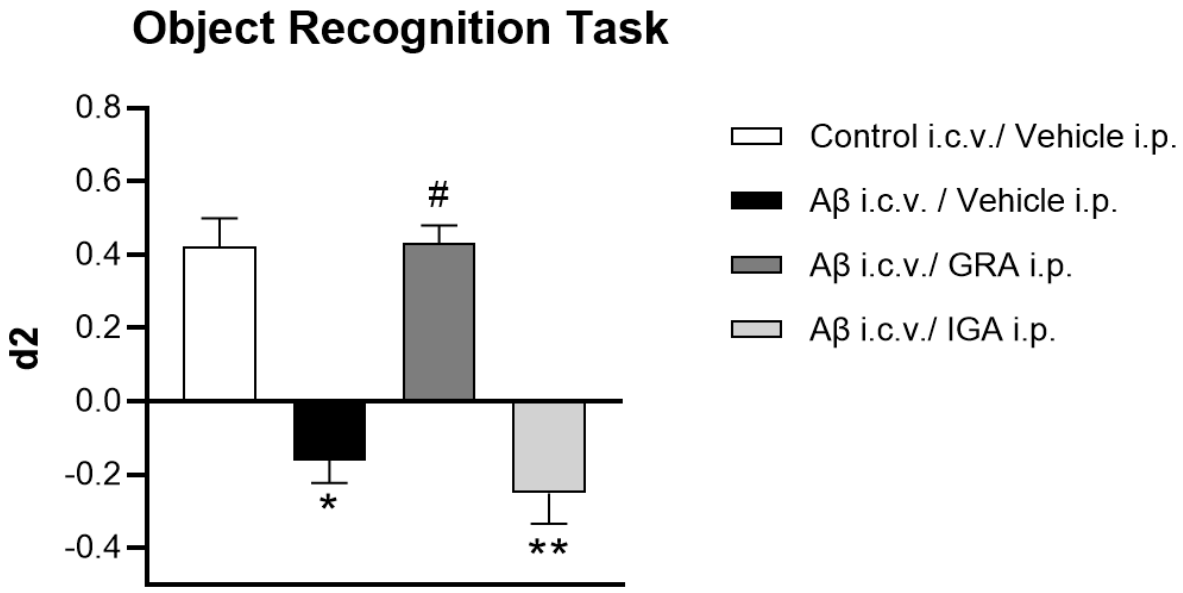

Fig. 3 Discrimination index (d2) in object recognition task (ORT). Experimental groups were as follows: Control intracerebroventricular (i.c.v.)/ Vehicle intraperitoneal (i.p.), n = 7; Aβ i.c.v./ Vehicle i.p., n = 7; Aβ i.c.v./ GRA i.p., n = 8; Aβ i.c.v./ IGA i.p., n = 7. Values are expressed as mean ± standard error of mean. *p < 0.05, **p < 0.01, and #p > 0.05 compared with the Control i.c.v./ Vehicle i.p. group. Data were subjected to nonparametric test followed by the Kruskal-Wallis post hoc test

5.2. GRA, but not IGA reduces myeloperoxidase activity in the hippocampus of AβO mice treated intraperitoneally

In this protocol, the activity of the enzyme myeloperoxidase (MPO), a marker of inflammatory and oxidative activity, was measured in the collected structures (Figure 4). In the HPC, we found that the Aβ i.c.v./ Vehicle i.p. and Aβ i.c.v./ IGA i.p. groups showed higher MPO activity compared to the Control i.c.v./ Vehicle i.p. group (Aβ-Veh: 18.9 ± 6.8; Aβ-IGA: 14.2 ± 2.4; Cont.-Veh: 4.7 ± 1.6; p< 0.05*) (Figure 4-A). The Aβ i.c.v./ GRA i.p. group exhibited MPO activity similar to the control group (Aβ-GRA: 7.1 ± 1.8; Cont.-Veh: 4.7 ± 1.6; p> 0.05#). MPO activity determinations in the CTX did not show significant differences between groups (Cont.-Veh: 16.9 ± 1.50; Aβ-Veh: 15.5 ± 1.44; Aβ-GRA: 15.0 ± 0.89; Aβ-IGA: 16.3 ± 1.19; p> 0.05#) (Figure 4-B).

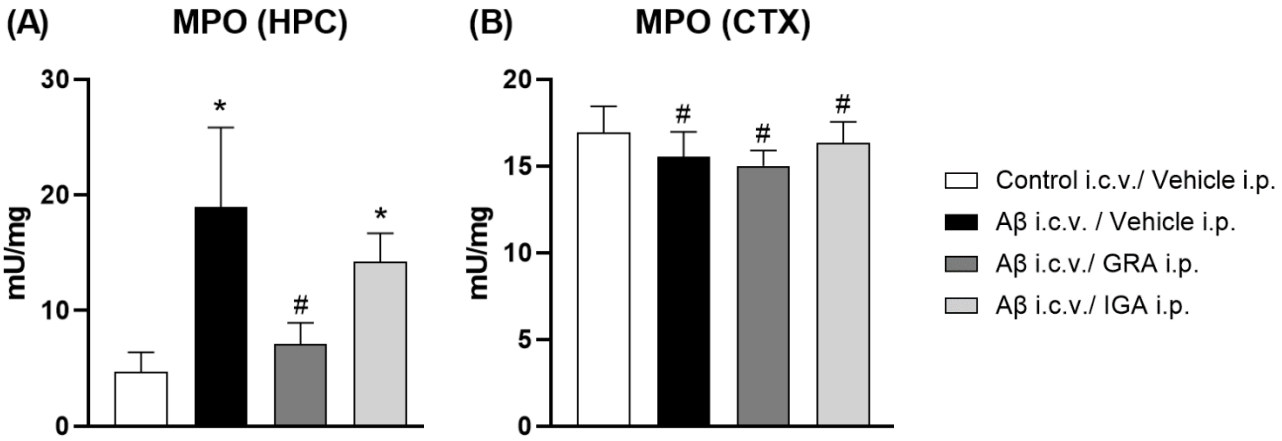

Fig. 4 Myeloperoxidase activity (mU/mg) content in **(A)** the hippocampus (HPC) and **(B)** cortex (CTX) of AβO-injected and control mice treated with vehicle or isoxazole grandisin analogue (IGA) for 7 days. Experimental groups were as follows: Control intracerebroventricular (i.c.v.)/ Vehicle intraperitoneal (i.p.), n = 7; Aβ i.c.v./ Vehicle i.p., n = 7; Aβ i.c.v./ GRA i.p., n = 8; Aβ i.c.v./ IGA i.p., n = 7. Values are expressed as mean ± standard error of mean. *p < 0.05, **p < 0.01, and #p > 0.05 compared with the Control i.c.v./ Vehicle i.p. group. Data were subjected to nonparametric test followed by the Kruskal-Wallis post hoc test

5.3. GRA, but not IGA prevents the increase of lipid peroxidation in the hippocampus of AβO mice treated intraperitoneally

Lipid peroxidation levels (thiobarbituric acid-reactive substances - TBARS) were also measured in the CTX and HPC of the animals (Figure 5). In the HPC (Figure 5-A), the Aβ i.c.v./ Vehicle i.p. and Aβ i.c.v./ IGA i.p. groups showed increased TBARS levels compared to the Control i.c.v./ Vehicle i.p. group (Cont.-Veh: 0.043 ± 0.003; Aβ-Veh: 0.065 ± 0.008; Aβ-IGA: 0.079 ± 0.024; p< 0.05*). The Aβ i.c.v./ GRA i.p. group displayed TBARS levels similar to the control group (Aβ-GRA: 0.048 ± 0.003; Cont.-Veh: 0.043 ± 0.003; p> 0.05#). In the CTX (Figure 5-B), no significant differences were observed between the experimental groups (Cont.-Veh: 0.104 ± 0.010; Aβ-Veh: 0.101 ± 0.012; Aβ-GRA: 0.103 ± 0.015; Aβ-IGA: 0.110 ± 0.004; p> 0.05#).

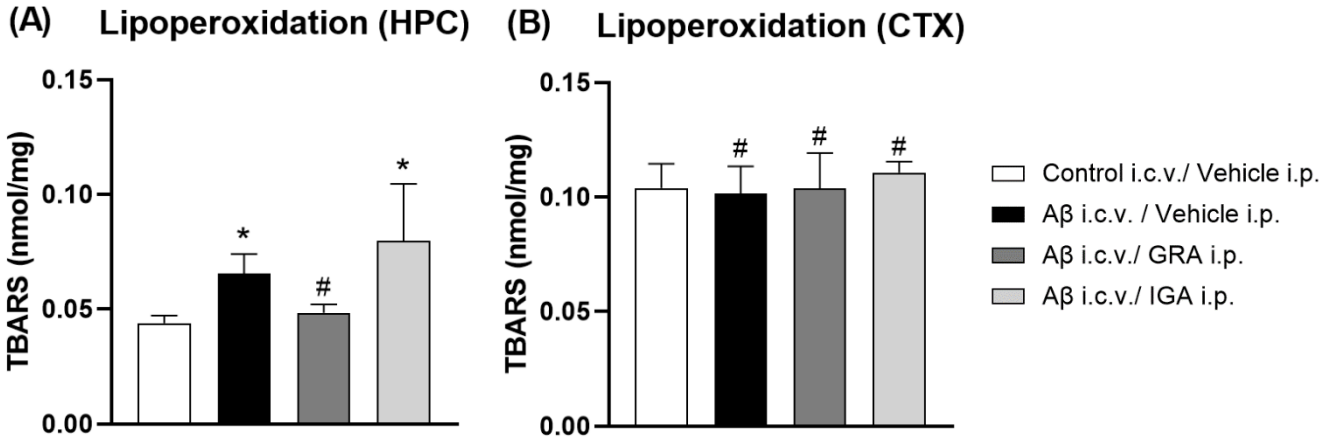

Fig. 5 Lipoperoxidation (nmol/mg) content in **(A)** the hippocampus (HPC) and **(B)** cortex (CTX) of AβO-injected and control mice treated with vehicle or isoxazole grandisin analogue (IGA) for 7 days. Experimental groups were as follows: Control intracerebroventricular (i.c.v.)/ Vehicle intraperitoneal (i.p.), n = 7; Aβ i.c.v./ Vehicle i.p., n = 7; Aβ i.c.v./ GRA i.p., n = 8; Aβ i.c.v./ IGA i.p., n = 7. Values are expressed as mean ± standard error of mean. *p < 0.05, **p < 0.01, and #p > 0.05 compared with the Control i.c.v./ Vehicle i.p. group. Data were subjected to nonparametric test followed by the Kruskal-Wallis post hoc test

5.4. GRA and IGA prevent the decrease in reduced glutathione levels in the hippocampus of AβO mice treated intraperitoneally

Additionally, we evaluated the reduced glutathione (GSH) levels in the hippocampus of these animals (Figure 6). A reduction in GSH levels were observed in the Aβ i.c.v./ Vehicle i.p. group compared to the Control i.c.v./ Vehicle i.p. group (Cont.-Veh: 0.042 ± 0.005; Aβ-Veh: 0.023 ± 0.003; p< 0.05*). In contrast, animals treated with GRA or IGA showed GSH levels similar to the control group (Aβ-GRA: 0.038 ± 0.005; Aβ-IGA: 0.032 ± 0.002; Cont.-Veh: 0.042 ± 0.005; p> 0.05#).

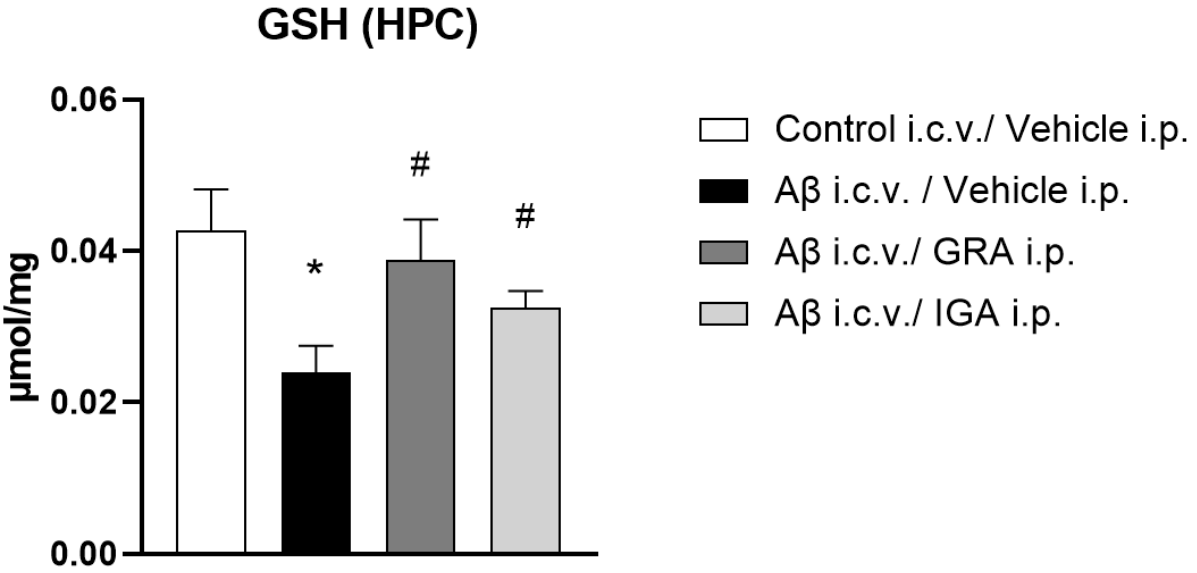

Fig. 6 Reduced glutathione activity (µmol/mg) content in the hippocampus (HPC) and cortex (CTX) of AβO-injected and control mice treated with vehicle or isoxazole grandisin analogue (IGA) for 7 days. Experimental groups were as follows: Control intracerebroventricular (i.c.v.)/ Vehicle intraperitoneal (i.p.), n = 7; Aβ i.c.v./ Vehicle i.p., n = 7; Aβ i.c.v./ GRA i.p., n = 8; Aβ i.c.v./ IGA i.p., n = 7. Values are expressed as mean ± standard error of mean. *p < 0.05, **p < 0.01, and #p > 0.05 compared with the Control i.c.v./ Vehicle i.p. group. Data were subjected to nonparametric test followed by the Kruskal-Wallis post hoc test

5.5. AβO injection has no effect on carbonylated protein levels in the hippocampus and cortex of intraperitoneally treated mice

Carbonylated protein levels were measured in the hippocampus (HPC) and cortex (CTX) of the animals (Figure 7). No significant differences were observed between the groups in either the HPC (Figure 7-A) or CTX (Figure 7-B) (HPC: Cont.-Veíc.: 19.2 ± 2.46; Aβ-Veíc: 19.1 ± 3.2; Aβ-GRA: 18.0 ± 2.07; Aβ-IGA: 17.4 ± 2.68; $p > 0.05$#) (CTX: Cont.-Veíc.: 12.5 ± 0.21; Aβ-Veíc: 13.1 ± 0.27; Aβ-GRA: 12.6 ± 0.49; Aβ-IGA: 13.9 ± 0.26; $p > 0.05$#).

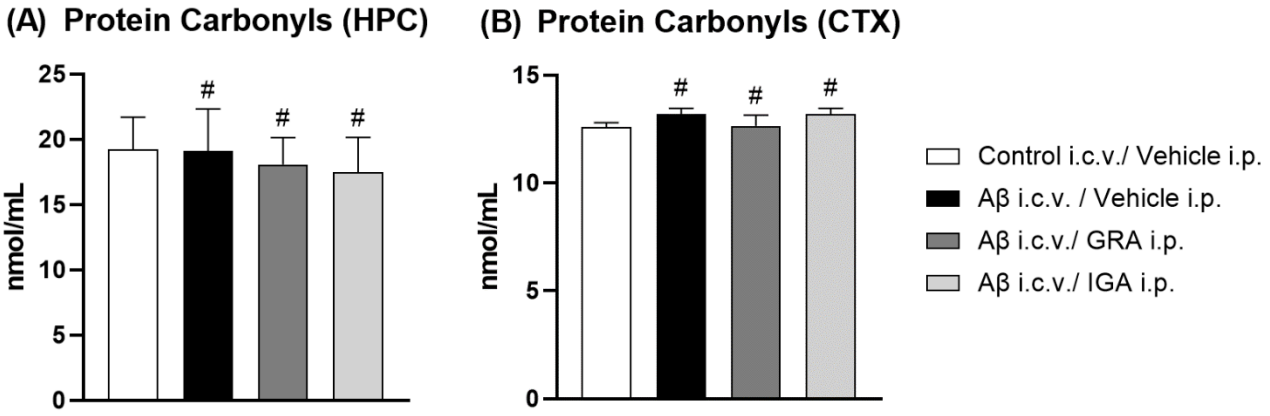

Fig. 7 Protein Carbonyl (nmol/mL) content in **(A)** the hippocampus (HPC) and **(B)** cortex (CTX) of AβO-injected and control mice treated with vehicle or isoxazole grandisin analogue (IGA) for 7 days. Experimental groups were as follows: Control intracerebroventricular (i.c.v.)/ Vehicle intraperitoneal (i.p.), n = 7; Aβ i.c.v./ Vehicle i.p., n = 7; Aβ i.c.v./ GRA i.p., n = 8; Aβ i.c.v./ IGA i.p., n = 7. Values are expressed as mean ± standard error of mean. *$p < 0.05$, **$p < 0.01$, and #$p > 0.05$ compared with the Control i.c.v./ Vehicle i.p. group. Data were subjected to nonparametric test followed by the Kruskal-Wallis post hoc test

5.6. Oral treatment with both GRA and IGA prevented recognition memory impairment in AβO mice

Furthermore, repeated oral treatment with the compounds was also evaluated (Figure 8). It was observed that the Aβ i.c.v./ Vehicle-oral group had a lower d2 index compared to the Control i.c.v./ Oral Vehicle group (Cont.-Veh. oral: 0.33 ± 0.09 vs Aβ-Veh. oral: -0.13 ± 0.06; p< 0.05*). On the other hand, the Aβ i.c.v./ GRA oral and Aβ i.c.v./ IGA oral groups showed similar levels to the control group (Aβ-GRA oral: 0.14 ± 0.06; Aβ-IGA oral: 0.46 ± 0.06; Cont.-Veh. oral: 0.33 ± 0.09; p> 0.05#). Furthermore, we observed that the group treated with IGA-oral showed significant higher d2 values than oral GRA (Aβ-GRA oral: 0.14 ± 0.06; Aβ-IGA oral: 0.46 ± 0.06; p< 0.05*).

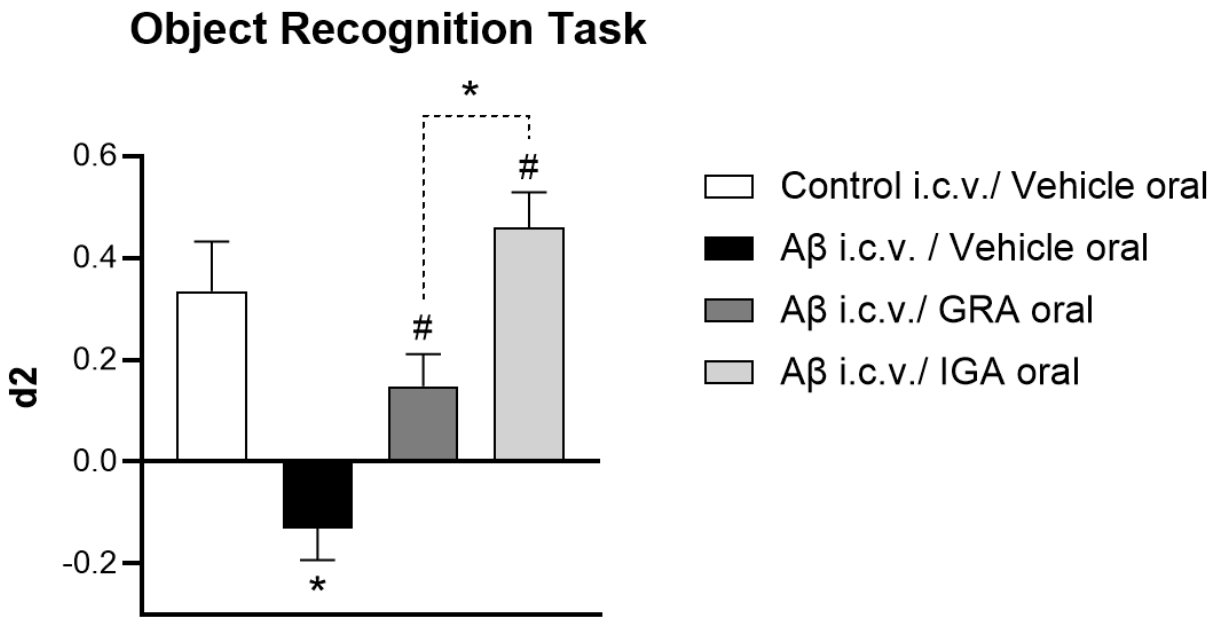

Fig. 8 Discrimination index (d2) in the Object Recognition Test (ORT) of treatment with Grandisin (GRA) and its isoxazole analog (IGA) administered orally for 7 days. Experimental groups were as follows: Control intracerebroventricular (i.c.v.)/ Vehicle oral, n = 4; Aβ i.c.v./ Vehicle oral, n = 4; Aβ i.c.v./ GRA oral, n = 8; Aβ i.c.v./ IGA oral, n = 5. The values are expressed as mean ± standard error of the mean. *p < 0.05, and #p > 0.05 compared to the Control i.c.v./ Vehicle i.p. group. Data were subjected to a non-parametric Kruskal-Wallis test

5.7. Oral treatment with both GRA and IGA reduce myeloperoxidase levels in the hippocampus of AβO mice

The evaluation of MPO activity for oral treatment was performed in the HPC and CTX (Figure 9). In the HPC (Figure 9-A), the Aß i.c.v./Vehicle-oral. group showed increased MPO activity compared to the Control i.c.v./Vehicle oral group (Cont.-Veíc. oral: 6.47 ± 1.37; Aß-Veíc. oral: 21.2 ± 1.87; p< 0.05*). On the other hand, the groups treated with GRA or IGA showed similar levels to the control group (Cont.-Veíc. oral: 6.47 ± 1.37; Aß-GRA oral: 6.91 ± 0.41; Aß-IGA oral: 7.62 ± 0.42; p> 0.05#). In the CTX (Figure 9-B), no statistical differences were observed between the groups (Cont.-Veíc. oral: 5.60 ± 0.53; Aß-Veíc. oral: 6.34 ± 0.10; Aß-GRA oral: 5.98 ± 0.31; Aß-IGA oral: 6.10 ± 0.25; p> 0.05#).

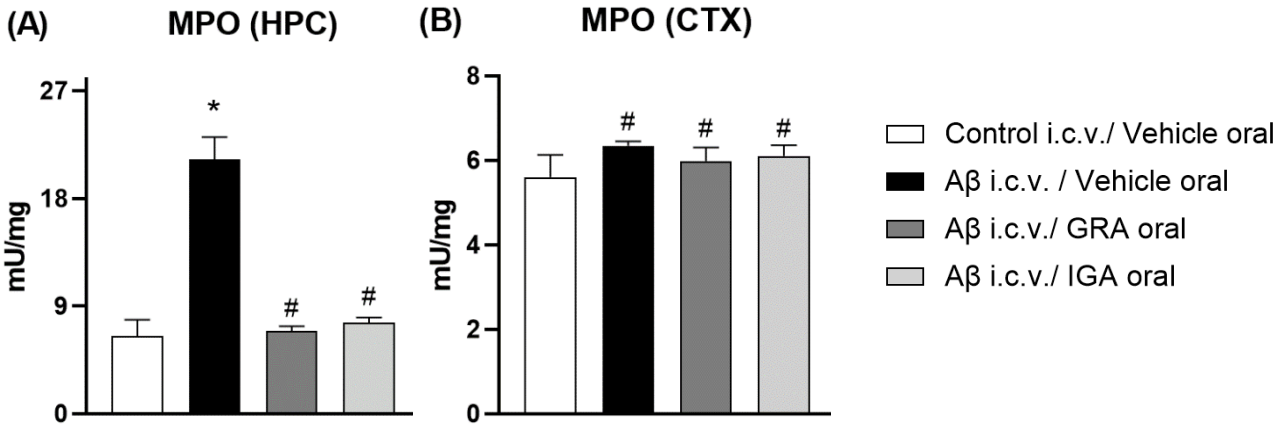

Fig. 9 Myeloperoxidase activity (mU/mg) content in **(A)** the hippocampus (HPC) and **(B)** cortex (CTX) of AβO-injected and control mice treated with vehicle or isoxazole grandisin analogue (IGA) administered orally for 7 days. Experimental groups were as follows: Control intracerebroventricular (i.c.v.)/ Vehicle oral, n = 4; Aβ i.c.v./ Vehicle oral, n = 4; Aβ i.c.v./ GRA oral, n = 8; Aβ i.c.v./ IGA oral, n = 5. Values are expressed as mean ± standard error of mean. *p < 0.05, **p < 0.01, and #p > 0.05 compared with the Control i.c.v./ Vehicle i.p. group. Data were subjected to nonparametric test followed by the Kruskal-Wallis post hoc test

5.8. Oral treatment with both GRA and IGA prevents the increase of lipid peroxidation in the hippocampus of AβO mice

In the oral treatment, lipid peroxidation was also measured in the HPC and CTX (Figure 10). In the HPC (Figure 10-A), the Aß i.c.v./Vehicle oral group showed an increase in TBARS levels compared to the control group (Cont.-Veíc. oral: 0.061 ± 0.01; Aß-Veíc. oral: 0.252 ± 0.04; p< 0.05*), whereas the groups treated with GRA or IGA exhibited levels similar to the control (Cont.-Veíc. oral: 0.061 ± 0.01; Aß-GRA oral: 0.064 ± 0.01; Aß-IGA oral: 0.068 ± 0.02; p> 0.05#). In the CTX (Figure 10-B), no significant differences were observed between the groups (Cont.-Veíc. oral: 0.050 ± 0.007; Aß-Veíc. oral: 0.049 ± 0.004; Aß-GRA oral: 0.045 ± 0.002; Aß-IGA oral: 0.043 ± 0.004; p> 0.05#).

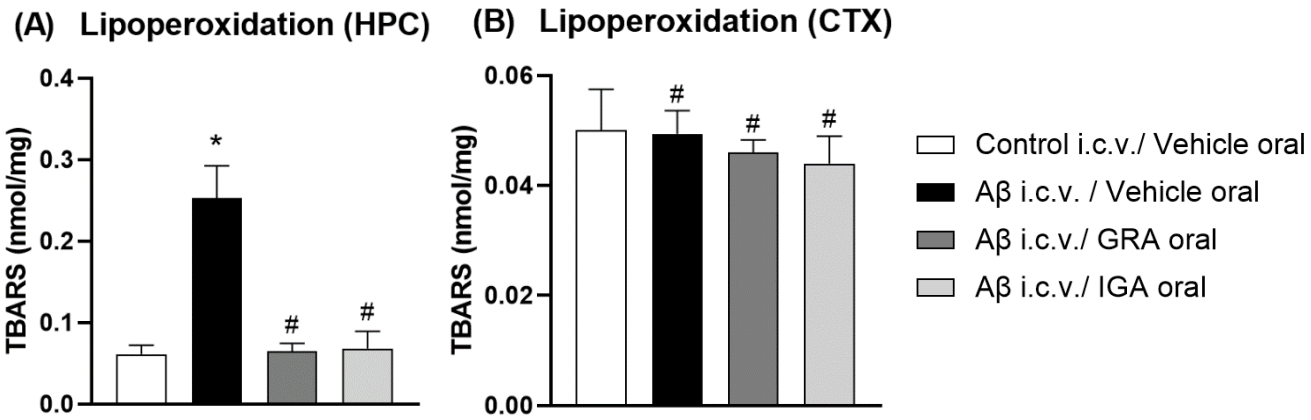

Fig. 10 Lipoperoxidation (nmol/mg) content in **(A)** the hippocampus (HPC) and **(B)** cortex (CTX) of AβO-injected and control mice treated with vehicle or isoxazole grandisin analogue (IGA) administered orally for 7 days. Experimental groups were as follows: Control intracerebroventricular (i.c.v.)/ Vehicle oral, n = 4; Aβ i.c.v./ Vehicle oral, n = 4; Aβ i.c.v./ GRA oral, n = 8; Aβ i.c.v./ IGA oral, n = 5. Values are expressed as mean ± standard error of mean. *p < 0.05, **p < 0.01, and #p > 0.05 compared with the Control i.c.v./ Vehicle i.p. group. Data were subjected to nonparametric test followed by the Kruskal-Wallis post hoc test

6. DISCUSSION

Several mechanisms influence the neurobiology of Alzheimer's disease (AD) [26]. Among these, oxidative and inflammatory processes stand out, playing a crucial role in cognitive decline and memory loss [27].

Furthermore, it was observed that repeated i.p. treatment with GRA, but not with its isoxazole derivative, was capable of preventing cognitive impairment and memory loss in mice that received i.c.v. injection of Aß (Figure 3). Similarly, oral administration of GRA, although with a less pronounced effect, also attenuated memory damage in animals treated with i.c.v. Aß (Figure 8). These properties may be associated with the compound antioxidant and anti-inflammatory effects [14, 15]. Such properties are promising, considering that in AD, the activation of extrasynaptic NMDA (E-NMDA) glutamatergic receptors promotes the production and release of oxidant substances, such as nitric oxide and superoxide anion. These substances contribute to neuronal death and consequent cognitive decline [30]. In addition to oxidative processes, AD is also characterized by inflammatory processes mediated by Aß oligomers, which induce the release of pro-inflammatory mediators [31]. This process activates microglia, leading to the production of pro-inflammatory cytokines such as IL-1ß, IL-6, and TNF, along with other mediators (ROS and NFkB), creating a neurotoxic environment that promotes disease progression [32, 42]. Given that neolignans, such as GRA, possess anti-inflammatory properties, it is plausible to suggest that this drug may reduce cytokine levels in affected structures. However, additional studies are needed to corroborate this hypothesis.

In the hippocampus, we observed that lipoperoxidation levels and MPO activity were elevated in the Aß i.c.v./Vehicle i.p. group compared to the control group (Figures 4-A and 5-A). Therefore, in this study, lipoperoxidation and MPO evaluations were used as markers of oxidative stress [34]. In the hippocampus, animals treated with GRA via intraperitoneal (i.p.) administration exhibited lipoperoxidation levels and MPO activity similar to those of the control group. Additionally, oral treatment with GRA or IGA revealed a reduction in oxidative markers, such as MPO (Figure 9) and lipoperoxidation (Figure 10), demonstrating that these substances also exhibit antioxidant activity when administered orally. These findings align with other studies that reported GRA ability to scavenge free radicals [15]. This antioxidant mechanism may contribute to reducing lipid peroxidation in both regions. On the other hand, neither i.p. nor oral treatment showed statistically significant differences among

experimental groups in lipoperoxidation levels and MPO activity in the cortex (Figures 4-B, 5-B, 9-B, and 10-B). A study by Figueiredo et al. (2013) demonstrated that persistent neural damage caused by Aß becomes evident 14 days after its injection. Therefore, as animals in our study were euthanized 10 days after surgery, it is possible that this time frame was insufficient to observe changes in the oxidative profile at the cortical level.

Furthermore, it was observed that the groups treated with i.p. GRA and its isoxazole derivative maintained GSH levels similar to those of the control group, whereas the group receiving i.c.v. Aß showed a reduction in these levels (Figure 6). Since GSH represents the first line of defense against oxidation, its levels decrease in the presence of oxidative processes. Thus, the data obtained indicate that both test drugs exhibit antioxidant effects, as evidenced by the maintenance of GSH levels in the treated groups. However, i.p. IGA did not demonstrate efficacy in reducing lipoperoxidation, MPO activity, or preventing Aß-induced memory impairment in other analyses. Therefore, we believe that the observed effect on GSH levels in animals treated with i.p. IGA may be attributed to a modest antioxidant pharmacological activity of the original compound, given that no active metabolites are formed through first-pass metabolism via the intraperitoneal route.

Due to the absence of pronounced effects from i.p. IGA, it was hypothesized that this substance could act as a prodrug, requiring hepatic metabolism for activation. Indeed, in previous studies where the anti-inflammatory activity of IGA was demonstrated, the compound was administered orally [35]. Therefore, in another group of animals, IGA was administered per oral to confirm its biological activity. When administered orally, IGA successfully prevented memory loss in animals that received Aß, suggesting that it works as a prodrug. Notably, IGA demonstrated superior efficacy compared to oral GRA treatment (Figure 8), highlighting its neuroprotective properties. Interestingly, GRA appears to be more effective when administered intraperitoneally compared to orally. This observation allows us to conclude that the observed neuroprotective activity is attributed to the original GRA structure. Additionally, it is presumed that during first-pass metabolism, metabolites with lower activity than GRA are formed. Valadares et al. (2011) demonstrated that GRA metabolism can produce the metabolite 4-O-demethylgrandisin [15]. Although this metabolite exhibits antioxidant activity, it is possible that it does not cross the

blood-brain barrier, which may explain the reduced efficacy of orally administered GRA in the present study.

In the measurements of protein carbonyls, no significant differences were observed between the groups, regardless of vehicle or AβO i.c.v. administration (Figure 7). We believe that the absence of differences in carbonylated protein levels among the experimental groups may be related to the duration of the experimental protocol. Some studies suggest that normalization of these levels occurs between 10 and 20 days after OßA injection, while in this study, animals were euthanized at 10 days [73]. Additionally, another factor that may have contributed to this result is the variability in carbonylated protein levels. Navigatore et al. (2024) reported that carbonylated protein concentrations in the hippocampus of animals injected with Aß oligomers fluctuate according to the photoperiod, showing higher or lower levels depending on the phase of the light-dark cycle (light or dark) at the time of measurement [28].

Furthermore, the effects on preventing cognitive impairment, as well as the reductions in oxidative markers observed in this study, may be associated with a neuroplastic effect of these substances. Zhai et al. (2005) demonstrated that tetrahydrofuran neolignans increased the viability of hippocampal neurons exposed to Aß oligomers. The same study showed that the application of these molecules resulted in an increased number of neurites in cortical neuron cultures [20]. Therefore, in addition to their neuroprotective effect against Aß, it is suggested that neolignans, such as GRA and IGA, may also exert a neurotrophic effect. In this regard, these substances could act through pathways involving neural growth factors, such as brain-derived neurotrophic factor (BDNF). Previous studies have shown that neolignans can enhance the expression and signaling pathways of this chemical mediator, as well as to promote the proliferation of neurons in culture [43]. Thus, the compounds tested in this study could support neuroprotection through this mechanism.

Additionally, in a recent study conducted by our group, it was demonstrated that a triazole derivative of grandisin exhibited neuroprotective effects in the same model used in the present study. This compound reduced the release of inflammatory cytokines, such as interferon and TNF, in the hippocampus of Aß-induced animals, as well as lipid peroxidation in the cortex and hippocampus of these animals [41]. Considering that this compound belongs to the same group as GRA and IGA, it is

possible that these substances act through similar neuroprotective pathways. However, IGA may offer pharmacokinetic advantages, as it is active via oral administration a route that is both safe and convenient.

7. CONCLUSION

These results suggest that repeated treatment with GRA or IGA has beneficial effects on oxidative and inflammatory markers in response to Aß and can attenuate memory impairment in an animal model of Alzheimer's disease. Furthermore, it is suggested that the synthetic compound IGA, despite being inactive via intraperitoneal administration, exhibits neuroprotective activity when administered orally, which is superior to that of GRA when delivered through the same route.

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