RESEARCH PAPER

Neuroprotective Effects of *Centella asiatica* and *Oroxylum indicum*, Alone and in Combination, Against Scopolamine-Induced Cognitive Impairment in Rats: Behavioural, Biochemical and Histological Evidence

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Abstract

Alzheimer's disease is characterised by progressive cognitive decline driven by cholinergic dysfunction, oxidative stress, neuroinflammation, impaired neurotrophic support and neurodegeneration. Scopolamine-induced cognitive impairment in rodents is a widely accepted pharmacological model that recapitulates several of these pathological features. The present study investigated the neuroprotective potential of *Centella asiatica* and *Oroxylum indicum*, administered alone and in combination, against scopolamine-induced cognitive deficits in Wistar rats. Animals were divided into six groups and treated for the experimental period, followed by a comprehensive battery of behavioural tests including Morris water maze, Y-maze, novel object recognition and passive avoidance. Biochemical analyses were performed to evaluate acetylcholinesterase activity, oxidative stress markers (MDA, GSH, SOD, catalase), pro-inflammatory cytokines (TNF-α, IL-1β, IL-6), NF-κB, BDNF, NRF2, HO-1, apoptotic markers (Bax, Bcl-2, Caspase-3), Aβ1–42 and phosphorylated tau. Glial activation was assessed by GFAP and Iba-1 immunohistochemistry, and hippocampal histoarchitecture was examined by haematoxylin and eosin staining. Scopolamine administration produced significant cognitive impairment accompanied by elevated oxidative stress, neuroinflammation, reduced neurotrophic signalling, increased apoptotic and pathological protein markers, enhanced gliosis and marked neuronal degeneration. Both plant extracts attenuated these alterations; however, the combination treatment consistently produced the most robust protective effects across behavioural, biochemical and histological parameters, often approaching the efficacy of the standard drug donepezil. These findings indicate that the combination of *Centella asiatica* and *Oroxylum indicum* exerts multi-target neuroprotective actions and holds promise as a potential therapeutic strategy for the management of cognitive disorders associated with cholinergic dysfunction and oxidative-inflammatory stress.

Keywords: *Centella asiatica*; *Oroxylum indicum*; scopolamine; cognitive impairment; oxidative stress; neuroinflammation; BDNF; neuroprotection; Alzheimer's disease

How to cite this article: Himanshu Panchal1* Pooja Arora1. Neuroprotective Effects of Centella asiatica and Oroxylum indicum, Alone and in Combination, Against Scopolamine-Induced Cognitive Impairment in Rats: Behavioural, Biochemical and Histological Evidence. Int J Drug Deliv Technol. 2026;16(89s):1612-1618. DOI: 10.25258/ijddt.16.89s.167.

1. Introduction

Alzheimer's disease (AD) is the most common cause of dementia and represents a major global health challenge. It is characterised by progressive memory loss, cognitive decline and behavioural changes [1,2]. The classical neuropathological hallmarks of AD include extracellular amyloid-β (Aβ) plaques, intracellular neurofibrillary tangles composed of hyperphosphorylated tau, synaptic loss and neurodegeneration [3,4]. In addition, cholinergic deficit, oxidative stress, chronic neuroinflammation and impaired neurotrophic support play critical roles in disease progression [5–7].

Scopolamine, a non-selective muscarinic receptor antagonist, is extensively used to induce transient cholinergic dysfunction and cognitive impairment in rodents. The scopolamine model reproduces several features of AD, including memory deficits, elevated acetylcholinesterase (AChE) activity, oxidative stress, neuroinflammation and reduced brain-derived neurotrophic factor (BDNF) levels [8,9].

Centella asiatica (L.) Urban (Apiaceae), commonly known as Gotu kola, has been used in traditional Ayurvedic and Chinese medicine for cognitive enhancement and wound healing. Its major bioactive constituents, asiaticoside, madecassoside, asiatic acid and madecassic acid, have been shown to improve memory, reduce oxidative stress and modulate amyloid pathology [10–12].

Oroxylum indicum (L.) Kurz (Bignoniaceae), known as Indian trumpet flower or Shyonaka, is rich in flavonoids such as baicalein, chrysin and oroxylin A. These compounds possess potent antioxidant, anti-inflammatory and neuroprotective properties [13–15]. Although both plants have individual reports of neuroprotection, studies evaluating their combined effect against scopolamine-induced cognitive impairment are limited. The present investigation was therefore designed to evaluate the neuroprotective potential of *C. asiatica* and *O. indicum*, alone and in combination, using a comprehensive battery of behavioural, biochemical and histological parameters.

2. Materials and Methods

2.1 Plant Material and Extract Preparation

Dried aerial parts of *Centella asiatica* and stem bark of *Oroxylum indicum* were collected, authenticated and extracted with hydroalcoholic solvent (70% ethanol) using Soxhlet apparatus. The extracts were concentrated under reduced pressure and stored at 4 °C until use.

2.2 Animals

Adult male Wistar rats (180–220 g) were housed under standard laboratory conditions (22 ± 2 °C, 12 h light/dark cycle) with free access to food and water. All experimental procedures were approved by the Institutional Animal Ethics Committee (IAEC) and conducted in accordance with CPCSEA guidelines.

2.3 Experimental Design

Animals were randomly divided into six groups (n = 6 per group):

Group	Treatment
I	Normal control (vehicle)
II	Scopolamine control (3 mg/kg, i.p.)
III	Donepezil (3 mg/kg, p.o.) + Scopolamine
IV	*C. asiatica* extract + Scopolamine
V	*O. indicum* extract + Scopolamine
VI	Combination (*C. asiatica* + *O. indicum*) + Scopolamine

Extracts and donepezil were administered orally for 14–21 days. Scopolamine was injected intraperitoneally 30 min before behavioural testing.

2.4 Behavioural Assessments

- **Morris Water Maze (MWM):** Escape latency during acquisition and time spent in target quadrant during probe trial.
- **Y-maze:** Percentage spontaneous alternation and total arm entries.
- **Novel Object Recognition (NOR):** Novel object exploration time.
- **Passive Avoidance:** Acquisition and retention latency.

2.5 Biochemical and Molecular Assays

Brain tissue (hippocampus/cortex) was homogenised for estimation of AChE, oxidative stress markers (MDA, GSH, SOD, Catalase), inflammatory cytokines (TNF-α, IL-1β, IL-6), NF-κB, BDNF, NRF2, HO-1, apoptotic markers (Bax, Bcl-2, Caspase-3), Aβ1–42 and p-Tau.

2.6 Immunohistochemistry and Histopathology

GFAP and Iba-1 immunoreactivity were quantified. Histopathological examination of the hippocampus was performed using Haematoxylin and Eosin (H&E) staining.

2.7 Statistical Analysis

Data are expressed as mean ± SEM (n = 6). Statistical analysis was performed using one-way ANOVA followed by Dunnett's multiple comparison test (GraphPad Prism). Significance levels: *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001 vs Scopolamine control.

3. Results

3.1 Body Weight

Scopolamine treatment reduced body weight gain. Treatment groups, particularly the combination, significantly restored body weight.

Table 1. Body weight on Day 28 (mean ± SEM)

Group	Body weight (g)
Normal	238.6 ± 4.2****
Scopolamine	191.4 ± 3.8
Donepezil	229.8 ± 4.1****
C. asiatica	224.5 ± 3.9***
O. indicum	221.3 ± 4.0***
Combination	233.7 ± 4.0****

3.2 Behavioural Performance

Table 2. Behavioural parameters (mean ± SEM)

Parameter	Normal	Scopolamine	Donepezil	*C. asiatica*	*O. indicum*	Combination
Time in target quadrant (s)	28.4 ± 1.1****	15.1 ± 0.9	24.7 ± 0.9****	22.8 ± 0.9****	21.2 ± 0.8****	26.5 ± 0.8****
Novel object exploration (s)	28.5 ± 1.2****	20.1 ± 0.9	25.8 ± 1.0****	24.2 ± 1.0*	23.1 ± 0.9	27.4 ± 1.0****
Retention latency (s)	268 ± 12****	58 ± 8	215 ± 12****	178 ± 11****	155 ± 10****	242 ± 13****
Acquisition latency (s)	18.2 ± 1.1	19.0 ± 1.0	18.5 ± 1.0	19.2 ± 1.0	18.8 ± 1.0	18.0 ± 1.0
Total arm entries	28.2 ± 1.2	26.8 ± 1.3	27.5 ± 1.2	27.2 ± 1.1	26.9 ± 1.3	28.0 ± 1.1
% Spontaneous alternation	78.5 ± 2.4****	42.6 ± 2.8	68.4 ± 2.6****	61.2 ± 2.5***	57.8 ± 2.4***	72.5 ± 2.3****

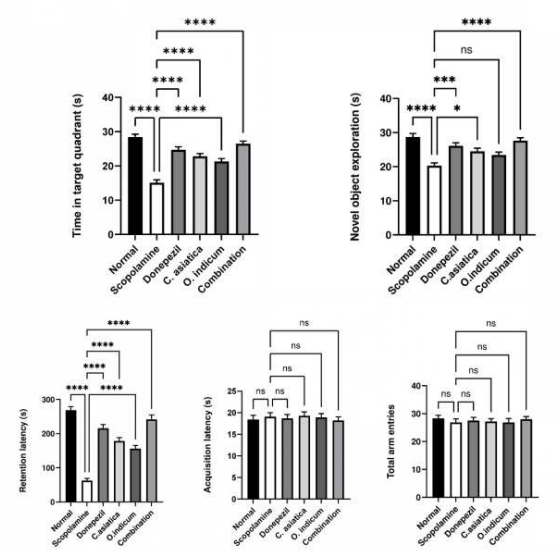


Figure 1. Effects of Centella asiatica, Oroxylum indicum, and their combination on behavioral and cognitive performance, assessed using the Y-maze, novel object recognition, and Morris water maze tests in scopolamine-induced experimental animal.

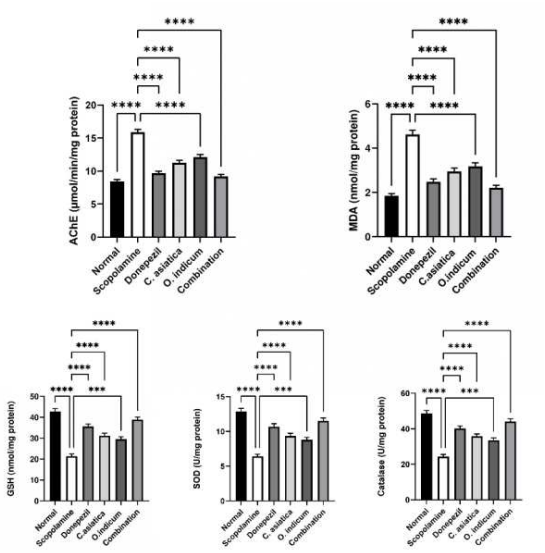


Figure 2. Effects of Centella asiatica, Oroxylum indicum, and their combination on acetylcholinesterase activity and oxidative stress markers, including malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), and catalase, in scopolamine-induced experimental animals

3.3 Cholinergic and Oxidative Stress Markers

Table 3. AChE and oxidative stress markers (mean ± SEM)

Parameter	Normal	Scopolamine	Donepezil	*C. asiatica*	*O. indicum*	Combination
AChE (µmol/min/mg)	8.4 ± 0.4****	15.9 ± 0.5	9.6 ± 0.4****	11.3 ± 0.4****	12.2 ± 0.5****	9.2 ± 0.4****
MDA (nmol/mg)	1.85 ± 0.12****	4.62 ± 0.25	2.48 ± 0.15****	2.95 ± 0.18****	3.18 ± 0.20****	2.25 ± 0.14****
GSH (nmol/mg)	42.8 ± 1.8****	21.4 ± 1.4	35.6 ± 1.6****	31.2 ± 1.5****	29.5 ± 1.4****	38.9 ± 1.5****
SOD (U/mg)	12.85 ± 0.55****	6.42 ± 0.40	10.68 ± 0.45****	9.35 ± 0.42****	8.78 ± 0.40****	11.65 ± 0.48****
Catalase (U/mg)	48.6 ± 2.2****	24.3 ± 1.5	40.2 ± 1.8****	35.8 ± 1.6****	33.5 ± 1.5****	44.8 ± 1.9****

3.4 Inflammatory Markers

Table 4. Inflammatory markers (mean ± SEM)

Parameter	Normal	Scopolamine	Donepezil	*C. asiatica*	*O. indicum*	Combination
TNF-α (pg/mg)	28.5 ± 1.6****	78.4 ± 3.2	42.6 ± 2.1****	50.8 ± 2.4****	55.2 ± 2.6****	37.5 ± 1.9****
IL-1β (pg/mg)	22.4 ± 1.3****	65.8 ± 3.0	34.7 ± 1.8****	42.5 ± 2.1****	46.2 ± 2.3****	30.1 ± 1.6****
IL-6 (pg/mg)	35.2 ± 2.0****	92.6 ± 4.1	52.8 ± 2.5****	64.5 ± 2.9****	69.8 ± 3.2****	45.6 ± 2.2****
NF-κB (ng/mg)	4.25 ± 0.28****	11.86 ± 0.55	6.42 ± 0.32****	7.85 ± 0.38****	8.35 ± 0.42****	5.45 ± 0.30****

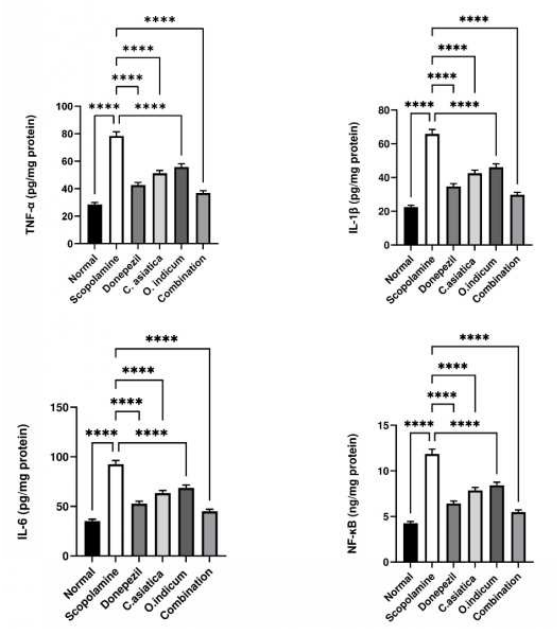


Figure 3. Effects of Centella asiatica, Oroxylum indicum, and their combination on pro-inflammatory markers, including TNF-α, IL-1β, IL-6, and NF-κB, in scopolamine-induced experimental animals.

3.5 Neurotrophic and Antioxidant Signalling

Table 5. BDNF, NRF2 and HO-1 (mean ± SEM)

Parameter	Normal	Scopolamine	Donepezil	*C. asiatica*	*O. indicum*	Combination
BDNF (pg/mg)	185.4 ± 7.5****	92.6 ± 5.2	152.8 ± 6.5****	135.2 ± 5.8***	128.5 ± 5.5***	168.5 ± 6.8****
NRF2 (ng/mg)	9.85 ± 0.45****	4.15 ± 0.28	7.65 ± 0.35****	6.85 ± 0.32****	6.45 ± 0.30****	8.75 ± 0.38****
HO-1 (ng/mg)	7.85 ± 0.35****	3.15 ± 0.22	6.15 ± 0.28****	5.45 ± 0.25****	5.15 ± 0.24****	7.05 ± 0.30****

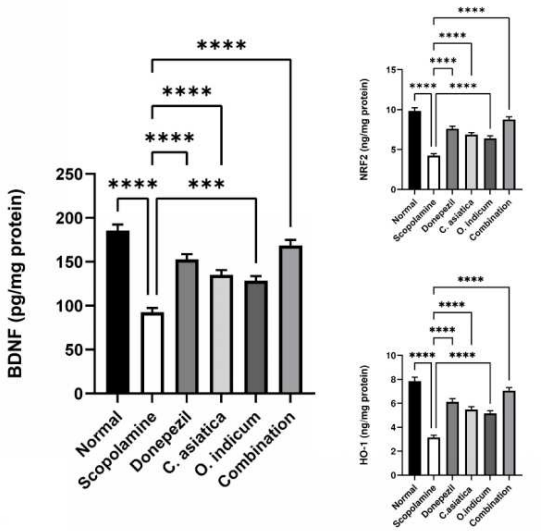


Figure 4. Effects of Centella asiatica, Oroxylum indicum, and their combination on neurotrophic and antioxidant defense markers, including BDNF, Nrf2, and HO-1, in scopolamine-induced experimental animals.

3.6 Apoptotic Markers

Table 6. Bax, Bcl-2 and Caspase-3 (mean ± SEM)

Parameter	Normal	Scopolamine	Donepezil	*C. asiatica*	*O. indicum*	Combination
Bax (relative)	1.00 ± 0.06****	3.45 ± 0.18	1.68 ± 0.09****	2.15 ± 0.12****	2.38 ± 0.14****	1.35 ± 0.08****
Bcl-2 (relative)	1.00 ± 0.05****	0.38 ± 0.04	0.78 ± 0.05****	0.65 ± 0.04**	0.58 ± 0.04**	0.88 ± 0.05****
Caspase-3 (relative)	1.00 ± 0.06****	3.85 ± 0.20	1.78 ± 0.10****	2.35 ± 0.13****	2.55 ± 0.14****	1.42 ± 0.09****

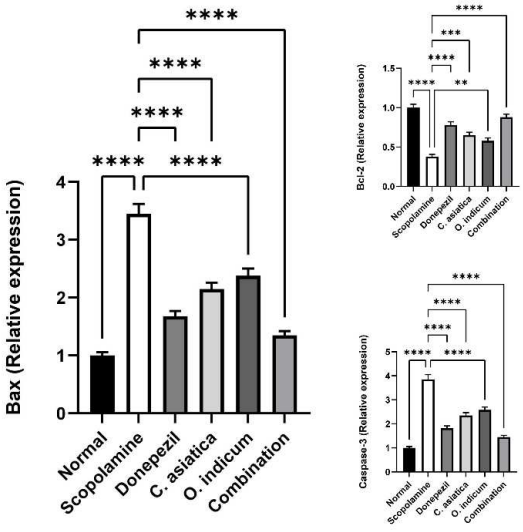


Figure 5. Effects of Centella asiatica, Oroxylum indicum, and their combination on apoptosis-related markers, including Bax, Bcl-2, and caspase-3, in scopolamine-induced experimental animals.

3.7 Pathological Proteins and Glial Activation

Table 7. Aβ1–42, p-Tau, GFAP and Iba-1 (mean ± SEM)

Parameter	Normal	Scopolamine	Donepezil	*C. asiatica*	*O. indicum*	Combination
Aβ1–42 (pg/mg)	42.5 ± 2.5****	128.6 ± 5.8	68.4 ± 3.5****	82.5 ± 4.0****	89.2 ± 4.2****	55.8 ± 2.8****
p-Tau (pg/mg)	18.5 ± 1.2****	58.6 ± 3.0	29.8 ± 1.8****	36.5 ± 2.0****	39.8 ± 2.2****	24.5 ± 1.5****
GFAP (% area)	8.25 ± 0.55****	28.45 ± 1.55	14.80 ± 0.95****	18.25 ± 1.10****	19.85 ± 1.20****	11.85 ± 0.75****
Iba-1 (% area)	6.85 ± 0.45****	24.65 ± 1.35	12.45 ± 0.75****	15.85 ± 0.90****	17.25 ± 1.00****	9.85 ± 0.65****

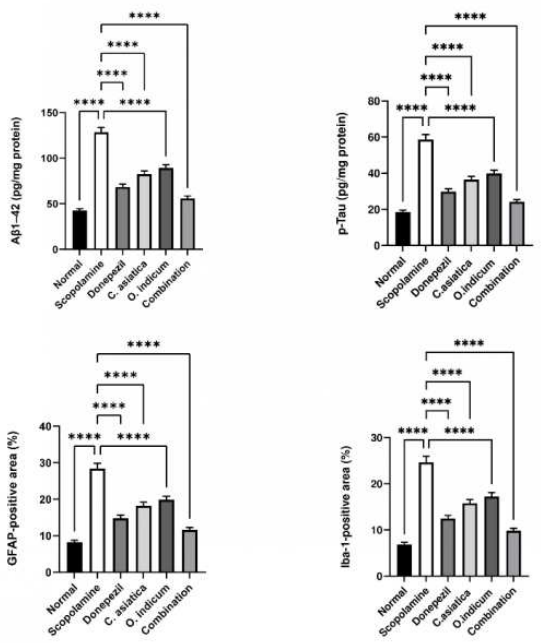


Figure 6. Effects of Centella asiatica, Oroxylum indicum, and their combination on Alzheimer's disease-associated pathological and neuroinflammatory markers, including Aβ1–42, phosphorylated tau (p-Tau), GFAP, and Iba-1 in scopolamine-induced experimental animals.

3.8 Histopathological Examination of Hippocampus

Histopathological evaluation of the hippocampal region was performed using Haematoxylin and Eosin (H&E) staining.

Normal control (Panel a): Showed normal histoarchitecture with intact pyramidal neurons having well-defined nuclei and prominent nucleoli. No neuronal degeneration or vacuolation was observed.

Scopolamine control (Panel b): Revealed marked neurodegenerative changes characterised by shrunken and darkly stained pyknotic neurons (black arrows), neuronal loss, cytoplasmic vacuolation (white arrowheads) and disrupted neuronal arrangement.

Donepezil (Panel c): Showed moderate protection with better preservation of neuronal morphology and reduced pyknotic cells.

***C. asiatica* (Panel d):** Exhibited partial restoration of normal neuronal architecture with fewer degenerating neurons.

***O. indicum* (Panel e):** Showed mild to moderate improvement with decreased pyknotic cells.

Combination (Panel f): Demonstrated near-normal hippocampal histoarchitecture with well-preserved pyramidal neurons, minimal pyknotic changes and almost complete absence of vacuolation, indicating superior neuroprotection.

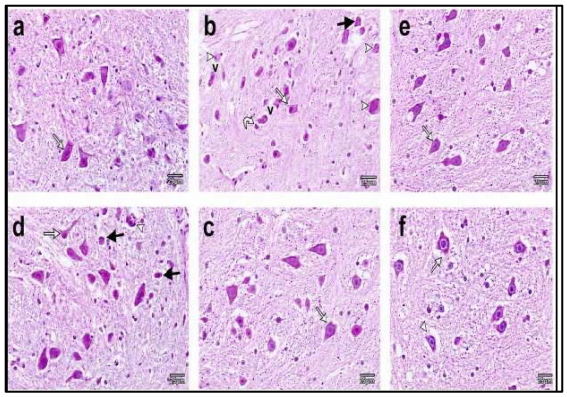


Figure 7. Representative photomicrographs of H&E-stained hippocampal sections (scale bar = 50 µm).

(a) Normal control – intact neurons. (b) Scopolamine control – pyknotic neurons (black arrows) and vacuolation (white arrowheads). (c) Donepezil. (d) *C. asiatica*. (e) *O. indicum*. (f) Combination (*C. asiatica* + *O. indicum*).

4. Discussion

The present study demonstrates that both *Centella asiatica* and *Oroxylum indicum* attenuate scopolamine-induced cognitive impairment, with the combination producing the most consistent and robust protection across behavioural, biochemical and histological endpoints. Scopolamine administration resulted in marked deficits in spatial, working, recognition and fear-motivated memory, accompanied by elevated AChE activity. These findings are consistent with the cholinergic hypothesis of cognitive dysfunction [8,9].

Oxidative stress was evidenced by increased MDA and depleted GSH, SOD and catalase. Both extracts, particularly the combination, effectively restored redox balance, attributable to the triterpenoid saponins of *C. asiatica* and flavonoids of *O. indicum* [10,13]. Neuroinflammation (elevated TNF-α, IL-1β, IL-6 and NF-κB) was strongly suppressed by the combination. Concurrent upregulation of the NRF2/HO-1 pathway and restoration of BDNF levels further support enhanced endogenous neuroprotective mechanisms.

Favourable modulation of the Bax/Bcl-2 ratio and reduction in Caspase-3 indicated attenuation of apoptosis. Significant reductions in Aβ1–42 and p-Tau, along with decreased GFAP and Iba-1 immunoreactivity, suggest disease-modifying potential. Histopathological findings corroborated the biochemical results. Scopolamine induced clear neuronal damage in the hippocampus, while the combination group showed near-normal neuronal architecture, confirming superior structural neuroprotection. The superior efficacy of the combination likely results from complementary mechanisms of the two plants acting on multiple pathological pathways simultaneously.

5. Conclusion

The combination of *Centella asiatica* and *Oroxylum indicum* produces multi-target neuroprotective effects against scopolamine-induced cognitive impairment in rats. It improves behavioural performance, restores cholinergic and redox homeostasis, suppresses neuroinflammation, enhances neurotrophic signalling, reduces apoptotic and pathological protein markers, attenuates glial activation, and preserves hippocampal neuronal architecture. These findings support further investigation of this combination as a potential therapeutic strategy for cognitive disorders.

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