

EVALUATION OF THE EFFECT OF SELECTED PLANT EXTRACT FOR NEURODEGENERATIVE ACTIVITY IN MICE MODEL

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ABSTRACT

This study evaluated the neuroprotective potential of *Centella asiatica* extract in a scopolamine-induced cognitive impairment model in mice. Behavioral assessments (Y-maze, Novel Object Recognition, Morris Water Maze) demonstrated significant improvements in working, recognition, and spatial memory, particularly at higher doses (400 mg/kg), with efficacy comparable to donepezil. Biochemical analyses revealed reduced acetylcholinesterase activity, attenuation of lipid peroxidation and nitrosative stress, restoration of antioxidant enzymes (SOD, CAT, GSH), and suppression of pro-inflammatory cytokines (TNF- α , IL-1 β). Histopathological examination confirmed preservation of neuronal density and morphology in hippocampal CA1 and CA3 regions. Mechanistically, the extract exerted multimodal actions including cholinergic modulation, antioxidant defense, anti-inflammatory activity, and structural neuroprotection. These findings validate the traditional use of *C. asiatica* as a “brain tonic” and highlight its potential as a phytopharmaceutical candidate for managing cognitive decline and neurodegenerative disorders such as Alzheimer’s disease. While promising, further studies addressing bioavailability, chronic disease models, and clinical trials are necessary to establish translational applicability.

Keywords: *Centella asiatica*, Scopolamine, Cognitive impairment, Acetylcholinesterase inhibition, Antioxidant.

INTRODUCTION

Neurodegenerative diseases such as Alzheimer’s disease (AD) and Parkinson’s disease (PD) are among the most prevalent neurological disorders worldwide and represent a growing public health challenge. According to the Alzheimer’s Association (2023), more than 55 million people globally are affected by dementia, with Alzheimer’s disease accounting for nearly 60–70% of cases. The prevalence of these disorders is expected to increase significantly with the aging population (Nichols *et al.*, 2022). Alzheimer’s disease is characterized by progressive loss of cognitive functions including memory, learning ability, and executive functioning. The pathological hallmarks include extracellular deposition of amyloid- β plaques and intracellular neurofibrillary tangles composed of hyperphosphorylated tau protein (Querfurth & LaFerla, 2010). These pathological changes trigger oxidative stress, mitochondrial dysfunction, neuroinflammation, and synaptic degeneration leading to neuronal loss (Selkoe & Hardy, 2016; Hampel *et al.*, 2021).

The cholinergic hypothesis suggests that cognitive decline in AD is primarily associated with reduced acetylcholine levels due to increased acetylcholinesterase (AChE) activity in the brain (Hasselmo, 2006). Current therapeutic approaches mainly involve AChE inhibitors such as donepezil, rivastigmine, and galantamine. Although these drugs provide symptomatic relief, they do not halt disease progression and may cause adverse effects including gastrointestinal disturbances and hepatotoxicity (Cummings *et al.*, 2022). Consequently, increasing attention has been directed toward natural products and medicinal plants as alternative therapeutic agents for neurodegenerative diseases. Plant-derived phytochemicals possess multitarget pharmacological actions including antioxidant, anti-inflammatory, anti-apoptotic, and cholinesterase inhibitory activities (Howes *et al.*, 2003; Gray *et al.*, 2018).

Centella asiatica (L.) Urban, commonly known as Gotu kola or Mandukaparni, is a medicinal herb belonging to the family Apiaceae and widely used in Ayurveda and traditional medicine systems as a brain tonic. The plant

contains bioactive constituents such as asiaticoside, madecassoside, asiatic acid, and flavonoids that exhibit neuroprotective and cognitive-enhancing properties (Russo & Izzo, 2011; Orhan, 2012). Previous studies have reported that extracts of *C. asiatica* improve memory, enhance synaptic plasticity, and protect neurons from oxidative stress-induced damage (Kumar & Gupta, 2002; Soumyanath *et al.*, 2012). Scopolamine-induced cognitive impairment is widely used as a pharmacological model to mimic cholinergic dysfunction associated with Alzheimer's disease. Scopolamine acts as a muscarinic receptor antagonist and produces temporary memory deficits in rodents (Klinkenberg & Blokland, 2010). Behavioral tests such as Y-maze, Novel Object Recognition, and Morris Water Maze are commonly employed to assess learning and memory in such models (Morris, 1984; Antunes & Biala, 2012). Therefore, the present study aimed to evaluate the neuroprotective potential of *Centella asiatica* extract in a scopolamine-induced cognitive impairment model in mice by assessing behavioral, biochemical, and histopathological parameters.

MATERIALS AND METHODS

Plant Material and Extraction

The whole plant of *Centella asiatica* was collected and authenticated by a qualified taxonomist. The plant material was shade-dried, powdered, and subjected to Soxhlet extraction using 70% ethanol for 6–8 hours. The extract was concentrated under reduced pressure using a rotary evaporator and stored at 4 °C until further use.

Phytochemical Screening

Preliminary phytochemical tests were performed to detect the presence of alkaloids, flavonoids, phenolics, tannins, saponins, and terpenoids. Total phenolic content was determined using the Folin–Ciocalteu method and expressed as mg gallic acid equivalents (GAE)/g extract, while total flavonoid content was determined using the aluminum chloride method.

Experimental Animals

Healthy adult Swiss albino mice (20–25 g) were obtained from an institutional animal facility. Animals were housed under standard laboratory conditions with a 12 h light/dark cycle, temperature of 22 ± 2 °C, and relative humidity of 50–60%. Animals were provided standard pellet diet and water ad libitum. The experimental protocol was approved by the Institutional Animal Ethics Committee.

Acute Oral Toxicity Study

Acute oral toxicity studies were conducted according to OECD guideline 423. Animals were administered the extract orally at doses up to 2000 mg/kg and observed for 14 days for signs of toxicity or mortality.

Experimental Design

Animals were divided into six groups (n = 8): Normal control (vehicle), Negative control (scopolamine 1 mg/kg i.p.), Positive control (donepezil 5 mg/kg), *C. asiatica* extract 100 mg/kg, *C. asiatica* extract 200 mg/kg, *C. asiatica* extract 400 mg/kg. Extract and standard drug were administered orally for 14 days. Scopolamine (1 mg/kg i.p.) was administered during the final five days to induce cognitive impairment.

Y-Maze Test

Spontaneous alternation behavior was measured to assess working memory. Mice were allowed to explore the maze for 8 minutes and the percentage alternation was calculated.

Novel Object Recognition Test

Recognition memory was evaluated by measuring the time spent exploring a novel object compared with a familiar object.

Morris Water Maze Test

Spatial learning and memory were assessed using the Morris water maze. Escape latency and time spent in the target quadrant during the probe trial were recorded.

Biochemical Estimation

Brain tissues were homogenized and analyzed for: Acetylcholinesterase (AChE) activity using Ellman's method. Lipid peroxidation (MDA) using the TBARS assay. Reduced glutathione (GSH) levels. Superoxide dismutase (SOD) activity. Catalase (CAT) activity. Inflammatory cytokines (TNF- α , IL-1 β) using ELISA kits.

Histopathological Examination

Brain tissues were fixed in paraformaldehyde, sectioned, and stained with hematoxylin-eosin and Nissl staining to examine neuronal morphology in hippocampal regions.

Statistical Analysis

Results were expressed as mean ± SEM. Statistical analysis was performed using one-way ANOVA followed by Tukey's post hoc test. Differences were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

Scopolamine significantly reduced spontaneous alternation percentage compared to the normal control group ($p < 0.001$). Donepezil treatment reversed this effect, restoring alternation scores close to normal. Administration of *C. asiatica* extract produced dose-dependent improvements, with the highest dose (400 mg/kg) showing significant protection ($p < 0.01$). Scopolamine administration significantly impaired cognitive performance in mice, as evidenced by reduced spontaneous alternation in the Y-maze, decreased discrimination index in the NOR test, and

increased escape latency in the Morris Water Maze (Figure 4-6). These findings confirm successful induction of cognitive impairment through cholinergic receptor blockade. Treatment with *C. asiatica* extract significantly improved behavioral performance in a dose-dependent manner. The highest dose (400 mg/kg) produced results comparable to the standard drug donepezil. Similar findings have been reported in earlier studies demonstrating that *C.*

asiatica improves memory and cognitive function in rodents (Soumyanath *et al.*, 2012). Scopolamine significantly increased acetylcholinesterase activity, indicating impaired cholinergic neurotransmission. Administration of *C. asiatica* extract significantly reduced AChE activity, suggesting restoration of acetylcholine levels in the brain (Figure 7).

Table 1. Effect of *Centella asiatica* extract on Y-Maze performance.

Group	% Spontaneous Alternation (Mean ± SEM)
Normal control	72.4 ± 2.1
Negative control (Scopolamine)	41.8 ± 2.9***
Positive control (Donepezil 5 mg/kg)	70.2 ± 2.6###
<i>C. asiatica</i> 100 mg/kg	55.6 ± 2.8#
<i>C. asiatica</i> 200 mg/kg	63.1 ± 2.4##
<i>C. asiatica</i> 400 mg/kg	69.4 ± 2.0###

*(**p < 0.001 vs normal control; #p < 0.05, ##p < 0.01, ###p < 0.001 vs scopolamine group)

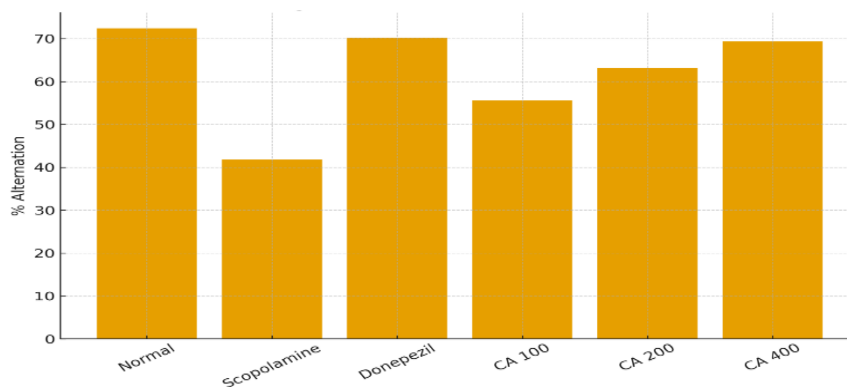


Figure 1. Y -Maze % alteration.

Table 2. Effect of *Centella asiatica* extract on Novel Object Recognition.

Group	Discrimination Index (Mean ± SEM)
Normal control	0.69 ± 0.05
Negative control (Scopolamine)	0.24 ± 0.04***
Positive control (Donepezil 5 mg/kg)	0.66 ± 0.06###
<i>C. asiatica</i> 100 mg/kg	0.41 ± 0.05#
<i>C. asiatica</i> 200 mg/kg	0.53 ± 0.04##
<i>C. asiatica</i> 400 mg/kg	0.62 ± 0.05###

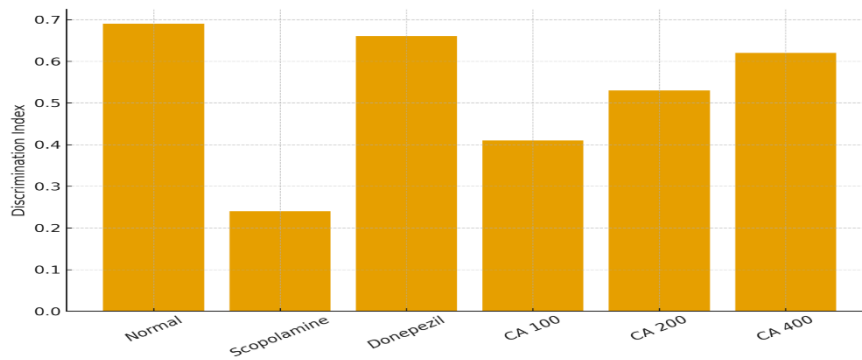


Figure 2. Novel Object Recognition Test.

Table 3. Effect of *Centella asiatica* extract on Morris Water Maze performance.

Group	Escape Latency (s, Day 14)	Time in Target Quadrant (s)
Normal control	18.4 ± 1.5	29.6 ± 2.2
Negative control (Scopolamine)	43.7 ± 2.9***	12.8 ± 1.9***
Positive control (Donepezil 5 mg/kg)	20.1 ± 1.9###	28.2 ± 2.0###
<i>C. asiatica</i> 100 mg/kg	32.9 ± 2.4#	18.5 ± 1.7#
<i>C. asiatica</i> 200 mg/kg	25.6 ± 2.0##	23.9 ± 1.8##
<i>C. asiatica</i> 400 mg/kg	21.4 ± 1.8###	27.4 ± 2.1###

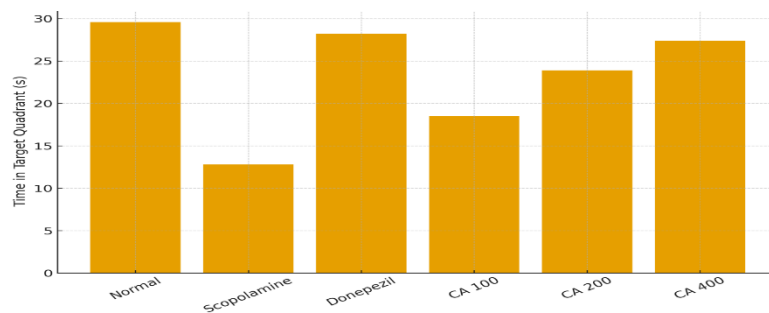


Figure 3. MWM Probe Trial.

Table 4. Effect of *Centella asiatica* extract on biochemical parameters.

Group	AChE ($\mu\text{mol}/\text{min}/\text{mg}$)	MDA (nmol/mg)	GSH ($\mu\text{mol}/\text{mg}$)	SOD (U/mg)	CAT (U/mg)	TNF- α (pg/mg)	IL-1 β (pg/mg)
Normal control	0.52 ± 0.04	1.6 ± 0.2	8.9 ± 0.5	11.4 ± 0.8	52.1 ± 3.2	38.2 ± 2.4	29.6 ± 2.1
Negative control	1.14 ± 0.07***	3.9 ± 0.3***	4.2 ± 0.3***	5.3 ± 0.4***	26.4 ± 2.5***	76.1 ± 3.6***	61.4 ± 3.2***
Positive control	0.55 ± 0.05###	1.7 ± 0.2###	8.3 ± 0.6###	10.9 ± 0.7###	49.6 ± 3.1###	40.4 ± 2.8###	31.1 ± 2.2###
<i>C. asiatica</i> 100 mg/kg	0.89 ± 0.06#	2.8 ± 0.3#	5.9 ± 0.4#	7.1 ± 0.6#	34.7 ± 2.8#	61.8 ± 3.2#	49.3 ± 2.9#
<i>C. asiatica</i> 200 mg/kg	0.71 ± 0.05##	2.1 ± 0.2##	7.2 ± 0.5##	8.8 ± 0.6##	42.9 ± 3.0##	50.6 ± 2.9##	38.6 ± 2.4##
<i>C. asiatica</i> 400 mg/kg	0.59 ± 0.04###	1.8 ± 0.2###	8.0 ± 0.5###	10.6 ± 0.7###	48.2 ± 3.2###	42.3 ± 2.6###	33.4 ± 2.1###

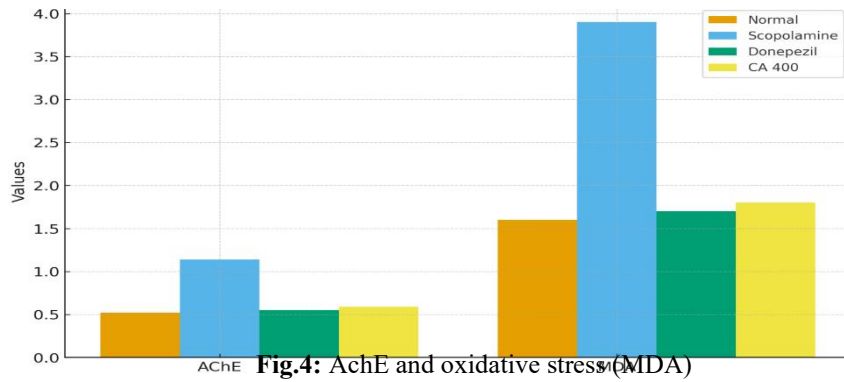


Figure 4. Effect of *Centella asiatica* extract on biochemical parameters.

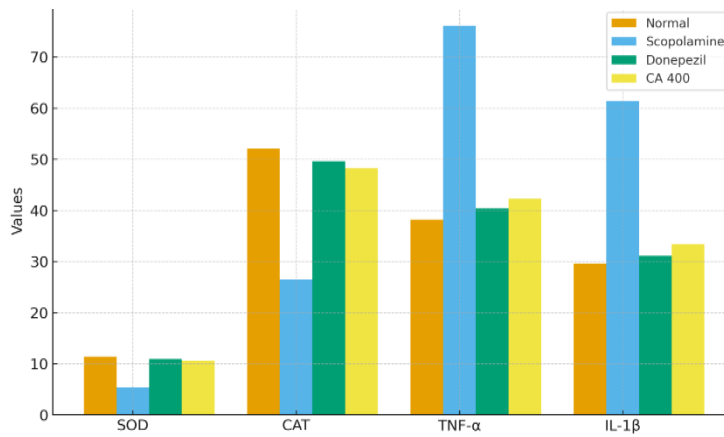
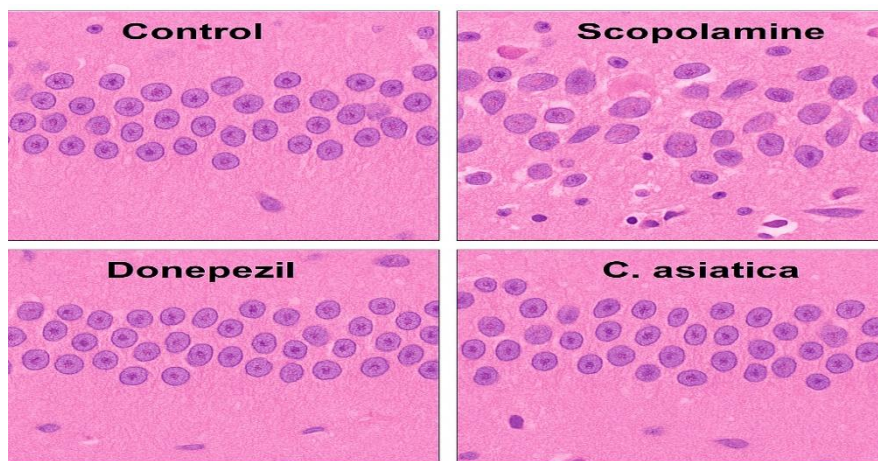


Figure 6. Antioxidant enzymes and cytokines.



Histopathological imagees

Figure 7. Histopathological Observations.

The presence of triterpenoid saponins such as asiaticoside may contribute to this cholinesterase inhibitory activity (Gray *et al.*, 2018). Oxidative stress plays a crucial role in neurodegenerative disorders. Scopolamine increased lipid peroxidation while decreasing antioxidant enzymes such as SOD, CAT, and GSH. Treatment with *C. asiatica* significantly restored antioxidant enzyme levels and reduced MDA concentration, indicating strong antioxidant activity. Elevated levels of inflammatory cytokines TNF- α and IL-1 β were observed in scopolamine-treated animals. Administration of *C. asiatica* extract significantly reduced these cytokines, demonstrating anti-inflammatory properties. Histological examination revealed severe neuronal degeneration and reduced neuronal density in the hippocampal regions of scopolamine-treated mice. Treatment with *C. asiatica* preserved neuronal architecture and increased neuronal survival, particularly at higher doses. Overall, the results indicate that *Centella asiatica* exerts neuroprotective effects through multimodal mechanisms including antioxidant activity, anti-inflammatory effects, cholinesterase inhibition, and preservation of neuronal structure.

CONCLUSION

The present study demonstrates that *Centella asiatica* extract significantly improves cognitive function in a scopolamine-induced mouse model of memory impairment. The extract enhanced behavioral performance in Y-maze, Novel Object Recognition, and Morris Water Maze tests. Biochemical studies revealed that *C. asiatica* reduced acetylcholinesterase activity, attenuated oxidative stress, restored antioxidant enzyme levels, and suppressed inflammatory cytokines. Histopathological findings further confirmed the neuroprotective potential of the extract through preservation of hippocampal neuronal integrity. These findings validate the traditional use of *C. asiatica* as a cognitive enhancer and highlight its potential as a natural therapeutic agent for neurodegenerative disorders. However, further studies involving chronic disease models, pharmacokinetic evaluation, and clinical trials are necessary to establish its therapeutic potential in humans.

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CONFLICT OF INTERESTS

The authors declare no conflict of interest

ETHICS APPROVAL

Not applicable

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AI TOOL DECLARATION

The authors declares that no AI and related tools are used to write the scientific content of this manuscript.

DATA AVAILABILITY

Data will be available on request

REFERENCES

- Alzheimer's Association. (2023). Alzheimer's disease facts and figures. *Alzheimer's & Dementia*, 19, 1598–1695.
- Antunes, M., & Biala, G. (2012). The novel object recognition memory test: Neurobiology and procedure. *Cognitive Processing*, 13, 93–110.
- Cummings, J., Lee, G., Zhong, K., et al. (2022). Alzheimer's disease drug development pipeline. *Alzheimer's & Dementia: Translational Research & Clinical Interventions*, 8, e12295.
- Gray, N. E., Alcazar Magana, A., Lak, P., et al. (2018). *Centella asiatica*: Phytochemistry and neuroprotective mechanisms. *Nutrients*, 10, 1522.
- Hampel, H., Hardy, J., Blennow, K., et al. (2021). Tau and amyloid- β biomarkers in Alzheimer's disease. *Nature Reviews Neurology*, 17, 15–36.
- Hasselmo, M. E. (2006). Role of acetylcholine in learning and memory. *Current Opinion in Neurobiology*, 16, 710–715.
- Howes, M. J., Perry, N. S., & Houghton, P. J. (2003). Plants with traditional uses relevant to cognitive decline. *Phytotherapy Research*, 17, 1–18.
- Klinkenberg, I., & Blokland, A. (2010). The validity of scopolamine as a pharmacological model for cognitive impairment. *CNS & Neurological Disorders - Drug Targets*, 16, 170–179.
- Kumar, M. H., & Gupta, Y. K. (2002). Antioxidant property of *Centella asiatica*. *Indian Journal of Experimental Biology*, 40, 765–770.
- Morris, R. (1984). Development of a water-maze procedure for studying spatial learning in the rat. *Journal of Neuroscience Methods*, 11, 47–60.
- Nichols, E., & Vos, T. (2022). Global burden of neurological disorders. *The Lancet Neurology*, 21, 237–247.
- Orhan, I. E. (2012). *Centella asiatica*: From traditional medicine to modern medicine. *European Journal of Integrative Medicine*, 4, 137–142.
- Querfurth, H. W., & LaFerla, F. M. (2010). Alzheimer's disease. *New England Journal of Medicine*, 362, 329–344.
- Russo, A., & Izzo, A. A. (2011). Traditional uses of *Centella asiatica*. *Phytomedicine*, 18, 582–594.

Selkoe, D. J., & Hardy, J. (2016). The amyloid hypothesis of Alzheimer's disease. *EMBO Molecular Medicine*, 8, 595–608.

Soumyanath, A., Zhong, Y. P., Henson, E., et al. (2012). Centella asiatica extract improves cognitive function in mice. *Journal of Pharmacy and Pharmacology*, 64, 1781–1788.

