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Assessment of Neuroprotective and Memory-Enhancing Activity of Donepezil Against Scopolamine-Induced Cognitive Impairment in Wistar Rats

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Abstract

Background: Cholinergic deficiency and oxidative stress are central to the cognitive decline observed in Alzheimer's disease (AD), and preclinical characterisation of acetylcholinesterase (AChE) inhibitors against these mechanisms remains valuable for validating their neuroprotective potential beyond symptomatic cholinergic enhancement. **Objective:** This study evaluated the memory-enhancing and neuroprotective activity of donepezil, administered at three oral dose levels, against scopolamine-induced cognitive impairment in adult male Wistar rats, using behavioural, biochemical and histopathological endpoints. **Methods:** Thirty adult male Wistar rats (180–200 g) were allocated to five groups (n = 6): normal control, scopolamine (3 mg/kg, i.p.) disease control, and three scopolamine-challenged groups pre-treated with oral donepezil at 1, 2 or 3 mg/kg for 21 consecutive days. Spatial learning and reference memory were assessed using the Morris water maze (MWM), retention memory using the elevated plus maze (EPM), and aversive memory using the passive avoidance (PA) paradigm. Brain AChE activity and oxidative stress indices (malondialdehyde [MDA], reduced glutathione [GSH], superoxide dismutase [SOD] and catalase) were quantified, and hippocampal CA1 cytoarchitecture was examined on haematoxylin and eosin (H&E)-stained sections using a semi-quantitative injury score. **Results:** Scopolamine significantly prolonged MWM escape latency and impaired PA/EPM retention, reduced target-quadrant time, elevated brain AChE activity and MDA, depleted GSH, SOD and catalase, and produced marked CA1 neuronal degeneration, oedema and pyknosis relative to normal controls ($p < 0.001$ for all measures). Donepezil produced a dose-dependent reversal of every deficit; the standard-dose group (3 mg/kg) consistently approached normal-control values and was statistically non-significant versus normal control on several endpoints, while remaining significantly different from disease control ($p < 0.001$). **Conclusion:** Donepezil conferred significant memory-enhancing and neuroprotective effects in the scopolamine model, an action attributable to combined augmentation of cholinergic transmission and attenuation of oxidative stress, with structural preservation of hippocampal CA1 neurons corroborating the behavioural and biochemical findings.

Keywords: *Donepezil; scopolamine; cognitive impairment; neuroprotection; acetylcholinesterase; oxidative stress; Wistar rats*

1. INTRODUCTION

Dementia, of which Alzheimer's disease (AD) is the leading cause, represents one of the foremost public-health challenges of the present era, with an estimated global burden of 55 million cases projected to rise to 139 million by 2050[1,42]. AD is neuropathologically defined by extracellular amyloid-β plaques, intracellular neurofibrillary tangles composed of hyperphosphorylated tau, synaptic loss and progressive neuronal death[2,3], and is clinically manifest as insidious, progressive impairment of memory and higher cognitive function[4].

Among the several interlinked mechanisms implicated in AD pathogenesis, cholinergic deafferentation remains one of the most therapeutically tractable. Degeneration of basal forebrain cholinergic neurons and consequent reduction in acetylcholine (ACh) availability compromise attention, encoding and consolidation of memory, a relationship first articulated in the cholinergic hypothesis[9,10] and subsequently exploited pharmacologically through acetylcholinesterase (AChE) inhibition. Donepezil, a reversible, centrally selective AChE inhibitor, remains a first-line agent for mild-to-moderate AD[11,12,13,14]; beyond cholinesterase inhibition, accumulating experimental evidence points to ancillary antioxidant, anti-inflammatory, anti-apoptotic and amyloid-modulating actions that may contribute to disease-modifying, neuroprotective effects[40].

Oxidative stress is closely intertwined with cholinergic dysfunction in AD: cholinergic neurons are disproportionately vulnerable to reactive oxygen species[15,19,20], and oxidative damage in turn impairs choline acetyltransferase activity and ACh synthesis, propagating a self-reinforcing cycle of neurodegeneration[16,17,18]. The scopolamine model, in which competitive muscarinic (principally M1) receptor blockade produces reproducible, reversible learning and memory deficits together with compensatory elevation of brain AChE activity and oxidative stress markers[21,22,35,36], is a well-validated platform for the preclinical screening of anti-amnesic and neuroprotective agents, notwithstanding its inability to recapitulate amyloid or tau pathology[21,23].

Although donepezil's cholinergic mechanism of action is well established clinically, further preclinical delineation of its neuroprotective contribution independent of, and complementary to, cholinesterase inhibition remains of value for understanding its full pharmacological profile and for benchmarking novel cognitive enhancers. Accordingly, the present study evaluated the dose-dependent memory-enhancing and neuroprotective activity of donepezil in a scopolamine-induced model of cognitive impairment in Wistar rats using an integrated battery of behavioural (Morris water maze, elevated plus maze, passive avoidance), biochemical (brain AChE activity, MDA, GSH, SOD, catalase) and histopathological (hippocampal CA1) endpoints.

2. MATERIALS AND METHODS

2.1 Animals and ethical approval

Healthy adult male Wistar rats (180-200 g, 8-10 weeks old) were procured from the Central Animal House Facility, Systemic Life Sciences, India, and acclimatised to standard laboratory conditions (22 ± 2 °C, 50–60% relative humidity, 12 h light/dark cycle) for 7 days prior to experimentation, with standard pellet diet and water available ad libitum. The protocol was approved by the Institutional Animal Ethics Committee (IAEC approval no. 26/IAEC- III/SLSRPL/2025), and the study is reported in accordance with the ARRIVE 2.0 guidelines for animal research[33].

2.2 Drugs and chemicals

Donepezil hydrochloride (Sigma-Aldrich, India), scopolamine hydrobromide (HiMedia Laboratories), 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB; Loba Chemie), acetylthiocholine iodide (SRL), thiobarbituric acid (Merck Life Science) and reduced glutathione standard (Sigma-Aldrich) were used. Formalin (10%, Rankem) was used for tissue fixation. Batch numbers of all reagents are on record with the corresponding laboratory.

2.3 Experimental design and treatment schedule

Animals were randomly allocated to five groups of six rats each. Donepezil and vehicle were administered orally, once daily, for 21 consecutive days. Scopolamine (3 mg/kg, i.p.) was administered to Groups II–V to induce cognitive impairment according to the behavioural testing schedule.

Group	Treatment
I	Normal control (vehicle p.o. + vehicle i.p.)
II	Disease control (vehicle p.o. + scopolamine 3 mg/kg, i.p.)
III	Donepezil low dose (1 mg/kg, p.o.) + scopolamine 3 mg/kg, i.p.
IV	Donepezil high dose (2 mg/kg, p.o.) + scopolamine 3 mg/kg, i.p.
V	Donepezil standard dose (3 mg/kg, p.o.) + scopolamine 3 mg/kg, i.p.

Table 1. Experimental grouping and treatment schedule (n = 6 per group).

2.4 Behavioural assessment

2.4.1 Morris water maze (MWM).

Spatial learning was assessed as escape latency to a hidden platform across a training period, and reference-memory retention was assessed as percentage time spent in the target quadrant during a probe trial conducted after removal of the platform[24].

2.4.2 Elevated plus maze (EPM).

Each rat was placed at the extremity of an open arm; the latency to enter a closed arm (transfer latency) was recorded on Day 1 (initial/acquisition) and again 24 h later (retention), with a cut-off of 90 s[25].

2.4.3 Passive avoidance (PA) test.

During the acquisition trial, latency to move from an illuminated to a dark compartment was recorded, and a mild foot-shock (0.5 mA, 2 s) was delivered on entry into the dark compartment. Retention step-through latency was recorded 24 h later, with a cut-off of 300 s[26].

2.5 Biochemical estimations

At the conclusion of behavioural testing, animals were sacrificed under appropriate anaesthesia and whole brain tissue was rapidly excised, rinsed in ice-cold saline, weighed and homogenised in chilled phosphate buffer; the resulting supernatant was used for biochemical assays. Brain AChE activity was determined by Ellman's colorimetric method using acetylthiocholine iodide as substrate and DTNB as chromogen (expressed as µmol/min/mg protein)[27]. Lipid peroxidation was quantified as malondialdehyde (MDA) by the thiobarbituric acid reactive substances method (nmol/mg protein)[28]. Reduced glutathione (GSH) was estimated using DTNB reagent (µmol/mg protein)[29]. Superoxide dismutase (SOD) activity was assessed from inhibition of pyrogallol auto-oxidation (U/mg protein)[30], and catalase activity from the rate of hydrogen peroxide decomposition at 240 nm (µmol H_2O_2 decomposed/min/mg protein)[31]. Total protein was estimated by the Lowry method[32].

2.6 Histopathological examination

Brain tissue was fixed in 10% neutral buffered formalin, routinely paraffin-embedded, sectioned at 5 µm and stained with haematoxylin and eosin. Hippocampal CA1 sections were examined by light microscopy for neuronal degeneration, cellular oedema and pyknotic nuclei, and scored semi-quantitatively (0 = no visible damage; 1 = mild, <25% neurons affected, minimal oedema; 2 = moderate, 25–50% neurons affected,

moderate oedema; 3 = severe, >50% neurons affected, marked oedema and pyknosis).

2.7 Statistical analysis

Data are expressed as mean ± SEM (n = 6 per group) and were analysed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison post-hoc test using GraphPad Prism version 9.0. A p value < 0.05 was considered statistically significant.

3. RESULTS

3.1 General observations and body weight

All animals survived the full protocol without overt signs of toxicity, sedation, tremor, convulsion or autonomic disturbance, and food/water intake remained within normal limits throughout. Body weight increased progressively in all groups over the 21-day period, with no statistically significant inter-group differences at any time point ($p > 0.05$), indicating that scopolamine and all donepezil doses were well tolerated.

Group	Treatment	Day 0	Day 7	Day 14	Day 21
I	Normal control	184.7 ± 4.1	191.8 ± 4.5	199.9 ± 4.8	207.6 ± 5.2
II	Disease control (scopolamine)	183.9 ± 3.8	190.6 ± 4.2	197.5 ± 4.6	203.8 ± 4.9
III	Donepezil low dose + scopolamine	185.2 ± 4.3	192.9 ± 4.7	201.3 ± 5.0	209.4 ± 5.4
IV	Donepezil high dose + scopolamine	184.5 ± 4.0	191.9 ± 4.4	200.1 ± 4.7	207.2 ± 5.1
V	Donepezil standard dose + scopolamine	184.1 ± 3.9	191.2 ± 4.3	199.4 ± 4.5	206.5 ± 4.8

Table 2. Effect of treatments on body weight (g). Values are mean ± SEM (n = 6). No statistically significant difference among groups at any time point (one-way ANOVA, p > 0.05).

3.2 Morris water maze

On Day 1, escape latency was comparable across all groups ($p > 0.05$), confirming equivalent baseline learning ability. Scopolamine-treated disease-control animals showed persistently prolonged escape latency relative to normal controls across the training period (Day 21: 47.17 ± 2.35 s vs. 11.83 ± 0.98 s), indicating impaired acquisition of spatial memory. Donepezil produced a dose-dependent reduction in escape latency, with the standard-dose group (16.83 ± 1.12 s on Day 21) approaching normal-control performance.

Group	Treatment	Day 1	Day 7	Day 14	Day 21
I	Normal control	57.83 ± 2.76	31.67 ± 2.09	17.83 ± 1.46	11.83 ± 0.98
II	Disease control	59.17 ± 2.94	53.83 ± 2.71†††	50.33 ± 2.48†††	47.17 ± 2.35†††
III	Donepezil low + scop.	58.50 ± 2.81	45.50 ± 2.36†††*	37.67 ± 2.02†††**	30.83 ± 1.76†††**

IV	Donepezil high + scop.	57.67 ± 2.69	40.33 ± 2.14†††***	27.67 ± 1.69†††***	20.67 ± 1.31†††***
V	Donepezil standard + scop.	57.92 ± 2.73	37.83 ± 2.01†***	23.83 ± 1.52†††**	16.83 ± 1.12†***

*Table 3. Effect of treatments on Morris water maze escape latency (s). Values are mean ± SEM (n = 6); one-way ANOVA with Tukey's post-hoc test. †p<0.05, ††p<0.01, †††p<0.001 vs. normal control; *p<0.05, **p<0.01, ***p<0.001 vs. disease control. Day 7: F=15.24, p<0.001; Day 14: F=39.81, p<0.001; Day 21: F=56.47, p<0.001.*

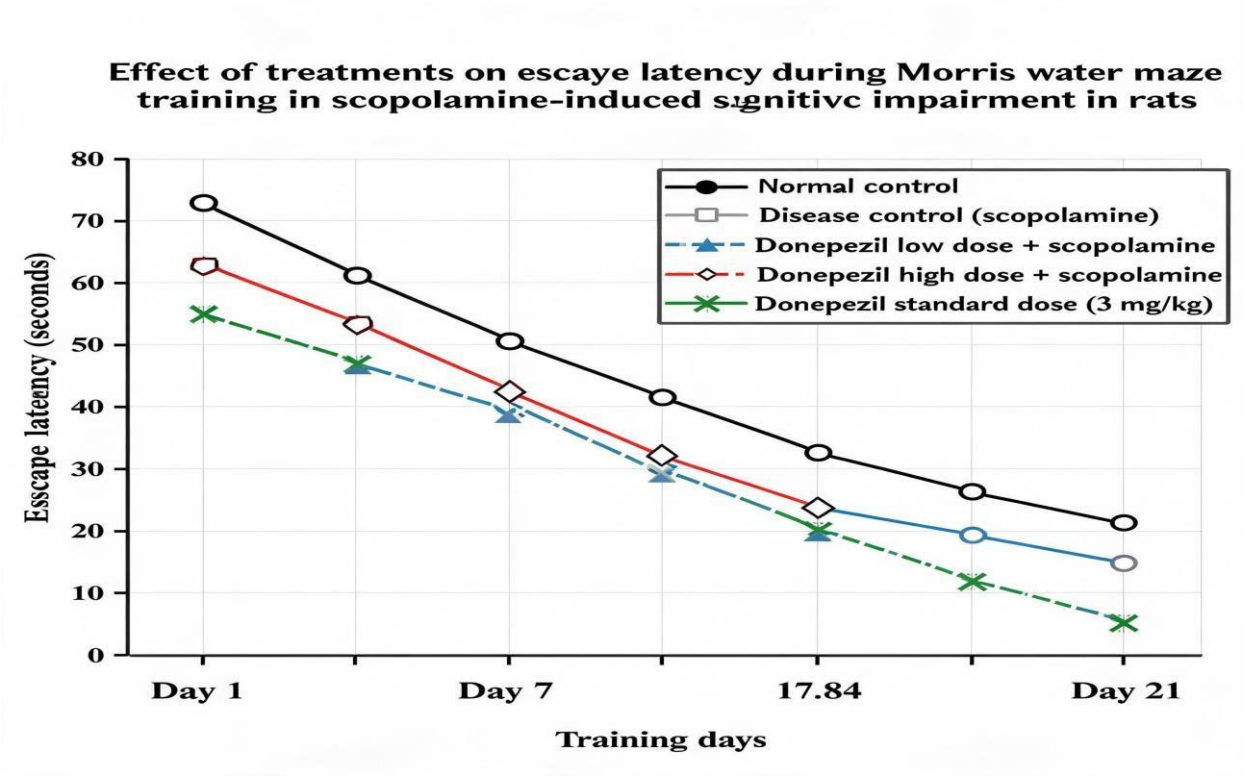

*Figure 1. Escape latency (s) of the five experimental groups on Days 1, 7, 14 and 21 of Morris water maze training. Values are mean ± SEM (n = 6). *p<0.05, **p<0.01, ***p<0.001 vs. disease control.*

Consistent with the acquisition data, probe-trial target-quadrant dwell time was markedly reduced in disease-control animals relative to normal controls (18.67 ± 1.35% vs. 41.83 ± 1.92%), reflecting impaired reference-memory retention. All donepezil doses significantly increased target-quadrant time relative to disease control, with the standard-dose group (37.83 ± 1.54%) statistically indistinguishable from normal control.

Group	Treatment	Target quadrant time (%)
I	Normal control	41.83 ± 1.92
II	Disease control (scopolamine)	18.67 ± 1.35†††
III	Donepezil low dose + scopolamine	27.50 ± 1.48††**
IV	Donepezil high dose + scopolamine	34.17 ± 1.67†***
V	Donepezil standard dose + scopolamine	37.83 ± 1.54 ns***

*Table 4. Effect of treatments on target-quadrant time (%) in the Morris water maze probe trial. Values are mean ± SEM (n = 6). †p<0.05, ††p<0.01, †††p<0.001 vs. normal control; **p<0.01, ***p<0.001 vs. disease control; ns = not significant vs. normal control. F=31.26, p<0.001.*

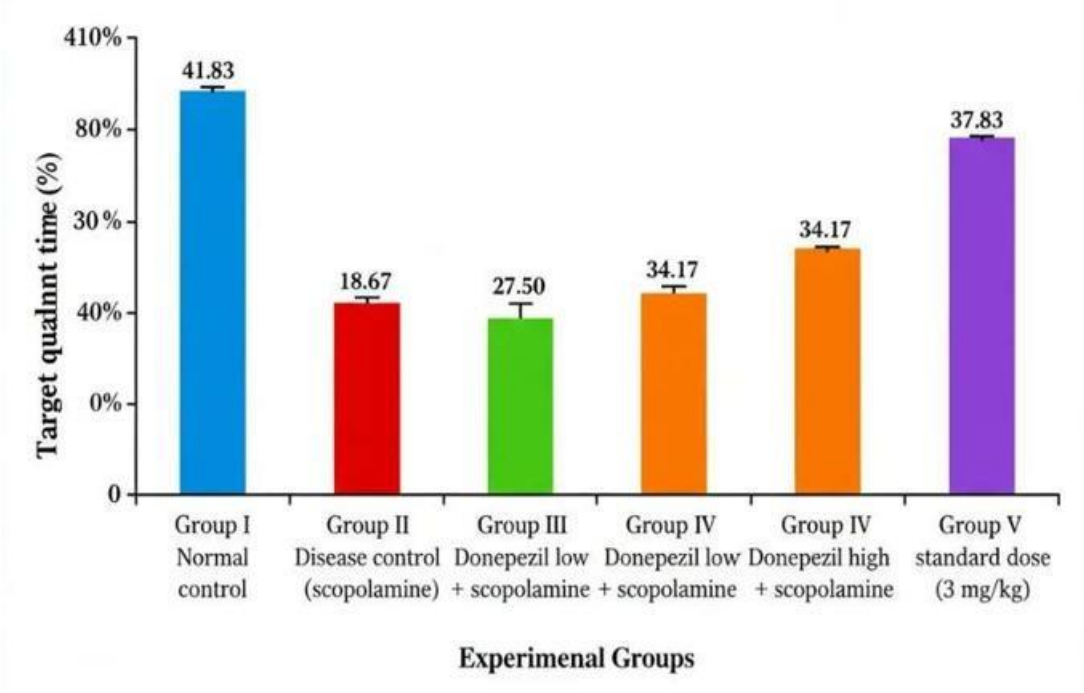

*Figure 2. Percentage of time spent in the target quadrant during the Morris water maze probe trial. Values are mean ± SEM (n = 6). **p<0.01, ***p<0.001 vs. disease control.*

3.3 Elevated plus maze

Initial (Day 1) transfer latency did not differ among groups (p > 0.05). On the retention day, disease-control animals showed significantly prolonged transfer latency relative to normal control (41.67 ± 1.89 s vs. 19.17 ± 1.24 s), indicating impaired retention memory. Donepezil dose-dependently reduced retention transfer latency, with the high- and standard-dose groups producing the largest reductions (25.83 ± 1.42 s and 22.50 ± 1.31 s, respectively), the latter not significantly different from normal control.

Group	Treatment	Initial TL, Day 1 (s)	Retention TL, Day 2 (s)
I	Normal control	46.33 ± 2.18	19.17 ± 1.24
II	Disease control (scopolamine)	45.83 ± 2.06	41.67 ± 1.89†††
III	Donepezil low + scopolamine	46.17 ± 2.11	33.50 ± 1.63†††*
IV	Donepezil high + scopolamine	45.67 ± 2.03	25.83 ± 1.42†***
V	Donepezil standard + scopolamine	46.00 ± 2.09	22.50 ± 1.31 ns***

*Table 5. Effect of treatments on transfer latency (s) in the elevated plus maze. Values are mean ± SEM (n = 6). †p<0.05, †††p<0.001 vs. normal control; *p<0.05, ***p<0.001 vs. disease control; ns = not significant vs. normal control. Day 2: F=29.44, p<0.001. TL = transfer latency.*

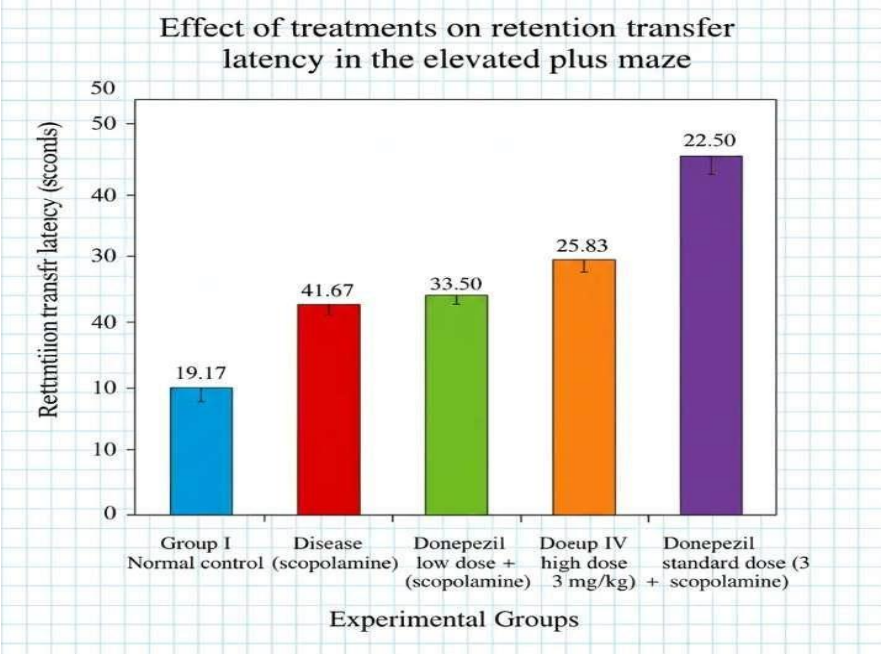

*Figure 3. Retention transfer latency (s) in the elevated plus maze on Day 2. Values are mean ± SEM (n = 6). *p<0.05, ***p<0.001 vs. disease control.*

3.4 Passive avoidance test

Acquisition latencies were equivalent across groups ($p > 0.05$). On retention testing, disease-control animals showed markedly reduced step-through latency relative to normal control (68.33 ± 4.27 s vs. 176.50 ± 5.84 s), consistent with impaired aversive memory. Donepezil significantly and dose-dependently increased retention latency, with the standard-dose group (158.33 ± 5.21 s) approaching the normal-control value.

Group	Treatment	Acquisition latency (s)	Retention latency (s)
I	Normal control	14.83 ± 1.02	176.50 ± 5.84
II	Disease control (scopolamine)	15.17 ± 0.94	68.33 ± 4.27†††
III	Donepezil low + scopolamine	14.67 ± 0.98	108.83 ± 5.13†††**
IV	Donepezil high + scopolamine	15.00 ± 1.05	142.67 ± 5.48††***
V	Donepezil standard + scopolamine	14.92 ± 0.96	158.33 ± 5.21†***

*Table 6. Effect of treatments on step-through latency (s) in the passive avoidance test. Values are mean ± SEM (n = 6). †p<0.05, ††p<0.01, †††p<0.001 vs. normal control; **p<0.01, ***p<0.001 vs. disease control. Retention latency: F=47.18, p<0.001.*

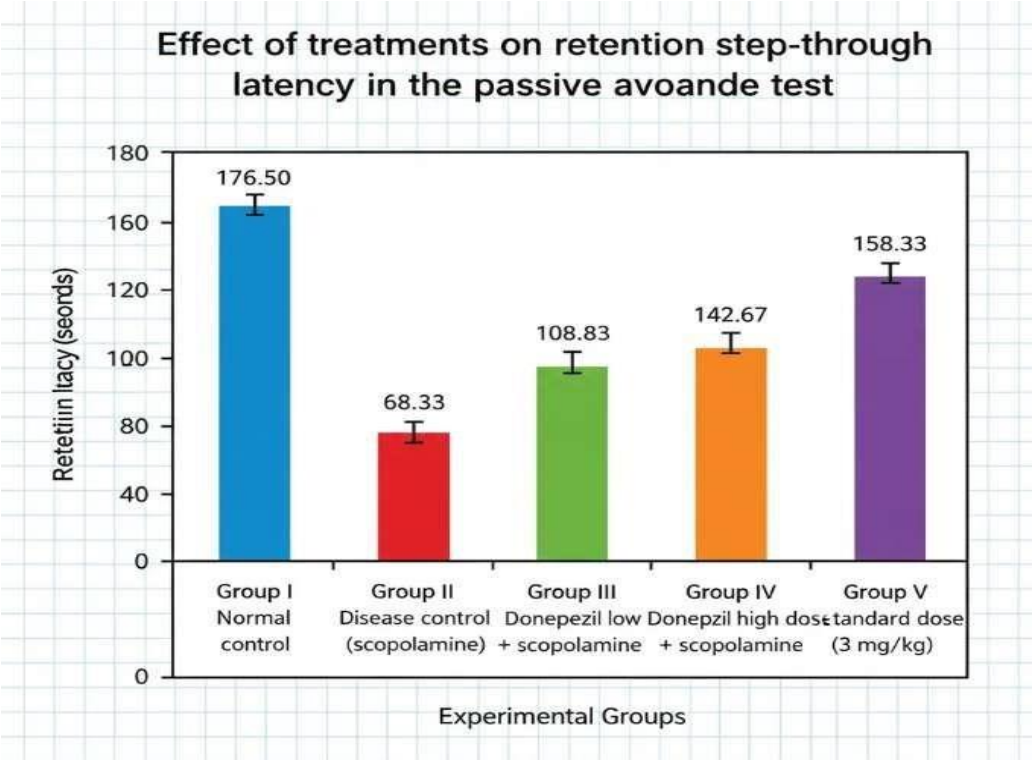

*Figure 4. Retention step-through latency (s) in the passive avoidance test. Values are mean ± SEM (n = 6). **p<0.01, ***p<0.001 vs. disease control.*

3.5 Brain acetylcholinesterase activity

Brain AChE activity was significantly elevated in disease-control animals relative to normal control (1.68 ± 0.06 vs. 0.94 ± 0.04 µmol/min/mg protein), consistent with scopolamine-induced cholinergic dysfunction. Donepezil produced a dose-dependent suppression of AChE activity, with the standard-dose group (1.01 ± 0.03 µmol/min/mg protein) not significantly different from normal control.

Group	Treatment	AChE activity (µmol/min/mg protein)
I	Normal control	0.94 ± 0.04
II	Disease control (scopolamine)	1.68 ± 0.06†††
III	Donepezil low dose + scopolamine	1.34 ± 0.05†††***
IV	Donepezil high dose + scopolamine	1.12 ± 0.04†***
V	Donepezil standard dose + scopolamine	1.01 ± 0.03 ns***

*Table 7. Effect of treatments on brain acetylcholinesterase (AChE) activity. Values are mean ± SEM (n = 6). †p<0.05, †††p<0.001 vs. normal control; **p<0.01, ***p<0.001 vs. disease control; ns = not significant vs. normal control. F=36.75, p<0.001.*

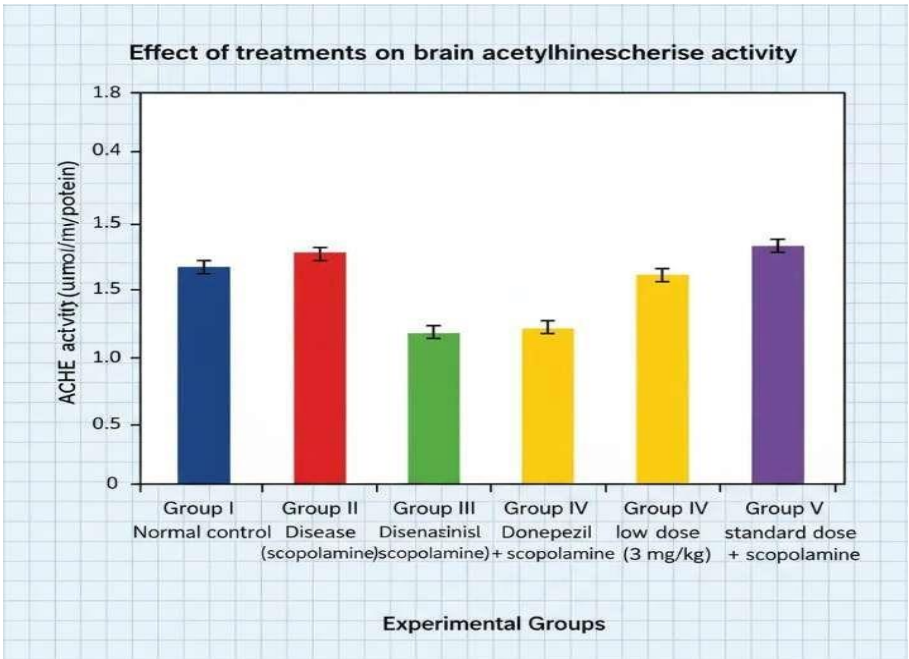

Figure 5. Brain acetylcholinesterase (AChE) activity (µmol/min/mg protein) across treatment groups. Values are mean ± SEM (n = 6).

3.6 Oxidative stress markers

Scopolamine produced a marked pro-oxidant shift in brain tissue: MDA was significantly elevated and GSH, SOD and catalase were significantly depleted in disease-control animals relative to normal control (all $p < 0.001$). Donepezil reversed each of these changes in a dose-dependent manner, with the standard-dose group not significantly different from normal control on any of the four markers.

Group	Treatment	MDA (nmol/mg protein)	GSH (µmol/mg protein)	SOD (U/mg protein)	Catalase (µmol H_2O_2/min/mg protein)
I	Normal control	2.18 ± 0.11	8.74 ± 0.29	9.46 ± 0.31	52.83 ± 1.84
II	Disease control	4.92 ± 0.18†††	4.21 ± 0.22†††	4.38 ± 0.19†††	26.17 ± 1.29†††
III	Donepezil low + scop.	3.86 ± 0.15†††**	5.96 ± 0.25†††*	6.41 ± 0.23†††**	35.83 ± 1.46†††**
IV	Donepezil high + scop.	2.94 ± 0.13†***	7.18 ± 0.24†***	7.98 ± 0.27†***	44.67 ± 1.63†***
V	Donepezil standard + scop.	2.51 ± 0.12 ns***	7.89 ± 0.21 ns***	8.71 ± 0.25 ns***	48.92 ± 1.58 ns***

*Table 8. Effect of treatments on brain oxidative stress markers. Values are mean ± SEM (n = 6). †p<0.05, †††p<0.001 vs. normal control; *p<0.05, **p<0.01, ***p<0.001 vs. disease control; ns = not significant vs. normal control. MDA: F=58.36, p<0.001; GSH: F=44.93, p<0.001; SOD: F=49.62, p<0.001; catalase: F=42.57, p<0.001. MDA, malondialdehyde; GSH, reduced glutathione; SOD, superoxide dismutase.*

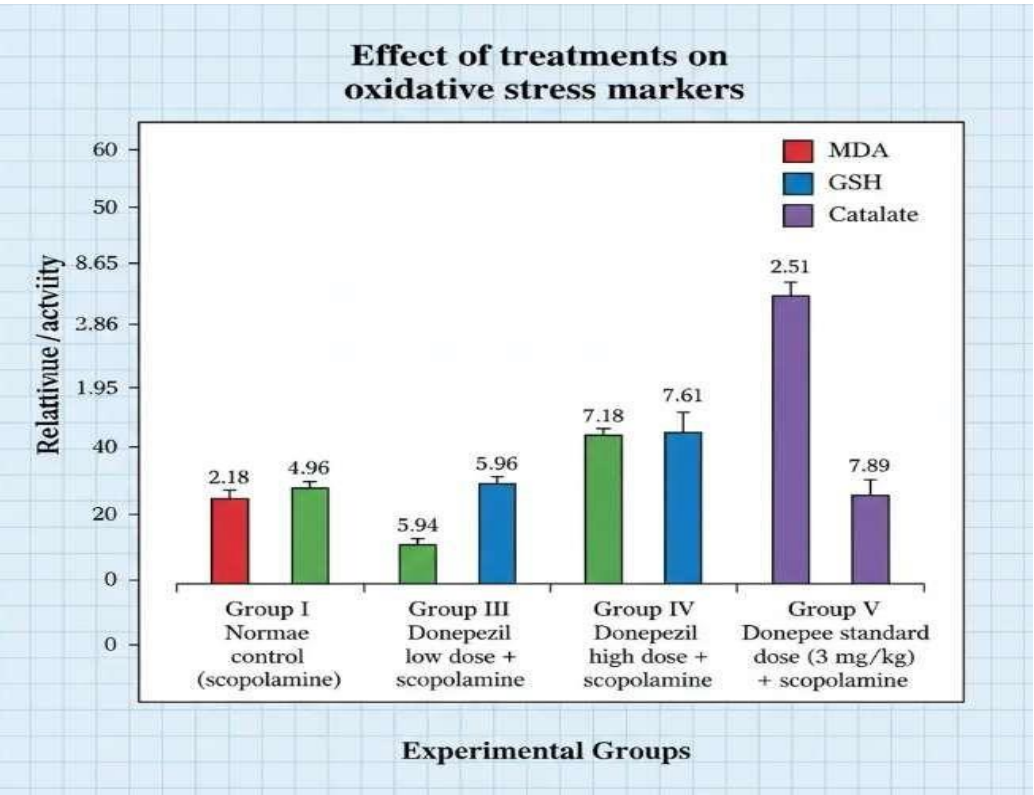

Figure 6. Brain oxidative stress markers across treatment groups. (A) Malondialdehyde (MDA), (B) reduced glutathione (GSH), (C) superoxide dismutase (SOD) activity, (D) catalase activity. Values are mean ± SEM (n = 6).

3.7 Hippocampal histopathology

Hippocampal CA1 sections from normal-control animals showed well-preserved pyramidal neurons with intact cytoarchitecture (total injury score 0.33 ± 0.21). Disease-control (scopolamine) sections showed severe neuronal degeneration, cellular oedema and pyknotic nuclei, yielding a markedly elevated total score (8.00 ± 0.52). Donepezil produced a dose-dependent reduction in histopathological injury across all three sub-scores (neuronal degeneration, cellular oedema, pyknotic nuclei), with the standard-dose group (total score 2.00 ± 0.37) showing near-normal hippocampal architecture.

Group	Treatment	Neuronal degeneration	Cellular oedema	Pyknotic nuclei	Total score
I	Normal control	0.17 ± 0.17	0.00 ± 0.00	0.17 ± 0.17	0.33 ± 0.21
II	Disease control	2.83 ± 0.17†††	2.67 ± 0.21†††	2.50 ± 0.22†††	8.00 ± 0.52†††
III	Donepezil low + scop.	2.00 ± 0.26†††*	1.83 ± 0.17†††*	1.67 ± 0.21†††*	5.50 ± 0.56†††**
IV	Donepezil high + scop.	1.17 ± 0.17†††***	1.00 ± 0.26†††***	0.83 ± 0.17†***	3.00 ± 0.45†††***
V	Donepezil standard + scop.	0.83 ± 0.17†***	0.67 ± 0.21***	0.50 ± 0.22***	2.00 ± 0.37†***

Table 9. Effect of treatments on hippocampal CA1 histopathological scores. Values are mean ± SEM (n = 6). Scoring: 0 = no visible damage; 1 = mild (<25% neurons affected); 2 = moderate (25–50% neurons affected); 3 = severe (>50% neurons affected, marked oedema/pyknosis). †p<0.05, ††p<0.01,

*†††p<0.001 vs. normal control; *p<0.05, **p<0.01, ***p<0.001 vs. disease control (one-way ANOVA, Tukey's post-hoc; p<0.001 for total score comparison among groups).*

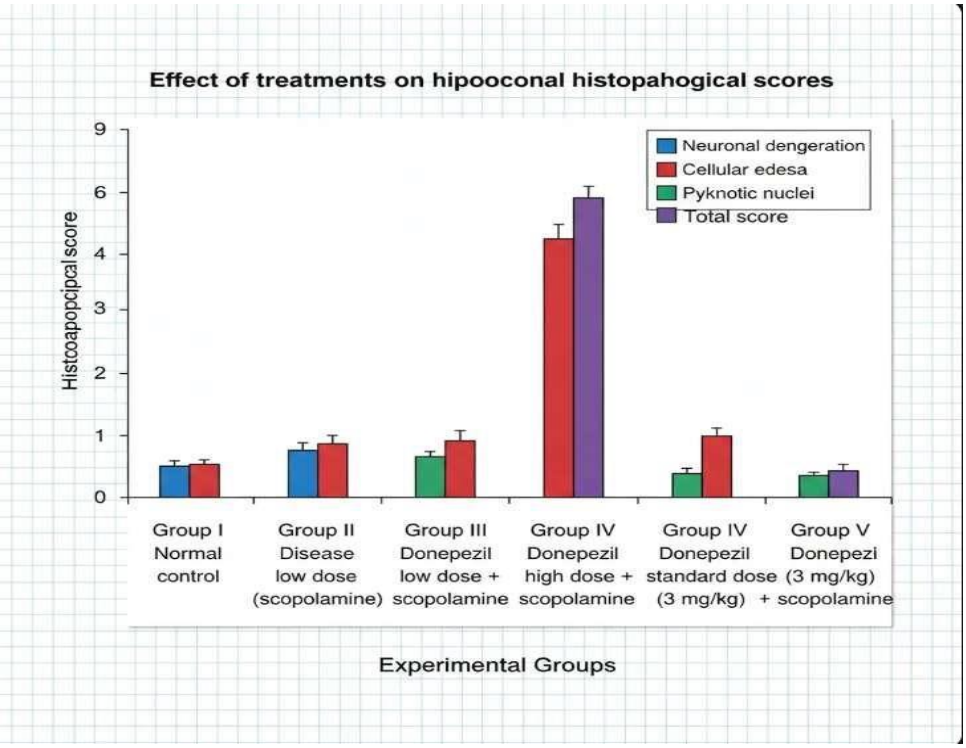

Figure 7. Representative photomicrographs of haematoxylin and eosin-stained hippocampal CA1 sections (magnification 400×; scale bar = 50 µm). Group I (normal control) shows intact pyramidal neurons with preserved cytoarchitecture; Group II (disease control) shows severe neuronal degeneration, oedema and pyknotic nuclei; Groups III–V (donepezil low, high and standard dose + scopolamine) show progressive, dose-dependent structural preservation.

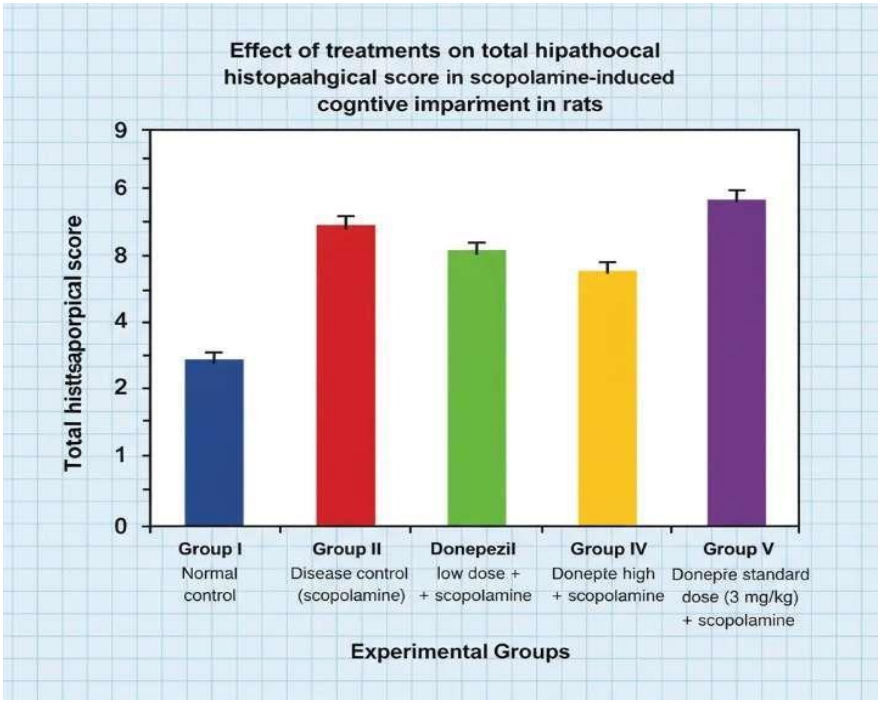

Figure 8. Total hippocampal CA1 histopathological injury score across treatment groups. Values are mean ± SEM (n = 6). Lower scores indicate better neuronal preservation.

4. DISCUSSION

The present study demonstrates that donepezil confers dose-dependent memory-enhancing and neuroprotective effects against scopolamine-induced cognitive impairment in Wistar rats, corroborated across independent behavioural, biochemical and structural endpoints.

Scopolamine, through competitive blockade of central muscarinic receptors, produced the expected profile of impaired spatial learning (prolonged MWM escape latency, reduced target-quadrant time), impaired retention memory (prolonged EPM transfer latency) and impaired aversive memory (reduced PA step-through latency), consistent with the established role of cholinergic transmission in acquisition, consolidation and recall[21,23]. Donepezil ameliorated each of these deficits, with the magnitude of benefit increasing with dose; the 3 mg/kg standard-dose group most closely approximated normal-control performance and was frequently statistically indistinguishable from it, while remaining significantly superior to disease control.

This behavioural recovery was paralleled by biochemical normalisation. Scopolamine elevated brain AChE activity, consistent with previously reported compensatory upregulation of the enzyme following cholinergic blockade[35]; donepezil, as expected of a centrally selective AChE inhibitor, suppressed this activity in a dose-related fashion, providing direct mechanistic support for the observed cognitive benefit. Independently of this cholinergic action, scopolamine also increased lipid peroxidation (MDA) and depleted the enzymatic and non-enzymatic antioxidant defences (SOD, catalase, GSH), indicating that oxidative stress accompanies and may amplify cholinergic blockade in this model[15,41]. Donepezil attenuated MDA accumulation and restored GSH, SOD and catalase toward normal values across all doses, an effect that cannot be attributed to AChE inhibition alone and is consistent with the antioxidant and anti-apoptotic actions of donepezil reported in other neurodegeneration models[40]. Comparable antioxidant-mediated

cognitive protection against scopolamine challenge has been reported for other agents evaluated alongside donepezil as a reference standard, including curcumin, quercetin and herbal formulations[37,38,39], supporting the general principle that attenuation of oxidative injury contributes meaningfully to anti-amnesic efficacy in this model, independent of the specific agent's primary mechanism.

The histopathological findings provide structural corroboration of these functional observations. Hippocampal CA1 neurons a region of particular vulnerability in both scopolamine challenge and human AD showed severe degeneration, oedema and pyknosis in disease-control animals, changes that were progressively attenuated across the donepezil dose range, with near-normal cytoarchitecture in the standard-dose group. The concordance between behavioural, biochemical and histological readouts strengthens the inference that donepezil's protective action in this model reflects a combination of enhanced cholinergic neurotransmission and attenuation of oxidative neuronal injury, rather than a behavioural artefact of AChE inhibition alone.

A limitation of the present study is that plasma and brain donepezil concentrations were not measured, precluding direct correlation of pharmacological exposure with the observed dose-response relationship; the study is also confined to male animals and a single scopolamine challenge paradigm. Future studies should include plasma and brain donepezil concentrations, dose-response modelling (e.g., ED50 values), sex-based comparisons, and chronic toxicity data to further strengthen the translational applicability of these findings.

5. CONCLUSION

Donepezil produced dose-dependent memory-enhancing and neuroprotective effects in a scopolamine-induced model of cognitive impairment in Wistar rats, evidenced by improved performance in the Morris water maze, elevated plus maze and passive avoidance tests; reduction of brain acetylcholinesterase activity and lipid peroxidation; restoration of glutathione, superoxide dismutase and catalase activity; and preservation of hippocampal CA1 neuronal architecture. These findings support a dual mechanism of action augmentation of cholinergic neurotransmission together with attenuation of oxidative stress underlying donepezil's

neuroprotective profile, and reinforce its relevance as a reference standard for preclinical evaluation of cognitive enhancers in cholinergic-deficit models of dementia.

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Author contributions

T.M.N. conducted the experiments, acquired and analysed the data, and drafted the manuscript.
N.N.J. conceived and supervised the study, provided resources, and critically revised the manuscript. Both authors read and approved the final manuscript.

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Conflict of interest

The authors declare no conflict of interest.

Ethical approval

All animal procedures were approved by the Institutional Animal Ethics Committee (approval no. 26/IAEC-III/SLSRPL/2025) and were conducted in accordance with institutional and national guidelines for the care and use of laboratory animals, and in accordance with the ARRIVE 2.0 guidelines for reporting animal research.

Data availability

The datasets generated and/or analysed during the current study are available from the corresponding author on reasonable request.

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