AMERICAN JOURNAL OF PHARMTECH RESEARCH
Journal home page: http://www.ajptr.com/

Evaluation of Nootropic Activity of Hydroalcoholic Fruit Extract of *Annona Reticulata* Linn. In Scopolamine induced Cognitive Impairment in Mice

Dr. R.L Manisha[1]*, Dr. Meesala Gowthami[2], Dr. K Sumalatha[3], R Kiran Kumar Reddy[4], Poluri Sri Samanvitha Reddy[4], Ponugupati Kamala Sree[4], Poluri Tejashwini[4], Rajigani Ravi Kumar[4]

1.Head of the Department, Associate Professor, Department of Pharmacology and Pharm D, Malla Reddy College of Pharmacy, Secunderabad

2.Assistant Professor, Department of Pharmacy Practice, Malla Reddy College of Pharmacy, Secunderabad

3.Professor, Department of Pathology, Malla Reddy Institute of Medical Sciences, Secunderabad.

4.Bachelor of Pharmacy, Malla Reddy College of Pharmacy, Secunderabad.

ABSTRACT

Memory and cognitive decline are hallmark features of Alzheimer's disease, and current synthetic cholinesterase inhibitors raise tolerability and toxicity concerns, prompting interest in plant-derived alternatives. This study evaluated the nootropic potential of the hydroalcoholic fruit extract of *Annona reticulata* in scopolamine-induced cognitive impairment. Thirty-six male Wistar mice were divided into six groups (n=6): normal control, piracetam (400 mg/kg), scopolamine (1 mg/kg, i.p.), and three extract doses (100, 200, 400 mg/kg, p.o.) with scopolamine. Spatial learning and memory were assessed using the Morris Water Maze, followed by hippocampal histopathology; data were analyzed by ANOVA (mean ± SEM). The extract produced significant, dose-dependent improvement in escape latency and target-quadrant occupancy, with the 400 mg/kg dose approaching piracetam-treated performance and preserving hippocampal architecture. These findings indicate that *A. reticulata* fruit extract possesses notable cognitive-enhancing and neuroprotective activity, warranting further mechanistic investigation.

Keywords: Annona reticulata, Nootropic activity, Cognitive impairment, Scopolamine, Morris water maze, Neuroprotection.

*Corresponding Author Name: Dr. R.L Manisha
Received 25 May 2026, Accepted 01 June 2026

Please cite this article as: Manisha RL *et al*., A Evaluation of Nootropic Activity of Hydroalcoholic Fruit Extract of Annona Reticulata Linn. In Scopolamine induced Cognitive Impairment in Mice. American Journal of PharmTech Research 2026.

INTRODUCTION

Age-associated cognitive deterioration and memory impairment represent defining clinical features of Alzheimer's disease and a spectrum of other neurodegenerative conditions. These disorders are characterised by a sustained and progressive erosion of mnemonic function, attentional capacity, and overall cognitive integrity.[1] Among the neurochemical substrates implicated in cognition, the cholinergic pathway occupies a position of central importance; dysfunction within this system — particularly at the level of muscarinic receptor-mediated signalling — is widely regarded as a primary contributor to memory failure observed across multiple neurological disease states.[2] Scopolamine, a competitive antagonist at muscarinic acetylcholine receptors, reliably replicates cholinergic hypofunction in rodent models by suppressing central cholinergic transmission, thereby generating reproducible and quantifiable cognitive deficits suitable for pharmacological intervention studies.[3] Whilst established synthetic nootropics such as piracetam exert measurable pro-cognitive effects, their chronic administration has been associated with a range of unwanted adverse outcomes, underscoring the clinical imperative to identify novel, plant-derived therapeutic candidates offering favourable safety–efficacy profiles.[5]

The exploration of medicinal plant extracts as sources of neuroprotective bioactives has gained considerable momentum in recent years, driven by accumulating evidence linking secondary metabolites — particularly polyphenols, alkaloids, and terpenoids — to antioxidant defense, mitochondrial preservation, and augmented acetylcholine availability.[6,7] *Annona reticulata* (family Annonaceae), commonly encountered across tropical regions of the Indian subcontinent, has an established ethnomedicinal heritage and has been subject to phytochemical characterisation revealing the presence of diverse bioactive constituents including tannins, phenolic acids, flavone glycosides, steroidal alkaloids, and saponins.[9,10] These phytoconstituents individually and synergistically contribute to antioxidant and cytoprotective mechanisms that may confer structural and functional resilience to hippocampal and cortical neurons vulnerable to cholinergic-deficit-induced degenerative insults.[6,7] Against this background, the present study was designed to evaluate the nootropic potential of the hydroalcoholic fruit extract of *Annona reticulata* employing the Morris Water Maze paradigm in a scopolamine-challenged murine cognitive impairment model.[3,4]

MATERIALS AND METHOD

The experimental work was carried out between November 2025 and February 2026. Thirty-six adult male Wistar mice (body weight 20–25 g) procured from Jeeva Life Sciences, Shanti Nagar, Uppal, were used throughout the study. Animals were maintained under controlled vivarium

conditions (ambient temperature 24 ± 2°C; 12-hour alternating light–dark photoperiod) with unrestricted access to standard rodent chow and potable water. All procedures involving animals were conducted in strict accordance with the regulatory framework established by the Committee for the Control and Supervision of Experiments on Animals (CCSEA), Government of India, and prior ethical clearance was obtained from the Institutional Animal Ethics Committee (IAEC), Malla Reddy College of Pharmacy, Maisammaguda, Secunderabad (Approval No.: **MRCP/CPCSEA/IAEC/2026/1/13**).

Preparation of Hydroalcoholic Extract

Mature fruits of *Annona reticulata* Linn. were collected during the fruiting season from the local region of Andhra Pradesh, India. The plant material was taxonomically identified and authenticated by a competent botanist, Dr. B Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateshwara University, Tirupati and was deposited in the Department of Pharmacognosy, Malla Reddy College of Pharmacy, for future reference. A representative photograph of the collected fruits is presented in Figure 01.

Figure 1: Fruit of *Annona reticulata*

Freshly harvested fruits of *Annona reticulata* were surface-cleaned under running water, rendered shadow-dry at ambient temperature to eliminate residual moisture, and subsequently reduced to a uniform fine powder using a mechanical grinder. The powdered plant material was subjected to exhaustive Soxhlet extraction employing a 70:30 (v/v) ethanol–water hydroalcoholic solvent system, with the extraction cycle sustained for a period of 72 hours.[8] The resultant extract was concentrated through rotary evaporation under reduced pressure conditions until a semi-solid consistency was attained, and the yield was documented prior to dissolution in the appropriate vehicle for in vivo administration.

Grouping:

The mice were randomly distributed across six experimental batches (n=6 per group) and

administered their respective treatments for six consecutive days, as described below:

Batch I: Normal control — distilled water (vehicle) per oral

Batch II: Standard drug — Piracetam 400 mg/kg per oral

Batch III: Disease control — Scopolamine 1 mg/kg intraperitoneal

Batch IV: HAFEAR 100 mg/kg per oral + Scopolamine 1 mg/kg intraperitoneal

Batch V: HAFEAR 200 mg/kg per oral + Scopolamine 1 mg/kg intraperitoneal

Batch VI: HAFEAR 400 mg/kg per oral + Scopolamine 1 mg/kg intraperitoneal

Spatial learning and memory were assessed using the Morris Water Maze (MWM) paradigm over a six-day experimental schedule.[4] On Day 1, all animals underwent a habituation trial in the absence of the hidden escape platform; each mouse was allowed sixty seconds of free exploration within the pool to familiarize itself with the aquatic environment and to attenuate novelty-induced anxiety that could otherwise confound cognitive measurements. Locomotor baseline parameters — including immobility duration, swimming initiation latency, and thigmotactic tendency — were recorded during this session. From Day 2 through Day 5, scopolamine-mediated cognitive impairment was confirmed and quantified through four consecutive trials per animal per day, each trial capped at a 60-second limit.[3] Animals failing to independently locate the submerged platform within the allotted time were gently guided onto it and permitted to remain stationary for 15 seconds to consolidate spatial memory through reinforced platform association. For each trial, the following indices were quantified: escape latency to the target quadrant, total number of entries into the target zone, latency to first entry into the target quadrant, and cumulative time spent therein. On Day 6, a probe trial was conducted following platform removal; each mouse swam freely for 60 seconds, and spatial reference memory retention was evaluated through analysis of swimming path, target quadrant dwell time, and frequency of crossings at the former platform location.[4]

Upon completion of the behavioural protocol, all animals were euthanised under deep ether anaesthesia. Brain tissue was immediately excised and rinsed in ice-cold isotonic saline to remove surface blood. Tissue specimens were fixed in 10% neutral buffered formalin for 24–48 hours to preserve cytoarchitectural integrity. Paraffin-embedded coronal sections of approximately 5 µm thickness were prepared using a rotary microtome and subsequently stained with haematoxylin and eosin (H&E) to facilitate histopathological assessment.[11] Stained sections were examined under bright-field light microscopy for evaluation of hippocampal neuronal morphology, cellular degeneration indices, cytoplasmic vacuolation, and degree of neuroprotection conferred by extract treatment.

A *P* value of <0.001 was adopted as the threshold for statistical significance throughout the study, and all quantitative data are presented as Mean ± SEM.[12]

RESULTS AND DISCUSSION

Table 01: Effect of the hydroalcoholic fruit extract of Annona reticulata on Morris Water Maze parameters (Day 1 habituation, Days 2–5 acquisition, and Day 6 probe trial) in scopolamine-induced cognitive impairment in mice.

Day 1

Group	Immobility (s)	Latency (s)	Thigmotaxis
Normal Control	4.70 ± 0.30	1.60 ± 0.15	42.0 ± 2.0
Scopolamine	4.90 ± 0.30	1.50 ± 0.14	43.0 ± 2.1
Standard	4.50 ± 0.25	1.70 ± 0.16	41.0 ± 1.9
Low dose	4.80 ± 0.30	1.60 ± 0.15	42.0 ± 2.0
Medium dose	4.60 ± 0.25	1.60 ± 0.15	41.0 ± 1.8
High dose	4.40 ± 0.25	1.50 ± 0.14	42.0 ± 2.0

Day 1 habituation results: Baseline locomotor parameters recorded on Day 1 — encompassing immobility duration, swimming onset latency, and thigmotactic behaviour — did not differ significantly across treatment groups ($p > 0.05$). This homogeneity in baseline motor performance confirms the absence of any group-level differences in locomotion or exploratory anxiety, validating that subsequent inter-group differences in cognitive parameters are attributable exclusively to pharmacological interventions rather than pre-existing motor confounds.

Day 2

Group	Latency to Target(s)	Entries	Latency to 1st Entry (s)	Time in Target (s)
Normal control	38.2 ± 2.1	3.0 ± 0.2	19.1 ± 1.3	24.6 ± 1.8
Scopolamine	52.4 ± 2.6$^{\$\$}$	3.2 ± 0.1$^{\$\$}$	27.8 ± 1.9$^{\$\$}$	15.3 ± 1.4$^{\$\$}$
Standard	30.1 ± 1.9 ***	1.8 ± 0.2 ***	17.6 ± 1.2 ***	27.9 ± 1.9 ***
Low dose	44.6 ± 2.4 ns	3.0 ± 0.2 *	22.4 ± 1.5 *	20.5 ± 1.6 *
Medium dose	36.2 ± 2.2 **	2.8 ± 0.2 **	19.8 ± 1.3 **	23.7 ± 1.7 **
High dose	32.4 ± 2.0 ***	2.4 ± 0.2 ***	18.3 ± 1.2 ***	26.1 ± 1.8 ***

Day 3

Group	Latency to Target (s)	Entries	Latency to 1st Entry (s)	Time in Target (s)
Normal control	28.6 ± 1.8	2.8 ± 0.2	14.7 ± 1.1	30.5 ± 2.0
Scopolamine	49.8 ± 2.5$^{\$\$}$	3.0 ± 0.1$^{\$\$}$	25.6 ± 1.8$^{\$\$}$	17.8 ± 1.5$^{\$\$}$
Standard	22.9 ± 1.6 ***	1.7 ± 0.2 ***	13.9 ± 1.0 ***	33.6 ± 2.1 ***
Low dose	35.7 ± 2.1 *	2.8 ± 0.2 *	18.6 ± 1.3 *	25.3 ± 1.8 *
Medium dose	27.3 ± 1.9 **	2.6 ± 0.2 **	15.2 ± 1.1 **	29.4 ± 1.9 **
High dose	24.8 ± 1.7 ***	2.2 ± 0.2 ***	14.3 ± 1.0 ***	31.8 ± 2.0 ***

Day 4

Group	Latency to Target (s)	Entries	Latency to 1st Entry (s)	Time in Target (s)
Normal control	20.9 ± 1.5	2.6 ± 0.2	11.6 ± 0.9	35.2 ± 2.1
Scopolamine	46.2 ± 2.4$^{\$\$}$	2.9 ± 0.1$^{\$\$}$	23.8 ± 1.6$^{\$\$}$	19.5 ± 1.6$^{\$\$}$
Standard	17.6 ± 1.3 ***	1.6 ± 0.2 ***	10.9 ± 0.8 ***	38.4 ± 2.2 ***
Low dose	28.4 ± 1.9 *	2.7 ± 0.2 *	15.7 ± 1.2 *	29.6 ± 1.9 *
Medium dose	22.8 ± 1.6 **	2.5 ± 0.2 **	12.8 ± 1.0 **	33.1 ± 2.0 **
High dose	19.9 ± 1.4 ***	2.1 ± 0.2 ***	11.7 ± 0.9 ***	36.6 ± 2.1 ***

Day 5

Group	Latency to Target (s)	Entries	Latency to 1st Entry (s)	Time in Target (s)
Normal control	15.6 ± 1.2	2.4 ± 0.2	8.4 ± 0.7	42.5 ± 2.4
Scopolamine	43.8 ± 2.3$^{\$\$\$}$	2.8 ± 0.1$^{\$\$\$}$	21.5 ± 1.5$^{\$\$\$}$	21.3 ± 1.7$^{\$\$\$}$
Standard	14.8 ± 1.1 ***	1.6 ± 0.2 ***	7.9 ± 0.6 ***	45.8 ± 2.6 ***
Low dose	23.6 ± 1.7 **	2.4 ± 0.2 **	12.6 ± 1.0 **	34.9 ± 2.1 **
Medium dose	18.9 ± 1.4 ***	2.2 ± 0.2 ***	9.8 ± 0.8 ***	39.6 ± 2.3 ***
High dose	16.5 ± 1.2 ***	2.0 ± 0.2 ***	8.6 ± 0.7 ***	43.2 ± 2.5 ***

Day 6 (Probe Trial)

Group	Latency to 1st Entry (s)	Entries into Target Zone	Time in Target Zone (s)
Normal control	11.8 ± 0.9	5.8 ± 0.4	38.6 ± 2.3
Scopolamine	22.6 ± 1.58$^{\$\$}$	6.2 ± 0.3$^{\$\$}$	18.4 ± 1.9$^{\$\$}$
Standard dose	10.9 ± 0.8 ***	2.1 ± 0.4 ***	41.2 ± 2.5 ***
Extract – Low dose	17.6 ± 1.2 *	4.7 ± 0.3 *	26.9 ± 2.1 *
Extract – Medium dose	13.9 ± 1.0 **	4.5 ± 0.4 **	34.1 ± 2.3 **
Extract – High dose	11.6 ± 0.9 ***	3.2 ± 0.4 ***	39.5 ± 2.4 ***

Mean ± SEM (n = 6). Tukey's post-hoc test and one-way ANOVA were utilized to examine acquisition trial data (Days 2–5). Extract-treated and standard drug groups were compared against the scopolamine control; the scopolamine group was compared against the normal control. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$; ns = not significant; $^{\$}p < 0.05$, $^{\$\$}p < 0.01$, $^{\$\$\$}p < 0.001$.

Throughout the acquisition phase (Days 2–5), animals in the scopolamine disease-control cohort consistently demonstrated markedly impaired spatial cognition relative to normally treated mice, characterised by substantially prolonged target-zone escape latency, elevated number of target quadrant entries, extended latency to initial platform-zone entry, and diminished time accumulated within the target sector. These behavioural signatures are indicative of compromised spatial encoding, memory consolidation, and navigational memory retrieval. By contrast, animals in the normal control group exhibited a progressive learning curve across training days, displaying systematically reduced escape latency and increased target dwell time consistent with intact spatial memory consolidation. The piracetam reference group demonstrated robust and statistically significant cognitive enhancement across all measured indices ($p < 0.001$).

Administration of the hydroalcoholic fruit extract of *Annona reticulata* yielded dose-graded and statistically significant improvements across all cognitive metrics evaluated in the Morris Water Maze. The 100 mg/kg dose produced a modest but discernible improvement, while the 200 mg/kg dose elicited more pronounced cognitive recovery with significant group differences relative to scopolamine controls. Most notably, the 400 mg/kg extract dose produced cognitive performance indices closely approximating those of both the piracetam reference group and the non-impaired normal control cohort, providing compelling evidence for potent, dose-dependent nootropic activity.

On Day 6 (probe trial), scopolamine-challenged animals exhibited marked reference memory deficits, reflected in substantially increased first-entry latency and markedly reduced time spent within the target zone where the escape platform had previously been located — findings consistent with failed long-term spatial memory consolidation. Extract-treated animals demonstrated progressive, dose-proportional improvements in probe trial performance, with the high-dose group achieving near-normalization of memory retention indices, underscoring the extract's capacity to restore hippocampal-dependent spatial memory following cholinergic disruption

PARAMETER WISE DATA

Table 02: Parameter-wise comparison of Morris Water Maze acquisition indices (time in target zone, number of entries, latency to target, and latency to first entry) across treatment groups over Days 2–5.

Time Spent in Target zone

Group	Day 2	Day 3	Day 4	Day 5
Normal control	38.2 ± 2.1	28.6 ± 1.8	20.9 ± 1.5	15.6 ± 1.2
Scopolamine	52.4 ± 2.6$^{\$\$}$	49.8 ± 2.5$^{\$\$}$	46.2 ± 2.4$^{\$\$}$	43.8 ± 2.3$^{\$\$\$}$
Standard	30.1 ± 1.9 ***	22.9 ± 1.6 ***	17.6 ± 1.3 ***	14.8 ± 1.1 ***
Extract – Low	44.6 ± 2.4 ns	35.7 ± 2.1 *	28.4 ± 1.9 *	23.6 ± 1.7 **
Extract – Medium	36.2 ± 2.2 **	27.3 ± 1.9 **	22.8 ± 1.6 **	18.9 ± 1.4 ***
Extract – High	32.4 ± 2.0 ***	24.8 ± 1.7 ***	19.9 ± 1.4 ***	16.5 ± 1.2 ***

No. of entries into Target zone

Group	Day 2	Day 3	Day 4	Day 5
Normal control	3.0 ± 0.2	2.8 ± 0.2	2.6 ± 0.2	2.4 ± 0.2
Scopolamine	3.2 ± 0.1$^{\$\$}$	3.0 ± 0.1$^{\$\$}$	2.9 ± 0.1$^{\$\$}$	2.8 ± 0.1$^{\$\$\$}$
Standard	1.8 ± 0.2 ***	1.7 ± 0.2 ***	1.6 ± 0.2 ***	1.6 ± 0.2 ***
Extract – Low	3.0 ± 0.2 *	2.8 ± 0.2 *	2.7 ± 0.2 *	2.4 ± 0.2 **
Extract – Medium	2.8 ± 0.2 **	2.6 ± 0.2 **	2.5 ± 0.2 **	2.2 ± 0.2 ***
Extract – High	2.4 ± 0.2 ***	2.2 ± 0.2 ***	2.1 ± 0.2 ***	2.0 ± 0.2 ***

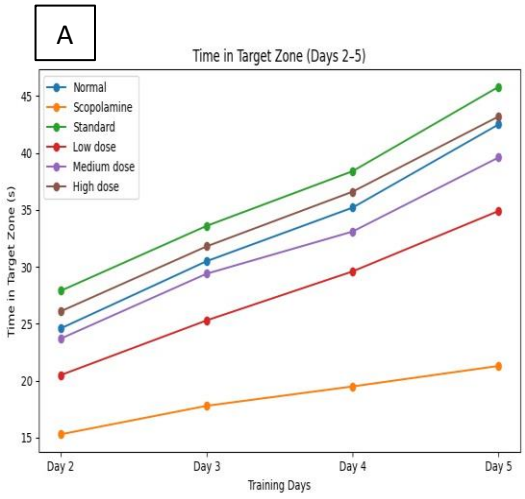
Latency to Target

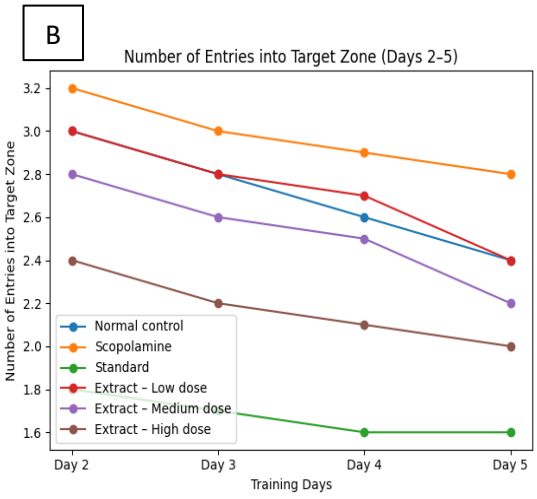
Group	Day 2	Day 3	Day 4	Day 5
Normal control	19.1 ± 1.3	14.7 ± 1.1	11.6 ± 0.9	8.4 ± 0.7
Scopolamine	27.8 ± 1.9$^{\$\$}$	25.6 ± 1.8$^{\$\$}$	23.8 ± 1.6$^{\$\$}$	21.5 ± 1.5$^{\$\$\$}$
Standard	17.6 ± 1.2 ***	13.9 ± 1.0 ***	10.9 ± 0.8 ***	7.9 ± 0.6 ***
Extract – Low	22.4 ± 1.5 *	18.6 ± 1.3 *	15.7 ± 1.2 *	12.6 ± 1.0 **
Extract – Medium	19.8 ± 1.3 **	15.2 ± 1.1 **	12.8 ± 1.0 **	9.8 ± 0.8 ***
Extract – High	18.3 ± 1.2 ***	14.3 ± 1.0 ***	11.7 ± 0.9 ***	8.6 ± 0.7 ***

Latency to First Entry

Group	Day 2	Day 3	Day 4	Day 5
Normal control	19.1 ± 1.3	14.7 ± 1.1	11.6 ± 0.9	8.4 ± 0.7
Scopolamine	27.8 ± 1.9$^{\$\$}$	25.6 ± 1.8$^{\$\$}$	23.8 ± 1.6$^{\$\$}$	21.5 ± 1.5$^{\$\$\$}$
Standard	17.6 ± 1.2 ***	13.9 ± 1.0 ***	10.9 ± 0.8 ***	7.9 ± 0.6 ***
Extract – Low	22.4 ± 1.5 *	18.6 ± 1.3 *	15.7 ± 1.2 *	12.6 ± 1.0 **
Extract – Medium	19.8 ± 1.3 **	15.2 ± 1.1 **	12.8 ± 1.0 **	9.8 ± 0.8 ***
Extract – High	18.3 ± 1.2 ***	14.3 ± 1.0 ***	11.7 ± 0.9 ***	8.6 ± 0.7 ***

Mean ± SEM (n = 6). Statistical analysis was performed using Tukey's post-hoc test and one-way ANOVA for acquisition trial data (Days 2–5). Comparisons were made between extract/standard drug groups versus scopolamine control, and scopolamine versus normal control. *p < 0.05, **p < 0.01, ***p < 0.001; ns = not significant.





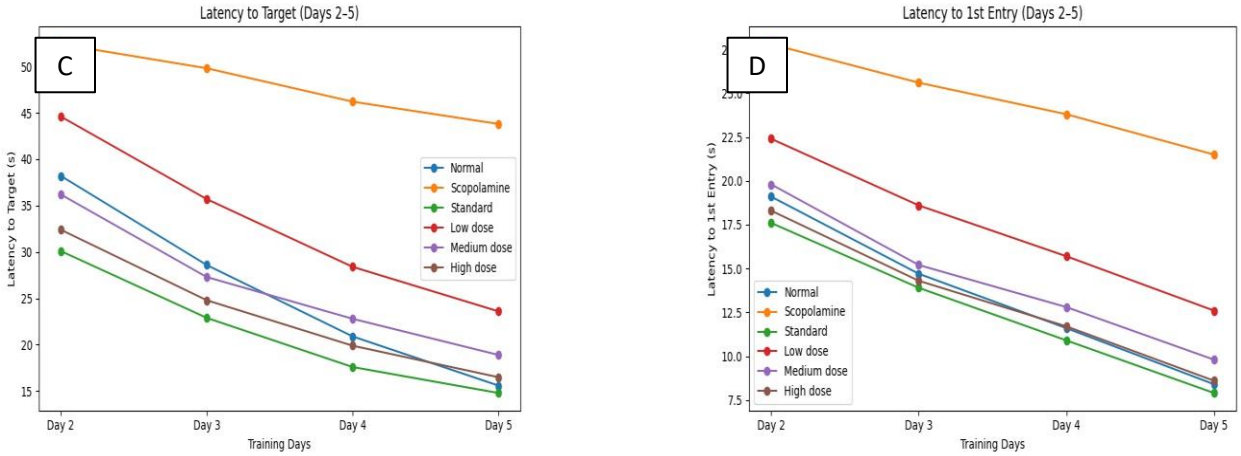


Figure 02: Graph showing (A) the amount of duration of stay in the target area, (B) the number of entries made there, and (C) the latency to the destination(D) First entry latency.

Across Days 2 to 5, the scopolamine-treated group showed significant cognitive impairment, as evidenced by longer latency to target, more entries, longer latency to initial entry, and less time spent in the target zone. These findings indicate impaired spatial learning, memory acquisition, and retention. In contrast, the normal control group showed progressive improvement, with increased time in the target zone and reduced latency parameters, reflecting normal learning and memory consolidation. The standard drug group exhibited significant cognitive enhancement, demonstrated by increased retention time and marked reduction in latency and entries ($p < 0.001$).

Treatment with the hydroalcoholic fruit extract of *Annona reticulata* produced significant, dose-dependent improvement in all cognitive parameters. The low dose showed moderate improvement, while the medium dose demonstrated greater enhancement in learning and memory performance. The high dose exhibited the most pronounced effect, showing increased duration in the target zone and decreased latency values that are on par with those of the conventional medication. Overall, the extract effectively reversed scopolamine-induced cognitive deficits and significantly improved spatial learning and memory, confirming its potent nootropic activity.

HISTOPATHOLOGICAL EXAMINATION

The current study's findings showed that histopathological sections of brain tissue from mice given Hydroalcoholic fruit extract of Annona reticulata and Scopolamine (inducing agent).

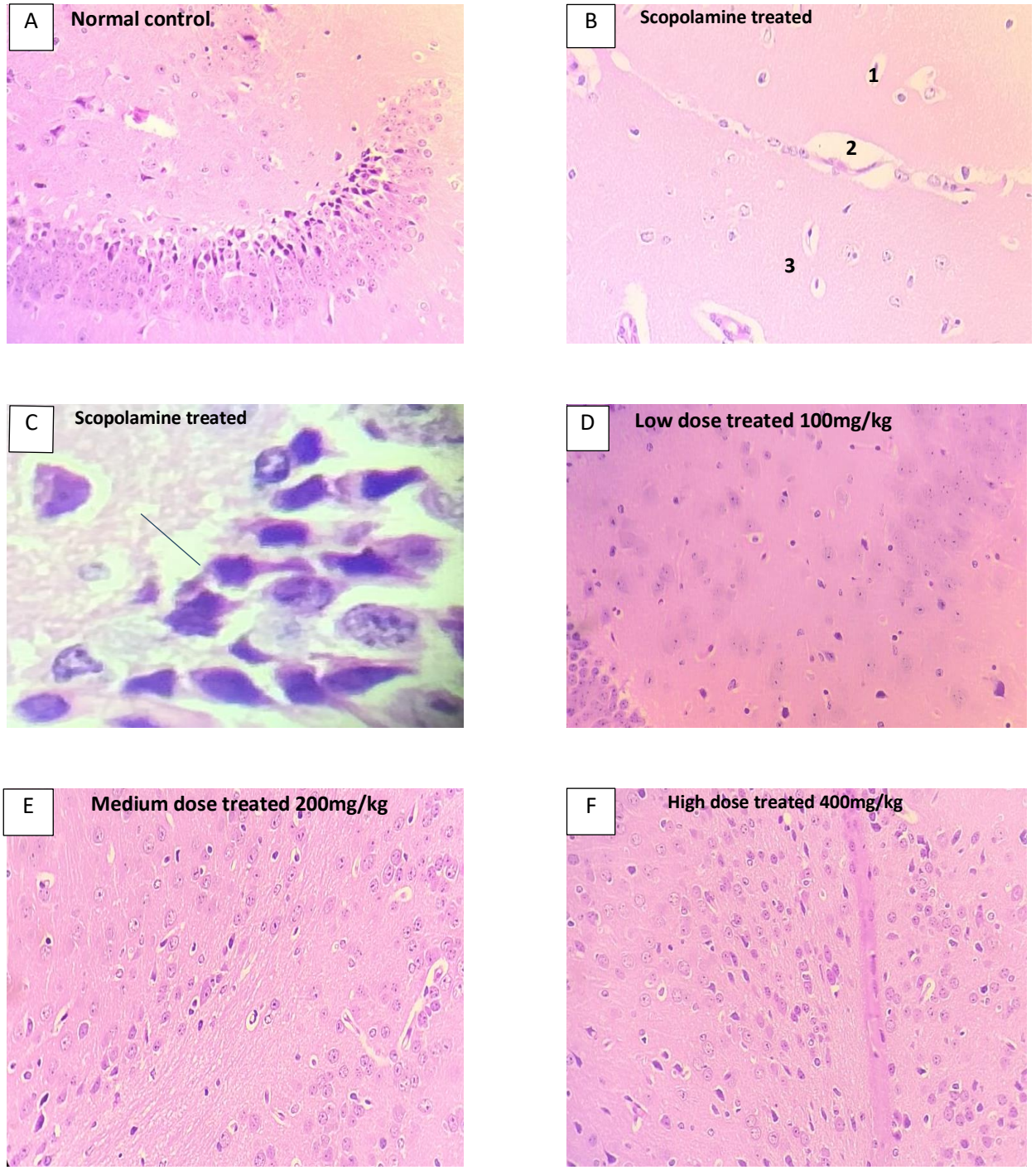


Figure 03: TS of the Mice brain treated with (A) Normal control: Normal hippocampal architecture with intact neurons and well-organized cell layers, indicating healthy brain tissue. **(B) Scopolamine treated:** (1) Neuronal swelling, (2) cytoplasmic vacuolation, and (3) degenerating neurons with condensed nuclei, indicating severe neuronal damage. **(C) Scopolamine treated (higher magnification):** Apoptotic cells. **(D) Dose LOW treated (100 mg/kg):** Mild improvement with partial preservation of neuronal structure and reduced degenerative changes. **(E) Dose MEDIUM treated (200 mg/kg):** Moderate neuroprotection with improved neuronal

arrangement and reduced cellular degeneration. **(F) Dose HIGH treated (400 mg/kg):** Marked preservation of neuronal architecture with intact neurons, indicating strong neuroprotective effect.

DISCUSSION:

The present investigation was designed to interrogate the cognitive-enhancing properties of the hydroalcoholic fruit extract of *Annona reticulata* within a scopolamine-mediated murine model of cholinergic cognitive impairment, evaluated using the validated Morris Water Maze spatial navigation paradigm.[4] Scopolamine exerts its amnesic action through competitive antagonism of central muscarinic M1 receptors, thereby suppressing acetylcholine-dependent hippocampal and cortical signalling cascades essential for memory encoding and consolidation; this pharmacological mechanism renders it the most widely adopted tool for generating reproducible, transient cognitive deficits in rodent pharmacological screening studies.[3] Acute oral toxicity profiling of the extract established its safety margin up to 2000 mg/kg,[13] providing a sufficient therapeutic index to justify selection of the three dose levels — 100, 200, and 400 mg/kg — employed in this efficacy study.

Quantitative behavioural analysis revealed that scopolamine challenge produced robust, statistically significant deficits in spatial learning and memory across all acquisition trial parameters — consistent with the well-characterised anticholinergic amnesic phenotype and in concordance with established literature documenting the cognitive consequences of cholinergic hypofunction.[4] Hydroalcoholic extract treatment produced a graded reversal of these deficits in a dose-proportional manner; the high-dose cohort (400 mg/kg) achieved cognitive performance metrics statistically indistinguishable from those of the piracetam reference group.[5] Piracetam is understood to potentiate acetylcholine neurotransmission and modulate AMPA receptor function to improve memory; the near-equivalent performance of the high-dose extract group thus suggests a comparable degree of cholinergic augmentation or neuroprotection.

Probe trial data corroborated the acquisition findings, with extract-treated animals demonstrating dose-related enhancement of spatial reference memory retention, further supporting hippocampal-dependent mnemonic recovery.[4] The neuroprotective mechanisms underlying these observations likely reflect the multifaceted phytochemical composition of the extract — notably its flavonoids, tannins, alkaloids, and phenolic acids — phytoconstituents with well-documented capacity to scavenge reactive oxygen species, attenuate neuroinflammatory cascades, and preserve mitochondrial membrane integrity.[6,10] It is postulated that attenuation of oxidative neuronal injury, combined with facilitation of cholinergic transmission through inhibition of acetylcholinesterase activity or upregulation of muscarinic receptor expression, collectively contributes to the observed

pro-cognitive outcomes.[2,7] The histopathological findings corroborated the behavioural data, demonstrating dose-dependent preservation of hippocampal neuronal architecture and attenuation of scopolamine-induced apoptotic and degenerative changes. Taken together, these data identify the hydroalcoholic fruit extract of *Annona reticulata* as a promising phytopharmacological candidate for the management of cognitive disorders, warranting advanced mechanistic characterization and clinical translation research.[9]

CONCLUSION:

The findings of this investigation collectively demonstrate that the hydroalcoholic fruit extract of *Annona reticulata* exerts significant, dose-dependent cognitive-enhancing and neuroprotective effects in a scopolamine-challenged murine model. The observed improvements in spatial learning acquisition, memory retention, and hippocampal histoarchitectural preservation are attributable, at least in part, to the concerted antioxidant activity and putative cholinergic facilitation provided by the extract's diverse phytochemical constituents — including flavonoids, phenolics, alkaloids, tannins, and saponins.[7] These bioactive classes are well-recognized for their capacity to attenuate oxidative neuronal stress, support mitochondrial function, and potentiate neurotransmitter availability, thereby creating an environment conducive to neuronal survival and synaptic plasticity.[2]

The extract's broad safety margin and robust efficacy at 400 mg/kg — yielding performance comparable to the reference nootropic piracetam — positions it as a scientifically credible candidate for further preclinical development. Future investigations should prioritise bioactivity-guided fractionation to identify principal active constituents, mechanistic in vitro studies to elucidate AChE inhibitory potential and antioxidant capacity, and in vivo evaluation in transgenic Alzheimer's disease models to strengthen translational relevance. Clinical validation through appropriate human trials will ultimately determine the extract's therapeutic applicability in the management of neurodegenerative cognitive disorders.

REFERENCES:

1. Bartus RT, Dean RL, Beer B, Lippa AS et al. The cholinergic hypothesis of geriatric memory dysfunction. Science. 1982;217(4558):408-14. doi.org/10.1126/science.7046051
2. Bachu B, Karnam P, Sunitha D, Rajaputana LM, Muvvala S. Review on day by day wrapping Dementia. World J Pharm Pharma Sci. 2023; 13(1): 293-304.
3. Terry AV, Buccafusco JJ et al. The cholinergic hypothesis of age and Alzheimer's disease-related cognitive deficits: recent challenges and their implications for novel drug

development. Journal of Pharmacology and Experimental Therapeutics. 2003;306(3):821-7. doi.org/10.1124/jpet.102.041616

4. Klinkenberg I, Blokland A et al. The validity of scopolamine as a pharmacological model for cognitive impairment: a review of animal behavioral studies. Neuroscience & Biobehavioral Reviews. 2010;34(8):1307-50. doi.org/10.1016/j.neubiorev.2010.04.001
5. Winblad B et al. Piracetam: a review of pharmacological properties and clinical uses. CNS Drug Reviews. 2005;11(2):169-82. doi.org/10.1111/j.1527-3458.2005.tb00268.x
6. Howes MJ, Perry NS, Houghton PJ et al. Plants with traditional uses and activities relevant to the management of Alzheimer's disease and other cognitive disorders. Phytotherapy Research. 2003;17(1):1-18. doi.org/10.1002/ptr.1280
7. Sultana R, Perluigi M, Butterfield DA et al. Oxidatively modified proteins in Alzheimer's disease: role of oxidative stress and antioxidants. Journal of Alzheimer's Disease. 2013;33(S1):S243-51. doi.org/10.3233/JAD-2012-129001
8. Rajaputana LM, Bachu B, Rajola MK Goud, Sabitha S, Sudhakar M. Screening of CNS stimulant activity of ethanolic fruit extract of Piper longum Linn in rats. TIJER Int J. 2023; 10(9): a862-869.
9. Badrie N, Schauss AG et al. Annona species: phytochemistry and biological activity. Food Research International. 2010;43(5):1256-67. doi.org/10.1016/j.foodres.2009.11.010
10. Gupta RK, Kesari AN, Murthy PS, Chandra R, Tandon V, Watal G et al. Hypoglycemic and antioxidant effects of Annona reticulata. Journal of Ethnopharmacology. 2005;99(1):75-81. doi.org/10.1016/j.jep.2005.01.055
11. Manisha RL, Mustala P, Bachu B, Padhy S, Muvvala S. Cassia auriculata: a comprehensive review of its phytochemistry, pharmacology, and therapeutic potential. World J Pharma Med Res. 2025; 11(11): 13-17.
12. Morris R et al. Developments of a water-maze procedure for studying spatial learning in the rat. Journal of Neuroscience Methods. 1984;11(1):47-60. doi.org/10.1016/0165-0270(84)90007-4
13. Manisha RL, Shaik R, Bellamkonda S, Shaik N, Rao BV, Kumar JV, et al. Evaluation of anxiolytic activity of flower extracts of Tagetes erecta Linn (Asteraceae) in rats. J Appl Pharm Sci. 2013; 3(12): 075-082.
14. Bakkali F, Averbeck S, Averbeck D, Idaomar M et al. Biological effects of essential oils: a review. Food and Chemical Toxicology. 2008;46(2):446-75. doi.org/10.1016/j.fct.2007.09.106

15. Rajaputana LM, Sai Divya M, Bachu B, Muvvala S. Nephroprotective activity of hydroalcoholic aerial plant extract of Amaranthus roxburghianus Nevski in gentamicin-induced nephrotoxicity in Wistar rats. World J Pharm Med Res. 2025; 11(3): 101-110.
16. Bancroft JD, Gamble M et al. Theory and Practice of Histological Techniques. 6th ed. Churchill Livingstone Elsevier. 2008; 121-134.
17. Motulsky HJ et al. Intuitive Biostatistics: A Nonmathematical Guide to Statistical Thinking. 3rd ed. Oxford University Press. 2014; 85-102.
18. OECD. OECD Guideline for Testing of Chemicals 423: Acute Oral Toxicity — Acute Toxic Class Method. OECD Publishing. 2001. doi.org/10.1787/9789264071001-en
19. Shaik R, Manisha RL, Sayana SB, Pal R. Hepatoprotective activity of alcoholic and aqueous extracts of Allium cepa Linn. (Liliaceae) in rats. Int J Pharm Sci Res. 2012; 3(9): 3189-3195.
20. Bachu B, Rajaputana LM, Muvvala S. Antiparkinsonian activity of methanolic extract of Acorus calamus rhizomes on paraquat-induced parkinsonism in rats. Int J Crea Res Thoughts. 2023; 11(9): e64-e70.