Ind. J. Pharm. Edu. Res., 2026; Krupapharmacon:112-122.
https://www.ijper.org

Original Article

Evaluation of the Neuroprotective Activity of Ethanolic Leaf Extract of Smilax glabra Roxb against Scopolamine Induced Dementia in Wistar Rats

Masali Aditya, Mayukh Sarkar*, Soumya K M

Department of Pharmacology, Rajiv Gandhi University of Health Sciences, Krupanidhi College of Pharmacy, Bengaluru, Karnataka, INDIA.

ABSTRACT

Background: Alzheimer's Disease (AD) is a common neurological condition characterised by progressive deficits in cognition, memory, and behavior. Its pathology involves cholinergic dysfunction, neuronal loss, and oxidative damage. Existing drugs provide only symptomatic relief without stopping progression, spurring interest in plants with antioxidant and neuroprotective effects. *Smilax glabra* Roxb., a traditional remedy, shows promise against inflammation and oxidative stress. **Objectives:** This study assessed the neuroprotective effects of ethanolic root extract from Smilax glabra Roxb. (SGE) in scopolamine-induced dementia using Wistar rats. **Materials and Methods:** Rats (5 groups) included normal control, scopolamine-induced dementia (2 mg/kg i.p.), rivastigmine standard, and three *Smilax glabra* Roxb. doses (oral, 30 days). Behavioral evaluations on days 15 and 30 used rotarod (motor coordination), elevated plus maze (anxiety), open field (exploration), closed chamber, and Morri's water maze (spatial memory). Brains were examined for total protein, antioxidant enzymes (catalase, superoxide dismutase), Acetylcholinesterase (AChE), lipid peroxidation, and histopathology. **Results:** *Smilax glabra* Roxb. significantly alleviated scopolamine-induced impulsivity, boosted home-return latency, curbed anxiety, and enhanced coordination. It elevated catalase, superoxide dismutase, and brain protein levels; lowered lipid peroxidation and AChE activity. Histology showed intact neurons and less degeneration in SGE groups. **Conclusion:** *Smilax glabra* Roxb. demonstrates neuroprotection in this dementia model via reduced oxidative stress, cholinesterase inhibition, and neuronal preservation, suggesting its value as a natural AD therapy candidate needing clinical validation.

Keywords: Acetylcholinesterase, Alzheimer's disease, Antioxidants, Behavioral tests, Neuroprotection, Scopolamine, *Smilax glabra* Roxb, Wistar rats.

Correspondence:
Mr. Mayukh Sarkar
Assistant Professor, Department of Pharmacology, Rajiv Gandhi University of Health Sciences, Krupanidhi College of Pharmacy, Bengaluru-560035, Karnataka, INDIA.
Email: mayukhsrkr4u@gmail.com

INTRODUCTION

Dementia is a progressive neurodegenerative syndrome marked by decline in memory, reasoning, language, judgement, and daily functioning. Alzheimer's Disease (AD) is the most common form, accounting for about 60-70% of cases worldwide.[1] The global burden of dementia is increasing, largely due to rising life expectancy. The World Health Organization estimates that about 55 million people are currently living with dementia, and this number may rise to 140 million by 2050.[2]

In AD, the Hippocampus and cerebral cortex undergo chronic neuronal degeneration and synaptic loss, which contributes to progressive impairment of learning and memory.[3] At the molecular level, AD is characterized by extracellular β-amyloid plaques and intracellular neurofibrillary tangles formed by hyperphosphorylated tau protein. These changes disrupt neuronal communication and cellular homeostasis.[4] Other important mechanisms involved in dementia progression include mitochondrial dysfunction, oxidative stress, neuroinflammation, and loss of cholinergic neurons. Reduced acetylcholine signaling is especially important because it is a major therapeutic target in AD.[5]

Current AD medications include acetylcholinesterase inhibitors such as donepezil, rivastigmine, and galantamine, as well as the NMDA receptor antagonist memantine. These drugs provide symptomatic relief but do not stop disease progression and may cause adverse effects.[6] Because of the limited effectiveness of current therapies, interest has increased in disease-modifying agents and phytochemicals with antioxidant, anti-inflammatory, and multi-target properties.[7]

Scopolamine-induced cognitive impairment is a widely used animal model of dementia because it blocks muscarinic receptors

DOI: 10.5530/ijper.krupapharmacon.28

and produces memory and attention deficits similar to early AD. It also increases oxidative stress and inflammation.[8]

Smilax glabra Roxb. is a medicinal plant traditionally used in Chinese and Ayurvedic medicine and reported to possess anti-inflammatory, antioxidant, hepatoprotective, and immunomodulatory activities. Its phytoconstituents include resveratrol, polyphenols, steroidal saponins, and flavonoids.[9] Based on these properties, *Smilax glabra* Roxb. may have potential neuroprotective effects, but more experimental evidence is needed. Therefore, the present study evaluates the ethanolic leaf extract of *Smilax glabra* Roxb. in a Scopolamine-induced dementia model in Wistar rats using behavioral, biochemical, and histopathological assessments.[10]

MATERIALS AND METHODS

Plant extraction and Authentication of Plant material

The leaves of *Smilax glabra* Roxb. were collected and authenticated from the Central Ayurveda Research Institute, Bengaluru, Karnataka, India. The leaves were shade dried for a week, ground into powder, and macerated for 72 hr in 90% ethanol and 20% distilled water. After filtering through Whatman paper, the extract was evaporated using a rotavapour at 80°C to remove ethanol, followed by drying in an oven at 50°C for 48 hr. The ethanolic extract yielded 8.2% (v/v).[11]

Dose Selection Criteria

The doses of *Smilax glabra* Roxb. ethanolic leaf extract (200 and 400 mg/kg) were selected based on previously published studies that evaluated acute toxicity of extracts in rodents. In an OECD 423 (acute toxic class method), with a single oral dose of up to 2000-5000 mg/kg body weight. No mortality or significant changes in general behavior, body weight, or gross organ pathology were observed during the 14-day observation period, indicating a relatively low acute toxicity and a wide safety margin for the leaf extract; Therefore, therapeutic doses were chosen well below these limits.[9]

Experimental Animals

Adult Male and Female Wistar Albino rats (200-250 g) was purchased from Krupanidhi College of Pharmacy located in Bangalore, India. Following acquisition, the animals were acclimated for ten days under controlled circumstances in an animal house. Environmental conditions were controlled by maintaining a temperature of 25±5°C and a relative humidity of 50% to 60% with a 12-hr light-dark cycle. The approved protocols were obtained from the Institutional Animals Ethics Committee (Approval No: KCP/IAEC/PCOL/166/JULY-2024). The care and handling of the animals met the animal welfare standards promulgated by the Committee for the Control and Supervision of Experiments on Animals (CCSEA). According to the ethical evaluation, rats were kept with *ad libitum* access to a pellet food and water prior to dietary restriction.

Experimental Procedure

Scopolamine-induced Alzheimer's Model

Scopolamine (1 mg/kg) was injected intraperitoneally once a day for six days in a row to cause Alzheimer's-like cognitive impairment. Behavioral assessments were completed before the initiation of scopolamine treatments and on day 15 and 30 post-induction. Following the induction period, the leaves of *Smilax glabra* Roxb were administered once daily orally, with varying medium (200 mg/kg) and high (400 mg/kg) doses. For the standard treatment, rivastigmine (2.5 mg/kg) was orally administered. Behavioral assessments were completed on Day 30; thereafter, the animals were euthanized in an anesthetized state. After being gently removed, the entire brain was cleansed with cold, isotonic saline. The brain was cut in half; one half was preserved in 10% formalin for biochemical assays, and the other half was preserved in 4% formaldehyde for histopathological analysis. A homogenate (10% w/v) in 0.1 M phosphate buffer (pH 7.4) was used to prepare brain tissue for biochemical investigations; each homogenate was centrifuged for 15 min at 3000 rpm. The supernatants were retained for further testing and appropriate use when assessing activity levels for selected markers of oxidative stress and enzyme functionality.[12,13]

Experimental Model

The Wistar Albino rats were weighed and divided into following five groups consisting of 6 animals in each group.

Group 1: Normal Group: Normal group rats (Rats will be feed on normal diet).

Group 2: Control Group: Scopolamine (1 mg/kg, *i.p*) induced dementia rats.

Group 3: Low dose group: Scopolamine (1 mg/kg, *i.p*) + *Smilax glabra* Roxb root extract (200 mg/kg, oral).

Group 4: High dose group: Scopolamine (1 mg/kg, *i.p*) + *Smilax glabra* Roxb root extract (400 mg/kg, oral).

Group 5: Standard Drug group: Scopolamine (1 mg/kg, *i.p*) + Rivastigmine (2.5 mg/kg, oral).

BEHAVIOURAL EVALUATION PARAMETERS

Behavioral Evaluation Parameters

Rotarod Apparatus (Motor Coordination and Learning)

The rotarod assessment was conducted to assess motor skills and balance by recording the time animals remained on the spinning rod. The animals were trained for 2 days, with the speed gradually raised from 4 to 20 rpm, and each trial lasted up to 300 sec. On days 15th and 30th, Scopolamine (1 mg/kg, i.p.) was administered

after the test compounds, and the latency to fall was measured 30 min later at 20 rpm.[14]

Morris Water Maze (Spatial Learning and Memory)

Spatial learning and memory were evaluated with the Morris water maze. The maze consisted of a circular pool filled with water (120-125 cm diameter, 50-55 cm deep) kept at 26°C, with platform placed just above the surface during acquisition and later submerged below the water level for the test phase. Animals were trained from different starting points, and on days 15th and 30th they were given up to 60 sec to find the hidden platform. If unable to do so, they were guided to it and allowed to stay there for 15-20 sec. Escape latency was recorded, and shorter latency indicated better spatial learning and memory.[13,15,16]

Elevated Plus Maze (EPM) [Cognitive Function]

Cognitive function was evaluated with the Elevated Plus Maze by recording transfer latency, the time taken for a rat to move from an open arm to a closed arm with all four paws inside. The maze consisted of two open arms and two closed arms (50 cm × 10 cm) with 40 cm-high walls arranged in a plus shape and elevated above the floor. After familiarization, animals were placed at the end of an open arm, and transfer latency was recorded on days 15th and 30th in a dark, quiet environment.[17]

Tissue Sample Preparation

Biochemical analyses were conducted 24 hr after the last behavioral testing on day 31. Animals were euthanised with an overdose ether inhalation and decapitated. The brains were swiftly removed and cleansed using ice-cold saline. For tissue homogenates (10% w/v), the brain tissue was placed in ice-cold 0.1 M phosphate buffer (pH 7.4) and subsequently centrifuged. Supernatant suspensions were saved for biochemical assays to determine total protein concentration and acetylcholinesterase activity. animals were euthanised with overdose anesthesia. The brains were harvested, washed in ice-cold saline, and fixed quickly in 10% neutral buffered formalin before histopathological examination.[18]

Biochemical Parameters

Total Brain Protein

Total protein in brain samples was estimated by the Biuret assay, which is based on the formation of a colored complex between cupric ions and peptide bonds in an alkaline medium. The absorbance of this compound was recorded spectrophotometrically at a wavelength of 578 nm, with sodium-potassium tartrate used to stabilize the reaction. Separately, a reagent blank, standard, and test samples were prepared, followed by the addition of 10 µL of reagent 2 and 1000 µL of reagent 1 to each tube. After thorough mixing, the tubes were incubated at 37°C for 5 min. The analyser was first blanked using the reagent blank, then the absorbance of the standard and test sample was measured, and the protein concentration was calculated using the provided formula.[18]

Acetylcholinesterase (AChE) Activity Measurement

AChE activity which is a cholinergic enzyme marker was measured in the whole brain homogenates using the Ellman colorimetric method. The reagent, 5,5'-Dithiobis(2- Nitrobenzoate) (DTNB), was made up in sodium citrate solution. Aliquots of the brain homogenate (0.1 mL) were mixed with phosphate buffer and DTNB, then acetylthiocholine iodide substrate was added to begin the enzymatic reaction. The change in absorbance was recorded at 412 nm, which corresponds to the production of thiocholine, and the AChE activity was calculated based on this measurement.[18,19]

Assessment of Enzymatic Antioxidants

Catalase Activity Assay

Catalase catalyses the decomposition of hydrogen peroxide to water and oxygen and thus protects against the effects of oxidative injury. Catalase activity was assessed by measuring the decrease in absorbance due to H_2O_2 degradation within the 210-240 nm range. The tissue homogenates were incubated with phosphate buffer at 37°C and the reaction started by the addition of hydrogen peroxide. The reaction was stopped by adding dichromate-acetic acid reagent, followed by measurement of the colorimetric change at 620 nm. One unit of catalase activity was defined as the micromoles of H_2O_2 decomposed per milligram of protein per minute.[19,20]

Superoxide Dismutase (SOD) Activity Determination

SOD is a primary enzymatic defense against reactive oxygen species by blocking the oxidation of epinephrine by oxygen radicals, which produces a chromogen in the presence of NBT. Tissue homogenate was incubated with sodium pyrophosphate buffer and NBT and then a solution of NADH was added to initiate the reaction. The reaction was terminated by adding glacial acetic acid after incubating the mixture at 30°C for an additional 90 sec. Absorbance of the formed chromogen was measured at 560 nm. SOD activity was expressed as nanomoles per minute per milligram of protein.[19,21]

Non-enzymatic Antioxidant

Lipid Peroxidation (LPO) Estimation

Lipid peroxidation was determined through measurement of the production of Malondialdehyde (MDA), using the Thio Barbituric Acid Reactive Substances (TBARS) assay. The brain homogenates were incubated with a solution of Thio barbituric acid, trichloroacetic acid and hydrochloric acid, and then placed in a water bath to heat. The samples were allowed to cool, and then were centrifuged. The absorbance of the supernatant was

read at 535 nm. MDA levels were expressed as nanomoles formed per minute per milligram of protein.[22]

Histopathological Studies of the brain:

At study termination (day 31), animals were euthanised with overdose anesthesia. The brains were harvested, washed in ice-cold saline, and fixed quickly in 10% neutral buffered formalin before histopathological examination.

Statistical Analysis

GraphPad Prism version 10.0 was used for data analysis. One-way ANOVA and Dunnett's post hoc test were used to assess statistical significance. For each set of six animals, the results are shown as mean±SEM, with $p<0.05$ considered statistically significant.

RESULTS

Interpretation of Rotarod Test Results

Scopolamine-treated rats (Group II) showed significantly shorter fall latencies on days 15 and 30 compared to Normal (Group I), indicating impaired motor coordination and reduced endurance consistent with cholinergic disruption. Treatment with *Smilax glabra* ethanolic extract improved motor performance in a dose-dependent manner: 200 mg/kg (Group III) produced a moderate but significant increase in fall latency by day 30, while 400 mg/kg (Group IV) produced larger, significant improvements on both test days. These benefits likely arise from *S. glabra's* active phytochemicals acting through antioxidant and neuroprotective mechanisms. The reference drug rivastigmine (Group V) also substantially increased fall latency compared to scopolamine, and the 400 mg/kg extract yielded effects similar to rivastigmine. Overall, *S. glabra* mitigated scopolamine- induced motor deficits and appears promising as a plant-derived candidate for treating neurodegenerative motor dysfunction (Figure 1).

Interpretation of Elevated Plus Maze (Open Arm Time) Results

Scopolamine significantly reduced time spent in the open arms of the elevated Plus Maze on days 15 and 30 compared to normal group, indicating increased anxiety like behavior and likely cholinergic dysfunction. *Smilax glabra* ethanolic extract reversed this effected dose-dependently: 200 mg/kg produced a modest but significantly increase in open arm time by day 30, while 400 mg/kg yielded larger, sustained increases across assessments, suggesting anxiolytic and neuroprotective effects. Rivastigmine similarly increased open-arm exploration, and the 400 mg/kg extract produced effects comparable to the standard drug. These results support *S. glabra's* potential to modulate cholinergic related anxiety like deficits, possibly through antioxidant and neuroprotective mechanisms (Figure 2).

Interpretation of Elevated Plus Maze (Closed Arm Time) Results

Scopolamine treated rats (Group II) spent significantly more time in the closed arms on days 15 and 30 compared to normal, indicating increased anxiety like behavior and cholinergic related cognitive impairment. *Smilax glabra* ethanolic extract reversed this effect in a dose-dependent manner: 200 mg/kg (Group III) produced a significant decrease in closed arm time by day 30, while 400 mg/kg (Group IV) produced larger, consistent reductions across the 30-day period, suggesting anxiolytic and cholinergic restorative effects. Rivastigmine (Group V) also reduced closed-arm occupancy, and the 400 mg/kg extract produced responses comparable to the standard drug. Overall, the findings support *S. glabra's* neuroprotective and antioxidant linked potential to alleviate anxiety like cognitive deficits in neurodegenerative models (Figure 3).

Interpretation of Morris Water Maze Test Results

Scopolamine treated rats (Group II) showed significantly longer escape latencies in the Morri's water maze on days 15 and 30 compared to normal group, indicating impaired spatial learning and memory consistent with cholinergic disruption and oxidative neurotoxicity. *Smilax glabra* ethanolic extract improved performance dose-dependently: 200 mg/kg (Group III) produced modest reductions in escape latency by day 30, while 400 mg/kg (Group IV) produced significant decrease by days 15 and 30, restoring performance toward normal levels. Rivastigmine (Group V) also markedly reduced escape times, and the 400 mg/kg extract yielded effects comparable to the standard drug. These results indicate *S.glabra* may enhance hippocampal-dependent learning and memory likely through antioxidant, anti-inflammatory, and cholinergic- modulating actions and support its potential as a plant derived candidate for addressing cognitive deficits in neurodegenerative disorders (Figure 4).

Interpretation of Catalase Levels in Brain

Scopolamine significantly reduced brain Catalase (CAT) activity compared to normal, indicating increased oxidative stress and impaired endogenous antioxidant defense. *Smilax glabra* ethanolic extract restored CAT activity in a dose-dependent manner: 200 mg/kg produced a moderate increase, while 400 mg/kg nearly normalized catalase levels. Rivastigmine also raised CAT activity, and the 400 mg/kg extract achieved levels comparable to the standard drug. These results indicate *S. glabra* protects against scopolamine -induced oxidative damage by enhancing antioxidant enzyme activity, supporting its potential neuroprotective role in neurodegenerative conditions (Table 1).

Interpretation of Superoxide Dismutase (SOD) Levels in Brain

Scopolamine significantly decreased brain SOD activity compared to normal, indicating disrupted redox balance and increased

oxidative stress akin to Alzheimer-like pathology. *Smilax glabra* ethanolic extract restored SOD levels dose-dependently: 200 mg/kg produced a modest improvement, while 400 mg/kg markedly increased SOD activity nearly to normal values. Rivastigmine similarly normalized SOD, and the 400 mg/kg extract achieved levels comparable to the standard drug. These results indicate *S. glabra* mitigates scopolamine-induced oxidative stress by enhancing endogenous antioxidant defences, supporting its neuroprotective potential in neurodegenerative conditions (Table 1).

Interpretation of Lipid Peroxidation (LPO) Levels in Brain

Scopolamine significantly raised brain Lipid Peroxidation (LPO), shown by increased MDA levels compared to normal, indicating enhanced oxidative damage to neuronal membranes. *Smilax glabra* ethanolic extract reduced LPO in a dose-dependent manner: 200 mg/kg produced an approximate 50% reduction in MDA relative to scopolamine, while 400 mg/kg markedly lowered LPO nearly to normal values. Rivastigmine also significantly decreased LPO, and the high-dose *S. glabra* effects were comparable to the standard drug. These results support *S. glabra's* antioxidant and membrane-protective actions and reinforce its potential as a neuroprotective candidate for conditions involving oxidative neuronal injury, such as Alzheimer's disease (Table 1).

Interpretation of Acetylcholinesterase (AChE) Activity in Brain

Scopolamine significantly increased brain AChE activity versus normal, reflecting enhanced acetylcholine breakdown and cholinergic dysfunction linked to memory impairment. *Smilax glabra* ethanolic extract inhibited AChE in a dose-dependent manner: 200 mg/kg produced moderate inhibition, while 400 mg/kg caused a pronounced reduction approaching normal levels. Rivastigmine also significantly lowered AChE activity, and the high-dose extract showed comparable effects. These findings indicate *S. glabra* may preserve cholinergic transmission by inhibiting AChE, which together with its antioxidant actions supports its potential as a natural candidate for improving cognitive deficits in neurodegenerative conditions (Table 1).

Interpretation of Total Protein Levels in Brain

Scopolamine-treated rats (Group II) showed a significant decrease in brain total protein versus normal, indicating neuronal injury, impaired protein synthesis, and cellular disruption consistent with neurodegeneration. *Smilax glabra* ethanolic extract restored total protein levels dose-dependently: 200 mg/kg produced a moderate recovery, while 400 mg/kg produced a significant restoration approaching normal values. Rivastigmine (Group V) also significantly restored protein levels, and the high-dose extract matched its effect. These results suggest *S. glabra* preserves neuronal integrity possibly by reducing oxidative damage and supporting protein homeostasis and further support its neuroprotective potential in models of Alzheimer's disease (Table 1).

Behavioral Observation and Assessment

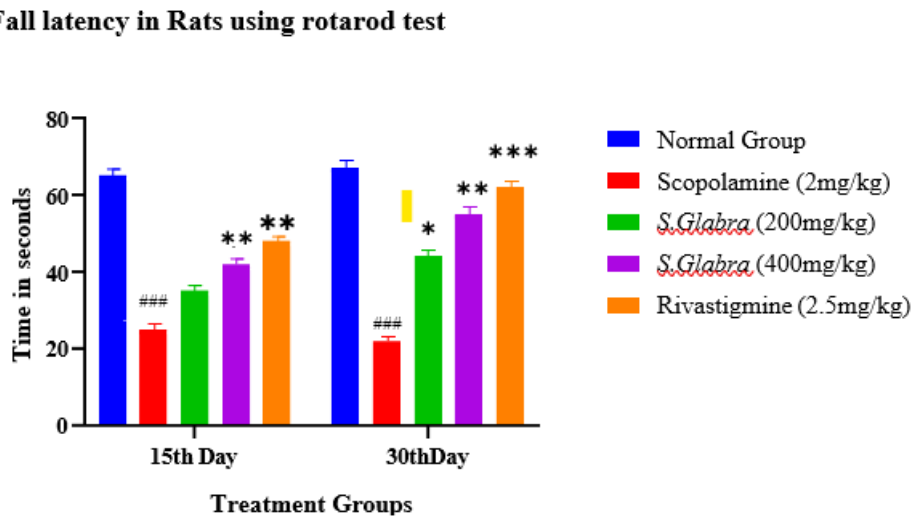

Figure 1: Fall latency in Rats using rotarod test. Data expressed as Mean±SD (n=6), ##p<0.01, ###p<0.001 when compared to normal group; *p<0.05, **p<0.01, ***p<0.001 when compared to the scopolamine-treated group.

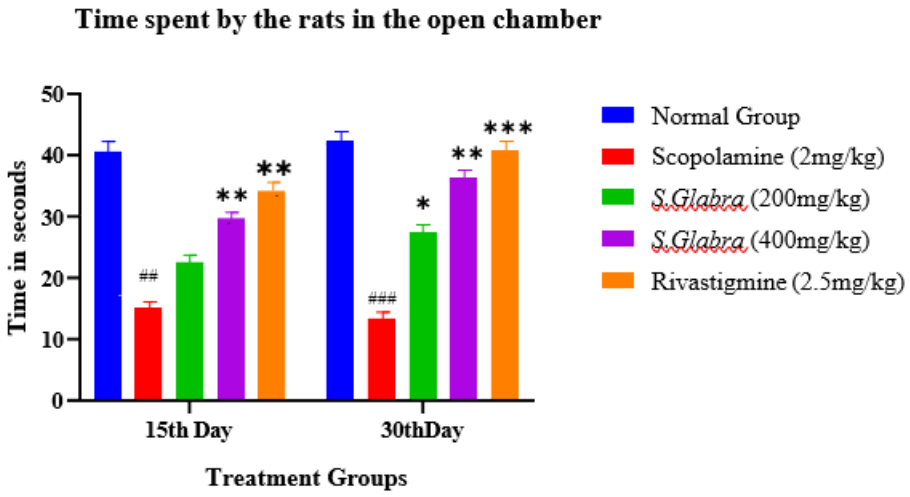

Figure 2: Time spent by the rats in the open chamber elevated plus maze test. Data expressed as Mean±SD (*n*=6), ##*p*<0.01, ###*p*<0.001 when compared to normal group; *p<0.05, **p<0.01, ***p<0.001 when compared to the scopolamine-treated group.

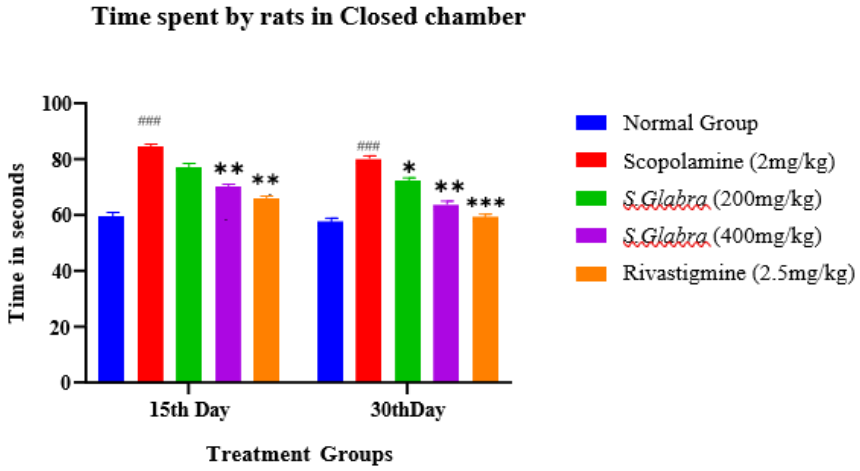

Figure 3: Time spent by the rats in the closed chamber elevated plus maze test. Data expressed as Mean±SD (*n*=6), ##*p*<0.01, ###*p*<0.001 when compared to normal group; *p<0.05, **p<0.01, ***p<0.001 when compared to the scopolamine-treated group.

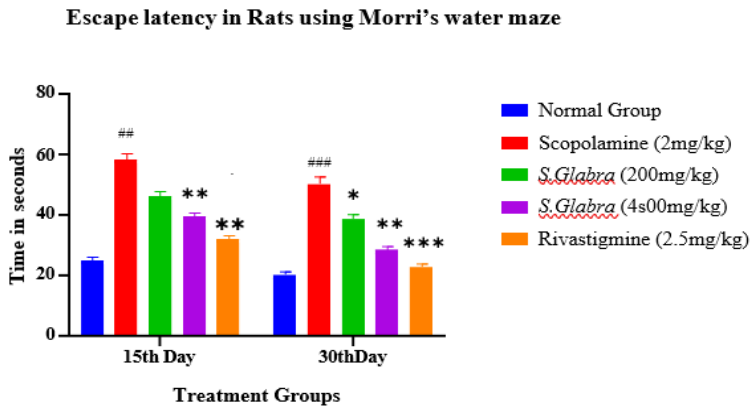

Figure 4: Escape latency in Rats using Morri's water maze test. Data expressed as Mean±SD (*n*=6), ##*p*<0.01, ###*p*<0.001 when compared to normal group; *p<0.05, **p<0.01, ***p<0.001 when compared to the scopolamine-treated group.

Histopathological studies

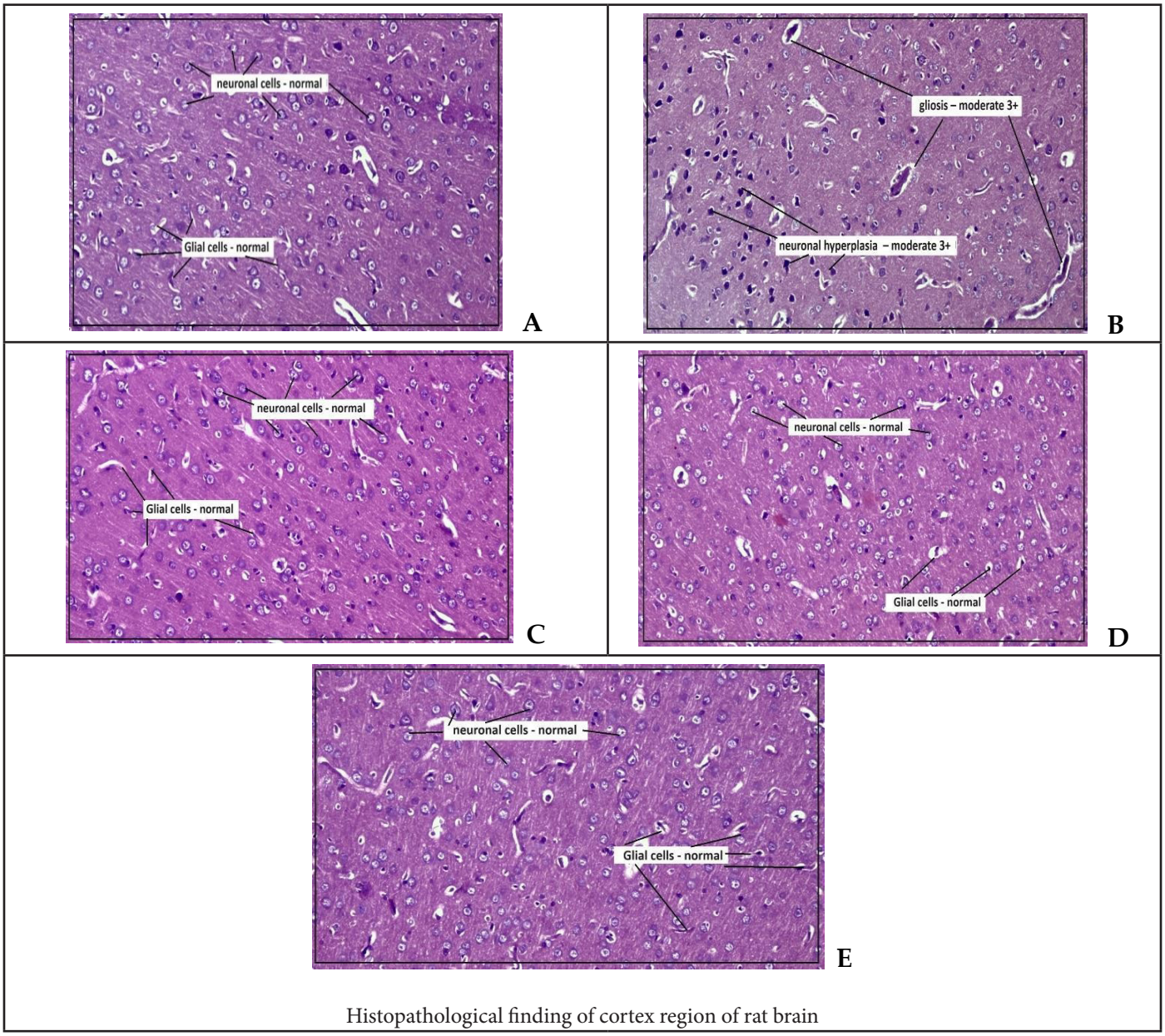

Figure 5: Histopathological finding of cortex region of rat brain. (A) Normal group, Rat Brain: Cortical region showing neuronal cells normal morphology (X100). (B) Scopolamine induced group, Rat Brain: Cortical region showing moderate Gliosis, apoptosis - moderate: 3+ (X100). (C) Low dose of SGR, Rat Brain: Cortical region showing neuronal cells normal, gliosis - focal -1+ morphology (X100). (D) High dose of SGR: Cortical region showing neuronal cells normal, gliosis normal morphology (X100). (E) Standard group, Rat Brain: Cortical region showing neuronal cells normal morphology (X100).

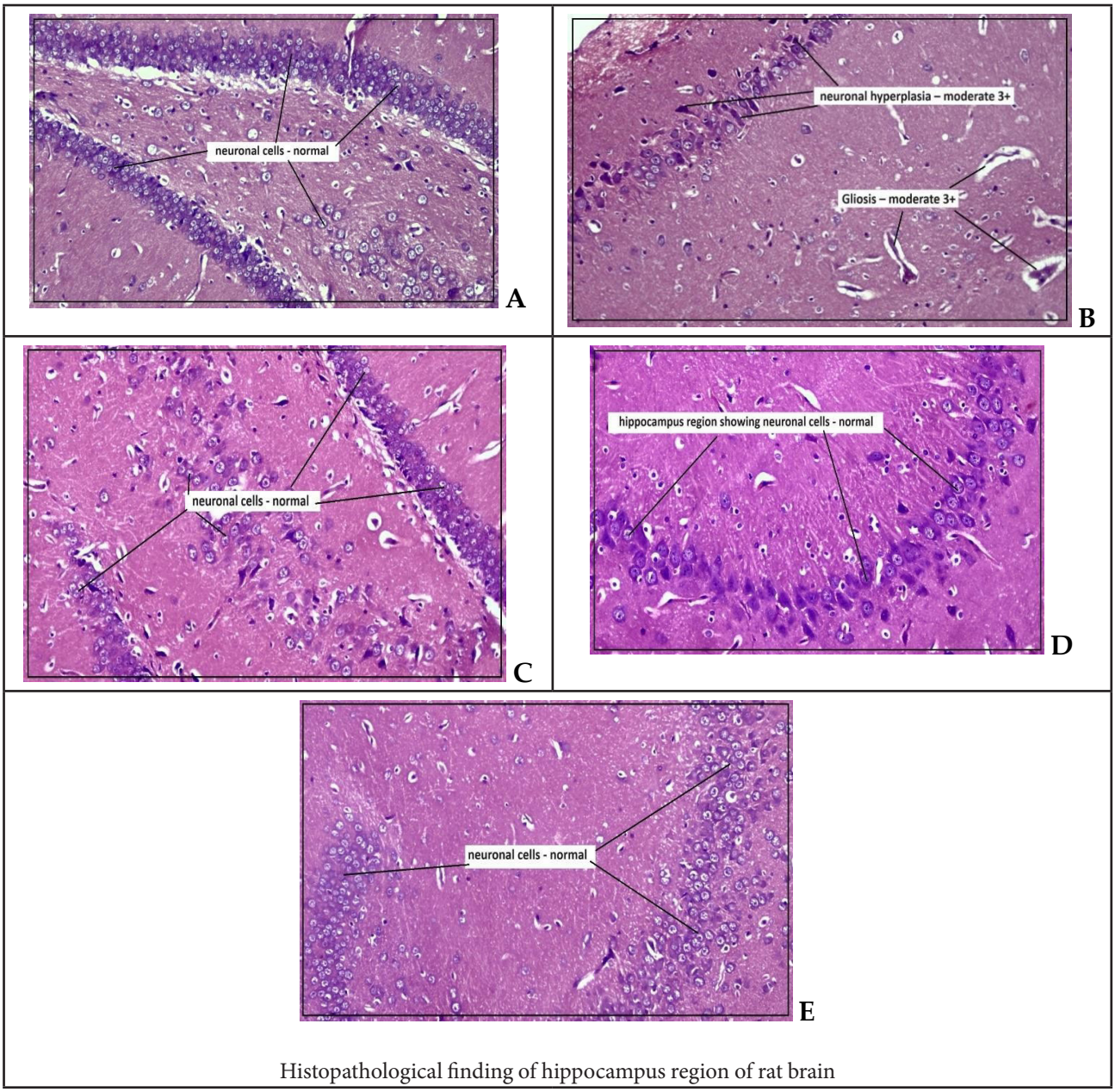

Figure 6: Histopathological finding of hippocampus region of rat brain. (A) Normal group, Rat Brain: hippocampus region showing neuronal cells normal morphology (X100). (B) Scopolamine induced group, Rat Brain: hippocampus region showing Pyknotic nuclei and gliosis - moderate 3+ (X100). (C) Low dose of SGR, Rat Brain: hippocampus region showing Neuronal cells - normal - NAD+ (X100). (D) High dose of SGR, Rat Brain: hippocampus region showing Neuronal cells - normal - NAD+ (X100). (E) Standard group, Rat Brain: hippocampus region showing neuronal cells normal morphology (X100).

DISCUSSION

Alzheimer's disease is an advancing neurodegenerative condition and the primary reason for dementia among elderly individuals. It is mainly marked by loss of memory, decline in cognitive functions, and alterations in behavior, and is associated with amyloid- β, tau pathology, cholinergic malfunction, oxidative stress, and neuroinflammation. The current medications like rivastigmine only alleviate symptoms, there is continued interest in natural compounds that may offer neuroprotective benefits.

In this research, scopolamine was utilized to create cognitive deficits in Wistar rats by inducing temporary cholinergic inhibition and oxidative damage similar to Alzheimer's disease. Repeated exposure to scopolamine resulted in noticeable impairments in learning and memory, evidenced by increased transfer latency in the Morris Water Maze. It also led to oxidative stress, reflected by heightened malondialdehyde levels and decreased activity of antioxidant enzymes, along with a decline in total protein content. Histopathological analysis highlighted

neuronal degeneration in both the cortex and hippocampus, including neuronal shrinkage, vacuolation, and pyknotic nuclei.

Administering *Smilax glabra* Roxb. extract resulted in improvements in these conditions in a dose-dependent manner. The higher dosage yielded superior behavioral outcomes, with shortened latency times in both maze assessments, indicating enhancements in memory and learning. From a biochemical perspective, the extract reduced lipid peroxidation and restored levels of superoxide dismutase, catalase and total proteins, highlighting improved antioxidant defense and diminished neurotoxicity. Histopathological evaluations also indicated better maintenance of neuronal structures in treated subjects. The results were comparable to those observed with rivastigmine across many parameters.

The advantages noted might be linked to the phytochemical components of the plant, which include flavonoids, phenolics, and saponins known for their antioxidant, anti-inflammatory, and potential acetylcholinesterase inhibitory effects. Therefore, the ethanolic leaf extract of *Smilax glabra* Roxb. displayed notable neuroprotective activity in scopolamine-induced dementia, indicating its promise as a natural option for Alzheimer's disease exploration.

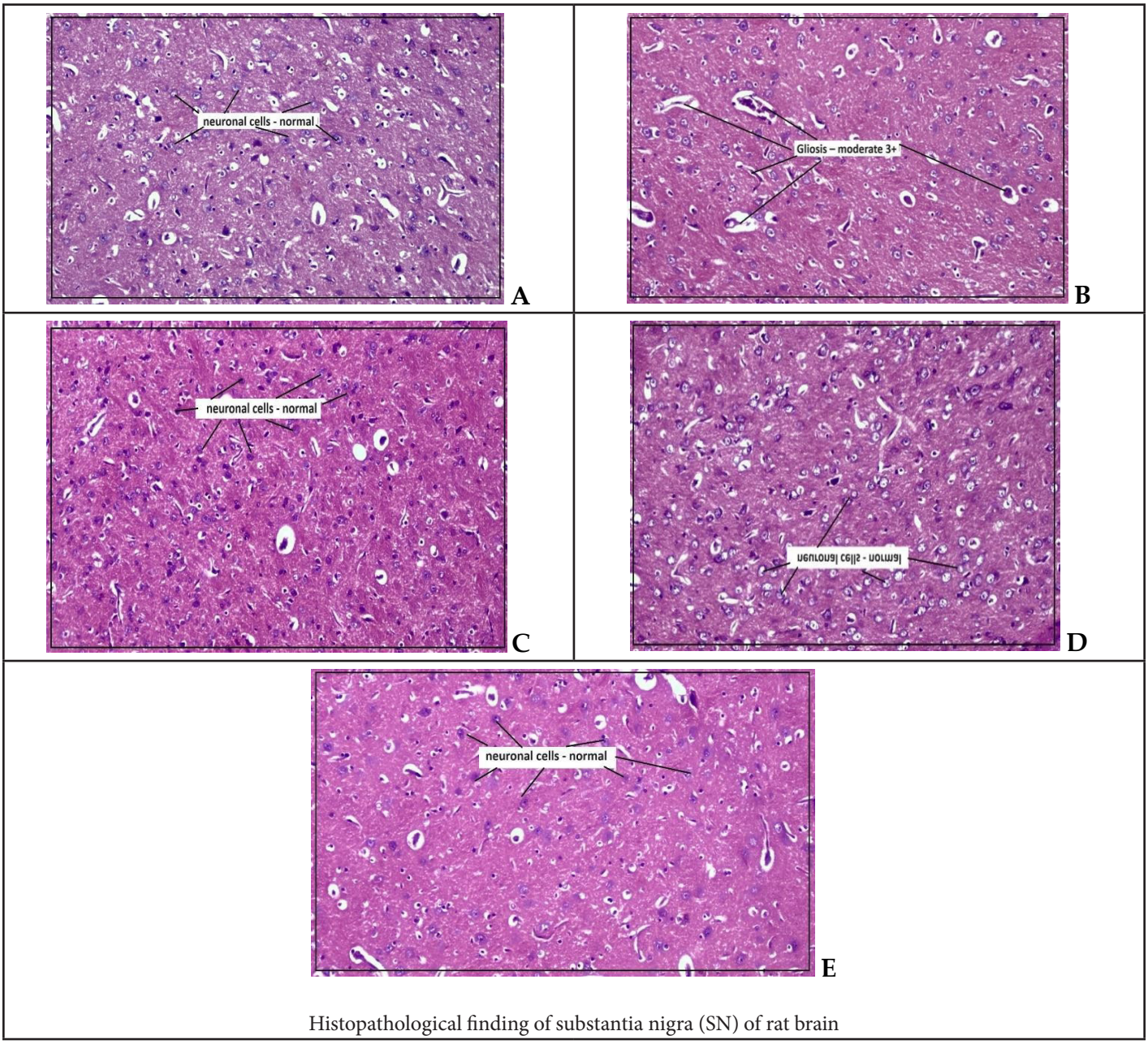

Figure 7: Histopathological finding of Substantia Nigra (SN) of rat brain. (A) Normal group, Rat Brain: hippocampus region showing neuronal cells normal morphology (X100). (B) Scopolamine induced group, Rat Brain: Cortical region showing moderate Gliosis, apoptosis - moderate: 3+ (X100). (C) Low dose of SGR, Rat Brain: Cortical region showing neuronal cells normal, gliosis - focal -1+ morphology(X100). (D) High dose of SGR: Cortical region showing neuronal cells normal, gliosis normal morphology (X100). (E) Standard group, Rat Brain: Cortical region showing neuronal cells normal morphology (X100).

Estimation of antioxidants of the brain

Table 1: Estimation of Catalase, SOD, LPO, Total Protein content, Acetylcholinesterase in brain of rats.

Groups	Treatment Groups	Catalase	SOD	Lipid peroxidation	Total Protein content	Acetylcholinesterase
Group 1	Normal Group (Saline *p.o*)	7.82±0.24	1.76±0.08	8.62±0.35	1.84±0.06	10.84±0.32
Group 2	Control Group (Scopolamine 2 mg/kg *i.p*)	3.46±0.18###	0.82±0.05###	18.47±0.42###	0.96±0.04###	18.26±0.41###
Group 3	*Smilax glabra* Roxb (Low Dose 200 mg/kg *p.o*) + Scopolamine	5.21±0.22*	1.12±0.07*	14.32±0.38*	1.28±0.05*	14.63±0.36*
Group 4	*Smilax glabra* Roxb (High Dose 400 mg/kg *p.o*) + Scopolamine	6.38±0.26***	1.48±0.06***	10.76±0.33***	1.62±0.06**	9.82±0.28**
Group 5	Standard Group (Rivastigmine 2.5 mg/kg *p.o*) + Scopolamine	6.72±0.19***	1.52±0.05***	9.24±0.29***	1.69±0.05***	9.21±0.26***

Estimation of **a)** Catalase, b) SOD, c) LPO, d) Total Protein, e) Acetylcholinesterase levels in brain. Results are expressed as Mean±SEM (n=6), ###p<0.001 when compared to the normal group, *p<0.05, ***p<0.001 when compared to the Scopolamine-induced group.

Future studies should focus on isolation of active constituents, *in vitro* AChE inhibition assays, and clinical validation to further support its therapeutic role (Figures 5-7).

CONCLUSION

The ethanolic extract from the leaves of *Smilax glabra* Roxb demonstrated neuroprotective properties in a rat model of Alzheimer's disease, a result likely attributed to its chemical components, including flavonoids, phenolics, saponins, glycosides, and tannins. This extract diminished lipid peroxidation and increased the activity of catalase and superoxide dismutase, suggesting enhanced antioxidant protection. Additionally, it improved behavioral outcomes by extending fall latency in the rotarod test, boosting spatial learning and memory in the Morris water maze, and demonstrating superior performance in the elevated plus maze. Moreover, the extract decreased the activity of acetylcholinesterase and restored total protein levels, further indicating its neuroprotective capabilities. *S. glabra* Roxb ethanolic leaf extract seems to be a valuable multi-target natural option for Alzheimer's disease due to its antioxidant, anti-inflammatory, and cholinergic effects. However, additional long-term studies are needed to establish its efficacy and safety for clinical use.

ABBREVIATIONS

AD: Alzheimer's Disease; **AChE:** Acetylcholinesterase; **CAT:** Catalase; **SOD:** Superoxide Dismutase; **MDA:** Malondialdehyde; **LPO:** Lipid Peroxidation; SGE: *Smilax glabra* Extract; **i.p.:** Intraperitoneal; **p.o.:** Per oral; **EPM:** Elevated Plus Maze; **MWM:** Morris Water Maze; **SEM:** Standard Error of the Mean; **ANOVA:** Analysis of Variance; **OECD:** Organisation for Economic Co-operation and Development; **DTNB:** 5,5'-dithi obis(2-nitrobenzoate); **NBT:** Nitroblue Tetrazolium; **NADH:** Nicotinamide Adenine Dinucleotide (reduced); **TBARS:** Thiobarbituric acid reactive substances.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

SUMMARY

Alzheimer's Disease (AD) is a progressive neurodegenerative disorder and the leading global cause of dementia, marked by cognitive decline, memory loss, and behavioral changes. Due to the limitations and side effects of the synthetic treatments available today, there is increased interest in natural compounds from medicinal plants with multi-targeting activity potential *Smilax glabra* Roxb. is one such candidate and is historically known for its anti-inflammatory, antioxidant, and detoxifying properties.

Smilax glabra Roxb., a perennial climbing shrub, is widely utilized in Traditional Chinese Medicine and Indian ethnomedicine. Its bioactive constituents-flavonoids, phenolics, saponins, glycosides, and tannins-exhibit diverse pharmacological effects, including antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, and neuroprotective properties. Of particular interest are flavonoids such as quercetin and kaempferol, which can cross the blood-brain barrier and exert influence over neurological pathways, suggesting potential utility in neurodegenerative

conditions associated with oxidative stress and cholinergic deficiencies.

This study aimed to assess the anti-Alzheimer's activity for a *S. glabra* ethanolic leaf extract through composite effects in a scopolamine-induced dementia model in Wistar rats. The scopolamine (1 mg/kg) was administered intraperitoneally for six continuous days in order to induce reversible memory impairment symptoms mirroring early events of AD, characterized by cholinergic blockade and oxidative stress.

Following scopolamine administration, rats were treated orally with *S. glabra* extract at doses of 200 mg/kg and 400 mg/kg for 24 days. Rivastigmine (2.5 mg/kg) served as the positive control. Behavioral assessments with the Elevated Plus Maze and Morris Water Maze conducted on days 15 and 30 revealed that scopolamine significantly impaired memory, as evidenced by increased transfer and escape latencies. Conversely, extract-treated groups, particularly at the higher dose, exhibited significant improvements in these parameters comparable to rivastigmine, indicating enhanced learning and memory.

Biochemical analyses demonstrated that scopolamine elevated brain Malondialdehyde (MDA) levels, indicative of lipid peroxidation, while reducing activities of key antioxidant enzymes, Superoxide Dismutase (SOD) and Catalase (CAT). Treatment with *S. glabra* significantly reversed these oxidative stress markers, restoring antioxidant enzyme activities and reducing lipid peroxidation. Total brain protein levels, diminished by scopolamine-induced neuronal damage, were similarly restored by the extract, suggesting neuroprotection and cellular preservation.

Histopathological evaluations corroborated these findings; scopolamine-treated rats displayed neuronal shrinkage, vacuolization, and pyknotic nuclei, indicating neurodegeneration predominantly in the hippocampus. Rats receiving *S. glabra* exhibited preserved neuronal morphology, confirming mitigation of neurodegenerative injury.

In conclusion, the neuroprotective effects of *Smilax glabra* Roxb in this scopolamine model may be explained by the ability of a range of potential phytochemicals to function as an antioxidant, anti-inflammatory, and cholinergic-modulating. The encouraging results from this study signal an important area of research and delineation of active compounds, mechanistic studies, and clinical trials to confirm whether this extract may provide a natural treatment for Alzheimer's disease.

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Cite this article: Aditya M, Sarkar M, Soumya KM. Evaluation of the Neuroprotective Activity of Ethanolic Leaf Extract of *Smilax glabra* Roxb against Scopolamine Induced Dementia in Wistar Rats. Indian J of Pharmaceutical Education and Research. Krupapharmacon 2026:112-22.