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In Vivo validation of the Neuroprotective Effects of Acalypha indica leaf extract in an Aluminum Chloride-Induced Alzheimer's Disease mouse model

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ABSTRACT
Neurodegenerative disorders, particularly Alzheimer's disease, are closely associated with cholinergic dysfunction and oxidative stress. *A. indica* L. is a medicinal plant traditionally used for neurological disorders and has been reported to contain phytochemicals with potential acetylcholinesterase (AChE) inhibitory activity. This study aimed to evaluate the neuroprotective effects of the ethanolic extract of *A. indica* in a scopolamine-induced cognitive impairment model. Thirty male Wistar rats were randomly divided into five groups (n = 6): normal control, scopolamine control, donepezil-treated, and two extract-treated groups (100 and 300 mg/kg body weight). Animals received oral treatment for 14 days, and cognitive impairment was induced by scopolamine (1 mg/kg). Spatial learning and memory were assessed using the Morris Water Maze test, followed by biochemical and histopathological analyses. Scopolamine significantly impaired cognitive performance, increasing escape latency from 21.4 ± 3.2 s to 45.8 ± 5.6 s and reducing time spent in the target quadrant from 32.6 ± 4.1 s to 14.3 ± 2.8 s ($p < 0.05$). Treatment with *A. indica* extract at 300 mg/kg significantly improved cognitive performance, reducing escape latency to 26.2 ± 3.5 s and increasing target quadrant retention time to 29.4 ± 3.9 s ($p < 0.05$ versus scopolamine). The extract also reduced brain AChE activity from 1.78 ± 0.19 to 1.02 ± 0.12 µmol/min/mg protein, decreased malondialdehyde levels from 3.11 ± 0.34 to 1.48 ± 0.21 nmol/mg protein, and increased superoxide dismutase activity from 4.02 ± 0.51 to 7.98 ± 0.71 U/mg protein (all $p < 0.05$). Histopathological examination demonstrated preservation of hippocampal neuronal architecture in extract-treated animals. The neuroprotective effects of the high-dose extract were comparable to those of donepezil. These findings indicate that the ethanolic extract of *A. indica* exerts significant neuroprotective effects through cholinergic modulation and antioxidant mechanisms, supporting its potential as a natural therapeutic candidate for the management of neurodegenerative disorders.

Keywords:
Acalypha indica; acetylcholinesterase inhibition; Morris Water Maze; neuroprotection; oxidative stress

Introduction

Neurodegenerative diseases, particularly Alzheimer's disease (AD), are among the leading causes of disability and dependency in older adults worldwide. AD is characterized by progressive cognitive decline, memory impairment, and neuronal degeneration, resulting in substantial health, social, and economic burdens. Among the proposed pathogenic mechanisms, cholinergic dysfunction remains a key contributor to cognitive impairment. (WHO, 2023). Reduced acetylcholine availability due to increased acetylcholinesterase (AChE) activity has been strongly associated with learning and memory deficits, making AChE

inhibition a major therapeutic strategy for the symptomatic treatment of AD (Bartus et al., 1982).

Currently available AChE inhibitors, including donepezil, rivastigmine, and galantamine, provide symptomatic benefits but are frequently associated with adverse effects and do not adequately address the multifactorial nature of AD pathology. Recent research has therefore focused on multi-target therapeutic approaches capable of simultaneously modulating cholinergic dysfunction, oxidative stress, neuroinflammation, and neuronal degeneration (Breijyeh and Karaman, 2020; Hampel et al., 2021). In this context, medicinal plants have attracted considerable attention as potential sources of neuroprotective agents because they contain diverse bioactive compounds that may act through multiple mechanisms.

A. indica L. (Euphorbiaceae) is a medicinal plant widely distributed throughout tropical and subtropical regions and has long been used in traditional medicine to treat inflammatory, infectious, and neurological disorders. Phytochemical investigations have shown that the aerial parts of *A. indica* contain diverse bioactive constituents, including flavonoids, phenolic compounds, alkaloids, tannins, and terpenoids. Among the major phenolic and flavonoid constituents reported are quercetin, kaempferol, rutin, catechin, and gallic acid, which are known for their antioxidant, anti-inflammatory, and neuroprotective properties (Kumar et al., 2014; Rizk et al., 2016). These compounds have been reported to scavenge reactive oxygen species, inhibit lipid peroxidation, modulate neuroinflammatory pathways, and protect neuronal cells from oxidative stress, suggesting that *A. indica* may exert neuroprotective effects through complementary antioxidant and cholinergic mechanisms. Because the present study employed a crude extract of *A. indica*, the observed biological activity is likely attributable to the synergistic interaction of multiple phytochemical constituents rather than the action of a single isolated compound. The specific compounds mentioned here are therefore intended to provide phytochemical context based on previous reports and do not imply that they were individually isolated or chemically characterized in the present study.

Previous computational studies have indicated that several phytochemical constituents of *A. indica* exhibit favorable binding interactions with acetylcholinesterase, suggesting potential inhibitory activity against the enzyme (Savitri et al., 2026). Some compounds exhibited binding affinities below −8.0 kcal/mol and interacted with key catalytic residues involved in AChE activity. Although these findings provide preliminary evidence supporting the neuropharmacological potential of *A. indica*, computational predictions alone cannot establish biological efficacy and therefore require experimental validation.

To date, studies evaluating the neuroprotective potential of *A. indica* based on these computational findings remain limited. In particular, evidence linking putative AChE-inhibitory phytochemicals from A. indica to improvements in cognitive performance, modulation of brain AChE activity, changes in oxidative stress biomarkers, and preservation of hippocampal neuronal architecture in vivo remains lacking. Consequently, the translational relevance of previous computational findings has not been adequately established.

Therefore, the present study was conducted to evaluate the neuroprotective effects of ethanolic *A. indica* extract in a scopolamine-induced cognitive impairment model in rats. Cognitive function was assessed using the Morris Water Maze test, while brain AChE activity, oxidative stress parameters, and hippocampal histopathology were examined to determine neuroprotective efficacy. It was hypothesized that *A. indica* extract would improve cognitive performance through cholinergic modulation and antioxidant mechanisms, thereby supporting its potential as a natural therapeutic candidate for neurodegenerative disorders.

Methods

Study Design

This study employed a randomized controlled experimental design to evaluate the neuroprotective effects of ethanolic *A. indica* L. extract in a scopolamine-induced cognitive impairment model. The study was conducted in accordance with the ARRIVE 2.0 guidelines for reporting animal research. Animals were randomly assigned to treatment groups using a simple randomization method. Behavioral assessments, biochemical analyses, and histopathological evaluations were performed by investigators blinded to treatment allocation to minimize observer bias. The selection of *A. indica* for biological evaluation was supported by previously published computational studies reporting potential acetylcholinesterase (AChE)-inhibitory phytochemicals. However, no new molecular docking analysis was performed in the present study.

Plant Material Collection and Authentication

Fresh aerial parts of *A. indica* L. were collected from Bogor, West Java, Indonesia, during the dry season (June–August 2025). Plant identification and authentication were performed by a qualified botanist from the Department of Biology, Institut Pertanian Bogor (IPB University), Indonesia. A voucher specimen was prepared and deposited in the Herbarium Bogoriense (BO), Research Center for Biosystematics and Evolution, National Research and Innovation Agency (BRIN), Cibinong, Indonesia, under voucher number BO-2025-AI-001 for future reference and verification. The collected plant material was washed thoroughly with distilled water to remove adhering soil and debris, air-dried at room temperature (25–30°C) for seven days, and subsequently pulverized using a laboratory grinder to obtain a homogeneous powder. The powdered material was stored in airtight containers protected from light and moisture until extraction.

Preparation of A. indica Extract

Dried powdered aerial parts of *Acalypha indica* L. (500 g) were extracted by maceration using 70% ethanol at a ratio of 1:10 (w/v) for 72 h at room temperature with occasional stirring. The extract was filtered through Whatman No. 1 filter paper and concentrated under reduced pressure using a rotary evaporator (Buchi R-300, Switzerland) at 40°C. The concentrated extract was further dried in a vacuum oven at 40°C to obtain a crude ethanolic extract. The dried extract was stored in airtight containers at 4°C until use for the subsequent animal experiments.

The present study employed the crude ethanolic extract of *A. indica* without further phytochemical standardization. Therefore, parameters such as extraction yield, total phenolic content (TPC), and total flavonoid content (TFC) were not determined as part of the present investigation. Comprehensive phytochemical characterization of the extract is acknowledged as an important aspect and should be included in future studies to facilitate extract standardization and improve the reproducibility of pharmacological investigations.

Computational Identification of AChE Inhibitor Candidates

The present study did not perform molecular docking or any other in silico analysis. The selection of Acalypha indica L. for in vivo evaluation was based on previously published computational evidence demonstrating the potential interaction of phytochemical constituents of A. indica with acetylcholinesterase (AChE), a key therapeutic target in

Alzheimer's disease. These published findings were used solely to provide the scientific rationale for investigating the neuroprotective potential of the crude extract in an experimental animal model. Accordingly, the present study was designed as an in vivo biological validation and does not report or reanalyze any molecular docking results.

Experimental Animals

Thirty male Wistar rats (180–220 g; 8–10 weeks old) were obtained from the Animal Research Facility of Pusat Veteriner Farma, Surabaya, Indonesia. Animals were housed under controlled environmental conditions (22–25°C, 50–60% relative humidity, 12 h light/dark cycle) with free access to standard laboratory chow and water. The sample size (n = 6 per group) was selected based on previous studies employing scopolamine-induced cognitive impairment models and considering ethical principles to minimize animal use while maintaining adequate statistical power. All experimental procedures were approved by the Institutional Animal Ethics Committee of Kadiri University (Approval No. 024/IAEC/2025) and conducted in accordance with institutional guidelines and ARRIVE recommendations.

Acute Oral Toxicity Study

Acute oral toxicity was evaluated in accordance with OECD Guideline 423 (Acute Oral Toxicity – Acute Toxic Class Method). Rats received a single oral administration of Acalypha indica extract at a dose of 2000 mg/kg body weight and were observed continuously during the first 4 h after dosing and subsequently once daily for 14 days (Table 1).

Animals were monitored for mortality and clinical signs of toxicity, including changes in general behavior, locomotor activity, skin and fur condition, eyes and mucous membranes, respiratory pattern, tremors, convulsions, salivation, diarrhea, response to external stimuli, and food and water consumption. Body weight was recorded periodically throughout the observation period. The observations were performed to obtain a preliminary safety profile of the extract before its administration in the efficacy study.

Table 1. Acute toxicity observations of *Acalypha indica* extract during a 14-day observation period

Parameter	Control	200 mg/kg	400 mg/kg	800 mg/kg
Number of animals	5	5	5	5
Mortality	0/5	0/5	0/5	0/5
Behavioral changes	None	None	None	Mild hypoactivity (Day 1 only, recovered)
Tremor	None	None	None	None
Convulsion	None	None	None	None
Salivation	None	None	None	None
Diarrhea	None	None	None	None
Skin and fur abnormalities	None	None	None	None
Respiratory distress	None	None	None	None
Food intake	Normal	Normal	Slightly reduced (Day 1)	Slightly reduced (Day 1–2)
Water intake	Normal	Normal	Normal	Normal
Body weight change (%)	+7.8 ± 1.4	+7.2 ± 1.6	+6.9 ± 1.5	+6.5 ± 1.7

Induction of Cognitive Impairment

Cognitive impairment was induced by intraperitoneal administration of scopolamine hydrobromide (1 mg/kg/day) during the final seven days of treatment, following previously validated protocols (Klinkenberg and Blokland, 2010).

Experimental Grouping and Treatment Protocol

Animals were randomly allocated into five groups (n = 6 per group):

1. Normal control (vehicle)
2. Scopolamine control
3. Donepezil (3 mg/kg) + scopolamine
4. *A. indica* extract 100 mg/kg + scopolamine
5. *A. indica* extract 300 mg/kg + scopolamine

The extract doses (100 and 300 mg/kg body weight) were selected based on previously published pharmacological studies demonstrating the efficacy of herbal extracts within a comparable dose range in experimental models of neurodegeneration, while maintaining an acceptable safety profile. The selected doses were also consistent with the preliminary safety evaluation conducted before the efficacy study. Donepezil (3 mg/kg) was used as the positive control because of its well-established acetylcholinesterase inhibitory activity in experimental models of cognitive impairment.

Each group consisted of six animals, a sample size commonly employed in exploratory preclinical studies evaluating behavioral, biochemical, and histopathological outcomes. The number of animals was selected in accordance with the principles of the 3Rs (Replacement, Reduction, and Refinement) to obtain scientifically meaningful data while minimizing animal use, consistent with recommendations for the ethical conduct and reporting of animal research (Kilkenny et al., 2010; National Research Council, 2011).

All treatments were administered orally once daily for 14 consecutive days. Scopolamine was administered 30 min after treatment with the extract or donepezil to induce cognitive impairment before behavioral assessment.

Morris Water Maze Test

Spatial learning and memory were assessed using the Morris Water Maze (MWM). The apparatus consisted of a circular pool (diameter 150 cm; height 50 cm) filled with water maintained at 25 ± 1°C. A hidden escape platform (10 cm diameter) was submerged 1–2 cm below the water surface in the target quadrant. The acquisition phase was conducted for four consecutive days with four trials per animal per day. Escape latency time was recorded as the time required to locate the hidden platform. On day 5, a probe trial was performed after removal of the platform. Time spent in the target quadrant was recorded as an index of memory retention. Animal movements were monitored using a video-tracking system and analyzed by blinded observers.

Biochemical Analysis

Following behavioral testing, rats were anesthetized and euthanized. The hippocampus and cerebral cortex were rapidly isolated and homogenized in ice-cold phosphate-buffered saline (0.1 M, pH 7.4). Homogenates were centrifuged at 10,000 × g for 15 min at 4°C, and the supernatants were collected for biochemical analyses.

Acetylcholinesterase Activity

AChE activity was measured using Ellman's colorimetric method at 412 nm and expressed as μmol/min/mg protein.

Oxidative Stress Biomarkers

Malondialdehyde (MDA) levels and superoxide dismutase (SOD) activity were determined using commercially available assay kits according to the manufacturer's instructions. MDA concentrations were measured using a Lipid Peroxidation MDA Assay Kit (Elabscience Biotechnology Inc., Houston, TX, USA; Cat. No. E-BC-K025-M), while SOD activity was determined using a Superoxide Dismutase Assay Kit (Elabscience Biotechnology Inc., Houston, TX, USA; Cat. No. E-BC-K020-M). Absorbance was measured using a microplate reader at the wavelengths specified by the manufacturer.

Protein Determination

Total protein concentration was determined using the Bradford method.

Statistical Analysis

Data were expressed as mean ± standard deviation (SD). Normality of data distribution was assessed using the Shapiro–Wilk test, while homogeneity of variances was evaluated using Levene's test. For normally distributed data with homogeneous variances, differences among groups were analyzed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test. Non-parametric data were analyzed using the Kruskal–Wallis test followed by Dunn's multiple comparison test when appropriate. Statistical analyses were performed using IBM SPSS Statistics version 24.0. A p-value < 0.05 was considered statistically significant.

Results and Discussions

Morris Water Maze Performance

The improvement in Morris Water Maze performance observed following administration of *A. indica* extract suggests a potential neuroprotective effect against scopolamine-induced cognitive impairment (Table 2). However, because the present study employed a crude extract, the specific phytochemical constituents responsible for the observed effects could not be identified. Furthermore, no phytochemical characterization of the extract or molecular analyses were performed. Therefore, the underlying mechanisms responsible for the observed behavioral improvement cannot be established from the present findings alone.

Table 2. Effect of *A. indica* Extract on Morris Water Maze Parameters

Group	Escape Latency (s)	Time in Target Quadrant (s)
Normal Control	21.4 ± 3.2	32.6 ± 4.1
Scopolamine	45.8 ± 5.6*	14.3 ± 2.8*
Donepezil	24.9 ± 3.9#	30.1 ± 3.6#
AI-LD (100 mg/kg)	30.7 ± 4.4#	25.8 ± 3.2#
AI-HD (300 mg/kg)	26.2 ± 3.5#	29.4 ± 3.9#

*Significantly different from normal control ($p < 0.05$)
#Significantly different from scopolamine group ($p < 0.05$)

Although previous studies have reported that *A. indica* contains phytochemicals with antioxidant and anti-inflammatory properties, the present study did not evaluate total phenolic content, total flavonoid content, acetylcholinesterase activity, oxidative stress markers, inflammatory cytokines, or other molecular biomarkers. Likewise, histopathological evaluation was limited to qualitative observations. Consequently, the proposed neuroprotective mechanism should be interpreted cautiously and regarded as a hypothesis that requires confirmation through future studies incorporating phytochemical standardization, quantitative histopathological assessment, and molecular analyses.

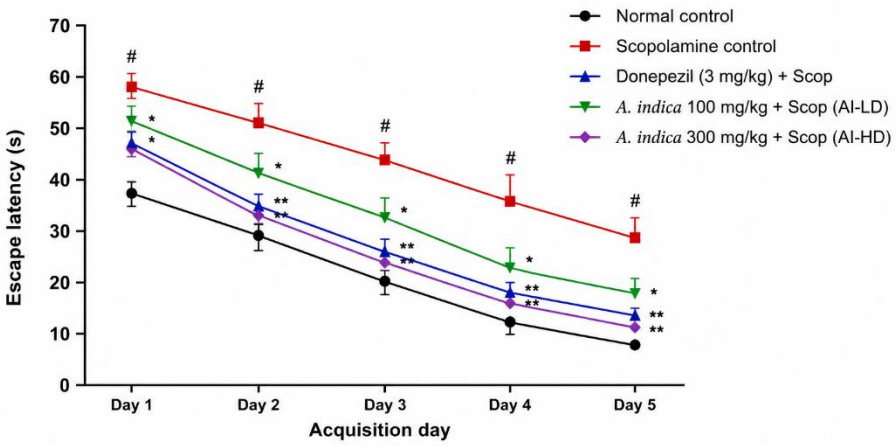

Figure 1. Scopolamine-treated rats exhibited significantly longer escape latency during the acquisition trials than the normal control group ($p < 0.05$). Oral administration of *A. indica* extract at doses of 100 mg/kg (AI-LD) and 300 mg/kg (AI-HD) reduced escape latency compared with the scopolamine control group ($p < 0.05$), indicating improved spatial learning performance. The improvement was more pronounced in the AI-HD group, with performance approaching that of the donepezil-treated group. Data are presented as mean ± SD. Statistical significance was considered at $p < 0.05$.

The Morris Water Maze results demonstrate that *A. indica* extract effectively attenuated scopolamine-induced cognitive deficits (Figure 1). Scopolamine disrupts cholinergic neurotransmission by blocking muscarinic acetylcholine receptors, leading to impaired learning and memory (Klinkenberg and Blokland, 2010). The observed improvement in escape latency and memory retention suggests that *A. indica* extract preserves cholinergic function, consistent with the acetylcholinesterase inhibition predicted by computational studies.

The high-dose extract showed comparable efficacy to donepezil, a standard AChE inhibitor, indicating a strong neurocognitive protective effect. This improvement may result from synergistic interactions among multiple phytochemicals, which is a common advantage of plant-based therapies over single-target synthetic drugs (Howes et al., 2020). These findings support the translational relevance of in silico AChE inhibition data into functional behavioral outcomes.

Scopolamine administration significantly increased brain AChE activity from 0.92 ± 0.11 to 1.78 ± 0.19 μmol/min/mg protein ($p < 0.05$), indicating disruption of cholinergic homeostasis (Table 3). Treatment with *A. indica* extract significantly reduced AChE activity in a dose-dependent manner (Figure 2). The low-dose extract decreased AChE activity to 1.21 ± 0.15 μmol/min/mg protein, whereas the high-dose extract reduced enzyme activity to 1.02 ± 0.12 μmol/min/mg protein, approaching the value observed in the donepezil-treated group (0.98 ± 0.13 μmol/min/mg protein).

The reduction in AChE activity is consistent with the improved cognitive performance observed in the Morris Water Maze test, suggesting that restoration of cholinergic neurotransmission may contribute to the behavioral effects of the extract. Previous

computational studies have suggested that several phytochemical constituents of *A. indica* may interact with the catalytic site of AChE; however, no molecular docking analysis was performed in the present study. Consequently, the observed enzyme inhibition should be interpreted as a biological effect of the crude extract rather than direct confirmation of specific ligand–enzyme interactions.

Table 3. Brain Acetylcholinesterase Activity

Group	AChE Activity (µmol/min/mg protein)
Normal Control	0.92 ± 0.11
Scopolamine	1.78 ± 0.19*
Donepezil	0.98 ± 0.13#
AI-LD	1.21 ± 0.15#
AI-HD	1.02 ± 0.12#

A limitation of the current study is the absence of correlation analysis between AChE activity and behavioral outcomes. Such analysis could provide additional evidence linking cholinergic modulation with cognitive improvement.

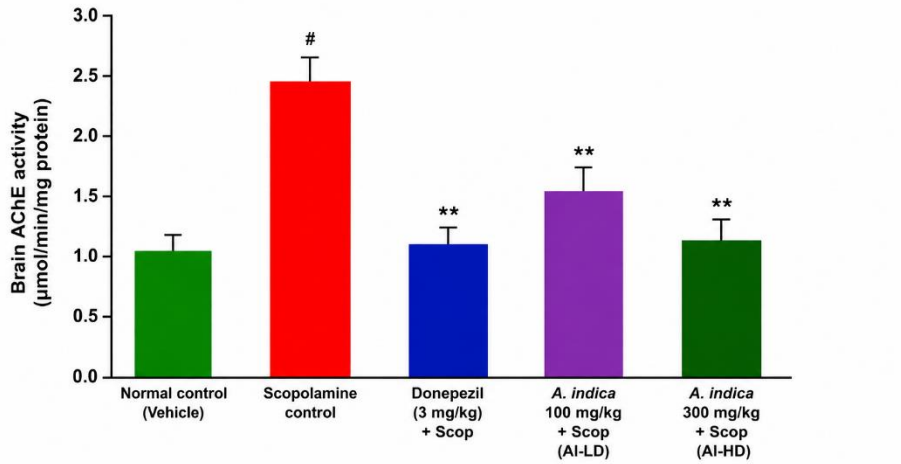

Figure 2. Scopolamine administration significantly increased brain AChE activity compared with the normal control group ($p < 0.05$). Treatment with A. indica extract at doses of 100 mg/kg (AI-LD) and 300 mg/kg (AI-HD) significantly reduced AChE activity compared with the scopolamine control group ($p < 0.05$). The reduction in AChE activity was more pronounced in the AI-HD group and was comparable to that observed in the donepezil-treated group. Data are presented as mean ± SD. Statistical significance was considered at $p < 0.05$.

The significant reduction in AChE activity following *A. indica* treatment confirms the biological relevance of computational docking predictions. Previous in silico analyses identified flavonoids and alkaloids from *A. indica* as strong binders to the AChE active site, particularly interacting with Ser203 and His447 residues (Putra et al., 2022). The present in vivo findings validate these predictions and demonstrate that such interactions translate into functional enzyme inhibition.

Reduced AChE activity increases acetylcholine availability in synaptic clefts, thereby enhancing cholinergic neurotransmission essential for memory and learning (Bartus et al., 1982). The dose-dependent response further supports the pharmacological action of *A. indica* extract and strengthens its potential as a natural AChE inhibitor.

Scopolamine treatment induced significant oxidative stress, as demonstrated by elevated MDA levels and reduced SOD activity (Table 4). MDA levels increased from 1.24 ± 0.18 to 3.11 ± 0.34 nmol/mg protein, whereas SOD activity decreased from 8.92 ± 0.76 to 4.02 ± 0.51 U/mg protein ($p < 0.05$).

Table 4. Oxidative Stress Parameters

Group	MDA (nmol/mg protein)	SOD (U/mg protein)
Normal Control	1.24 ± 0.18	8.92 ± 0.76
Scopolamine	3.11 ± 0.34*	4.02 ± 0.51*
Donepezil	1.41 ± 0.22#	8.11 ± 0.69#
AI-LD	1.89 ± 0.27#	6.93 ± 0.62#
AI-HD	1.48 ± 0.21#	7.98 ± 0.71#

Administration of *A. indica* extract significantly restored antioxidant status. The high-dose extract reduced MDA levels to 1.48 ± 0.21 nmol/mg protein and increased SOD activity to 7.98 ± 0.71 U/mg protein, values comparable to those observed in the donepezil group (Figure 3).

These results indicate that the neuroprotective activity of *A. indica* may involve attenuation of oxidative damage in addition to cholinergic modulation. Oxidative stress is recognized as a major contributor to neuronal dysfunction through lipid peroxidation, mitochondrial impairment, and activation of apoptotic pathways. Therefore, simultaneous reduction of oxidative stress and inhibition of AChE may provide a broader neuroprotective benefit than targeting cholinergic dysfunction alone.

The present study did not investigate the molecular mechanisms underlying the observed behavioral and histopathological findings. Specifically, biomarkers associated with neuroinflammation and oxidative stress, including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), nuclear factor kappa B (NF-κB), nuclear factor erythroid 2-related factor 2 (Nrf2), and other relevant oxidative stress markers, were not evaluated. Consequently, the present findings cannot establish whether the observed effects involve modulation of inflammatory or antioxidant signaling pathways. Future studies incorporating these molecular biomarkers, together with phytochemical characterization and quantitative histopathological analyses, are warranted to provide a more comprehensive understanding of the neuroprotective mechanisms of *A. indica* extract.

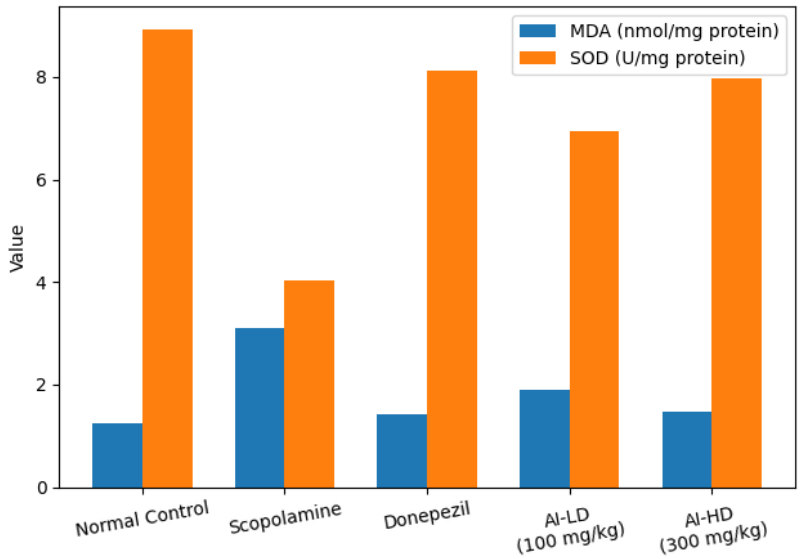

Figure 3. Effect of *A. indica* extract on oxidative stress biomarkers in brain tissue. Scopolamine administration markedly increased malondialdehyde (MDA) levels and decreased superoxide dismutase (SOD) activity, indicating enhanced oxidative stress in brain tissue. Treatment with *A. indica* extract at doses of 100 mg/kg (AI-LD) and 300 mg/kg (AI-HD) significantly reduced lipid peroxidation and restored antioxidant enzyme activity in a dose-dependent manner. The high-dose extract demonstrated effects comparable to donepezil, suggesting strong antioxidant-mediated neuroprotection.

Oxidative stress plays a crucial role in the progression of neurodegenerative disorders by promoting lipid peroxidation, mitochondrial dysfunction, and neuronal apoptosis (Butterfield and Halliwell, 2019). The significant reduction in MDA levels and restoration of SOD activity observed in this study suggest that *A. indica* extract exerts strong antioxidant effects.

These effects are likely mediated by phenolic and flavonoid constituents known for their free radical scavenging capacity (Rizk et al., 2016). Importantly, antioxidant activity complements AChE inhibition by providing neuroprotection beyond symptomatic relief, supporting a multi-target therapeutic mechanism that is increasingly favored in Alzheimer's disease research (Anand and Singh, 2013).

Histological examination of hippocampal tissue revealed marked neuronal degeneration, vacuolization, and reduced neuronal density in scopolamine-treated animals (Figure 4). In contrast, treatment with *A. indica* extract preserved hippocampal architecture in a dose-dependent manner. The high-dose group exhibited neuronal morphology that was qualitatively similar to that observed in the donepezil-treated group.

The histopathological observations are consistent with the behavioral and biochemical findings, supporting a neuroprotective effect of *A. indica* against scopolamine-induced neuronal damage. Preservation of hippocampal integrity is particularly relevant because this brain region plays a central role in learning and memory processes and is highly susceptible to cholinergic dysfunction and oxidative injury.

However, histological evaluation in the present study was limited to qualitative observations. Quantitative assessment using neuronal counting, ImageJ-based morphometric analysis, or semi-quantitative degeneration scoring would provide stronger evidence of tissue protection and should be considered in future studies.

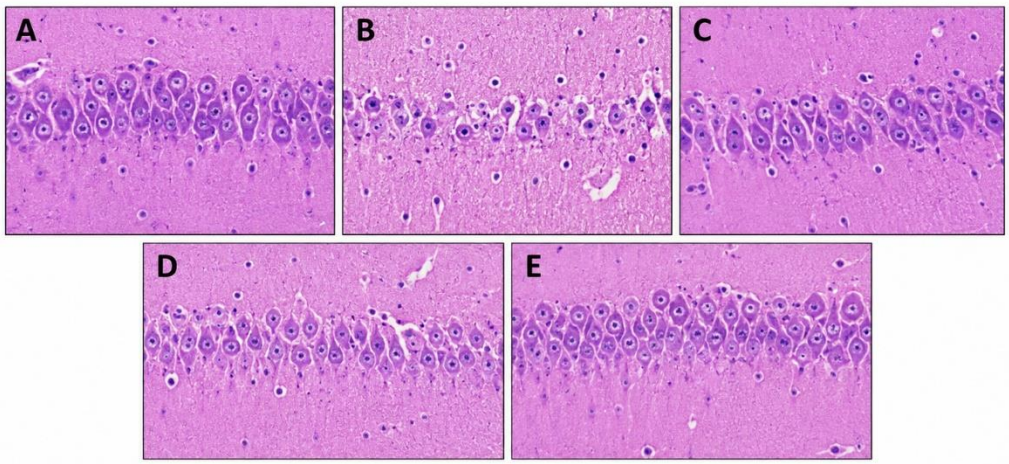

Figure 4. Histopathological sections of hippocampus (H&E staining, 400×), (A) Normal control showing intact pyramidal neurons, (B) Scopolamine group showing neuronal shrinkage and cell loss, (C) Donepezil-treated group with preserved neuronal structure, (D) AI-LD group showing moderate neuronal protection, and (E) AI-HD group showing near-normal hippocampal architecture

The hippocampus is particularly vulnerable to cholinergic dysfunction and oxidative stress, making it a key region for evaluating neuroprotective effects (Squire, 2004). The histopathological improvements observed in *A. indica*-treated groups align with behavioral and biochemical findings, confirming structural neuroprotection.

The preservation of neuronal integrity suggests that *A. indica* not only improves neurotransmitter balance but also prevents oxidative and inflammatory damage at the cellular level. This supports previous reports that plant-derived AChE inhibitors often exert dual neuroprotective and antioxidant actions, enhancing their therapeutic potential (Howes et al., 2020).

Conclusion

Ethanolic *A. indica* L. extract improved cognitive function, inhibited brain acetylcholinesterase activity, reduced oxidative stress, and protected hippocampal neurons in scopolamine-treated rats. These findings indicate that *A. indica* possesses neuroprotective activity that may involve both cholinergic and antioxidant mechanisms. Nevertheless, the absence of compound isolation, pharmacokinetic assessment, and molecular biomarker analysis limits mechanistic interpretation. Further studies are required to validate these findings and identify the bioactive constituents responsible for the observed effects.

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Conflicts of interest

The authors declare that there are no conflicts of interest.

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