Research Article **Open Access**

Passiflora ligularis extract improved acetylcholinesterase activity, oxidative stress markers, and neurocognitive outcomes in scopolamine-induced Alzheimer's model in zebrafish

Rosa Martha Pérez Gutiérrez*[3], Julio Téllez Gómez[1] Diana Maylet Hernández Martínez[2], Alethia Muñiz Ramirez[3], José María Mota Flores[1]

[1]Natural Products Research Laboratory, Department of Organic Chemistry, School of Chemical Engineering and Extractive Industries, National Polytechnic Institute (IPN), Adolfo López Mateos Professional Unit S/N, Av. National Polytechnic Institute, Mexico City, CP 07708, Mexico; [2]Pharmacology Research Laboratory, Department of Biophysics, National School of Biological Sciences, National Polytechnic Institute, Carpio S/N, Mexico City, Mexico; [3]SECIHTI - IPICYT / Advanced Materials Division, San Luis Potosí, 76216, Mexico

Corresponding Author: Rosa Martha Pérez Gutiérrez, SECIHTI -- IPICYT / Advanced Materials Division, Road to San José Dam, Lomas 4th Section, Zip Code 76216, San Luis Potosí, Mexico

Submission Date: June 17th, 2025, **Acceptance Date:** July 18th, 2025, **Publication Date:** July 24th, 2025

Please cite this article as: Gutiérrez R. M. P., Gómez J. T., Martínez D. M. H., Ramirez A. M., Flores J. M. M. *Passiflora ligularis* extract improved acetylcholinesterase activity, oxidative stress markers, and neurocognitive outcomes in scopolamine-induced Alzheimer's model in zebrafish. *Functional Foods in Health and Disease* 2025; 15(7): 479-494. DOI: https://doi.org/10.31989/ffhd.v15i7.1684

ABSTRACT

Background: *Passiflora ligularis* (granadilla) is known for its health benefits, including its potential in managing obesity. inflammation, and diabetes. This study aimed to evaluate the effects of an extract from *P. ligularis* fruit (PAS) o neuroinflammation, n cognitive function, and oxidative stress in a zebrafish model of scopolamine (SCO)-induced neurodegeneration.

Methods: Flavonoid profile was evaluated using high-performance liquid chromatography (HPLC-UV-MS). Zebrafish (*Danio rerio)* received oral PAS at dose of 4, 6, and 8 µg/ml or donepezil used as positive control (0.65 mg/kg) for 19 days, followed by SCO administration (100 µg/ml) one hour before behavioral tests. These included the novel tank

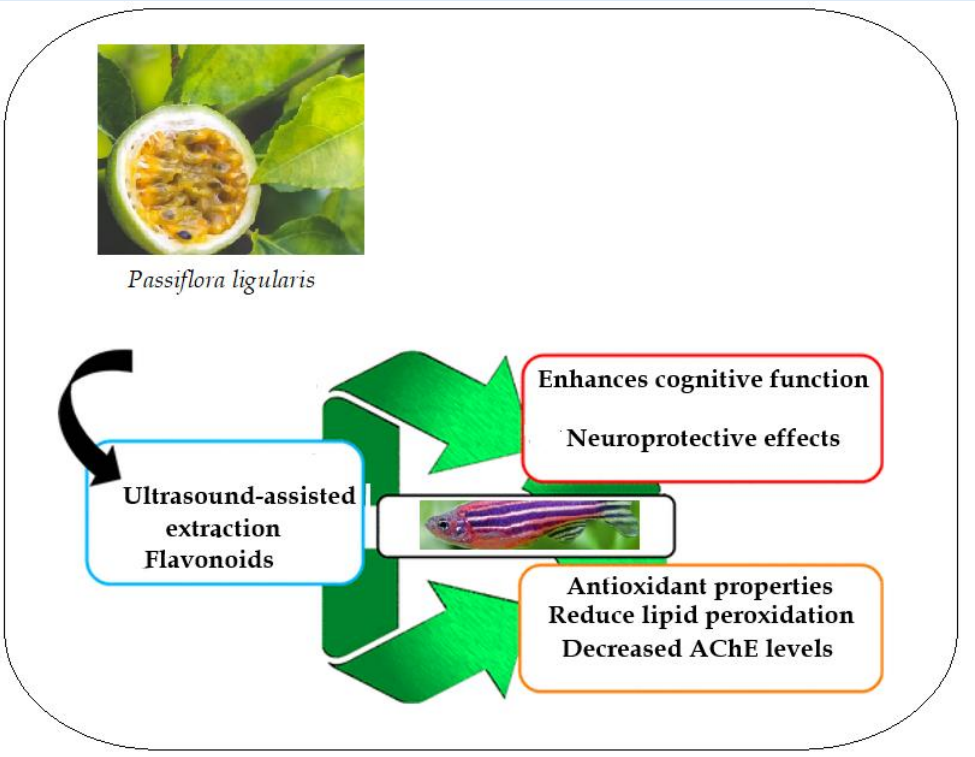
diving test (NTT), Y-maze test, and novel object recognition test (NOR). Post-experiment biochemical brain parameters were analyzed.

Results: HPLC-UV-MS identified 11 phenolic compounds previously reported for their neuroprotective potential. PAS treatment significantly improved acetylcholinesterase (AChE) activity, oxidative stress markers, and neurocognitive impairments, demonstrating effects comparable to the donepezil group.

Conclusions: These findings suggest that *Passiflora ligularis* extract may be a promising phytotherapeutic approach for alleviating memory impairment and symptoms associated with Alzheimer's disease.

Novelty of the study: This study investigates the effect of *Passiflora ligularis* extract on neurodegenerative disease in zebrafish model, revealing its potential to alleviate neurocognitive impairments mitigate neuroinflammation, reactive oxygen species and acetylcholinesterase.

Keyword: *Passiflora ligularis;* Alzheimer´s disease; neurodegenerative disease; oxidative stress



Graphical Abstract: The efficacy of *Passiflora ligularis* extract in the restoration and enhancement of neurocognitive disease

INTRODUCTION

Alzheimer's disease (AD) is a neurodegenerative condition characterized by a decline in biological functions, including the neurotransmitter acetylcholine (ACh), which naturally decreases with aging. This reduction is significantly more pronounced in individuals

with AD and has been linked to elevated acetylcholinesterase (AChE) activity. AChE has been associated with βA-fibrils and the aggregation of these structures, contributing to disease progression [1]. Several pharmacological treatments target these mechanisms to alleviate clinical symptoms. Donepezil and Galantamine function as acetylcholinesterase inhibitors, while Rivastigmine inhibits both acetylcholinesterase and butyrylcholinesterase. Additionally, Galantamine modulates nicotinic receptors [2], and Memantine acts as an N-methyl-D-aspartate (NMDA) receptor antagonist [3]. AD manifests as a progressive decline in cognitive abilities, initially appearing as memory loss and difficulty forming new memories. Over time, individuals experience significant impairment in recalling past events. Neurodegeneration also affects brain regions responsible for motor control and language [4].

Neuroinflammation has an important role in maintaining brain homeostasis and can be triggered by various stimuli. If sustained, it may contribute to neurodegeneration. Microglia, immune cells in the central nervous system (CNS), actively monitor these stimuli and execute protective functions to preserve neural equilibrium [4]. However, an imbalance between phagocytosis and pro-inflammatory factors can initiate an inflammatory response, activating NF-κB signaling [5]. This leads to the production of pro-inflammatory cytokines, such as tumor necrosis factor (TNF)-α, and interleukin IL-6, IL-1β exacerbating AD pathology [6].

Reactive oxygen species (ROS) are very reactive molecules involved in redox processes due to the presence of an unpaired electron in their atomic orbitals [7]. The link between oxidative stress and Alzheimer's disease (AD) progression is evidenced by several factors, including mitochondrial dysfunction, the redox potential of Aβ metal ions, macromolecular peroxidation, and the upregulation of p-tau and Aβ amyloid formation [8]. Currently pharmacological treatments exist for AD, they primarily slow disease progression rather than prevent or reverse it, as they do not address its underlying cause. In recent years, researchers have explored innovative treatments through multi-faceted and multi-level studies to develop more effective pharmaceuticals [9]. Thus, there is a pressing need to identify a novel and reliable neuroprotective agent characterized by safety, and stability against neurotoxicity.

Scopolamine (SCO) in Alzheimer's disease model is widely used for studying Alzheimer's disease (AD) due to its ability to induce damage in the cellular antioxidative system, increase oxidative stress, inhibit acetylcholinesterase (AChE) activity, impair mitochondrial function, trigger neuroinflammation, and promote apoptosis. These processes contribute to the accumulation of amyloid plaques and tau protein, which are hallmarks of AD [10].

Zebrafish (*Danio rerio*), owing to their genetic similarities with humans, serve as a valuable animal model for studying neurodegenerative diseases [11]. *Passiflora ligularis* fruit contains a diverse range of phenolic compounds, including tannins, anthocyanins, and flavonoids [12]. Research has highlighted its antidiabetic [12], anti-inflammatory [13], antilipidemic [14], and inhibitory effects on alpha-amylase and alpha-glucosidase [14]. Additionally, it has shown neuroprotective potential against autism [15] and exhibits hepatoprotective and nephroprotective properties [16To further investigate the neuroprotective effects of *Passiflora ligularis*, we examine its hydroethanolic extract in models of SCO-induced neurotoxicity in zebrafish.

Materials and Methods

Ethical considerations: All procedures involving the fish were ethically approved by the Ethics Committee of the Escuela Nacional de Ciencias Biológicas-IPN (ENCB-

CEI/2024) and conducted in accordance with the guidelines established by the Committee on Care and Use of Laboratory Animals (NIH publication 85-23, revised 1985). Additionally, all procedures complied with the Mexican Official Standard (NOM-062-Z00-1999).

Chemicals: All reagents were obtained from Sigma-Aldrich (St. Louis, MO, USA).

Ultrasonic-Assisted Extraction (UAE): *Passiflora ligularis* fruit was sourced from Mercado de Jamaica, CDMX, and authenticated at the herbarium of the Escuela Nacional de Ciencias Biológicas - Instituto Politécnico Nacional, CDMX. A voucher specimen (4578) was deposited for reference. The fruit was dried at 45 °C in a hot air oven, then ground using an electric grinder to achieve a particle size of 0.5 mm. Phenolic compounds were extracted using an ultrasonic cleaning bath (Bransonic, Stemart, NY, USA), operating at 3 × 50W, 40 kHz, and 40°C for 20 minutes with an ethanol-water (50%/50%) solvent. The extracts were separated from the sediment via filtration

Total Flavonoid Content (TF): TF was analyzed using a spectrophotometer (Model 1800, Shimadzu, Kyoto, Japan). The measurement involved mixing 1 mL of aluminum chloride with 25 mL of the ethanol-water mixture (50%/50%) used during extraction, followed by the addition of 1 mL of the stock solution. A blank was prepared using 1 mL of the aluminum chloride solution. Absorbance was recorded at 311 nm, with isoquercetin serving as the reference standard [17]. The assay data were expressed in terms of isoquercetin concentration, following the calibration curve equation: $y = 4.687x - 0.0046$ ($R^2 = 0.9995$).

HPLC-UV-MS Analysis: High-performance liquid chromatography (HPLC) analysis was conducted using an Agilent 1100 LC system (Waldbronn, Germany) with a SunFire™ C18 reversed-phase column (5.0 µm, 150 mm × 4.6 mm; Waters) maintained at 30 °C. The mobile phase consisted of methanol (A) and 0.2% acetic acid (B), with the following gradient profile: 0–5 min: 55% A 5–20 min: 55%–70% A 20–30 min: 70%–100% A 30–40 min: 100% A The flow rate was 1.0 $mL \cdot min^{-1}$, and UV absorbance was monitored at 310 nm. Compound identification was based on unique mass and fragmentation spectra, compared with reference data from PubChem, MassBank, NIST, and mzCloud.

Zebrafish Model: Sixty adult wild-type *Danio rerio* zebrafish (males, 8 months old) were housed under a 14:10-hour light/dark cycle at 28.5 ± 0.5 °C in a tank with filtered and aerated drinking water at a density of two fish per liter. They were fed daily with brine shrimp and commercial flake fish food (Ocean Nutrition, Newark, U.S.A.). Cognitive testing was performed after a two-week acclimation period.

Experimental protocol: Group I: control without exposure to any treatment. Group 2: scopolamine control (SCO, 100 µM, Sigma–Aldrich, St Louis, MO, USA) Group 3: PAS treatment (4 µg/L) Group 4: PAS treatment (6 µg/L) Group 5: PAS treatment (8 µg/L) Group 6: donepezil treatment as positive control (20 µg/L)

The doses of SCO, PAS, and donepezil were selected based on previous studies. SCO (100 µM) was administered 30 minutes before the behavioral experiments [20]. PAS (4, 6, and 8 µg/L) and donepezil (20 µg/L) were delivered via immersion in a 500 mL glass container for 1 hour once daily. Following treatment, the animals were transferred to a separate water tank system for 5 minutes before undergoing behavioral testing. To minimize the number of fish used, only one behavioral assay battery was conducted at a time. All behavioral assessments took place in the morning

between 8:00 and 11:00 a.m.

Neurocognitive Function Assessment: After exposure to SCO, the animals underwent neurocognitive function evaluation using various tests, including the light/dark chamber test, Y-maze test, and novel object recognition (NOR) test.

Light and Dark Chamber Test: The Light and Dark Chamber Test assesses spatial memory using a specialized tank with dimensions of 23 cm in height, 10 cm in width, and 20 cm in length. The tank is divided into two equal segments of 10 cm each and maintains a water level of 12 cm. One segment, designated as the dark chamber, is covered with black paper, while the other remains exposed to natural light (50 lux). Typically, fish prefer the illuminated chamber, spending only a brief period in the dark chamber. Following treatment, a fish's choice of the light chamber suggests an improvement in memory function. Conversely, selecting the dark chamber indicates impaired memory performance [18].

Test (NOR): Novel Object: The novel object recognition test is a widely used behavioral assay for evaluating memory function in zebrafish models [19]. The experiment begins with a three-day acclimation phase, during which fish are introduced to a novel tank (30x30x30 cm) filled with 6 cm of water—free of objects—for 5 minutes each day. On the fourth day, the training phase commences, where fish are presented with two identical objects for a duration of 10 minutes. One hour later, during the test phase, one of the familiar objects (FO) is replaced with a novel object (NO), and the fish's interactions are observed for 10 minutes. Memory performance is quantified by calculating preference percentages.

$$[(time\ of\ exploration\ of\ NO)/(time\ of\ exploration\ of\ FO) + time\ of\ exploration\ of\ NO\ \ x\ 100\ ]$$

Y-Maze Task: The Y-maze task was conducted to evaluate the fish's response to a novel environment. A Y-shaped glass tank (dimensions: 25x8x15 cm per arm; total volume: 5 L) was used. The three arms of the maze were designated as the start arm, the always open arm, and the novel arm. During the training session, the novel arm was blocked, while all arms were accessible during the test session. Each fish was individually placed in the start arm for a 5-minute training session, with the novel arm closed. After a 1-hour interval, the 5-minute test session commenced, during which the fish were placed back in the start arm, now with the novel arm open. Locomotor activity was assessed based on turning angle (%) and distance traveled (m), while response to novelty was determined by measuring the time spent in the novel arm (% of total arm time). This test served as an index of memory [20]. Following the completion of the test, brain tissue was collected for biochemical analyses.

Biochemical Parameter Test Brain Tissue Preparation: Immediately after the neurocognitive assessment, the fish were euthanized via hypothermia, and their brains were swiftly extracted. The brain tissue was homogenized in ice (1:10) with **1**.15% KCl and potassium phosphate buffer (K_3PO_4; 0.1 M; pH 7.4). The homogenates were subjected to centrifugation at 1000 × g for 15 minutes, and the resulting supernatant was used for biochemical analysis

Determination of Biochemical Parameters: The enzymatic activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and reduced glutathione (GSH), as well as the malondialdehyde (MDA) level, were quantified using commercial kits, following the manufacturer's protocols (Abcam, Cambridge, UK). Additionally, acetylcholinesterase (AChE) activity was assessed using a

fluorometric red kit (Abcam, Cambridge, UK).

Statistical Analysis: Data were analyzed through one-way analysis of variance (ANOVA), followed by Tukey's post hoc multiple comparison test, with statistical significance set at $p < 0.05$. Analyses were performed using GraphPad Prism 7.0 (GraphPad, La Jolla, CA, USA). All behavioral tests were video recorded using a Logitech C525 and analyzed with ANYmaze® tracking software (Stoelting Co., USA).

RESULTS

Ultrasound-Assisted Extraction of Total Flavonoids (TF): An ultrasound-assisted extraction method was employed to obtain total flavonoids from *P. ligularis*. The content of TF, expressed as isoquercetin-equivalents per mg of extract, was determined in a hydroalcoholic extract. The results revealed that the TF yield from ultrasound extraction was 148.21 µg isoquercetin-equivalent/mg, surpassing the values obtained using water (60.983 µg isoquercetin-equivalent/mg) and ethanol combined with XAD-2 resin (134.99821 µg isoquercetin-equivalent/mg) [23].

Profiles of Phenolic Compounds: The hydroalcoholic extract of *Passiflora ligularis* was analyzed using HPLC-UV-MS (Figure 1), revealing a phenolic profile characteristic of the *Passiflora* species. The identified compounds included isovitexin (1), rutin (2), catechin (3), epicatechin (4), orientin (5), apigenin (6), luteolin (7), quercetin (8), ferulic acid (9), vitexin (10), and chrysin (11). Among them, ferulic acid was the most abundant. All these compounds have been previously reported [11-14].

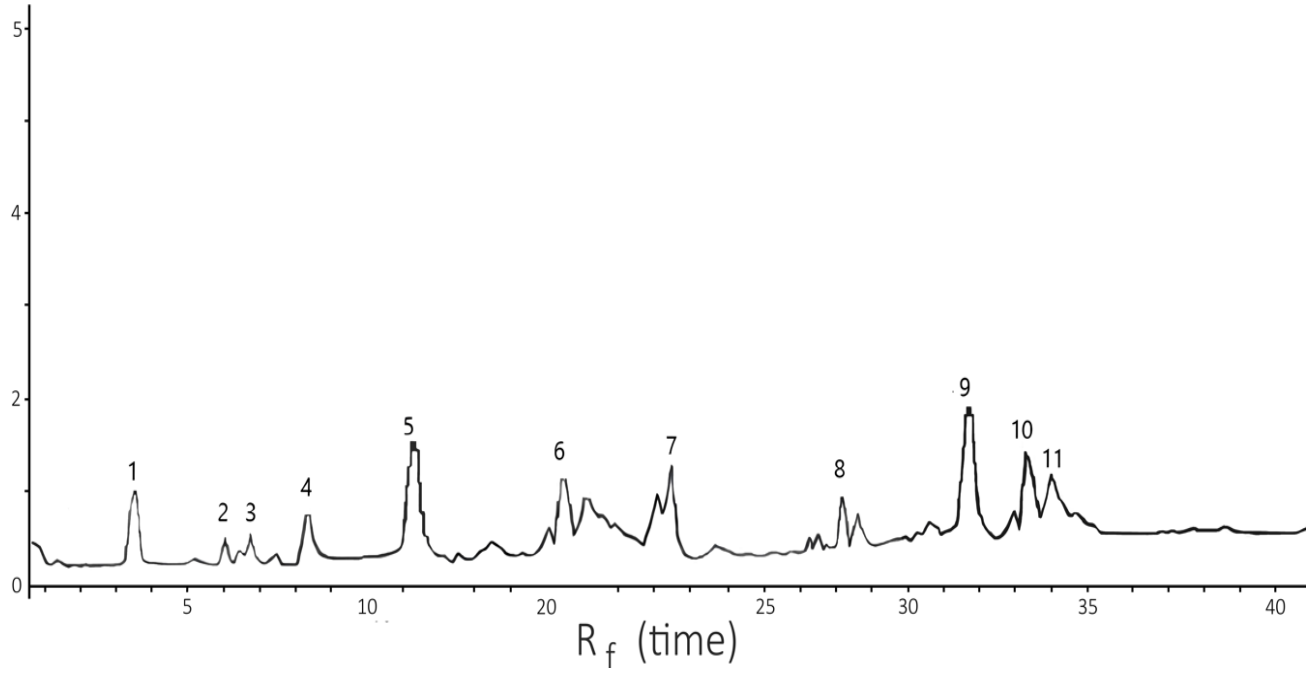


Figure 1. Chromatograms of *extract from P. ligularis* by HPLC-UV-MS analysis. The main phytochemicals are marked by numbers: isovitexin, (1), rutin, (2), catechin (3), epicatechin (4), orientin (5), apigenin (6), luteolin (7), quercetin (8) ferulic acid (9), vitexin, (10), chrysin (11).

Evaluation of Neurocognitive Functions

Effects of PAS in the Light and Dark Chamber Test: AD primarily affects short-term memory in its early stages, eventually leading to long-term memory deficits. Experimentally, cognitive responses to pharmacological treatments for AD can be examined in animal models through behavioral and cellular assessments. Previous studies have validated zebrafish as a reliable model for investigating cognitive phenotypes. Their memory performance is frequently evaluated using T- and Y-maze tests, which assess spatial memory deficits. These tests are particularly relevant in non-genetic models, such as those induced by scopolamine (SCO), a cholinergic receptor antagonist that disrupts short-term memory and learning while also affecting locomotor activity [21].

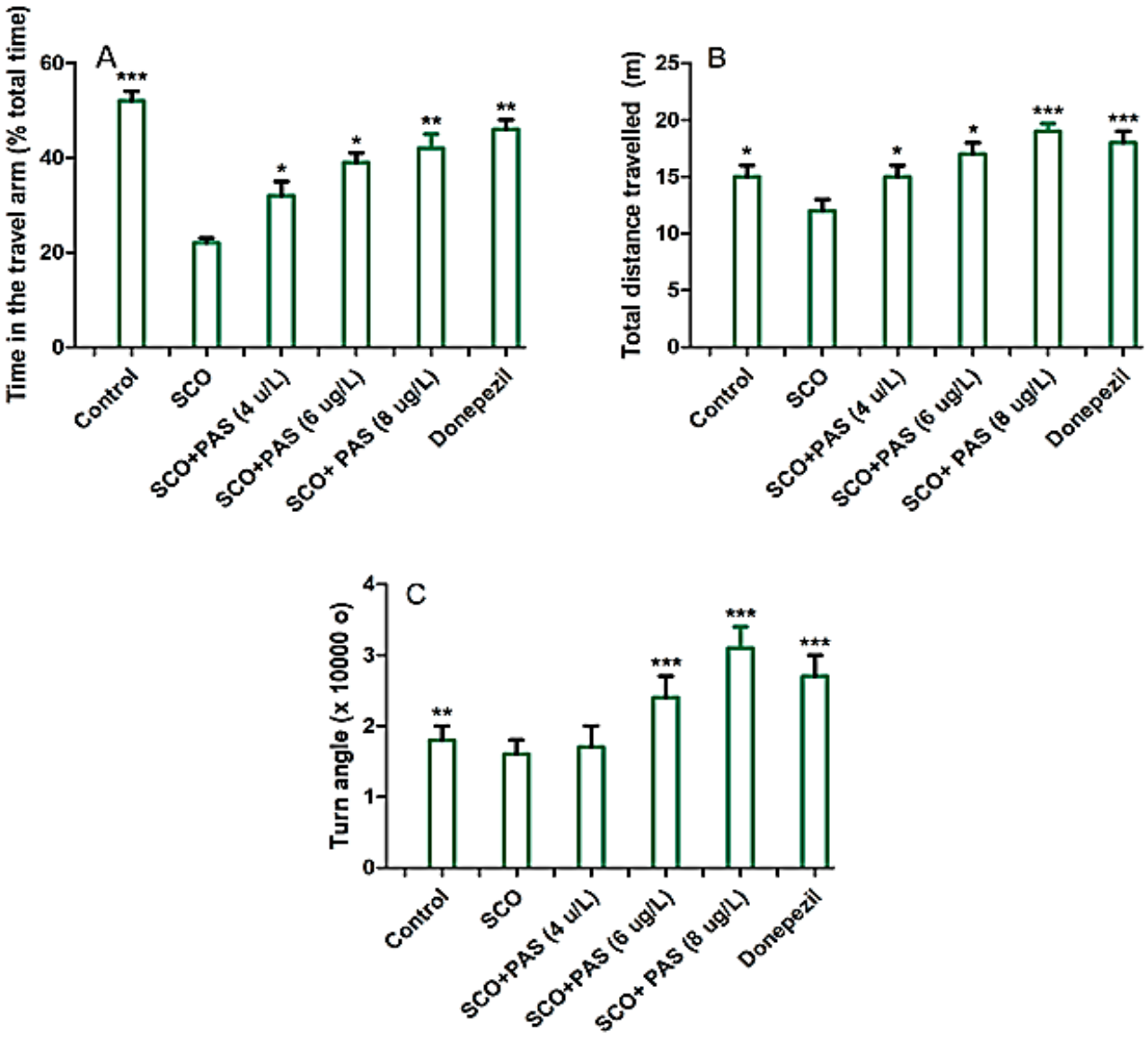


Figure 2. Schematic representation of *P. ligularis* in the light-dark transition test in zebra fish. **(A**) the light zone (TSLC); (**B**) the dark zone (TSDC). Results demonstrated a significant difference between the time spent in the dark and light chambers. Data are expressed as the mean ± SD (n=10), ***p < 0.001, **p < 0.01, and *p < 0.05 compared with SCO.

Effect of PAS in the Novel Object Recognition (NOR) Test: Long-term memory was evaluated using the NOR test, which measures the ability to differentiate a novel object from a familiar one [22]. Fish treated with SCO 8 mg/L significantly mitigated SCO-induced memory impairment ($p < 0.05$), enhancing exploration of the novel object (Figure 3). Our findings align with previous studies demonstrating the cognitive benefits of flavonoids in SCO-induced AD models [22].

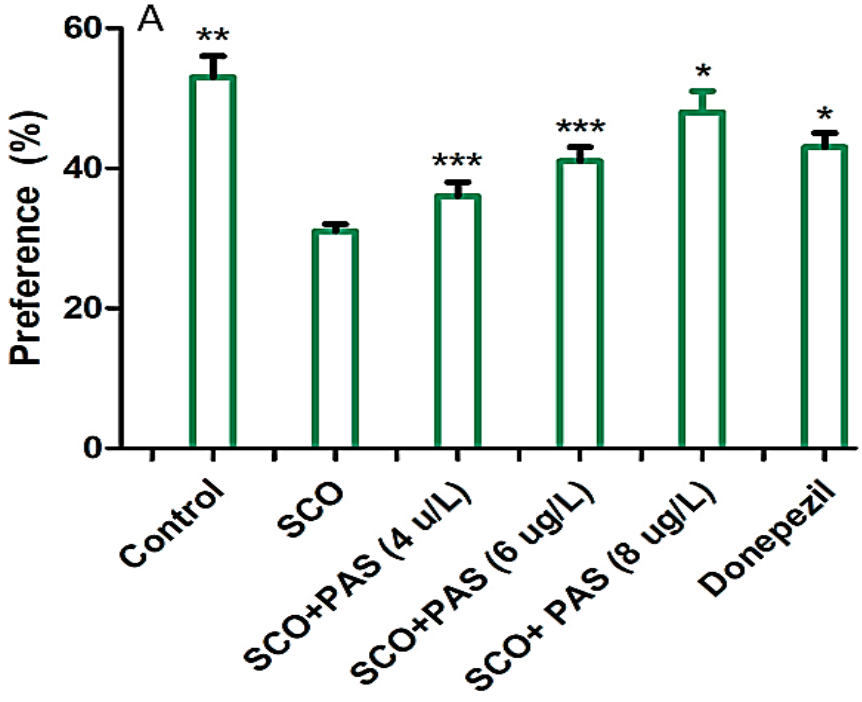


Figure 3. *Effect of* extract From *P. ligularis* in exploration time of the new objects during the testing day of the novel object recognition test (NOR). All data are shown as % exploration. Data are expressed as the mean ± SD (n=10), ***p < 0.001, **p < 0.01, and *p < 0.05 compared with SCO.

Effect of PAS in the Y-Maze Test: SCO treatment impaired spatial memory, as evidenced by a substantial reduction in time spent in the novel arm of the Y-maze (Figure 4A). While animals in the normal group explored all three arms, SCO treatment induced hyperlocomotion, leading to reduced activity in the novel arm, a lower total distance traveled (Figure 4B), and a decreased absolute angle of turn (Figure 4C) compared to controls. PAS administration significantly improved locomotor activity compared to the SCO-treated group. Specifically, PAS at doses of 6 mg/L and 8 mg/L alleviated SCO-induced memory deficits, enhancing cognitive performance in zebrafish.

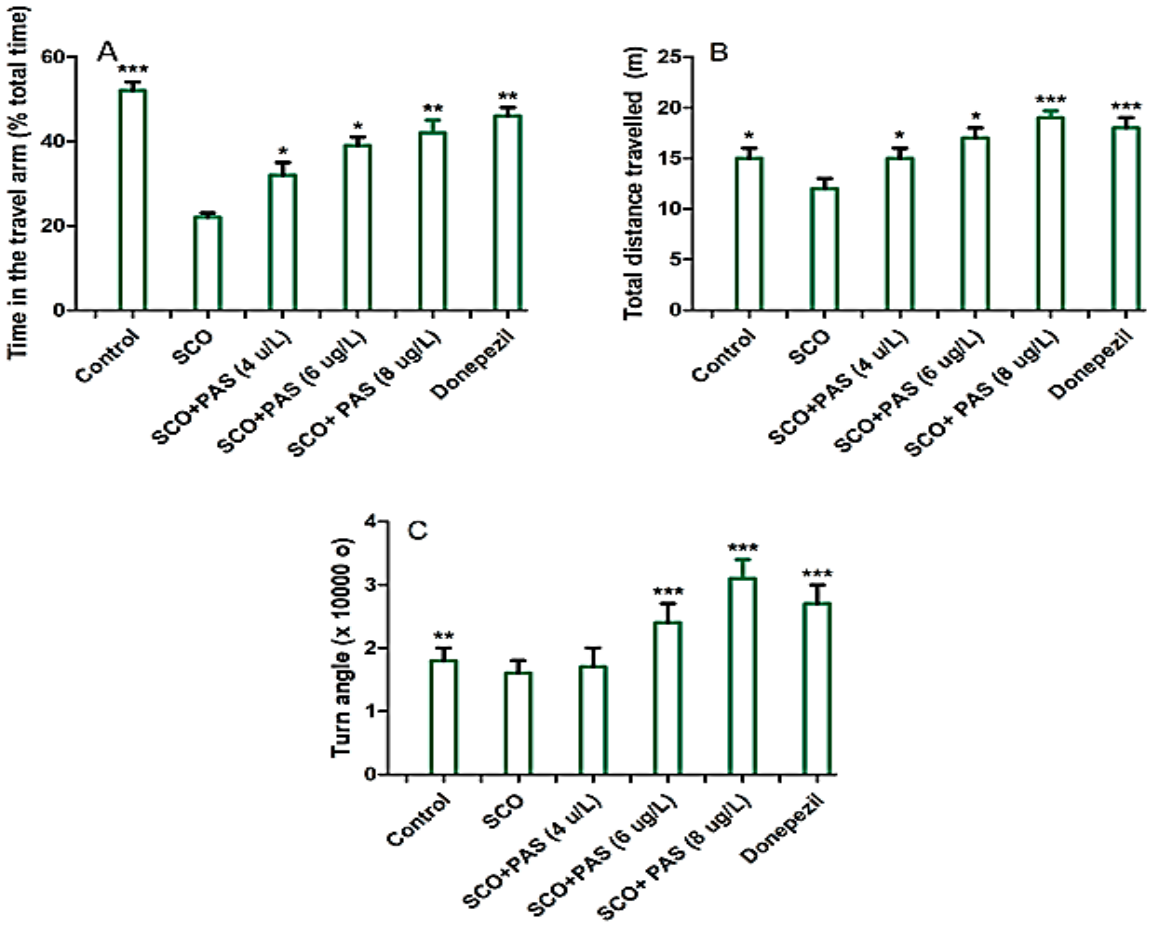


Figure 4. Y-maze test of the hydroethanolic extract Fom *P. ligularis* (PAS) at concentrations of 4 µg/L, 6 µg/L and 8µg/L in memory and locomotion; (**A**) Time spent in the novel arm; (**B**) Total distance traveled; (**C**) Turn angle. Data are expressed as the mean ± SD (n=10), ***p < 0.001, **p < 0.01, and *p < 0.05 compared with SCO.

ChE evaluation: AChE activity plays a crucial role in the cholinergic system, which is essential for memory processing. Increased AChE activity leads to alterations in cholinergic neurons, resulting in dysfunctions. In Alzheimer's disease (AD), memory and learning enhancement are associated with elevated AChE activity. Our results demonstrated a significant increase in AChE activity in zebrafish treated with scopolamine (SCO) compared to the control group. However, treatment with PAS extract significantly reduced AChE activity across all tested doses. Figure 5 illustrates the effect of PAS on AChE activity levels in comparison to donepezil. PASPAS extract exhibited strong inhibitory activity against AChE, thereby improving cholinergic function and mitigating cognitive impairment. These fruits have abundant bioactive compounds and can serve as nutritious food or medicinal resources, depending on the intended application. Ethnopharmacological strategies combined with bioassay-guided isolation have proven effective in identifying plant-derived compounds with potential acetylcholinesterase (AChE) inhibitory activity, particularly for memory-related disorders [23].

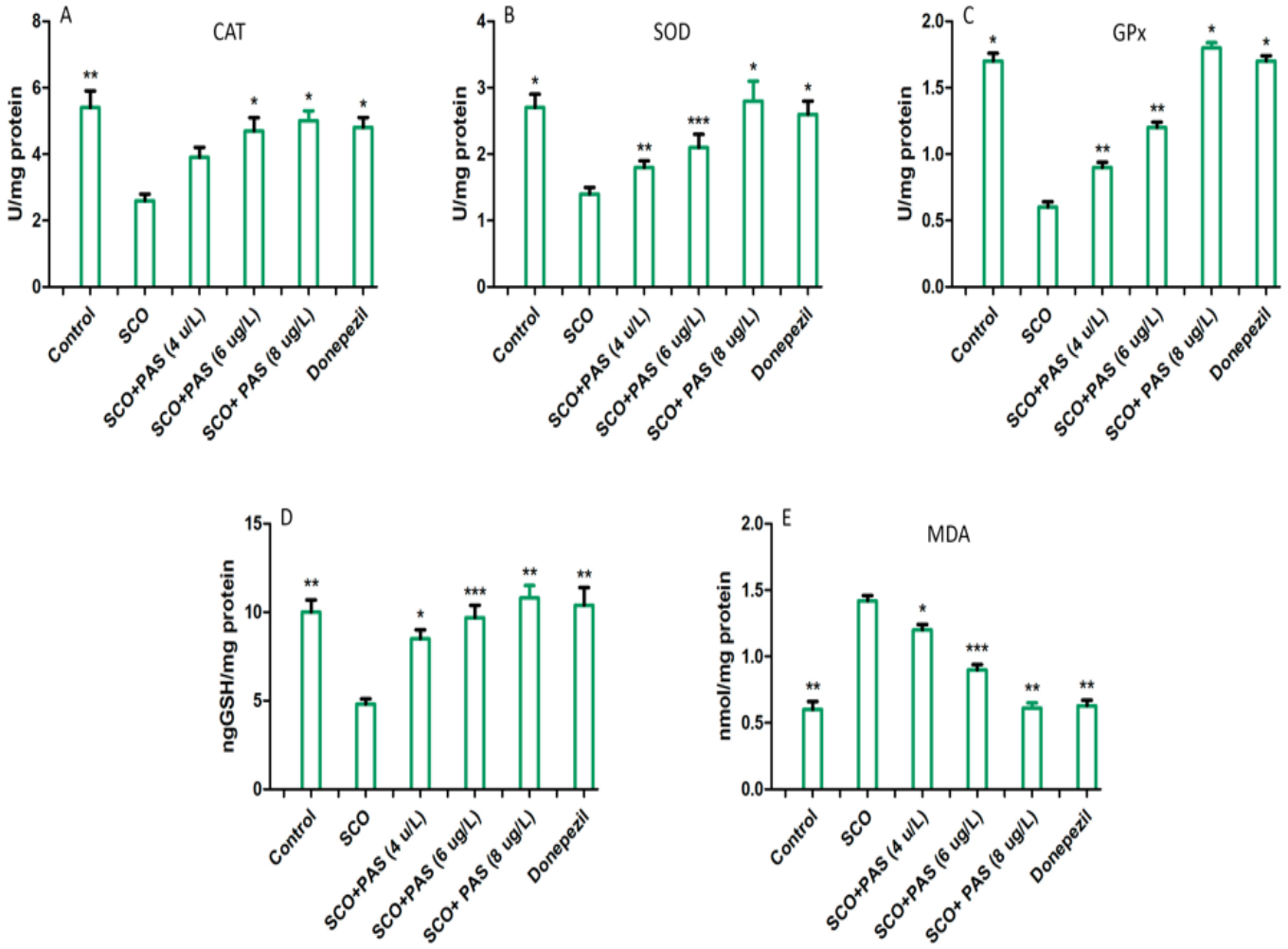


Figure 5. *P. ligularis* (PAS) hydroethanolic extract (4 µg/L, 6 µg/L and 8µg/L) alleviate oxidative stress in the zebrafish brain. Impact of PAS on: (**A**) CAT; (**B**) SOD; (**C**) GPx; (MDA (**E**). Values are means ± DS (N=10) of three replicates. Different asterisks indicated significant difference ***p < 0.001, **p < 0.01, and *p < 0.05 compared with SCO.

Antioxidant Activity: The results suggest that PAS extract may have potential applications in treating cognitive impairment. SCO administration exhibits strong prooxidant effects, as evidenced by increased reactive oxygen species (ROS) levels. This is reflected in the reduction of antioxidant enzyme activities, including superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX), and reduced glutathione (GSH) (Figure 6A-D). Additionally, there is a significant increase in malondialdehyde (MDA) levels (Figure 5E) and carbonylated proteins (Figure 6A), leading to lipid and protein oxidation. Previous studies indicate that SCO induces oxidative stress and contributes to memory impairment [24]. Oxidative stress is a key contributor to the onset of numerous diseases. Edible plants known for their antioxidant properties offer promising potential in preventing or reducing the formation of reactive species [25-26]

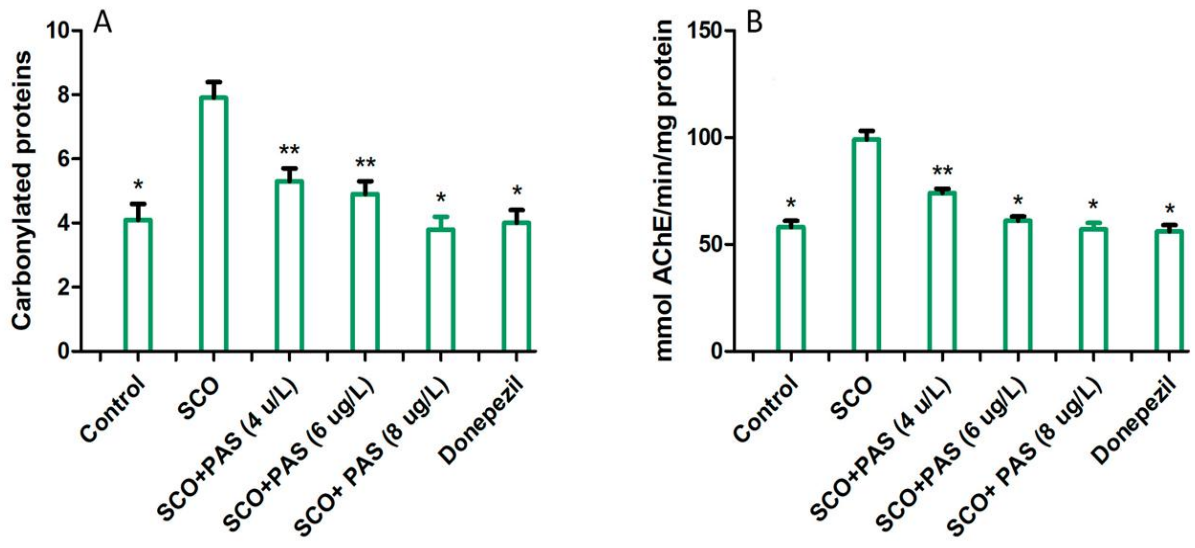


Figure 6. Impact of PAS on: (**A**) carbonylated proteins levels in different groups; (**B**) exhibited an anti-AChE effect in the zebrafish brain. Values are means ± DS (N=10). analyses: **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, and * $p < 0.05$.

DISCUSSION

A total of 11 phenolic compounds were identified through HPLC-UV-MS analysis, confirming that the hydroalcoholic extract has a chemical composition comparable to previous reports [11-14]. These flavonoids support antioxidant activity and memory enhancement. The neuroprotective properties of key compounds including isovitexin have been shown to improve spatial memory, decrease amyloid-beta levels, reduce neuroinflammation, and alleviate autophagic dysfunction via miR-107 signaling [27]. Rutin helps ameliorate amyloid-beta burden, plasticity impairment, synaptic loss, and neuroinflammation [28]. Catechin enhances neuronal processes and prevents tau oligomer aggregation [29]. Epicatechin reduces neurofibrillary tangles, extracellular amyloid accumulation, and improves synaptic plasticity [30]. Furthermore, orientin inhibits oxidative stress, acetylcholinesterase (AChE) activity, and inflammatory cytokines [31]. Apigenin mitigates reactive oxygen species (ROS), suppresses inflammatory pathways, and slows disease progression [32]. Luteolin alleviates memory and learning deficits, reduces neuroinflammation, and downregulates endoplasmic reticulum (ER) stress markers GRP78 and IRE1α in brain tissues [33]. Lastly, quercetin enhances cognitive function, inhibits amyloid-beta aggregation and tauopathy, and possesses anti-inflammatory and antioxidant properties that improve mitochondrial function [34]. Ferulic acid exhibits anti-inflammatory, antioxidant, and anti-Aβ aggregation properties, preventing hyperphosphorylated tau protein accumulation and inhibiting AChE activity. Additionally, it provides neuroprotection by preventing Aβ-fibril formation [35]. Vitexin has been found to alleviate neuroinflammation in animal models of Alzheimer's disease (AD) [36]. Chrysin counteracts lipid peroxidation, enhances antioxidant enzyme activity, and mitigates learning impairment, neuroinflammation, and memory

loss by modulating Na+/K+-ATPase activity and glutamate [37].

SCO exposure leads to significant memory impairment ($p < 0.05$) compared to the control group. However, PAS pretreatment significantly improves cognitive performance ($p < 0.01$), increasing the number of entries into the light zone (TSLC) reducing the time spent in the dark chamber (TSDC). The beneficial effects of PAS appear dose-dependent, resembling the neuroprotective impact of Donepezil. The results are depicted in Figure 2A-B. PAS extract significantly ameliorates SCO-induced cognitive dysfunction, increasing TSLC while reducing NEDC values, thus suggesting improved locomotor function.

In the NOR test PAS extract exhibited a reduction in amnesic profile in zebrafish mainly associated with the presence of flavonoids compared with SCO group. Our results are supported by previous investigations that demonstrated that flavonoids improve cognitive impairment in AD animal models [38].

To evaluate the effect of flavonoids in locomotor activity were assessed by Y-maze test. Our results suggest that the time spent and the number of entries exceeding a threshold of one indicate healthy memory function. The behavioral findings from the Y-maze test demonstrate that PAS extract at doses of 4, 6, and 8 µg/L enhances retention memory and cognitive function in a zebrafish model of scopolamine-induced AD.

Scopolamine (SCO) is an anticholinergic ligand that blocks the binding of acetylcholine (ACh) to its receptor [39thereby contributing to cognitive impairment in zebrafish [39]. Consequently, SCO is widely recognized as a key model for studying cognitive enhancement in zebrafish. SCO increases ACh levels through various mechanisms, including AChE inhibition and an agonistic effect on nicotinic ACh receptors [39]. Our findings suggest that PAS extract competitively reduces AChE activity, leading to an increase in ACh levels. Further studies are needed to confirm this mechanism. SCO-induced exacerbation of AChE activity was observed across all tested concentrations. The decline in ACh levels is likely due to increased ACh hydrolysis, resulting in altered behavioral responses and neuromuscular activity. In the NOR and Y-maze tests, PAS demonstrated a cholinesterase-inhibitory effect, contributing to memory enhancement in zebrafish.

Biochemical analysis of brain homogenates treated with PAS extract reveals a significant restoration of antioxidant enzyme activity in zebrafish exposed to SCO. This suggests that PAS extract possesses antioxidant properties, as demonstrated by the reduction in lipid peroxidation products, including MDA levels. The findings indicate that PAS extract exerts neuroprotective effects through its antioxidant action, enhancing antioxidant enzyme activity and reducing MDA levels. Excess ROS can damage nucleic acids, proteins, and lipids, resulting in DNA oxidation, protein oxidation, and lipid peroxidation. SCO exposure is reported to induce memory loss due to increased oxidative stress in the brain [40]. Zebrafish exposed to SCO (100 µM) show a significant ($p < 0.05$) activity ameliorates the effects of SCO on antioxidant enzymes by reducing the increase in MDA and preventing the decrease in GSH levels in the zebrafish brain. Our findings suggest that PAS exhibits neuroprotective activity against oxidative stress due to its high phenolic compound content. In groups treated with PAS extract, flavonoids provide protection against oxidative stress. Several flavonoids have demonstrated significant antioxidant properties in animal studies [41].

The treated fish show an increase in antioxidant enzyme activity, enhancing their resistance to pro-oxidative compounds induced by SCO exposure.

This study's findings are consistent with previous research on donepezil, which served as positive control. Donepezil is known for its neuroprotective and antioxidant properties. Similarly, pretreatment with PAS extract inhibits AChE and MDA activity while enhancing SOD, CAT, GPX, and GSH levels in the zebrafish brain. These biochemical changes help mitigate cognitive impairments caused by SCO exposure. The neuroprotective effects of PAS extract may stem from flavonoids, which interact with toxic compounds and reduce their toxicity.

Bridging to Functional Food Science: Plants have long held a dual role in human history, serving both nutritional and medicinal purposes. Literature suggests that with the advent of hunting practices, Plants began to assume a significant place in the human diet. Among natural resources, a wide range of products are utilized for medicinal purposes, including crude drugs, traditional remedies, and medicinal plants. Some of these serve dual roles as both therapeutic agents and edible items, often classified as health or functional foods. These functional foods are recognized for their tertiary role—modulating physiological functions with the aim of supporting and enhancing human health through dietary means. Building on this background, our research in pharmaceutical food science has focused on identifying bio-functional products derived from natural resources that may help prevent lifestyle-related diseases or alleviate their early symptoms.

Scientific Innovation and Practical Implications: The aim of this study was to demonstrate the potential of *Passiflora ligularis* as a medicinal plant, owing to its rich content of phytochemicals such as polyphenols, sterols and lipids. Bioactive compounds may contribute to the treatment and as an agent in the treatment and prevention of neurodegenerative disease. The consumption of *P. ligularis* has increased in recent years due to its diverse functional properties including anti-inflammatory potential, antidiabetic, antilipidemic and antioxidant activities. Regular intake of fruit may support overall health and reduce the risk or alleviate symptoms of memory impairment associated with Alzheimer's disease.

CONCLUSION

Our findings indicate that PAS extract enhances cognitive function in a scopolamine-induced zebrafish model of Alzheimer's disease (AD). It significantly improves retention memory, as demonstrated by the Y-maze test, and boosts locomotor function, as assessed by the light and dark chamber test. Additionally, PAS extract enhances recognition memory, as shown in the NOR test. Beyond cognitive benefits, the extract exhibits direct and indirect endogenous antioxidant properties, including increased levels of reduced glutathione and antioxidant enzymes, reduced lipid peroxidation, decreased AChE levels, and neurotransmitter regulation. Furthermore, PAS extract demonstrates potential neuroprotective effects in the zebrafish model, suggesting its possible role in treating neurodegenerative disorders such as Alzheimer's disease.

ABBREVIATION: DN: AChE: acetylcholinesterase, AD: Alzheimer's disease, CAT: catalase, CNS: central nervous system, CDMX: ciudad de México, GPX: glutathione peroxidase, MDA: malondialdehyde NMDA: N-methyl-D-aspartate, ROS: reactive oxygen species; SCO: Scopolamine, SOD: superoxide dismutase,

Acknowledgments and Funding: This research was supported by the Instituto Politécnico Nacional (No.20245671).

AUTHOR CONTRIBUTIONS: Conceptualization, R.M.P.G. and A.M.R.; writing—original draft, J.T.G.; K.L.P.G.; writing-review and editing, A.M.R.; and D.M.H.M.; supervision, A.M.R.; and J.T.G. All authors have read and agreed to the published version of the manuscript.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper

REFERENCES

1. Singh B, Day CM, Abdella S, Garg S.J Control Release. Alzheimer's disease current therapies, novel drug delivery systems and future directions for better disease management. *J. Control Release.* 2024; 367:402-424 DOI: https://doi.org/10.1016/j.jconrel.2024.01.047.
2. Varadharajan A, Davis AD, Ghosh A, Jagtap T, Xavier A, Menon AJ, Roy D, Gandhi S, Gregor T.J Guidelines for pharmacotherapy in Alzheimer's disease A primer on FDA-approved drugs. *Neurosci Rural Pract.* 2023;14(4):566-573. DOI: https://doi.org/10.25259/JNRP_356_2023
3. Dubois B, López-Arrieta J, Lipschitz S, Doskas T, Spiru L, Moroz S, Venger O, Vermersch P, Moussy A, Mansfield CD, Hermine O, Tsolaki M. Masitinib for mild-to-moderate Alzheimer's disease: results from a randomized, placebo-controlled, phase 3, clinical trial tudy Group Investigators. *Alzheimers Res Ther.* 2023; 28;15(1):39. DOI: https://doi.org/10.1186/s13195-23-01169
4. Moussavi Z, Uehara M, Rutherford G, Lithgow B, Millikin C, Wang X, Saha C, Mansouri B, Omelan C, Fellows L, Fitzgerald PB, Koski L Repetitive transcranial magnetic stimulation as a treatment for Alzheimer's disease: A randomized placebo-controlled double-blind clinical trial. *Neurotherapeutics.* 2024;21(3): e00331. DOI: https://doi.org/10.1016/j.neurot.2024.e00331.
5. Kim Y, Lim K, Oh T. Taming neuroinflammation in Alzheimer's disease: The protective role phytochemicals through the gut-brain axis. *J Biomed Pharmacother.* 2024; 78: 117277. DOI: https://doi.org/10.1016/j.biopha.2024.117277
6. Resveratrol Attenuates CSF Markers of Neurodegeneration and Neuroinflammation in Individuals with Alzheimer's Disease. Liu X, Baxley S, Hebron M, Turner RS, Moussa C. *Int J* Mol *Sci.* 2025;26(11):5044. DOI: https://doi.org/10.3390/ijms26115044.
7. Chen L, Chen S, Yang XF, Min JW. J Antioxidants attenuate mitochondrial oxidative damage through the Nrf2 pathway: A promising therapeutic strategy for stroke. Neurosci Res. 2023;101(8):1275-1288. DOI: https://doi.org/1002/jnr.25194
8. Pappolla MA, Martins RN, Poeggeler B, Omar RA, Perry G. Oxidative Stress in Alzheimer's Disease: The Shortcomings of Antioxidant Therapies. J Alzheimers Dis. 2024;101(s1): S155-S178. DOI: https://doi.org/10.3233/JAD-240659.
9. Devanarayan V, Charil A, Horie K, Doherty T, Llano DA, Andreozzi E, Sachdev P, Ye Y, Murali LK, Zhou J, Reyderman L, Hampel H, Kramer LD, Dhadda S, Irizarry MC; Alzheimer's Disease Neuroimaging Initiative (ADNI). *Alzheimers Dement.* 2025;21(2): e14411. DOI: https://doi.org/0.1002/alz.14411
10. Taheri M, Ghafoori H, Sepehri H, Mohammadi A neuroprotective effect of thiazolidine-2,4-dione derivatives on memory deficits and neuropathological symptoms of dementia on a scopolamine-induced Alzheimer's Model in Adult Male Wistar Rats. *ACS Chem Neurosci.* 2023 Sep 6;14(17):3156-3172. DOI: https://doi.org/10.1021/acschemneuro.3c00294.
11. Zucolotto SM, Fagundes C , Reginatto FA, Ramos, L, Castellanos C, Duque EP. Analysis of C-glycosyl flavonoids from South American *Passiflora* Species by HPLC-DAD and HPLC-MS. *Phytochem. Anal.* 2021; 23(3): 2232–239. DOI: https://doi.org/1010.1002/pca.1348.
12. Angel-Isaza J, Carmona-Hernandez JC, González-Correa CH, Narváez-Solarte WV. Potential hypoglycemic and antilipidemic activity of polyphenols from Passiflora ligularis (Granadilla). *Molecules.* 2023; 28:3551. DOI: https://doi.org/10.3390/molecules28083551.
13. Angel-Isaza L, Carmona-Hernandez JC, Narváez-Solarte W, Gonzalez-Correa CE. Polyphenols from Passiflora ligularis regulate inflammatory markers and weight gain. BioMol Concepts. 2021;12: 36–45.

14. Daza MG, Macías MC, Forero AM, Rodríguez J, Aragón M, Jiménez C, Ramos FA, Castellanos L. Identification of alpha-Amylase and alpha-Glucosidase Inhibitors and Ligularoside A, a new triterpenoid saponin from Passiflora ligularis Juss (Sweet (Granadilla) leaves, by a nuclear magnetic resonance-based metabolomic study. J Agric Food Chem. 2021; 69(9): 2919-2931.
DOI: https://doi.org/10.1021/acs.jafc.0c07850.
15. Al-Radadi NS, Al-Bishri WM, ElShebiney NA. Plant-mediated green synthesis of gold nanoparticles using an aqueous extract of Passiflora ligularis, optimization, characterizations, and their neuroprotective effect on propionic acid-induced autism in Wistar rats. Saudi Pharm J. 2024;32(2): 101921.
DOI: https://doi.org/10.1016/j.jsps.2023.101921.
16. Nerdy N, Ritarwan K. Hepatoprotective Activity and Nephroprotective Activity of Peel Extract from Three Varieties of the Passion Fruit (Passiflora Sp.) in the Albino Rat Maced J Med Sci. 2019 27;7(4):536-542.
DOI: https://doi.org/10.3889/oamjms.2019.153.
17. Ndaba N, Fotsing MCD, Govender PP. Assessment of Drimia delagoensis (Jessop) Baker Total Phenol, Flavonoids Content and Antioxidant Activity of Both Bulb and Leaves. Chem Biodivers. 2024;21(1): e202301402.
DOI: https://doi.org/10.1002/cbdv.202301402.
18. Cho A, Xu BY, Friedman DS, Foster PJ, Jiang Y, Pardeshi AA, Jiang Y, Aung T, He M. Role of static and Dynamic Ocular Biometrics Measured in the Dark and Light as Risk Factors for Angle Closure Progression. Am. J. Ophthalmol. 2023; 256:27-34. DOI: https://doi.org/10.1016/j.ajo.2023.07.032.
19. Szczepańska, K, Bojarski, A.J, Popik, P. Novel object recognition test as an alternative approach to assessing the pharmacological profile of sigma-1 receptor. Pharmacol. Rep. 2023;75: 1291–1298.
DOI: https://doi.org/10.1007/s43440-023-00516-x
20. Alafifi SA, Wahdan SA, Elhemiely AA, Elsherbiny DA, Azab SS. Modulatory effect of liraglutide on doxorubicin-induced testicular toxicity and behavioral abnormalities in rats: role of testicular-brain axis. Naunyn Schmiedebergs Arch Pharmacol. 2023;396(11):2987-3005.
DOI: https://doi.org1007/s00210-023-02504-7
21. Gravato C, be SR, Oliveira DP, Soares AMX, Domingues I. acetylcholinesterase (AChE) Activity in Embryos of Zebrafis. Methods Mol Biol. 2021; 2240:119-124.
DOI: https://doi.org/10.1007/978-1-0716-1091-6_10.
22. Swiercz AP, Tsuda MC, Cameron HA. The curious interpretation of novel object recognition. Trends Neurosci. 2025; 48(4): 250-256.
DOI: https://doi.org/10.1016/j.tins.2025.02.003
23. Reemst K, Shahin H, Shahar OD. Learning and memory formation in zebrafish: Protein dynamics and molecular tools. Front. Cell Dev. Biol., 2023; 11.
DOI: https://doi.org/10.3389/fcell.2023.1120984
24. Ghalachyan L, Mairapetyan S, Tadevosyan A, Hovhannisyan L, Mairapetyan K, Hakobjanyan A, Daryadar M, Siseryan R, Tovmasyan A, Ghahramanyan A. The study of bioactive compounds and gross β-radioactivity of some grain vegetables and medicinal plants in outdoor hydroponic systems and soil culture in Ararat Valley. Functional Food Sci. 2025;5(5):170-181.
DOI: https://doi.org/10.31989/ffs.v5i5.1616
25. HakobjanyanA, Karapetyan A, Mairapetyan S, Ghahramanyan A, Yeghiazaryan A, Mayrapetyan K. Medicinal potential of Thuja occidentalis and its essential oil. Bioact. Compd. Health Dis. 2025; 8(5): 166-176.
DOI: https://doi.org/10.31989/bchd.v8i5.1626
26. Kim A, Balanov P, Smotraeva I. Study of changes in antioxidant activity during fermentation of various types of legumes. Functional Foods Health Dis 2024; 14(11): 751-765
DOI: https://doi.org/10.31989/ffhd.v14i11.1452
27. Heng J, G Wang N, Zhang, F, Li L, Shi H, Li. Retraction of Isovitexin modulates autophagy in Alzheimer's disease via miR-107 signalling. Transl. Neurosci. 2022; 13, (1): 407.
DOI: https://doi.org/10.1515/tnsci-2022-0262.
28. Pan RY, Ma XX, Wang XF, Li XL, Qi YH, Cheng J, Liu QW, Tan CH, Yuan Z. Sodium rutin ameliorates Alzheimer's disease-like pathology by enhancing microglial amyloid-clearance. Sci Adv. 2019; 5(2): eaau6328.
DOI: https://doi.org/10.1126/sciadv.aau6328.
29. Andrade V, Cortés N, Pastor G, Gonzalez A, Ramos-Escobar N, Pastene E, Rojo LE, Maccioni RB. n-acetyl cysteine and catechin-derived polyphenols: a path toward multi-target compounds against Alzheimer's disease. J Alzheimers Dis. 2020; 75(4): 1219- 1227.
DOI: https://doi.org/10.3233/JAD-200067.
30. Zhao S, Zhang LZ, Yang C, Li S, Rong C. Procyanidins and Alzheimer's disease". Mol Neurobiol. 2019;56(8): 5556-5567. DOI: https://doi.org/10.1007/s12035-019-1469-6.
31. Rasool MA, Malik S, Waquar Q, Tul-Ain TH, Jafar R, Rasool A, Kalsoom MA, Ghafoor SA, Sehgal K, Gauthaman PN. In-silico characterization and in-vivo validation of albiziasaponin-a, iso-orientin, and salvadorin using a rat model of Alzheimer's disease. Front Pharmacol. 92018; 9:730.
DOI: https://doi.org/10.3389/fphar.2018.00730.

32. Nabavi SF, Kha H, D'onofrio G, Samec D, Shirooie S, Dehpour AR, Argüelles S, Habtemariam S, Sobarzo-Sanchez E. Apigenin as neuroprotective agent: Of mice and men. Pharmacol Res. 2018; 128:359-365. DOI: https://doi.org/10.1016/j.phrs.2017.10.008.
33. Kou JJ, Shi JZ, He YY, Hao JJ, Zhang HY, Luo DM, Song K, Yan JY, Xie X, Du M, Pang X.B. Luteolin alleviates cognitive impairment in Alzheimer's disease mouse model via inhibiting endoplasmic reticulum stress-dependent neuroinflammation. Acta Pharmacol Sin. 2022; 43(4): (840-849. DOI: https://doi.org/10.1038/s41401-021-00702-8.
34. Zhang XW, Chen JY, Ouyang D, Lu JH, Quercetin in Animal Models of Alzheimer's Disease: A Systematic Review of Preclinical Studies. Int J. Mol. Sci. 2020; 21(2): 493. DOI: https://doi.org/10.3390/ijms21020493.
35. Zhou S, Dong, X. Neuroprotective properties of ferulic acid in preclinical models of Alzheimer's disease: a systematic literature review. Curr Med Chem. 202330; 24: 2796-2811. DOI: https://doi.org/10.2174/0929867329666220906110506.
36. Yahaya MAF, Zolkiffly ZI, Moklas MAMm, Hamid HA, Stanislas J, Zainol M, Mehat MZ. Possible epigenetic role of vitexin in regulating neuroinflammation in alzheimer's disease. J. Immunol. Res. 2020; 9: 2020:9469210. DOI: https://doi.org/10.1155/2020/9469210.
37. Mishra A, Mishra PS, Bandopadhyay R, Khurana N, Angelopoulou E, Paudel YN, Piperi C. Neuroprotective potential of chrysin: mechanistic insights and therapeutic Potential for neurological disorders. Molecules. 2021; 26(21):6456. DOI: https://doi.org/10.3390/molecules26216456
38. Mhalhel K, Sicari M, Pansera L, Chen J, Levanti M, Diotel N, Germanà H, Giuseppe SA. Zebrafish: A model deciphering the impact of flavonoids on neurodegenerative Disorders. Cells. 2023;12(2):252. DOI: https://doi.org/10.3390/cells12020252.
39. Karunakaran KB, Thiyagaraj A, Santhakumar K. Novel insights on acetylcholinesterase inhibition by Convolvulus pluricaulis, scopolamine and their combination in zebrafish. Nat. Prod. Bioprospect. 2022;12(6). DOI: https://doi.org/10.1007/s13659-022-00332-5
40. Asadi Rizi A, Amjad L, Shahrani M, Amini Khoei H. Investigating the Mechanisms Involved in Scopolamine induced Memory Degradation. Arch Razi Inst. 2024;30;79(3):555-564. DOI: https://doi.org/10.32592/ARI.2024.79.3.555.
41. Bazyar H, Moradi L, Zaman F, Zare Javid A. The effects of rutin flavonoid supplement on glycemic status, lipid profile, atherogenic index of plasma, brain-derived neurotrophic factor (BDNF), some serum inflammatory, and oxidative stress factors in patients with type 2 diabetes mellitus: A double-blind, placebo-controlled trial. Phytother Res. 2023;37(1):271- 284. DOI: https://doi.org/10.1002/ptr.7611