doi: http://dx.doi.org/10.11606/issn.1679-9836.v103i2e-218782 Rev Med (São Paulo). 2024 Mar-Apr;103(2):e-218782.

Review Article

Therapeutic potential of phytoconstituents from Pilocarpus microphyllus Stapf ex Wardlew. and propolis in Alzheimer's disease: an Integrative Review

Potencial terapêutico de fitoconstituintes de Pilocarpus microphyllus Stapf ex Wardlew. e da própolis na doença de Alzheimer: uma Revisão Integrativa

José Gustavo Queiroz do Nascimento[1], Izadora Alves Coelho[2], Francisco das Chagas Sousa Rocha[3], Maria Francilene Souza Silva[4], Jociane Sthefanny Tavares Silva[5], João Antônio Leal de Miranda[6]

Nascimento JGQ, Coelho IA, Rocha FCS, Silva MFS, Silva JST, Miranda JAL Therapeutic potential of phytoconstituents from Pilocarpus microphyllus Stapf ex Wardlew. and propolis in Alzheimer's disease: an Integrative Review / *Potencial terapêutico de fitoconstituintes de Pilocarpus microphyllus Stapf ex Wardlew. e da própolis na doença de Alzheimer: uma Revisão Integrativa*. Rev Med (São Paulo). 2024 Mar-Apr;103(2):e-218782.

ABSTRACT | Introduction: Alzheimer's disease (AD) is an irreversible neurodegenerative disorder characterized by the deterioration of cognitive functions, for which the available pharmacotherapy is not capable of effecting a cure. **Objectives:** To investigate the neuroprotective properties of phytoconstituents from Jaborandi (Pilocarpus microphyllus) and propolis as potential agents in the therapy of AD. **Methodology:** This is an integrative literature review study, in which the PICO strategy was followed in formulating the guiding question. Five databases were used: PubMed, Web of Science, Scopus, Embase, and Lilacs. The analysis was conducted with the descriptors "epiisopiloturine", "episopilosin", "pilocarpine", "macaubine", "propolis", "alzheimer" extracted from the DeCS (Health Sciences Descriptors) and Medical Subject Headings (MeSH). Keywords were isolated and combined using boolean operators AND and OR. **Results:** The search resulted in 602 studies identified in the databases, 337 were duplicates, leaving 265 for title and abstract reading, of which 250 were excluded. In the end, 10 studies were read in full and 08 studies were included in the scope of the review. The research evidenced neuroprotective activity for propolis, through antioxidative, anti-inflammatory, and anticholinesterase action, configuring it with therapeutic potential in AD. For pilocarpine, a substance with various clinical applications, few studies demonstrated a correlation with AD, in cognitive and behavioral improvement. **Conclusion:** Therefore, benefits for the use of propolis in cognitive dysfunctions are concluded, while for pilocarpine, this relationship was not established. It is necessary to develop research that elucidates the possible functional roles for these substances.

KEY WORDS: Azheimer; Jaborandi; Neuroprotection; Propolis; Pilocarpine.

RESUMO| Introdução: A doença de Alzheimer (DA) é uma enfermidade neurodegenerativa irreversível, caracterizada pela deterioração das funções cognitivas, cuja farmacoterapia disponível não é capaz de efetivar a cura. **Objetivos:** Averiguar as propriedades neuroprotetoras dos fitoconstituintes do Jaborandi (*Pilocarpus microphyllus*) e da própolis como possíveis agentes na terapêutica da DA. **Metodologia:** Trata-se de um estudo do tipo revisão integrativa da literatura, em que seguiu a estratégia PICO na formulação da pergunta norteadora. Utilizou-se cinco bases de dados: *Pubmed*, *Web of Science, Scopus, Embase* e *Lilacs*. A análise foi conduzida com os descritores "epiisopiloturine", "episopilosin", "pilocarpine", "macaubine", "propolis", "alzheimer" extraídos do DeCS (Descritores em Ciências da Saúde) e do Medical Subject Headings (MeSH). As palavras-chave foram isoladas e combinadas entre si por meio dos operadores *booleanos* AND e OR. **Resultados:** A busca resultou em 602 estudos identificados nos bancos de dados, 337 eram duplicados, restando 265 para leitura do título e resumo, os quais 250 foram excluídos. Ao final, 10 estudos foram lidos na íntegra e 08 estudos incluídos no escopo da revisão. A pesquisa evidenciou atividade neuroprotetora para a própolis, através de ação antioxidativa, anti-inflamatória e anticolinesterásica, configurando-se com potencial terapêutico na DA. Para a pilocarpina, substância com diversas aplicabilidades clínicas, poucos trabalhos demonstraram correlação com a DA, na melhora cognitiva e de comportamento. **Conclusão:** Conclui-se, portanto, benefícios para o uso da própolis nas disfunções cognitivas, enquanto para a pilocarpina não se estabeleceu essa relação. Faz-se necessário o desenvolvimento de pesquisas que elucidem os possíveis papéis funcionais para essas substâncias.

PALAVRAS-CHAVE: Alzheimer; Jaborandi; Neuroproteção; Propolis; Pilocarpina.

[1.] Universidade Federal do Piauí, Campus Senador Helvídio Nunes de Barros, Picos, Piauí, Brasil. ORCID: https://orcid.org/0009-0000-3918-137X. E-mail: jgustavoqueiroz@ufpi.edu.br.
[2.] Universidade Federal do Piauí, Campus Senador Helvídio Nunes de Barros, Picos, Piauí, Brasil. ORCID: https://orcid.org/0009-0006-7899-7981. Email: izadoracoelho36@gmail.com.
[3.] Universidade Federal do Piauí, Campus Senador Helvídio Nunes de Barros, Picos, Piauí, Brasil. ORCID: https://orcid.org/0000-0002-6616-1291. Email: franciscorocha@ufpi.edu.br.
[4.] Universidade Federal do Ceará, Departamento de Fisiologia e Farmacologia, Faculdade de Medicina, Laboratório de Neuropsicofarmacologia e psiquiatria translacional, Fortaleza, Ceará, Brasil. ORCID: https://orcid.org/0000-0002-4509-1159. Email: lenolysilva@hotmail.com.
[5.] Universidade Federal da Paraíba, Campus II, Areia, Paraíba, Brasil. ORCID: https://orcid.org/0009-0007-8385-5109. Email: jociane.sthefanny@academico.ufpb.br.
[6.] Universidade Federal do Piauí, Campus Senador Helvídio Nunes de Barros, Picos, Piauí, Brasil. ORCID: https://orcid.org/0000-0002-0273-4169. Email: joaoantonio@ufpi.edu.br.
Correspondence: José Gustavo Queiroz do Nascimento. Rua Cícero Duarte, 349 – Bairro Junco. Picos, PI. CEP: 64607-670. E-mail: jgustavoqueiroz@ufpi.edu.br.

INTRODUCTION

Alzheimer's disease (AD) is a progressive and irreversible neurodegenerative disorder characterized by the deterioration of cognition and behavior. Clinically, AD is understood as dementia, defined as a deficit in memory function (amnesia) and loss of at least one other cognitive domain[1]. Currently, AD affects 50 million people, with an expected tripling by 2050 due to population aging, especially in lower-income countries, resulting in high healthcare costs and loss of functionality[2].

With a complex and not fully elucidated pathophysiology, Alzheimer's disease is characterized by neuropathological changes, believed to involve the accumulation of proteins such as β-amyloid (Aβ) and hyperphosphorylation of the tau protein, as well as neuronal and synaptic atrophy. Additionally, factors such as inflammation, oxidative stress, infection, and cholinergic neurons may contribute to the development of the disease[3-5].

The search for natural products with neuroprotective properties gains prominence as a promising approach for the treatment and prevention of AD. This is due to the multifactorial nature of this pathology, which has suggested the need to explore multiple therapeutic targets and possibly combined approaches[6], as well as the pharmacological properties of natural products and phytoconstituents due to their ability to interact with biological molecules and provide optimized interaction with target proteins, as well as good tolerance and wide safety[7].

Phytoconstituents from Jaborandi (*Pilocarpus microphyllus* Stapf ex Wardlew.), such as pilocarpine, and beehive products, such as propolis, stand out as potential therapeutic targets for AD. Jaborandi is a plant native to the North and Northeast of Brazil, a source of substances such as pilocarpine, epiisopiloturine, epiisopilosine, and macaubine, with therapeutic properties such as anthelmintic, anti-inflammatory, and antiapoptotic[8-9]. Pilocarpine, extensively studied, is used in the treatment of glaucoma and xerostomia, acting as a non-selective muscarinic agonist, suggesting the potential to counteract age-related cognitive decline[10].

Furthermore, propolis, a bee product, has a history of use as food and medicine, with a wide range of beneficial activities[11]. This is a complex substance used by bees to protect the hive, with antioxidant properties, standing out in combating degenerative diseases[12]. Research has explored its potential in the treatment of neurological diseases, reducing inflammatory and oxidative markers[13-15].

Given the complexity of this scenario and the lack of effective treatments for Alzheimer's Disease (AD), this study aims to evaluate the neuroprotective properties of natural components from Jaborandi (*Pilocarpus microphyllus*) and propolis in AD, with the intent of exploring their therapeutic potential, considering the absence of effective treatments and the biological properties of these substances.

METHODOLOGY

This study adopts an integrative literature review approach, employing both descriptive and quantitative methods. Integrative review methodology enables a comprehensive analysis of a phenomenon, allowing for the inclusion of both experimental and non-experimental studies. This approach relies on a meticulous selection of methods, aiming for a broad and in-depth understanding of the topic under analysis[16].

The research was conducted between November 2022 and August 2023, following the typical six stages of integrative review: formulating the research question, systematically searching for articles in the literature, data collection, analysis of selected studies, discussion of results, and presentation of the review[17].

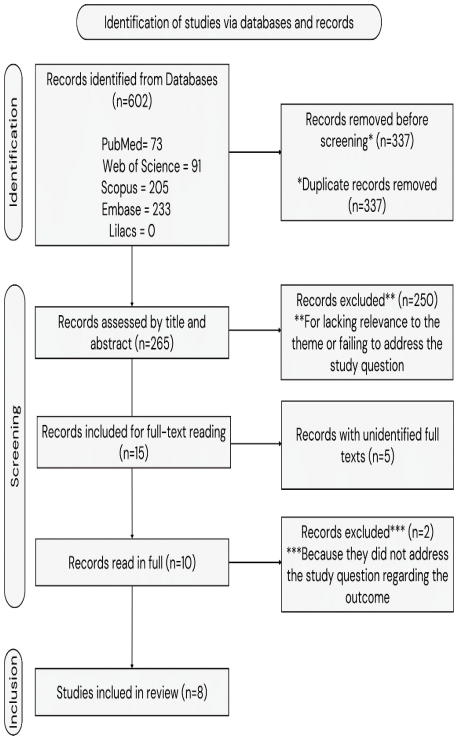
In formulating the guiding question, the PICO strategy was used. Its characters refer respectively to P – Problem/ Population; I – Intervention; C – Comparison; O – Outcomes[18]. Thus, the organization of the characters was: P – Alzheimer's (animals induced with dementia or patients with AD), I – phytoconstituents in Alzheimer's/dementia treatment, C – Cholinesterase inhibitors / Glutamatergic system modulators; O – reduction of dementia/improvement in cognition/delay of AD. In the end, the formulated question was: In what way do phytoconstituents from *Pilocarpus microphyllus* and propolis act in the treatment of Alzheimer's disease?

To conduct the research, PRISMA recommendations for the key items reported in integrative systematic reviews were followed[19]. Five databases were utilized: Medline/Pubmed, Web of Science, Scopus, Embase, and Lilacs. The analysis was conducted using the following descriptors and their respective synonymous terms: "epiisopiloturine," "episopilosin," "pilocarpine," "macaubine," "propolis," "Alzheimer," extracted from the DeCS (Health Sciences Descriptors) and the Medical Subject Headings (MeSH). Keywords were isolated and combined using the boolean operators AND and OR. In the article selection process, those meeting the following inclusion criteria were considered: fully published in a peer-reviewed journal; experimental studies involving animals and/or humans; and published in English. Excluded were conference proceedings, case reports, literature reviews, thesis studies, dissertations, and conference papers, as well as works inaccessible in full. Screening and selection of articles were independently performed by two authors, with conflicts resolved by a third researcher when necessary. Additionally, the time frame was set from 2002 to 2022.

RESULTS

As evidenced in Figure 1, the initial search resulted in a total of 602 studies identified in the databases, applying time and language filters. Out of this total, 337 references were duplicates, leaving 265 for title and abstract screening. Of these, 250 were excluded for not being relevant to the topic or not addressing the research question. Thus, 15 studies were selected, however, in five cases, the full text was not available. In the end, ten studies were reviewed in full, of which 02 were discarded for not establishing a relationship between the researched substance and its benefits in Alzheimer's disease. Consequently, eight studies were included in the scope of this review.

.**Figure 1** - Flowchart of the search, screening, and inclusion process of studies in the integrative review.



The qualitative analysis of the eight included articles was conducted and separated according to the substances analyzed. Among the researched substances, "propolis" and "pilocarpine" yielded results in the databases, with 75% (n=6) and 25% (n=2) of the included articles, respectively, while "epiisopiloturine", "episopilosin", and "macaubine" did not yield any results.

Table 1 illustrates the types of studies included, according to reference, objective, study population and sample size, intervention developed, bioactive compound of the substances, dose, frequency of exposure, route of administration, standard treatment, and main results obtained.

Table 1 - Main aspects of studies on the effect of pilocarpine and propolis in Alzheimer's disease models, in chronological order, in articles published from 2003 to 2022 in the Medline/Pubmed, Web of Science, Scopus, Embase, and Lilacs databases.

N	Study Data	Objective	Population	Sample Size	Comparison Group	Intervention	Dose	Exposure Frequency	Outcome
1	WANG, 2003	Evaluate the therapeutic effect of pilocarpine in SCO-induced dementia	Kunming mice	60	Saline solution	Pilocarpine treatment	20 mg/kg and 40 mg/kg Intraperitoneal	2 times/day for 11 days	Pilocarpine attenuated scopolamine-induced cognitive decline through muscarinic action.
2	PREDIGER, 2006	Evaluate the effect of pilocarpine on age-related cognitive deficit	Wistar rats	32	Saline solution	Pilocarpine treatment	30 mg/kg Intraperitoneal	Daily for 21 days	Pilocarpine attenuated age-related deficits in social recognition and olfactory discrimination through muscarinic action.
3	CHEN, 2008	Assess the protective effect of propolis on SCO-induced amnesia	Kunming mice	50	Piracetam 100mg/kg	Hydroethanolic extract of propolis treatment	50 mg/kg and 100 mg/kg oral	Daily for 4 days	Propolis mitigated amnesia through AChE inhibition.
4	NANAWARE, 2008	Evaluate the protective effect of propolis on β-amyloid peptide-induced dementia	Wistar rats	56	Donepezil 0,75mg/kg	Ethanol extract of propolis treatment	100, 200 e 300mg/kg oral	Daily for 21 days	Propolis mitigated β-amyloid peptide-induced dementia through AChE inhibition, regulation of monoamines, and oxidative stress.

continue

continuation

N	Study Data	Objective	Population	Sample Size	Comparison Group	Intervention	Dose	Exposure Frequency	Outcome
5	HUSSEIN, 2017	Assess the neuroprotective effect of propolis on MSG-induced dementia	Wistar rats	28	Saline solution	Unspecified propolis treatment	600 mg/kg Oral	Daily for 2 months	Propolis attenuated glutamate-induced impairments by reducing oxidative stress, β-amyloid plaque size, and AChE activity.
6	ZHU, 2018	Evaluate the effects of propolis on preventing cognitive decline in elderly individuals living at high altitudes	Human subjects	60	Placebo	Propolis capsules treatment (ethanol extract)	0.83g oral	Daily for 24 months	Propolis attenuated cognitive decline after 24 months by controlling systemic cytokine levels.
7	OMAR, 2019	Assess the protective effect of propolis on AIS-induced neurotoxicity	Rats (Rattus norvegicus)	40	Saline solution	Hydroethanolic extract of propolis treatment	200ml/kg oral	Alternate days for 8 weeks	Propolis mitigated aluminum-induced toxicity by preserving internal organelles and macromolecule content.
8	MORIGUGHI, 2022	Evaluate the effects of propolis in animals genetically modified for dementiaParte superior do formulário	APP-KI and wild-type mice	30	Memantine 1mg/Kg	Unspecified propolis treatment Parte superior do formulário	1mg, 10mg and 100mg/ kg oral	Daily for 8 weeks Parte superior do formulário	Propolis enhanced memory in APP-KI mice tested, via increased ATP content and inhibition of Kir6.2 channel in the CA1 region. Parte superior do formulário

SCO: scopolamine; AChE: acetylcholinesterase; MSG: monosodium glutamate; AIS: aluminum salicylate

From the literature search, a final analysis of eight studies was obtained, with 25% (n=2) regarding pilocarpine and 75% (n=6) on propolis. The study data varied between the years 2003 to 2022.

Concerning the objective of the studies, the aim was to evaluate the effects of pilocarpine and propolis therapy on experimental models of Alzheimer's dementia. For this, 25% (n=2) of the works used Scopolamine (SCO), 12.5% (n=1) used β-amyloid peptide, 12.5% (n=1) monosodium glutamate (MSG), 12.5% (n=1) aluminum salicylate (AIS), 12.5% (n=1) transgenic animals, and finally, 25% of the studies (n=2) used age-related cognitive deficit models. The predominant sample in the studies consisted of animals, with 37.5% (n=3) being mice, 50% (n=4) being rats, and 12.5% (n=1) being humans. The sample size ranged from 28 to 60, with a mean of 44.5.

Regarding comparison groups, 25% of the studies (n=2) utilized standard treatment for Alzheimer's, including Donepezil (acetylcholinesterase inhibitor) and Memantine (glutamatergic inhibitor). One study (n=1) (12.5%) used Piracetam (a nootropic drug). The remaining (n=5) employed saline or placebo as a comparison method in previously reported dementia models, totaling 62.5%.

For the types of propolis extracts used in the studies, the following results were obtained: hydroethanolic extract (33.3%), ethanolic extract (33.3%), and unspecified forms (33.3%). The geographical location of propolis sources, the following relation was obtained: Brazil (25%), China (12.5%), India (12.5%), Egypt (12.5%), and unspecified origin (12.5%). Among the main phytoconstituents of propolis from different geographical diversities, 50% of the studies (n=3) analyzed the bioactive compounds, with results highlighting Caffeic Acid, Artepillin C, Galangin, Pinocembrin, and Chrysin. The other half (n=3) of the studies did not specify or conduct this type of assay. For pilocarpine, a chemical constituent of Jaborandi, its origin in the two present studies was not specified.

About administration dose, studies with rats or mice using propolis ranged from 1 mg/kg to 600 mg/kg. As for the dose used in humans, the only study reported here was by Zhu et al.[20], with the administration of 0.83 g of propolis/day, present in six capsules. Furthermore, for propolis, the only route of administration was oral (n=6). Regarding the frequency of exposure, most studies (83.3%) had a single daily administration (n=5), and 16.7% had alternate-day administration (n=1). The duration of treatment varied between 4 days and 24 months; however, there was a predominance of studies with 8 weeks (n=3), totaling 37.5%.

The dosage of pilocarpine, the assays were more restricted, ranging from 20-40 mg/kg through the intraperitoneal route, with administration frequency of once or twice daily and a period of 11 to 21 days. When evaluating the outcomes found for pilocarpine and its relation to dementia models, it was found that it managed to attenuate cognitive deficits in elderly rats of 24 months of age, associated with social recognition capacity and olfactory discrimination, compared to untreated rodents of the same age[10]. The study by Wang et al.[21] corroborates the benefits of pilocarpine on rat dementia in cognitive tests, through its muscarinic activity on M1 receptors – more present in the Central Nervous System (CNS). However, it is noteworthy that

peripheral cholinergic effects were relevant, such as increased salivation, intestinal propulsion (diarrhea), and bradycardia. In the same study, the combination with the muscarinic antagonist, anisodamine, is indicated as an adjuvant to attenuate side effects and maintain selectivity over the CNS[21].

When evaluating the results regarding the use of propolis for dementia models, several benefits were evidenced through variable physiological mechanisms. Among them, the cholinergic pathway stood out prominently, being highlighted in 50% (n=3) of the six propolis studies, through histopathological studies of the cortex, which demonstrated dose-dependent inhibition of acetylcholinesterase, comparable or superior to standard treatment or saline solution[22-24].

Additionally, another elucidated action pathway was on oxidative stress, reported in 2 studies, totaling 33.3%. Thus, in anatomopathological studies with animals, propolis consistently demonstrated to reduce the expression of oxidative markers, such as malondialdehyde (MDA) and nitric oxide (NO), while preventing the decrease in antioxidants, such as glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT). Moreover, it demonstrated to activate the Brain-Derived Neurotrophic Factor (BDNF) and reduce stress biomarkers, such as 8-OHdG (DNA oxidation marker) and MDA (lipid peroxidation biomarker)[23,24]. These results are compatible with those found in other studies involving propolis for antioxidant purposes[25,26].

The studies by Hussein et al.[24] and Nanaware et al.[23] also demonstrated another important mechanism of action of propolis on the pathophysiology of AD: it evidenced the reduction in the size of β-amyloid plaques in the cortex and hippocampus of rodents. Furthermore, Omar et al.[27] found a neuroprotective activity of propolis by preserving macromolecule contents (glycogen, carbohydrate, and proteins) and cellular organelles after aluminum-induced toxicity, avoiding greater tissue deterioration.

In the dementia model with genetically modified animals, propolis combined with memantine treatment managed to increase intracellular ATP content and alleviate cognitive deficits through the inhibition of the Kir6.2 channel in the CA1 region. However, the use of propolis alone was not sufficient to mitigate memory impairments in these animals[28]. The Kir6.2 channel is primarily found in neurons and plays a crucial role in glucose detection and regulation of neuronal excitability within the metabolic cycle[29].

It is also noteworthy that significant results regarding propolis were elucidated in a randomized, placebo-controlled clinical trial involving humans. In the study by Zhu et al.[20], propolis was demonstrated to significantly attenuate cognitive decline in elderly individuals after 24 months. For this, researchers assessed patients using the Mini-Mental State Examination (MMSE) cognitive test, comparing the results between the placebo and propolis groups. The study correlated cognitive improvement with the control of systemic levels of inflammatory cytokines, such as IL-1β, IL-6, TNF-α, and IL-10.

DISCUSSION

The literature extensively documents the use of pilocarpine for various purposes. For instance, pilocarpine has been widely studied in models of epileptic status in animal experiments, as well as in ophthalmological applications, such as a pupil dilator, glaucoma treatment, dry eye syndrome, and xerostomia, often associated with Sjögren's syndrome[30,31]. This greater availability of results in other areas may explain the scarcity of research linking pilocarpine to Alzheimer's disease therapy.

Regarding the development of experimental models, they play a crucial role in the therapeutic study of diseases. In the case of Alzheimer's disease, which involves various pathophysiological mechanisms, experiments seek to simulate effects on cognitive function, often using drugs with cholinergic, neurotoxic, oxidative, or amyloid β-protein accumulation action. Among the main chemical induction models of dementia, scopolamine, streptozotocin, alcohol, metals, and β-amyloid peptides stand out[32]. Aged animals can serve as experimental models of dementia, with the advantage of being non-invasive and not involving the use of chemicals. The use of transgenic animals, genetically modified to express genetic mutations responsible for Alzheimer's disease pathological changes in humans, is also promising. Therefore, chemically induced dementia was the preferred approach in this study, likely due to ease of reproduction. However, it is worth noting that age-related spontaneous models may be more reliable, as they are natural, especially in studies aiming to test in humans[33].

Concerning the sample characteristics, it is important to consider that the use of animals in scientific research may vary, but rodents are often chosen due to ease of reproduction and handling, small size, and widely studied and recognized behavior[34]. However, the fact that only one study used humans highlights the scarcity of information involving pilocarpine and propolis in AD treatment.

Currently, the standard treatment for Alzheimer's disease consists of symptomatic drugs, following the cholinergic hypothesis as the main reference. According to this theory, Alzheimer's disease leads to a decrease in acetylcholine (ACh) biosynthesis. Therefore, inhibition AChE increases acetylcholine availability in the synaptic cleft, which improves cognitive functions. Among the drugs in this class, donepezil and rivastigmine are prominent[35,36]. Additionally, N-methyl-D-aspartate (NMDA) receptor antagonists are believed to play a significant role in Alzheimer's disease pathophysiology, with memantine being a representative used in moderate to severe cases of the disease[37]. The use of piracetam (a nootropic drug) for Alzheimer's disease is not well understood, and some clinical trials have failed to demonstrate cognitive improvement, with some evidence disapproving its use in Alzheimer's disease[38,39].

In this study, most of the works did not use standard treatment for Alzheimer's disease as a comparison group with propolis and pilocarpine, limiting the evaluation of the positive effects found for these substances. Organic solvents (hydroethanolic and ethanolic extracts) are often employed due to their ability to interact with both lipophilic and hydrophilic bioactive molecules. Among them, ethanol is preferred option due to its non-toxic nature, economic advantages, and the possibility of reuse[40]. Thus, describing the organic solvents used

is crucial; however, many studies inadequately characterize the extract types.

Brazilian propolis stood out in the research, followed by propolis of Asian origin. Zulhendri et al.[15] also highlighted the relevance of studies with Brazilian propolis in a systematic review, which presented the second-highest number of works found. This demonstrates the worldwide recognition of the benefits of Brazilian propolis and its phytoconstituents, especially regarding neuroprotective activity[41].

As many articles did not specify the main phytoconstituents of propolis from different geographical regions, the analysis of the substances present and their relationship with the presented results is limited, since therapeutic properties are directly correlated with their various organic compounds.

Regarding the administration dose of propolis in rodents' models, the literature does not record research that has established exact or specific doses for the use of propolis in Alzheimer's disease or other neurodegenerative diseases. Research in this field is still quite limited, and there is a wide variability in dosing and therapeutic regimens used. However, positive results were prevalent and the use of doses in the range of 100-300 mg/kg in rodents' models.

Furthermore, the administration of propolis extract was found to be safe during the evaluated clinical trials, even at very high doses, such as 2000 mg/kg, with no reported side effects, signs of toxicity, or mortality[20,23]. Similarly, in other clinical trials involving humans in various areas of propolis-related research, safety in its use was indicated[42,43].

Regarding the dosage of pilocarpine, different studies present varying doses. Wang et al.[21] reported that the dose of 40 mg/kg yielded the best results compared to 20 mg/kg. Prediger et al.[10] used a dose of 30 mg/kg, which also led to satisfactory outcomes, aligning with the dose employed by De Sarno et al.[44] On the other hand, De-Mello et al.[45] opted for higher doses, reaching 300 mg/kg, considered subconvulsive, to assess the long-term prophylactic effect on cognitive decline. However, the peculiar characteristics of this study, such as the frequency of single-dose administration, may complicate the comparison of concentrations, evaluation of results, and definition of a therapeutic threshold for this substance.

Furthermore, concerning pilocarpine, it is essential to highlight that the applicability of muscarinic agonists in neuroprotective activity has been evaluated in other studies correlating with the cholinergic pathway. The study by De Sarno et al.[44] elucidated that substances like pilocarpine can stimulate serine phosphorylation in GSK3, serving as a possible therapeutic agent in neurodegenerative diseases. However, research with muscarinic agonists has not yielded satisfactory results when tested for Alzheimer's disease, only progressing to some clinical phases. Among the main reasons, the prevalence of various side effects due to low selectivity for central nervous system receptors stands out. This may explain the limited availability of studies correlating pilocarpine and Alzheimer's disease. Additionally, some works, albeit with limited success, seek to develop selective M1 agonists with fewer peripheral side effects, aiming to improve cognitive and behavioral disorders, which could represent a treatment option for Alzheimer's disease[46-48].

Upon evaluating the neuroprotective capacity of propolis, consistent results emerged across various mechanisms including its anticholinesterase, antioxidant, and anti-inflammatory capacities. Inhibition of AchE constitutes an important therapeutic pathway for Alzheimer's disease (AD), as corroborated by other studies on propolis[49,50]. Additionally, the antioxidative findings involving propolis by Taysi et al.[25] and Shang et al.[26], align with this study. Furthermore, Zhu et al.[20], linked cognitive improvement to the control of systemic inflammatory cytokine levels, a finding supported by research indicating that propolis use may improve comorbidities associated with decreased inflammatory activity[26,43].

Therefore, the evidence from the literature supports the neuroprotective activity of propolis and its phytoconstituents through various pathways, potentially reshaping the therapeutic landscape for neurodegenerative diseases, particularly Alzheimer's disease. Concerning pilocarpine, a muscarinic agonist, its benefits for AD are limited, although experimental models suggest some cognitive improvement.

CONCLUSION

Based on the literature analysis, it can be inferred that propolis has demonstrated neuroprotective activity through various pathways, which may render it relevant in the context of therapeutic substances for neurodegenerative diseases, particularly Alzheimer's disease. Regarding pilocarpine, a muscarinic agonist, its benefits in treating Alzheimer's disease appear limited, although some studies indicate cognitive improvement in experimental models. Therefore, given the lack of standardization in studies and the necessity for further investigations into the therapeutic properties of propolis and pilocarpine, it is essential to undertake additional research to clarify their potential functional roles.

ACKNOWLEDGMENT: We would like to thank the Fundação de Amparo à Pesquisa do Estado do Piauí (FAPEPI) for the grant awarded.

FINANCING: Fundação de Amparo à Pesquisa do Estado do Piauí (FAPEPI).

AUTHOR CONTRIBUTION: José Gustavo Queiroz do Nascimento, Izadora Alves Coelho, Francisco das Chagas Sousa Rocha - Conception and design of the study, bibliographic research, analysis and interpretation of data and construction of the manuscript; Maria Francilene Souza Silva, João Antônio Leal de Miranda – Guidance and review of the manuscript.

REFERENCES

1. Gulisano W, Maugeri D, Baltrons MA, Fà M, Amato A, Palmeri A, et al. Role of amyloid-β and tau proteins in Alzheimer's disease: confuting the amyloid cascade. J Alzheimer's Dis. 2018;64(s1):S611-S631. Doi: https://doi.org/10.3233/jad-189015

2. Zhang XX, Tian Y, Wang ZT, Ma YH, Tan L, Yu JT. The epidemiology of Alzheimer's disease modifiable risk factors and prevention. J Prev Alzheimers Dis. 2021;8:313-21. Doi: https://doi.org/10.14283/jpad.2021.15

3. Breijyeh Z, Karaman R. Comprehensive review on Alzheimer's disease: causes and treatment. Molecules. 2020;25(24):5789. Doi: https://doi.org/10.3390/molecules25245789

4. Khan S, Barve KH, Kumar MS. Recent advancements in pathogenesis, diagnostics and treatment of Alzheimer's disease. Curr Neuropharmacol. 2020;18(11):1106-25. Doi: https://doi.org/10.2174/1570159X18666200528142429

5. Rostagno AA. Pathogenesis of Alzheimer's Disease. Int J Mol Sci. 2022;24(1):107. Doi: https://doi.org/10.3390/ijms24010107

6. Habtemariam S. Natural products in Alzheimer's disease therapy: would old therapeutic approaches fix the broken promise of modern medicines? Molecules. 2019;24(8):1519. Doi: https://doi.org/10.3390/molecules24081519

7. Singh AK, Rai SN, Maurya A, Mishra G, Awasthi R, Shakya A, et al. Therapeutic potential of phytoconstituents in management of Alzheimer's disease. Evid Based Complement Alternat Med. 2021;2021:1-19. Doi: https://doi.org/10.1155/2021/5578574

8. Sabá RT, Lameira OA, Luz JM, Gomes AP, Innecco R. Micropropagação do jaborandi. Hortic Bras. 2002;20(1). Doi: https://doi.org/10.1590/S0102-05362002000100021

9. Rocha JA, Andrade IM, Véras LM, Quelemes PV, Lima DF, Soares MJ, et al. Anthelmintic, antibacterial and cytotoxicity activity of imidazole alkaloids from Pilocarpus microphyllus leaves. Phytother Res. 2017;31(4):624-30. Doi: https://doi.org/10.1002/ptr.5771

10. Prediger RD, De-Mello N, Takahashi RN. Pilocarpine improves olfactory discrimination and social recognition memory deficits in 24-month-old rats. Eur J Pharmacol. 2006;531(1-3):176-82. Doi: https://doi.org/10.1016/j.ejphar.2005.12.032

11. Escobar AL, Xavier FB. Propriedades fitoterápicas do mel de abelhas. Revista Uningá. 2013;37(1). Doi: https://doi.org/10.46311/2318-0579.37.eUJ1115

12. Citó AM, Barros ED, Moura AK, Cruz ES, Lima JS Neto. Caracterização de compostos voláteis e da fração apolar do mel, própolis e cera de abelha (Apis mellifera) de Picos – Piauí. In: Oliveira-Junior JM, Calvão LB, organizators. A interface do conhecimento sobre abelhas 2. Ponta Grossa, PR: Atena; 2020. p. 139-153. Doi: https://doi.org/10.22533/at.ed.368200110

13. Sforcin JM. Biological properties and therapeutic applications of propolis. Phytother Res. 2016;30(6):894-905. Doi: https://doi.org/10.1002/ptr.5605

14. Ni J, Wu Z, Meng J, Zhu A, Zhong X, Wu S, et al. The neuroprotective effects of Brazilian green propolis on neurodegenerative damage in human neuronal SH-SY5Y cells. Oxid Med Cell Longev. 2017;2017. Doi: https://doi.org/10.1155/2017/7984327

15. Zulhendri F, Perera CO, Tandean S. Can propolis be a useful adjuvant in brain and neurological disorders and injuries? A systematic scoping review of the latest experimental evidence. Biomedicines. 2021;9(9):1227. Doi: https://doi.org/10.3390/biomedicines9091227

16. Souza MT, Silva MD, Carvalho R. Revisão integrativa: o que é e como fazer. Einstein (São Paulo). 2010;8:102-6. Doi: https://doi.org/10.1590/S1679-45082010RW1134

17. Mendes KD, Silveira RC, Galvão CM. Uso de gerenciador de referências bibliográficas na seleção dos estudos primários em revisão integrativa. Texto & Contexto Enferm. 2019;28:e20170204. Doi: https://doi.org/10.1590/1980-265X-TCE-2017-0204

18. Santos CM, Pimenta CA, Nobre MR. A estratégia PICO para a construção da pergunta de pesquisa e busca de evidências. Rev Latino-Am Enfermagem. 2007;15:508-11. Doi: https://doi.org/10.1590/S0104-11692007000300023

19. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: An updated guideline for reporting systematic reviews. BMJ. 2021;71:1-9. Doi: https://doi.org/10.1016/j.ijsu.2021.105906

20. Zhu A, Wu Z, Zhong X, Ni J, Li Y, Meng J, et al. Brazilian green propolis prevents cognitive decline into mild cognitive impairment in elderly people living at high altitude. J Alzheimers Dis. 2018;63(2):551-60. Doi: https://doi.org/10.3233/jad-170630

21. Wang H, Lu Y, Chen H-Z. Differentiating effects of anisodamine on cognitive amelioration and peripheral muscarinic side effects induced by pilocarpine in mice. Neurosci Lett. 2003;344(3):173-6. Doi: https://doi.org/10.1016/S0304-3940(03)00444-0

22. Chen J, Long Y, Han M, Wang T, Chen Q, Wang R. Water-soluble derivative of propolis mitigates scopolamine-induced learning and memory impairment in mice. Pharmacol Biochem Behav. 2008;90(3):441-6. Doi: https://doi.org/10.1016/j.pbb.2008.03.029

23. Nanaware S, Shelar M, Sinnathambi A, Mahadik KR, Lohidasan S. Neuroprotective effect of Indian propolis in β-amyloid induced memory deficit: Impact on behavioral and biochemical parameters in rats. Biomed Pharmacother. 2017;93:543-53. Doi: https://doi.org/10.1016/j.biopha.2017.06.072

24. Hussein UK, Hassan NE, Elhalwagy ME, Zaki AR, Abubakr HO, Venkata KC, et al. Ginger and propolis exert neuroprotective effects against monosodium glutamate-induced neurotoxicity in rats. Molecules. 2017;22(11):1928. Doi: https://doi.org/10.3390/molecules22111928

25. Taysi S, Demir E, Cinar K, Tarakcioglu M. The Radioprotective effects of Propolis and Caffeic acid Phenethyl Ester on radiation-induced oxidative/nitrosative stress in brain tissue. Free Radic Biol Med. 2016;100:S111. Doi: https://doi.org/10.1016/j.freeradbiomed.2016.10.286

26. Shang H, Bhagavathula AS, Aldhaleei WA, Rahmani J, Karam G,Rinaldi G, et al. Effect of propolis supplementation on C-reactive protein levels and other inflammatory factors: A systematic review and meta-analysis of randomized controlled trials. J King Saud Univ Sci. 2020;32(2):1694-701. Doi: https://doi.org/10.1016/j.jksus.2020.01.003

27. Omar NA, Abu-Almaaty AH, El-Aziz YM, Abdeen AM, Mohamed FE, Hashem MM, et al. Impacts of Egyptian propolis extract on rat cerebellum intoxicated by aluminum silicate: histopathological studies. Environ Sci Pollut Res. 2019;26:22061-8. Doi: https://doi.org/10.1007/s11356-019-05469-4

28. Moriguchi S, Inagaki R, Saito T, Saido TC, Fukunaga K. Propolis promotes memantine-dependent rescue of cognitive deficits in APP-KI mice. Mol Neurobiol. 2022;59(7):4630-46. Doi: https://doi.org/10.1007/s12035-022-02876-6

29. Thomzig A, Laube G, Prüss H, Veh RW.. Pore-forming subunits of K-ATP channels, Kir6.1 and Kir6.2, display prominent differences in regional and cellular distribution in the rat brain. J Comp Neurol.

2005;484(3):313-30. Doi: https://doi.org/10.1002/cne.20469

30. Vivino FB, Al-Hashimi I, Khan Z, LeVeque FG, Salisbury III PL, Tran-Johnson TK, et al. Pilocarpine tablets for the treatment of dry mouth and dry eye symptoms in patients with Sjögren syndrome: a randomized, placebo-controlled, fixed-dose, multicenter trial. Arch Intern Med. 1999;159(2):174-81. Doi: https://doi.org/10.1001/archinte.159.2.174

31. Juvale II, Has AT. The evolution of the pilocarpine animal model of status epilepticus. Heliyon. 2020;6(7). Doi: https://doi.org/10.1016/j.heliyon.2020.e04557

32. Rios AT. Síndrome demencial e modelos experimentais para o estudo de doenças neurodegenerativas [undergraduate thesis]. Fortaleza. Curso de Ciências Biológicas, Universidade Federal do Ceará; 2017. 69 p. http://www.repositorio.ufc.br/handle/riufc/48178

33. Neha, Sodhi RK, Jaggi AS, Singh N. Animal models of dementia and cognitive dysfunction. Life Sci. 2014;109(2):73-86. Doi: https://doi.org/10.1016/j.lfs.2014.05.017

34. Balmus IM, Ciobica A, Negura A, Negura L, Anton E. Basic aspects in selecting a suitable transgenic rodent model for Alzheimer's disease. Psychiatr Danub. 2015;27(4):338-45.

35. Cacabelos R. Donepezil in Alzheimer's disease: From conventional trials to pharmacogenetics. Neuropsychiatr Dis Treat. 2007;3(3):303-33. Doi: https://doi.org/10.2147/ndt.s12160181

36. Müller T. Rivastigmine in the treatment of patients with Alzheimer's disease. Neuropsychiatr Dis Treat. 2007;3(2):211-8. Doi: https://doi.org/10.2147/nedt.S32211

37. Folch J, Busquets O, Ettcheto M, Sánchez-López E, Castro-Torres RD, Verdaguer E, et al. Memantine for the treatment of dementia: a review on its current and future applications. J Alzheimers Dis. 2018;62(3):1223-40. Doi: https://doi.org/10.3233/jad-170672

38. Croisile B, Trillet M, Fondarai J, Laurent B, Mauguière F, Billardon M. Long-term and high-dose piracetam treatment of Alzheimer's disease. Neurology. 1993;43(2):301. Doi: https://doi.org/10.1212/wnl.43.2.301

39. Flicker L, Grimley EJ; Cochrane Dementia and Cognitive Improvement Group. Piracetam for dementia or cognitive impairment. Cochrane Database of Syst Rev. 1996;2012(2). Doi: https://doi.org/10.1002/14651858.CD001011

40. Gonçalves AC, Rodrigues M, Santos AO, Alves G, Silva LR. Antioxidant status, antidiabetic properties and effects on Caco-2 cells of colored and non-colored enriched extracts of sweet cherry fruits. Nutrients. 2018;10(11):1688. Doi: https://doi.org/10.3390/nu10111688

41. Shimazawa M, Chikamatsu S, Morimoto N, Mishima S, Nagai H, Hara H. Neuroprotection by Brazilian green propolis against in vitro and in vivo ischemic neuronal damage. Evid Based Complement Alternat Med. 2005;2:201-7. Doi: https://doi.org/10.1093/ecam/neh078

42. El-Sharkawy HM, Anees MM, Van Dyke TE. Propolis improves periodontal status and glycemic control in patients with type 2 diabetes mellitus and chronic periodontitis: a randomized clinical trial. J Periodontol. 2016;87(12):1418-26. Doi: https://doi.org/10.1902/jop.2016.150694

43. Silveira M, Teles F, Melo E, Borges V, Miranda F, Dutra F, et al. Effects of Brazilian green propolis extract (Epp-Af) on inflammation in hemodialysis patients. Nephrol Dial Transplant. 2020;35(suppl. 3):1894. Doi: https://doi.org/10.1093/ndt/gfaa142.P1574

44. De Sarno P, Bijur GN, Zmijewska AA, Li X, Jope RS. In vivo regulation of GSK3 phosphorylation by cholinergic and NMDA receptors. Neurobiol Aging. 2006;27(3):413-22. Doi: https://doi.org/10.1016/j.neurobiolaging.2005.03.003

45. De-Mello N, Souza IQ Junior, Carobrez AP. Pilocarpine prevents age-related spatial learning impairments in rats. Behav Brain Res. 2005;158(2):263-8. Doi: https://doi.org/10.1016/j.bbr.2004.09.006

46. Messer WS. Cholinergic agonists and the treatment of Alzheimer's disease. Curr Top Med Chem. 2002;2:353-8. Doi: https://doi.org/10.2174/1568026024607553

47. Foster DJ, Choi DL, Conn PJ, Rook JM. Activation of M1 and M4 muscarinic receptors as potential treatments for Alzheimer's disease and schizophrenia. Neuropsychiatr Dis Treat. 2014;10:183-91. Doi: https://doi.org/10.2147/NDT.S55104

48. Scarpa M, Hesse S, Bradley SJ. M1 muscarinic acetylcholine receptors: a therapeutic strategy for symptomatic and disease-modifying effects in Alzheimer's disease? Adv Pharmacol. 2020;88:277-310. Doi: https://doi.org/10.1016/bs.apha.2019.12.003

49. Tamfu AN, Tagatsing FM, Talla E, Ozturk M, Mbafor JT, Duru ME. Chemical composition and evaluation of anticholinesterase activity of essential oil from Cameroonian propolis. Issues Biol. Sci. Pharm. Res. 2019;7(3):58-63. Doi: https://doi.org/10.15739/ibspr.19.007

50. Boulechfar S, Zellagui A, Chemsa AE, Bensouici C, Segueni N, Lahouel M, et al. Investigation of Antioxidant and Anticholinesterase Potential of Essential Oil and Methanolic Extract of Propolis from Mila Region. Journal of Biologically Active Products from Nature. 2019 Nov 2;9(6):434–44. Doi: https://doi.org/10.1080/22311866.2019.1703816

Received: 2023, November 14
Aceptted: 2024, April 16