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Original Research Article

Neuroprotective Potential of Glaucine Against Scopolamine-Induced Cognitive Dysfunction in Rat model

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Conflict of interest: Nil

Abstract

Background: Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive impairment, cholinergic dysfunction, and oxidative stress. Glaucine, an aporphine alkaloid isolated from *Pennisetum purpureum*, has previously demonstrated promising acetylcholinesterase (AChE) binding affinity through molecular docking studies, warranting further pharmacological investigation.

Objective: The present study aimed to evaluate the neuroprotective and anti-Alzheimer's potential of glaucine against scopolamine-induced cognitive impairment in rats through behavioural, biochemical, and histopathological assessments.

Methods: *In vitro* antioxidant activity was determined using the DPPH radical scavenging assay, while AChE inhibitory activity was evaluated using Ellman's method. Acute oral toxicity was assessed according to OECD 423/425 guidelines. Alzheimer's disease was induced in Wistar rats using scopolamine (2 mg/kg, i.p.). Glaucine (1 and 2.5 mg/kg, p.o.) and galantamine (2.5 mg/kg) were administered, and cognitive performance was evaluated using the Elevated Plus Maze and Rectangular Maze tests. Brain homogenates were analysed for AChE, superoxide dismutase (SOD), catalase (CAT), and malondialdehyde (MDA) levels. Histopathological examination of brain tissues was also performed.

Results: Glaucine exhibited moderate *in vitro* antioxidant activity (DPPH IC_{50} >320 µg/mL) and significant AChE inhibitory activity (IC_{50} = 77.51 µg/mL). *In vivo*, glaucine significantly improved learning and memory in behavioural models, reduced brain AChE activity, restored SOD and CAT levels, and markedly decreased MDA concentration in a dose-dependent manner. Histopathological studies demonstrated preservation of neuronal architecture and reduced neurodegenerative changes, particularly at 2.5 mg/kg, with effects comparable to galantamine.

Conclusion: Glaucine exerts significant neuroprotective effects against scopolamine-induced Alzheimer's-like neurodegeneration through dual mechanisms involving cholinesterase inhibition and attenuation of oxidative stress. These findings suggest that glaucine is a promising multitarget natural therapeutic candidate for the management of Alzheimer's disease.

Keywords: Alzheimer's disease; Glaucine; *Pennisetum purpureum*; Acetylcholinesterase; Oxidative stress; Scopolamine; Neuroprotection; Behavioural studies.

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Introduction

Neurodegenerative diseases are a group of progressive disorders characterized by the gradual and irreversible loss of neuronal structure and function, ultimately leading to deterioration of the

central and/or peripheral nervous system[1]. Among these conditions, Alzheimer's disease (AD) is the most common and severe form of dementia—a clinical syndrome marked by progressive cognitive decline that impairs memory, reasoning, and daily functioning[2,3].Globally, neurological disorders affect over 3.4 billion individuals, accounting for approximately 9–11 million deaths and contributing to more than 440 million disability-adjusted life years (DALYs), underscoring their growing public health and socioeconomic burden[4,5]. AD represents the leading neurodegenerative disorder, responsible for 60–70% of all dementia cases, and currently affects 34–40 million people worldwide. Mortality from AD and related dementias reached nearly 2 million deaths in 2021 and is projected to exceed 6.6 million annually by 2050, reflecting the urgent need for improved therapeutic strategies [6-8].The pathogenesis of AD is complex and multifactorial, with oxidative stress emerging as a central mechanism. Excessive production of reactive oxygen species (ROS) and impaired antioxidant defenses contribute to amyloid-β (Aβ) aggregation, tau hyperphosphorylation, mitochondrial dysfunction, synaptic impairment, and neuronal death, all of which accelerate cognitive decline[9]. Clinical studies have demonstrated that elevated oxidative stress biomarkers such as lipid peroxidation products, protein carbonyls, and 8-hydroxy-2′-deoxyguanosine positively correlate with increased acetylcholinesterase (AChE) activity[10].The cholinergic hypothesis remains a cornerstone of AD research, proposing that cognitive decline results from marked reductions in acetylcholine (ACh), a neurotransmitter critical for learning, memory, and attention. Elevated activity of AChE and butyrylcholinesterase (BuChE) in AD accelerates ACh degradation, exacerbating cholinergic deficits and promoting disease progression[11]. Collectively, these findings position oxidative stress markers and cholinesterase activity as key pathological biomarkers in AD, highlighting their value for both diagnostic assessment and therapeutic targeting.Currently available medications for AD primarily function by enhancing cholinergic signalling and modulating glutamatergic activity. However, their therapeutic benefits are limited, and they are frequently associated with adverse effects, particularly gastrointestinal complications such as nausea, vomiting, diarrhoea, anorexia, and abdominal discomfort[12,13]. These limitations have prompted growing interest in natural sources of bioactive compounds as alternative or complementary therapeutic options.

In recent years, medicinal plants and herbal remedies have attracted increasing scientific attention for their potential as reservoirs of neuroprotective agents. While only a fraction of these plants have been extensively studied, several species—including Curcuma longa, Ginkgo biloba, Withania somnifera, Convolvulus pluricaulis, Tinospora cordifolia, Allium sativum Linn., and Azadirachta indica—have demonstrated significant anti-Alzheimer's effects. These activities are largely attributed to their antioxidant, anti-inflammatory, and cholinesterase-inhibitory properties[14-17]. Beyond crude plant extracts, numerous secondary metabolites have been isolated and characterized for their neuroprotective potential. Examples include karanjin and embelin[14], 4-[(Z)-(hydroxyimino)-methyl]-N, N-dimethylaniline and phenylacetic acid from Solanum mauritianum [15], and indole alkaloids such as geissoschizoline, geissoschizone, geissospermine, and 3,4,5,6-tetradehydrogeissospermine from Geissospermum vellosii[16]. Other compounds, including magnolol, matrine, stigmasterol, liquiritigenin, gelsemine, nobiletin, mogrol, and honokiol, have also been reported to exert potent neuroprotective and anti-Alzheimer's effects[17].Importantly, galantamine, an alkaloid originally isolated from Galanthus (snowdrop) and Narcissus species, exemplifies the success of a plant-derived secondary metabolite as a clinically approved drug for mild to moderate AD [18]. This success validates the therapeutic potential of natural compounds and underscores the importance of continued exploration of plant-derived metabolites. Despite galantamine's proven efficacy, AD remains a multifactorial neurodegenerative disorder with no definitive cure, and current treatments primarily provide symptomatic relief. Therefore, identifying novel plant-derived compounds with complementary or improved mechanisms of action is essential to address unmet therapeutic needs in AD management.

In our earlier research, *Pennisetum purpureum* (Napier grass) was identified as a medicinal plant rich in flavonoids, alkaloids, and phenolic compounds, exhibiting promising neuroprotective properties. In a previous *in vitro* investigation, gas chromatography–mass spectrometry (GC-MS) profiling combined with molecular docking studies revealed that glaucine, an isoquinoline alkaloid isolated from *pennisetum purpureum*, demonstrated strong binding affinity toward AChE, suggesting its potential as an anti-Alzheimer's candidate[19]. Glaucine is known to possess diverse pharmacological activities, including bronchodilatory effects[20], *in vitro* acetylcholinesterase inhibitory activity and antioxidant properties, inhibition of breast cancer cell migration[21], and antitussive effects[22]. Despite these pharmacological attributes, no *in vivo* evidence currently supports its efficacy against AD. Therefore, the present study was designed as a continuation of our earlier work to evaluate the *in vivo* anti-Alzheimer's potential of glaucine, with a focus on its cholinesterase-inhibitory activity. Additionally, histopathological and biochemical

analyses were performed to further elucidate its neuroprotective mechanisms.

Materials and Methods

Reagents and Chemicals: The test compound glaucine and the standard galantamine were procured from Sisco Research Laboratories Pvt. Ltd., Andheri (East), Mumbai – 400 099, Maharashtra, India (Batch No.: [to be added]). The standard antioxidant ascorbic acid, 2,2-diphenyl-1-picrylhydrazyl (DPPH), acetylcholinesterase (AChE), and 5,5'-dithiobis (2-nitrobenzoic acid) (DTNB) were purchased from SRL Chemicals, Mumbai, India. Scoplamine was purchased from Dhamtech pharma & consultants, India. All other reagents and chemicals used in the study were of analytical grade and obtained from SRL Chemicals, Mumbai, India.

In Vitro Screening Methods

DPPH Radical Scavenging Assay: The antioxidant potential of glaucine was determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging method as described by Perumal et al. (2018). A 0.135 mM DPPH solution was prepared in methanol, and varying concentrations of the test compound and standard ascorbic acid (5–320 µg/mL) were mixed with 2.5 mL of the DPPH solution. After incubation at room temperature for 30 minutes, absorbance was measured at 517 nm [23]. The percentage inhibition was calculated using the equation:

$$\%\ DPPH\ Inhibition = \frac{OD\ of\ control - OD\ of\ test}{OD\ of\ control} X\ 100$$

Acetylcholinesterase (AChE) Inhibition Assay: AChE inhibitory activity was evaluated using a modified Ellman's spectrophotometric method (Ellman et al., 1961). The reaction mixture contained DTNB (3 mM), acetylthiocholine iodide (15 mM), phosphate buffer (0.1 M, pH 8), and varying concentrations of the test compound (25–400 µg/mL). The enzymatic reaction was initiated by adding AChE (0.16 U/mL) and the formation of the yellow 5-thio-2-nitrobenzoate anion was measured at 405 nm for 5 minutes[24]. The percentage inhibition was calculated as:

$$\%\ Inhibition = \frac{OD\ of\ control - OD\ of\ test}{OD\ of\ control} X\ 100$$

Ethical Approval: All experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC) of The Erode College of Pharmacy, Tamil Nadu, India (Approval No.: ECP/IAE/041/2025-2026), under the registration of the Committee for the Control and Supervision of Experiments on Animals (CCSEA), Government of India (Reg. No.: 2025/GO/Re/S/18/CCSEA).

Acute toxicity test

Acute Oral Toxicity (OECD 423/425 Method): Acute oral toxicity of Glaucine hydrobromide was evaluated in Wistar rats as per OECD guidelines 423 and 425. Animals (180–220 g) were fasted overnight and administered the test compound orally in 0.5% CMC at doses of 50, 200, and 300 mg/kg, followed by observation for 14 days. Behavioral and clinical signs such as salivation, convulsion, catalepsy, locomotor activity, grooming, and respiration were recorded periodically during the first 4 h and daily thereafter, Mortality, body weight, and food intake were monitored, and necropsy was performed on all animals at the end of the observation period. Dose-dependent neurotoxic signs were observed at ≥50 mg/kg, whereas 5 mg/kg caused only mild CNS depression without mortality, and was therefore considered the No Observed Adverse Effect Level (NOAEL). Based on OECD recommendations for safe exploratory dosing, sub-NOAEL fractions of 2.5 mg/kg (50%) and 1 mg/kg (20%) were selected for in-vivo cognitive studies[25,26].

Induction of Alzheimer's Disease: Scopolamine is widely used to induce cholinergic dysfunction and cognitive deficits in rodent models of Alzheimer's disease. Although many studies use doses of 0.5–1 mg/kg i.p. , higher doses such as 2 mg/kg i.p. have been used in chronic models to exacerbate oxidative stress and neuroinflammation. In this study, we adopted a 2 mg/kg i.p. dose to robustly induce scopolamine-mediated amnesia for evaluating the neuroprotective efficacy of glaucine[27,28].

Experimental Design:

Animals were randomly divided into five groups (n = 6 per group) as shown in Table no. 1.

- Group I: Normal control – received 0.5% carboxymethyl cellulose (CMC), p.o.
- Group II: Alzheimer's control – received scopolamine (2 mg/kg, i.p.).
- Group III: Glaucine (1 mg/kg, p.o.) + Scopolamine (2 mg/kg, i.p.).
- Group IV: Glaucine (2.5 mg/kg, p.o.) + Scopolamine (2 mg/kg, i.p.).
- Group V: Standard – Galantamine (2.5 mg/kg, p.o.) + Scopolamine (2 mg/kg, i.p.).

Behavioral assessments were performed for two consecutive days following drug administration. At the end of the experimental period, the rats were humanely euthanized, and the brains were carefully excised. A portion of the brain was used for biochemical estimations (AChE, SOD, CAT, and MDA), while the remaining tissue was fixed in 10% neutral buffered formalin for histopathological evaluation.

Table 1: Treatment Groups

Groups (n=6)	Treatment received
1	Normal Control -CMC (0.5%), p.o. 1st day.
2	AD Control -Scopolamine (2mg/kg body weight), i.p. 1st day.
3	Test 1-Glaucine (1mg/kg b.w), p.o. 1st day+ scopolamine (2mg/kg b.w.), i.p. 1st day.
4	Test 2- Glaucine (2.5mg/kg b.w), p.o. 1st day+ scopolamine (2mg/kg b.w), i.p. 1st day.
5	Standard-Galantamine (2.5 mg/kg b.w), p.o. 1st day+ scopolamine (2mg/kg b.w), i.p. 1st day.

p.o-per oral, i.p- intraperitoneal, b.w- body weight, CMC-carboxy methyl cellulose

Behavioural Assessment

Elevated Plus Maze (EPM): The EPM test was employed as an exteroceptive Behavioural model to evaluate learning and memory. The apparatus consisted of a central platform (10 × 10 cm) connected to two open arms (50 × 10 cm) and two closed arms (50 × 40 × 10 cm), elevated 50 cm above the floor. Each rat was placed at the end of an open arm facing away from the central platform.

The transfer latency (TL)—time taken to enter a closed arm with all four paws—was recorded (cut-off time: 180 s). Retention was tested 24 h later, and a decrease in TL was considered an indicator of improved memory performance[29].

Rectangular Maze Test: The rectangular maze is a reward-based Behavioural model for assessing working memory. The maze consists of an entry chamber (A) and a reward chamber (B) connected by a twisting corridor. On day 1, rats were habituated for 10 min. On subsequent test days, the time required to reach the reward chamber (TRC) was recorded, with a shorter TRC indicating better learning ability.

Acquisition (learning) and retention (memory) phases were performed on consecutive days under controlled conditions[29].

Histopathological assessment: The brains were removed and post fixed in the same fixative overnight at 48°C. The brains were embedded in paraffin and stained with Haematoxylin-Eosin. The hippocampus lesions were assessed microscopically at 10X and 40X magnifications[30]

Biochemical Estimations

Preparation of Brain Homogenate: Brains were rapidly excised, rinsed with ice-cold saline, and homogenized (10% w/v) in 0.1 M phosphate buffer (pH 7.4) using a Teflon-glass homogenizer at 4 °C. The homogenates were centrifuged at 10,000 rpm for 15 min at 4 °C, and the supernatants were used for biochemical assays (AChE, SOD, CAT, and MDA)[24].

Acetylcholinesterase (AChE) Activity: AChE activity was measured according to Ellman et al. (1961) using 10 µL of brain supernatant in a total assay volume of 200 µL. The formation of the yellow 5-thio-2-nitrobenzoate anion was monitored at 405 nm using a microplate reader[24]. The percentage inhibition was calculated as:

$$\%\ Inhibition = \frac{OD\ of\ control - OD\ of\ test}{OD\ of\ control} X\ 100$$

Antioxidant Parameters

Superoxide Dismutase (SOD): SOD activity was assayed by the reduction of nitro blue tetrazolium (NBT) as described by Misra and Fridovich (1972)[31]. The absorbance of the reaction mixture was measured at 560 nm, and enzyme activity was expressed as units/min/mg protein.

Catalase (CAT): Catalase activity was estimated by monitoring the decomposition of hydrogen peroxide at 240 nm (Aebi, 1984)[32]. Results were expressed as µmol of H_2O_2 decomposed per minute per milligram of protein.

Oxidative Stress Marker

Lipid Peroxidation (LPO): The extent of lipid peroxidation was measured by the thiobarbituric acid reactive substances (TBARS) method (Ohkawa et al., 1979)[33]. The absorbance of the developed pink chromogen was read at 532 nm, and results were expressed as nmol of malondialdehyde (MDA)/mg protein.

Statistical Analysis: Data were expressed as mean ± SEM. Statistical comparisons between groups were performed using one-way analysis of variance (ANOVA) followed by Bonferroni's multiple comparison test in GraphPad Prism (version 10.6.0). Differences were considered statistically significant at $p < 0.05$, $p < 0.01$, and $p < 0.001$.

Results

***In-Vitro* Screening Methods**

DPPH scavenging assay: The DPPH radical scavenging activity of glaucine and ascorbic acid showed a dose-dependent increase in inhibition. Ascorbic acid exhibited strong antioxidant activity with an IC_{50} value of 17.92 µg/mL, while glaucine showed comparatively moderate activity with an IC_{50} value >320 µg/mL, indicating moderate free radical scavenging potential. The results illustrated in Table no. 2 & Figure no. 1.

Table 2: *In vitro* antioxidant activity - DPPH assay

S. No.	Conc. (µg/ml)	% Inhibition (Mean ± SD)	
		Ascorbic acid	Glaucine
1	5(µg/mL)	7.93 ± 0.19	1.84 ± 0.27
2	10(µg/mL)	32.32 ± 0.29	1.76 ± 0.27
3	20(µg/mL)	68.80 ± 0.32	3.26 ± 0.20
4	40(µg/mL)	79.94 ± 0.23	3.47 ± 0.56
5	80(µg/mL)	89.03 ± 0.29	5.74 ± 0.44
6	160(µg/mL)	94.00 ± 0.73	5.06 ± 0.27
7	320(µg/mL)	96.19 ± 0.95	6.73 ± 0.27
IC_{50} (µg/mL)		**17.92**(µg/mL)	**>320**(µg/mL)

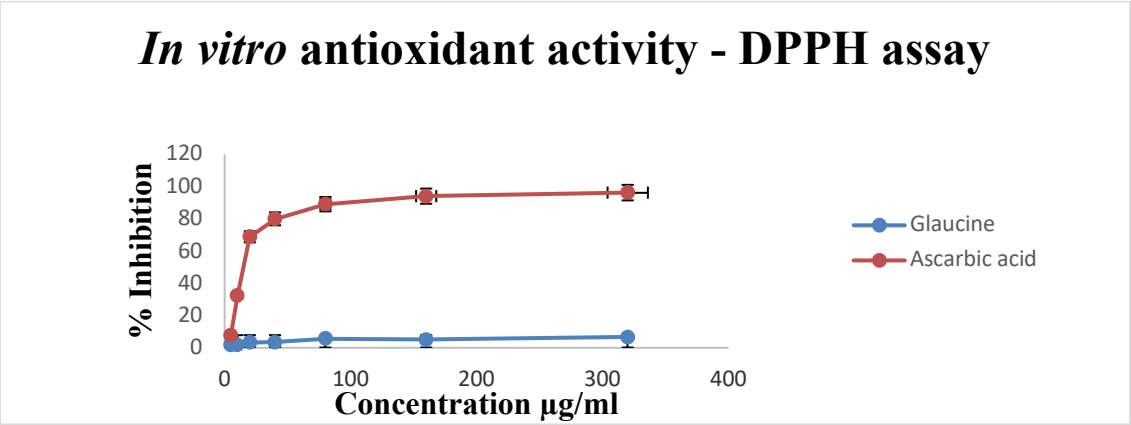

Figure 1: *In vitro* antioxidant activity - DPPH assay

AchE inhibition Assay: The *In-vitro* acetylcholinesterase (AChE) inhibition assay revealed an IC_{50} of 31.28 µg/mL for galantamine, while glaucine exhibited an IC_{50} value of 77.51 µg/mL, the results are illustrated in Table no. 3 and Figure no. 2.

Table 3: *In-vitro* AchE inhibition assay results

S. No.	Conc (µg/ml)	% Inhibition (Mean ± SD)	
		Galantamine	Glaucine
1	**25(µg/mL)**	40.28 ± 0.31	19.92 ± 0.20
2	**50(µg/mL)**	60.13 ± 0.31	39.70 ± 0.31
3	**100(µg/mL)**	76.06 ± 0.26	64.18 ± 0.52
4	**250(µg/mL)**	84.44 ± 0.66	77.97 ± 0.93
5	**500µg/mL)**	90.09 ± 0.27	87.03 ± 0.27
IC_{50} (µg/mL)		**31.28(µg/mL)**	**77.51(µg/mL)**

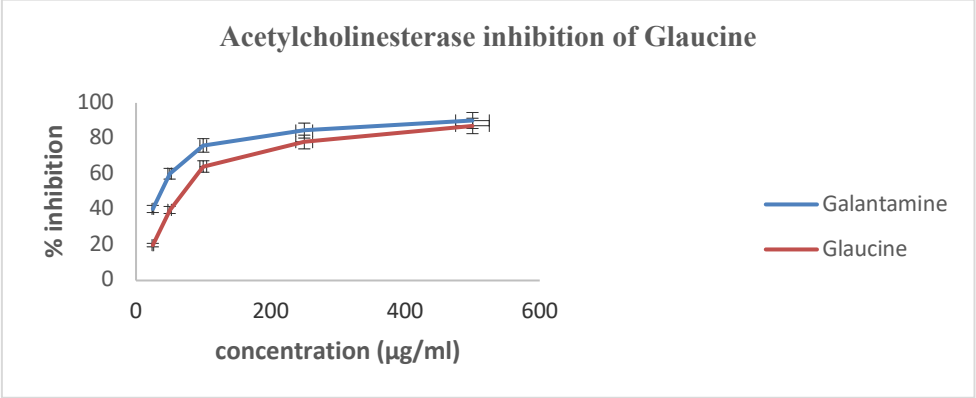

Figure 2: *In-vitro* AchE inhibition of glaucine

Acute toxicity test

Table 4: Acute Oral Toxicity (OECD 423/425)

Guidelines	Dose Administered	No of Animals	No of Death	Toxicity Signs
Acute Oral Toxicity (OECD 423)	300 mg/kg	5	0	Observed (Catalepsy like action, Salivation)
Acute Oral Toxicity (OECD 423)	50 mg/kg	5	0	Observed (Catalepsy like action, Salivation)
Acute Oral Toxicity (OECD 423)	5 mg/kg	5	0	No severe toxicity

Behavioural assessment

Elevated Plus Maze: The results of *In vivo* Behavioural assessment using Elevated plus maze illustrated in the Table no. 5, Figure no. 3.

Table 5: Transfer latency in Elevated plus maze on day 1 and day 2

Group	Transfer latency (TL) in secs	
	Day 1	Day 2
1. Normal Control	40.25 ± 20.68	17.50 ± 6.00 **
2. AD Control (SCO) (2mg/kg)	39.50 ± 12.96	43.30 ±15.90
3. SCO + Glaucine (1mg/kg)	35.50 ± 10.14	15.50 ±4.50 *
4. SCO + Glaucine (2.5mg/kg)	39.75 ± 15.71	11.80 ±3.50 ***
5. SCO + Galantamine (2.5mg/kg)	28.25 ± 5.74	8.50 ± 2.10 *

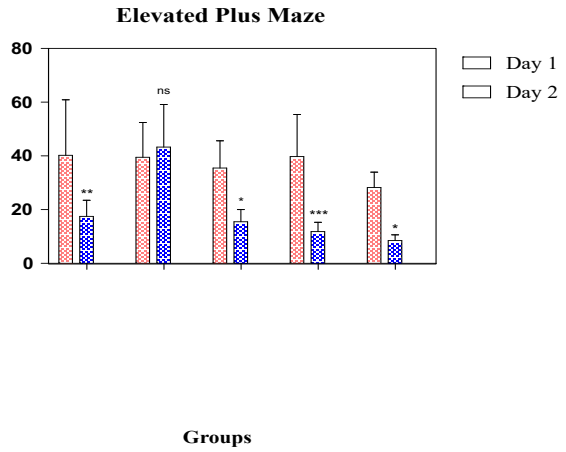

Figure 3: Effect of Glaucine treatments on elevated plus maze.

Effect of Glaucine treatments at different dose levels on the elevated plus maze method. AD control = Alzheimer's Disease control, SCO = Scopolamine. Data are expressed as mean ± SEM, n = 6.

*, **, *** indicates significances *p<0.05, p<0.01, p<0.001* when compared to day 1.

Statistical analysis was done with two -way ANOVA Followed by Bonferroni's multiple comparison test using graph pad prism.

AD control group showed a significant (P < 0.05) increase in transfer latency (TL) on day 1 & day 2 compared to the normal control, indicating scopolamine-induced memory impairment. In contrast, Glaucine (1mg/kg & 2.5 mg/kg) & Galantamine group exhibited a significant (P < 0.05) reduction in TL compared to the Alzheimer's control, reflecting improved cognitive function.

Rectangular Maze: The results of *In vivo* Behavioural assessment using rectangular maze illustrated in the Table no. 6, Figure no. 4.

Table 6: Time to reach Reward Chamber in Rectangular maze on day 1 and 2

Group	Time to reach reward chamber (TRC) in secs	
	Day 1	Day 2
1.Normal Control	111.00 ± 23.12	76.50 20.71
2.AD Control (SCO) 2mg/kg	131.33 ± 25.63	105.50 ± 22.79
3.SCO + Glaucine 1mg/kg	99.67 ± 18.35	65.33 ± 17.22
4.SCO + Glaucine 2.5mg/kg	107.50 ± 22.67	58.00 ± 22.37 **
5.SCO + Galantamine (2.5mg/kg)	126.67 ± 24.00	75.17 ± 29.49 **

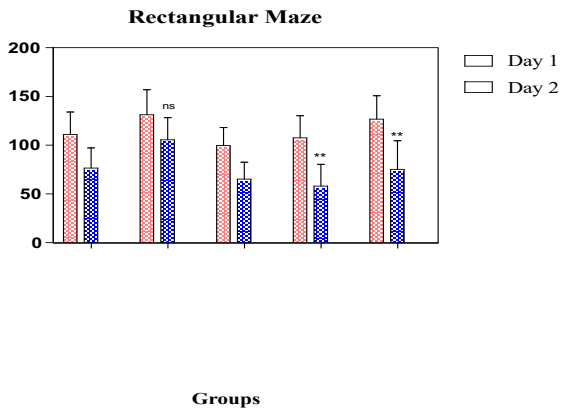

Figure 4: Effect of Glaucine treatments on rectangular maze

Effect of Glaucine treatments at different dose levels on the rectangular maze method. AD control = Alzheimer's Disease control, SCO = Scopolamine. Data are expressed as mean ± SEM, n = 6.

**, ** indicates significances *p<0.01* when compared to day 1.

Statistical analysis was done with two -way ANOVA Followed by Bonferroni's multiple comparison test using graph pad prism.

AD Control showed a significant *(P < 0.05)* increase in the time to enter the reward chamber (TRC) on day 1 & day 2 compared to the normal control, indicating scopolamine-induced memory impairment. In contrast, Glaucine (1mg/kg & 2.5 mg/kg) & Galantamine group exhibited a significant *(P < 0.05)* decrease in TRC time compared to the Alzheimer's control, suggesting improved learning and memory.

Biochemical Evaluation:

AchE Inhibition: The AChE inhibition assay in brain tissue homogenate brain tissue homogenate is illustrated in Table no. 7 and Figure no. 5.

Table 7: AchE inhibition in brain tissue homogenate

Group	% Inhibition (Mean ± SD) Mean ± SEM
1.Normal Control	1.852 ± 0.004
2.AD Control (SCO) (2mg/kg)	5.136 ± 0.0145****
3.SCO + Glaucine (1mg/kg)	3.577 ± 0.0075****
4.SCO + Glaucine (2.5mg/kg)	3.048 ± 0.009****
5.SCO + Galantamine (2.5mg/kg)	2.467 ± 0.002****

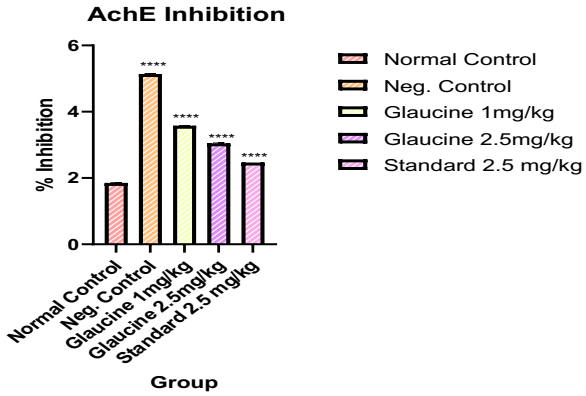

Figure 5: Effect of Glaucine treatments on AchE level in brain tissue homogenate

Effect of Glaucine treatments on AchE level in brain tissue homogenate. Neg control- negative control. Data are expressed as mean ± SEM, n = 6.

**** indicates significances *p<0.0001* when compared to Normal control group.

**** indicates significances *p<0.0001* when compared to AD control group.

Statistical analysis was done with two -way ANOVA Followed by Bonferroni's multiple comparison test using graph pad prism.

The AChE inhibition assay showed a significant *(P < 0.05)* elevation in AChE activity in the AD control group compared to the normal control. Treatment with glaucine (1 mg/kg and 2.5 mg/kg) produced a dose-dependent reduction in AChE activity, while the standard galantamine (2.5 mg/kg) showed a marked decrease, confirming glaucine's neuroprotective and cholinesterase inhibitory potential.

Antioxidants (SOD, CAT) and oxidate stress (MDA/LPO) parameters: The activities of antioxidant enzymes (SOD, CAT) and the oxidative stress marker (MDA/LPO) in brain tissue homogenate are illustrated in Table no. 8 and Figure no. 6.

Table 8: Antioxidants (SOD, CAT) and oxidate stress (MDA/LPO) parameters

Group	SOD (unit/mg prot ein)	CAT (µmoles of H_2O_2 used/ min/ mg protein)	MDA (mM/ mg protein)
1.Normal Control	0.309±0.011	0.309±0.011	0.334±0.029
2.AD Control (SCO) (2mg/kg)	0.251±0.022	0.251±0.022	2.488±0.239 ****
3.SCO + Glaucine (1mg/kg)	0.436±0.025 ****	0.436±0.025	0.415±0.035 ****
4.SCO + Glaucine (2.5mg/kg)	0.557±0.015 ****	0.557±0.015 ****	0.585±0.036 ****
5.SCO + Galantamine (2.5mg/kg)	0.362±0.018 **	0.362±0.018 **	0.376±0.027 ****

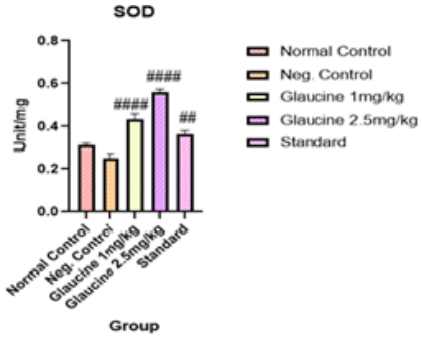

Figure 6(a): Effect of Glaucine treatments on Antioxidant SOD

Effect of Glaucine treatments at different dose levels on the elevated plus maze method. AD control = Alzheimer's Disease control, SCO = Scopolamine Data are expressed as mean ± SEM, n = 6.

##, #### indicates significance *p<0.001, p<0.0001* when compared to AD control group.
Statistical analysis was done with one -way ANOVA Followed by Bonferroni's multiple comparison test.

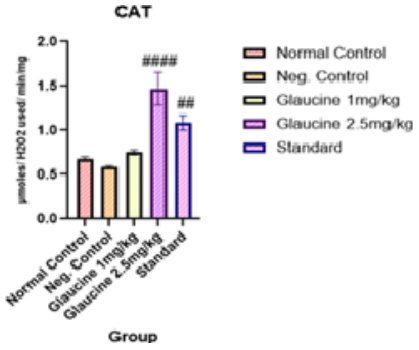

Figure 6(b): Effect of Glaucine treatments on Antioxidant CAT

Effect of Glaucine treatments at different dose levels on the elevated plus maze method. AD control = Alzheimer's Disease control, SCO = Scopolamine Data are expressed as mean ± SEM, n = 6.

##, #### indicates significance *p<0.01, p<0.0001* when compared to AD control group.
Statistical analysis was done with one -way ANOVA Followed by Bonferroni's multiple comparison test.

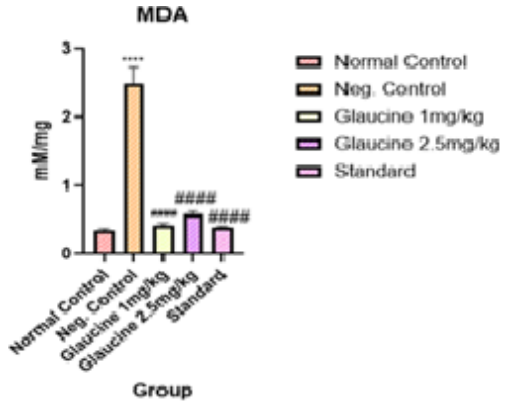

Figure no. 6(c): Effect of Glaucine treatments on Oxidative stress MDA/LPO

Effect of Glaucine treatments at different dose levels on the elevated plus maze method. AD control = Alzheimer's Disease control, SCO = Scopolamine Data are expressed as mean ± SEM, n = 6.

**** indicates significance *p<0.0001* when compared to normal control group.

indicates significance *p<0.0001* when compared to AD control group

Statistical analysis was done with one -way ANOVA Followed by Bonferroni's multiple comparison test.

The scopolamine-treated AD control group exhibited significantly reduced SOD and CAT activities with a marked rise in MDA levels, indicating oxidative stress. Glaucine treatment significantly *(P < 0.05)* restored SOD and CAT activities and reduced MDA levels in a dose-dependent manner, comparable to galantamine, confirming its strong antioxidant and neuroprotective efficacy.

Histopathological Studies:
Group I: (Normal Control + Vehicle)

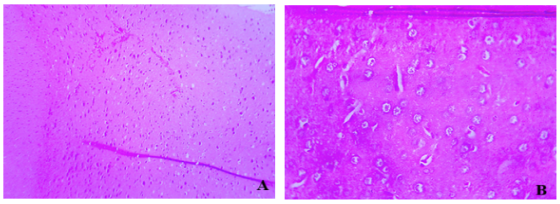

Figure 7 (A): 10x shows cerebral cortex shows normal (B) 40x shows normal ganglionic cell layer.

Group II: (Alzheimer's Control + SCO 2mg/kg)

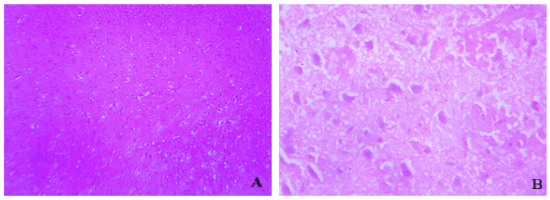

Figure 8 (A): 10x shows mild neuronal loss with eosinophilic deposition (B) 40x shows cortical areas with atrophied.

Group III: (SCO + Glaucine 1mg/kg)

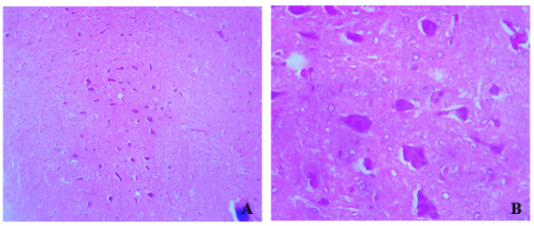

Figure 9 (A): 10x shows focal neuronal loss with extracellular eosinophilic deposition (B) 40x shows focal neuronal loss with eosinophilic deposition.

Group IV: (SCO + Glaucine 2.5 mg/kg)

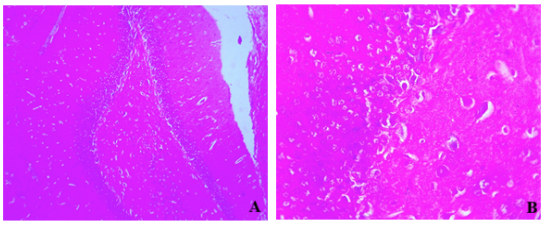

Figure 10 (A): 10x shows hippocampus shows focal mild neuronal loss (B) 40x shows focal mild neuronal degeneration

Group V: (SCO + Galantamine 2.5mg/kg)

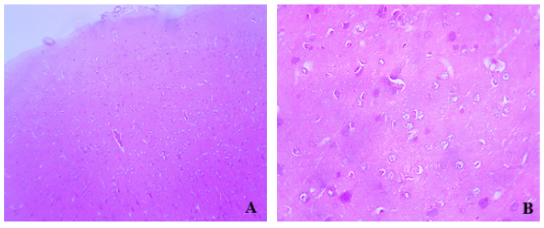

Figure 11 (A): 10x shows focal mild neuronal loss (B) 40x shows focal mild neuronal loss.

Discussion

Alzheimer's disease is a complex and multifactorial neurodegenerative disorder involving severe pathological mechanisms, including cholinergic dysfunction, amyloid β aggregation, oxidative stress and neuroinflammation [34]. Among the available therapeutic strategies, AchE inhibition remains widely accepted approach to enhance neurotransmission and improve cognitive function[35]. Furthermore, increasing evidence suggests that oxidative stress plays a pivotal role in AD pathogenesis, and antioxidants may exert neuroprotective effects by alternating reactive oxygen species (ROS)-mediated neuronal damage[36].

In our previous molecular docking, phytoconstituents from Pennisetum purpureum, glaucine an aporphine alkaloid was identified as a lead compound [37], due to its high binding affinity towards AchE. Move from computational prediction from experimental validation, a continuation study explored the lead compound's multitarget approach on AchE inhibition. And their impact on markers of oxidative stress. This evaluation represents the final validation step in our systematic drug discovery pipeline—progressing from molecular docking screening of Pennisetum purpureum, through *in vitro* validation (DPPH and AChE inhibition assays), to comprehensive *in vivo* characterization including Behavioural, biochemical, and now morphological assessment.

In-Vitro Studies

DPPH scavenging activity: In AD, the overproduction of reactive oxygen species (ROS) overwhelms the brain's defence mechanisms—like enzymes and non-enzymatic antioxidants—leading to oxidative stress and neuronal damage. oxidative stress as a pivotal contributor to AD, closely associated with hallmark pathologies such as amyloid-β (Aβ) plaques, tau hyper phosphorylation, and synaptic dysfunction[38].Studies have shown that higher intake of natural antioxidants from plant source improves on cognitive function and reduce the risk of development AD[39], through the mechanism of increased neuronal communication with reduced oxidative stress. Proton-radical scavenging activity which is an important attribution of antioxidants, which can be measured by DPPH radical scavenging activity. In our DPPH assay, the IC_{50} value of glaucine was found to be moderate[40], whereas the standard antioxidant, ascorbic acid, exhibited an IC_{50} of ascorbic acid shows powerful antioxidant activity, while glaucine demonstrates appreciable but significantly lower antioxidant activity compared to the standard. Furthermore, our findings are in agreement with recent literature suggesting that alkaloids such as glaucine inherently possess lower antioxidant activity than ascorbic acid, particularly in DPPH-based assays. This

disparity can be explained by structural differences: glaucine lacks phenolic hydroxyl groups required for efficient hydrogen-atom donation, which is a key mechanism of radical scavenging in ascorbic acid's enediol structure. Ascorbic acid can rapidly donate electrons, resulting in markedly superior antioxidant activity, whereas glaucine shows weaker radical scavenging potential.

Previous studies have similarly reported that aporphine alkaloids, including glaucine, exhibit lower antioxidant potential than phenolic compounds, while derivatization with hydroxycinnamic acid moieties significantly enhances their activity[41,42]Additionally, recent reviews of natural-product-inspired alkaloids confirm that despite their broad bioactivity, alkaloids generally trail phenolic antioxidants in radical scavenging capacity[43]. Furthermore, plant-extract study by Erica et al. attributes the diminished DPPH activity of O-substituted alkaloids to their limited functional groups available for effective radical stabilization, especially when compared with potent standards like ascorbic acid[44].

***IN-VITRO* AchE inhibition:** There is a strong correlation between severity of Alzheimer's disease with relation of cholinergic transmission. The AchE is an enzyme which is responsible to terminate the neuronal and signalling transmission of Ach, is an important and crucial neurotransmitter with in the neurons [45]. Inhibiting both AchE and BuchE can prevent the transmission & signalling of Ach and thus it is recommended as therapeutic agents for the treatment of AD. AchE inhibitors are the primary class of drug currently approved for AD. The approved AchE inhibitors are donepezil, rivastigmine and galantamine[46], in which galantamine is naturally occurring plant alkaloid, that selectively inhibit AchE and restore the reduced cholinergic system. In our *in vitro* study both glaucine and standard galantamine showed dose dependent % inhibition of AchE. Galantamine has low IC50 indicating highest anti-cholinesterase activity, while glaucine shows high IC50 indicating moderate AchE activity.Previous reports also documented the presence of moderate AchE compounds in various plant samples which goes parallel with our current study[47,48].

OECD Toxicity Studies: Glaucine hydrobromide exhibited dose dependent neurotoxicity in rats, with convulsion, salivation and catalepsy at 50-300 mg/kg.Studies have shown that glaucine provides CNS and behavioural effects at 7-28 mg/kg in rodents, indicating locomotor suppression and neurotransmitter alterations[49], this supports the dose at 7-28 mg/kg is pharmacologically safe for investigation of memory and learning with confounding toxicity.While, in our study 5 mg/kg caused only mild depression without mortality, hence 5 mg/kg considered as No Observed Adverse Effect Level (NOAEL), based on OECD 423 and 425[50,51] for safety exploratory dosing, Sub-NOAEL fraction of 50% (2.5 mg/kg) and 20% (1 mg/kg) were selected for *in-vivo* cognitive studies. Our results in agreement with earlier reports that glaucine alter locomotor and cognitive functions at low doses (0.5-2.5 mg/kg) without causing leathality[52].

Behavioural Studies: Based on invitro results, we further screened *in vivo* models in scopolamine induced AD models in rats. Studies have shown that Scopolamine is psychopharmacological model to induce AD and useful for to functional connectivity analysis for brain activity [53]. single dose of IP injection of scopolamine induces cognitive deficits, resembles amnesia and also scopolamine increases in AchE and BuchE activities[54].Scopolamine antagonizes muscarinic acetylcholine (Ach) receptors involved in working memory, working memory deficits are known feature of AD. Single dose of IP injection (2mg/kg) induces significant decrease in the choline neurotransmitter Ach level, detoriation of central cholinergic neuron impairs the cognition, studies have shown scopolamine is excellent neuro behavioural model that it impairs memory particularly short-term memory and learning acquisition[55].The present study evaluated the effect of glaucine hydrobromide on scopolamine-induced memory impairment using two validated Behavioural models: the Elevated Plus Maze (EPM) and the Rectangular Maze. Scopolamine, a muscarinic receptor antagonist, is widely employed to induce transient cognitive deficits resembling Alzheimer's disease pathology by impairing cholinergic neurotransmission[56].

In the EPM test, transfer latency (TL) is used as an index of learning and memory, where a significant reduction in TL on the retention trial (Day 2) compared with the acquisition trial (Day 1) reflects improved memory consolidation and retrieval[57]. In this study, scopolamine significantly impaired retention, whereas treatment with glaucine (1 and 2.5 mg/kg) produced a marked reduction in TL compared to the AD control group. Notably, glaucine at 2.5 mg/kg showed comparable efficacy to galantamine, a standard acetylcholinesterase inhibitor, suggesting that glaucine may enhance cholinergic neurotransmission and facilitate memory retention. Similarly, the Rectangular Maze task assessed spatial learning by measuring the time required to reach the reward chamber (TRC). Animals treated with scopolamine displayed prolonged TRC, consistent with impaired spatial memory. However, glaucine-treated groups demonstrated a significant reduction in TRC, with the 2.5 mg/kg dose exhibiting robust improvement, again comparable to galantamine. These findings align with earlier reports that alkaloids with structural similarity to glaucine, such as

galantamine, improve memory through cholinergic modulation [58]. The results suggest that glaucine hydrobromide possesses cognition-enhancing properties at sub-NOAEL doses (1 and 2.5 mg/kg), as evidenced by its ability to reverse scopolamine-induced amnesia. This effect may be attributed to its potential interaction with central neurotransmitter systems, consistent with earlier studies demonstrating glaucine's neuroactive properties[59]. Together, the maze-based paradigms confirm that glaucine has therapeutic promise for cognitive impairment, which is comparable to standard (alkaloid) drug galantamine.

Biochemical Analysis

AchE inhibition assay: *In vitro* AchE inhibition assay provided that glaucine possesses moderate inhibitory activity, such *in vitro* assays cannot fully predict its ability to act within the central nervous system. Therefore, *in vivo* evaluation was undertaken to verify whether glaucine could exert similar AchE inhibitory effect within brain tissue under physiological conditions. Estimation of AchE activity was selected as a key biochemical endpoint because of enhanced AchE activity is major pathological hallmark of AD, leading to rapid hydrolysis of Ach and progressive cognitive decline. Scopolamine induces significant decrease in the choline neurotransmitter Ach level, and detoriate the central cholinergic neuron which impairs the cognition. Hence, assessing AchE inhibition in brain tissue homogenate provided mechanistic insight to glaucine's ability to restore cholinergic balance and improve cognitive performance. Treatment groups both glaucine (1mg/kg and2.5 mg/kg) and standard galantamine showed a marked decrease in AchE activity in brain homogenate indicates effective inhibition of the enzyme and subsequent enhancement of cholinergic transmission, which plays a critical role in learning and memory process. In this study, AchE activity was quantified from rat brain homogenate, providing direct evidence of its effect on central cholinergic regulation. The observed effect is dose dependent, the higher dose of glaucine produces more inhibition, which is closely approaching the response seen in galantamine.

Galantamine is an alkaloid, which reversibly block the acetylcholinesterase, thereby it enhances the cholinergic signalling, improves memory. Studies have shown natural compound can be potential ChEI's, for example alkaloid[60], flavonoids and coumarin. (Glaucine is an alkaloid-mechanism) Our results are in agreement with previous studies that lobeline is multifunctional alkaloid modulates the cholinergic & glutamatic activities[61].

Antioxidant and oxidative stress evaluation: The brain exhibits heightened vulnerability to oxidative stress due to its high metabolic rate, abundant lipid content, and relatively limited antioxidant defenses[62-64]. Oxidative stress in Alzheimer's disease is characterized by excessive production of reactive oxygen species (ROS) that overwhelms endogenous antioxidant mechanisms, leading to neuronal membrane damage, protein oxidation, and ultimately cognitive decline.In our study, scopolamine-induced AD model rats demonstrated a significant decrease in antioxidant enzyme activities (SOD and CAT) accompanied by elevated MDA levels, confirming the establishment of oxidative stress in brain tissue. These findings are consistent with previous reports demonstrating that scopolamine administration disrupts the balance between oxidative and antioxidative systems in the brain[65].Superoxide dismutase (SOD) and catalase (CAT) constitute the primary enzymatic defence system against oxidative damage in neurons. SOD catalyses the dismutation of superoxide radicals (O_2^-) into hydrogen peroxide (H_2O_2), which is subsequently converted to water and molecular oxygen by CAT[62-64]. The reduction in SOD and CAT activities observed in the scopolamine-treated group indicates compromised antioxidant defence mechanisms, rendering neurons vulnerable to oxidative damage. Malondialdehyde (MDA), an end-product of lipid peroxidation, serves as a reliable biomarker for oxidative damage to neuronal membranes. The significantly elevated MDA levels in the AD control group reflect extensive lipid peroxidation and membrane integrity disruption, which contributes to neuronal dysfunction and cognitive impairment[65].Treatment with glaucine (1 mg/kg and 2.5 mg/kg) demonstrated dose-dependent restoration of antioxidant enzyme activities. Notably, glaucine at 2.5 mg/kg exhibited robust antioxidant effects, significantly increasing SOD and CAT activities while simultaneously reducing MDA levels compared to the AD control group. The 2.5 mg/kg dose showed comparable efficacy to the standard drug galantamine in preventing oxidative stress-induced neuronal damage.The neuroprotective antioxidant effects of glaucine may be attributed to multiple mechanisms: direct free radical scavenging activity, potential upregulation of endogenous antioxidant enzyme expression, and modulation of oxidative stress-related signalling pathways. Previous studies have reported that alkaloids can enhance cellular antioxidant defences through various mechanisms[66-68]. The dose-dependent protective effect observed with glaucine treatment, particularly at 2.5 mg/kg, supports its therapeutic potential. The improvement in antioxidant status correlates with the enhanced cognitive performance observed in Behavioural studies, suggesting that mitigation of oxidative stress contributes significantly to the memory-enhancing effects of glaucine.Our results suggest that glaucine exerts neuroprotective effects not only through AChE inhibition and cholinergic modulation but also via antioxidant mechanisms.

This dual action—addressing both cholinergic dysfunction and oxidative stress[69-71].

Enhanced oxidative stress and AchE activity are known to facilitate amyloid plaque formation and tau pathology. In our study glaucine observed both the cholinergic and oxidative stress. Therefore, glaucine's dual action- AchE inhibition and antioxidant enhancement and directly supress amyloidogenic and tau related neuro degeneration. Similar dual action was reported for other isoquinoline alkaloids[79], suggesting this structural class can modulate multiple molecular targets involved in Alzheimer's disease.

Histopathological Analysis: Histopathological analysis provides morphological confirmation of neurochemical and Behavioural findings by directly assessing neuronal integrity. The cerebral cortex and hippocampus—key regions for learning and memory—are highly vulnerable to early degeneration in Alzheimer's disease[72-74]. Hence, microscopic evaluation of these areas offers valuable insight into the neuroprotective efficacy of glaucine. Scopolamine administration produced marked neurodegenerative changes, including neuronal loss, cortical atrophy, and prominent eosinophilic deposition, indicating cellular injury and neuronal death—hallmark features of cholinergic dysfunction and oxidative stress-mediated neurotoxicity. The presence of extracellular eosinophilic material signifies degeneration and debris accumulation following apoptotic or necrotic cell death[74,75]. These observations are consistent with reports that scopolamine-induced muscarinic blockade triggers neurotransmission deficits, oxidative damage, and neuronal apoptosis[76,77], supporting the validity of this AD model. Glaucine treatment produced dose-dependent preservation of neuronal architecture. At 1 mg/kg, partial protection was observed, while 2.5 mg/kg exhibited substantial neuroprotection with preserved cortical morphology, minimal neuronal loss, and markedly reduced eosinophilic deposits. The neuroprotective efficacy of glaucine 2.5 mg/kg was comparable to galantamine, an established cholinesterase inhibitor[78], highlighting its therapeutic potential. Histopathological improvements strongly correlated with functional recovery in Behavioural assays and biochemical normalization. This structure–function concordance confirms that glaucine's cognitive benefits arise from genuine neuroprotection rather than symptomatic relief. The 2.5 mg/kg dose demonstrated optimal safety and efficacy, comparable to galantamine, underscoring glaucine's potential as a natural multitarget therapeutic for Alzheimer's disease through AChE inhibition, it maintains acetylcholine levels and prevents cholinergic neuronal loss. Additionally, enhanced antioxidant defences (SOD, CAT) and reduced lipid peroxidation (MDA) limit oxidative injury, while decreased eosinophilic deposition indicates possible anti-apoptotic activity[78]. These multimodal effects align with the current view that effective AD therapies must target multiple pathological pathways.

Conclusion

The present study demonstrated the neuroprotective potential of glaucine, an aporphine alkaloid from *Pennisetum purpureum*, against scopolamine-induced Alzheimer's-like neurodegeneration. Glaucine showed moderate in vitro acetylcholinesterase (*AChE*) inhibitory and antioxidant activities, validating its predicted binding affinity toward *AChE*. *In vivo*, glaucine (1 and 2.5 mg/kg) significantly improved learning and memory in behavioural models, with the higher dose showing efficacy comparable to galantamine. Biochemical analysis confirmed dose-dependent *AChE* inhibition and restoration of antioxidant enzymes (*SOD* and *CAT*), along with reduced lipid peroxidation (*MDA* levels). Histopathological findings further supported its neuroprotective effect through preservation of neuronal architecture. Overall, glaucine acts via dual mechanisms—*AChE* inhibition and oxidative-stress modulation—highlighting its potential as a natural multitarget therapeutic candidate for Alzheimer's disease.

Future Aspects: In future, molecular docking and mechanistic studies targeting AchE, β- amyloid (Aβ) interactions and tau phosphorylation cascade may further elucidate these associations.

Further to evaluate its long-term neuroprotective efficacy and safety to enable the rational development of glaucine- based analogues with improved and blood brain barrier protection and disease inhibiting potential in Alzheimer's therapy.

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