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Research Article

Evaluation of the Nootropic Activity of the Ethanolic Leaf Extract of *Neolamarckia cadamba* in Experimental Animal Models

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Abstract

This study aimed to evaluate the nootropic activity of the ethanolic leaf extract of *Neolamarckia cadamba* (ELENC) in mice. Nootropic activity was assessed using ELENC at selected doses. Donepezil (100 mg/kg, p.o.) was used as the standard reference drug, and scopolamine (1 mg/kg, i.p.) was administered to induce amnesia. Learning and memory were evaluated using the Elevated Plus Maze, Radial Arm Maze, and Y-maze paradigms in scopolamine-induced amnesic mice. In addition, acetylcholinesterase (AChE) levels were estimated in whole-brain homogenates to assess cholinergic function. ELENC treatment significantly increased behavioral and spontaneous alternation in the Y-maze task, indicating improved spatial working memory. In the elevated plus-maze model, ELENC significantly reduced transfer latency in scopolamine-treated mice, suggesting enhanced learning and cognition. The extract also increased locomotor activity and markedly decreased brain acetylcholinesterase levels compared with the normal control, negative control, and standard drug groups, indicating restoration of cholinergic neurotransmission disrupted by scopolamine. Overall, these findings suggest that ELENC improves learning and memory across multiple behavioral models, and the reduction in brain AChE activity further supports its neuroprotective potential. Therefore, *Neolamarckia cadamba* leaf extract may serve as a promising therapeutic candidate for the management of Alzheimer's disease and related cognitive impairments.

Keywords: Nootropic activity, *Neolamarckia cadamba*, Alzheimer's disease, Donepezil, scopolamine

1 INTRODUCTION

According to the World Health Organization, in 2021, 57 million people had dementia worldwide, over 60% of whom lived in low-and middle-income countries. Every year, there are nearly 10 million new cases According to the World Health Organization's 2022 blueprint for dementia research, an estimated 55.2 million individuals globally are affected [1–3]. Dementia, an age-related mental disorder, is a major symptom of Alzheimer's disease (AD). Alzheimer's disease (AD) is a progressive neurodegenerative condition associated with cerebrovascular dysfunction. It gradually destroys brain cells, leading to memory impairment, abnormal behavior, difficulty in thinking, personality alterations, and ultimately, death. The disease is characterized by neuronal loss and the presence of neurofibrillary tangles and neuritic plaques in the brain. The cholinergic system, particularly the neurotransmitter acetylcholine (ACh), plays a crucial role in learning and memory. A decline in acetylcholine levels in the brain is considered a key factor in the development of dementia in patients with AD [4,5]. Substances such as scopolamine induce learning and memory deficits in both animals and humans. Memory loss, amnesia, dementia, anxiety, schizophrenia, and Alzheimer's disease can arise due to various factors, including aging, stress, and emotional disturbances [3,6].

Nootropic agents are a group of psychotropic medications used to manage Alzheimer's disease (AD). The term nootropic originates from the Greek words noon (mind) and tropos (turn), reflecting their role in enhancing cognitive functions. Often referred to as "smart drugs," nootropics improve memory, promote cerebral blood flow, and enhance oxygen delivery to the brain [5,7]. Synthetic nootropics, such as tacrine, donepezil, aniracetam, piracetam, and rivastigmine, are widely prescribed for cognitive impairment and memory decline

associated with AD. Although clinically beneficial, these drugs are often associated with adverse effects, dependency concerns, and limited bioavailability. To address these limitations, scientific interest has shifted toward herbal formulations with nootropic potential. Many plant-based compounds exhibit strong antioxidant properties that help protect neuronal cells from oxidative damage and may serve as safer and more effective alternatives to conventional synthetic agents [8]. A wide range of medicinal herbs have been extensively investigated, and many have demonstrated notable memory-enhancing properties. Neolamarckia cadamba is an important medicinal plant traditionally used in the Ayurvedic system. It has been prescribed for diverse health conditions, including fever, uterine disorders, blood and skin diseases, tumors, anemia, inflammation of the eye, and diarrhea. Pharmacological studies have reported its antioxidant, anti-hepatotoxic, antimalarial, analgesic, anti-inflammatory, antipyretic, diuretic, and laxative activities [9].

Previous research has also highlighted the strong antioxidant potential and in silico anti-Alzheimer properties of this plant, suggesting its possible therapeutic relevance in managing dementia. In light of this evidence, the present study aimed to evaluate the nootropic activity of the ethanolic leaf extract of N. cadamba (ELENC) using an experimental animal model.

2 MATERIALS AND METHODS

2.1Experimental animals

Swiss albino mice of either sex weighing between 22 and 30 g, respectively, were used in the present study. They were housed under standard laboratory conditions (temperature 25°C ± 1°C), relative humidity 55% ± 5%, and 12.00:12.00 h dark: light cycle) with a standard pellet diet and water ad libitum. The experiment was conducted according to the standard procedure prescribed by the CPCSEA, India. The study protocol was approved by the Ethical Committee of the IAEC (Regd.No:1362/C/10/CPCSEA) at AKRG College of Pharmacy, Nallajerla, Andhra Pradesh, India.

2.2Drugs and chemicals

Donepezil (Dr. Reddy's, India), scopolamine hydrobromide (APP Pharmaceuticals, India), and normal saline were used in this study. All other reagents and chemicals were of analytical grade and procured from Loba Chemie (Mumbai, India).

2.3Plant material and preparation of extracts

Fresh leaves of *N. cadamba* were collected from the Andhra Pradesh region of India in October 2025. The plant material was authenticated by Dr M. Madhavi, Y.S.R. Horticultural University, Venkata Ramannagudem, Andhra Pradesh. The leaves were shade-dried, powdered, and sieved to obtain a uniform fine powder. The powdered material (700 g) was Soxhlet-extracted with 99.9% ethanol, selected for its efficiency as a universal solvent for phytochemical extraction. The extract was concentrated using a rotary evaporator, yielding a dark green, sticky mass with an 8.8% yield. The extract was designated as the ethanolic leaf extract of *Neolamarckia cadamba* (ELENC)[10].

2.4Preliminary phytochemical investigation

The ethanolic extract (ELENC) was screened for major phytochemical constituents, including alkaloids, flavonoids, glycosides, saponins, carbohydrates and tannins [11].

2.5Acute toxicity study

An acute toxicity study was conducted in accordance with OECD Guideline 420 (Fixed Dose Procedure). Six female Swiss albino mice (20–25 g) were administered the test extract at a dose of 2,000 mg/kg. Following administration, the animals were monitored at 30 min, 1 h, 2 h, 4 h, 24 h, 48 h, and 72 h, and thereafter, daily for 14 days to assess clinical signs, behavioural changes, and mortality [12].

2.6Behavioural study

The mice underwent one week of behavioural training without receiving any extract or drug. Only animals that demonstrated complete training responses were included in this study. All behavioral assessments were conducted during the light phase between 9:00 a.m. and 4:00 p.m. in a soundproof environment [13].

2.7Scopolamine-induced amnesia in mice

Scopolamine, a muscarinic antagonist that crosses the blood–brain barrier, impairs short-term memory and learning by blocking acetylcholine receptors. A dose of 1 mg/kg is widely used to induce cognitive deficits without causing severe peripheral anticholinergic effects and is considered a suitable model for mimicking Alzheimer-like memory impairment [7,14].

For the evaluation of nootropic activity, the animals were divided into five groups:

Group 1: Normal control

Group 2: Scopolamine control (1 mg/kg, i.p.)

Group 3: Standard (Donepezil 10 mg/kg, p.o.)

Group 4: ELENC 100 mg/kg

Group 5: ELENC 400 mg/kg

Animals were trained in the maze for 7 days without treatment, and fully trained animals were selected for the study. All groups received their respective treatments once daily for 14 days. On the final day, scopolamine (1 mg/kg, i.p.) was administered intraperitoneally to all groups except the normal control group 60 min after dosing. Behavioural assessments of nootropic activity were performed on day 14 and the following day.

2.7.1 Transfer latency test by The Elevated Plus Maze

The Elevated Plus Maze (EPM) served as an exteroceptive behavioral model for assessing memory in mice. The apparatus consisted of two open arms (50 × 10 cm) and two closed arms (50 × 10 × 40 cm) arranged perpendicular to each other, connected by a central platform (5 × 5 cm), and elevated 50 cm above the floor

in a dimly lit room. Each animal was placed individually at the end of an open arm, and the time taken to move from the open arm to one of the closed arms was recorded as transfer latency (TL). A cutoff time of 120 s was applied. A significant reduction in TL during retention assessment was considered indicative of improved memory performance [15].

2.7.2 Radial arm maze:

The radial arm maze is an exteroceptive behavioral model used to evaluate learning and memory in rodents. It consists of an octagonal central platform with eight radial arms, each containing food cups. The animals were food-restricted to 85% of their normal intake to ensure motivation. Before treatment, the mice were trained to explore all eight arms within 300 seconds, and training was considered complete when each animal successfully visited all arms. The time taken to locate food and the number of re-entries into previously visited arms were recorded as the baseline performance [16].

Mice were then treated with donepezil for 14 days, followed by scopolamine (1 mg/kg, i.p.) to induce amnesia on day 15. 60 minutes later, the animals were retested in the maze. Learning and memory were assessed using the number of days required to learn the task, latency to find food, and the number of initial correct entries. Errors were categorized as Working Memory Errors (re-entering visited arms) and Reference Memory Errors (entering arms that never contained food) [17].

2.7.3 Y-maze task

The Y-maze task is a simple method for evaluating memory-enhancing activity in laboratory animals. It is generally used to check the behavioral patterns of animals. A wooden Y-maze was used in this study. It consists of three arms with an angle of 120° between each of the two arms. Each arm was 8 cm wide, 30 cm long, and 15 cm deep. The arms were designated as the start arm (A), the novel arm with food stimuli (B), and the other arm (C). In the first trial, the mice were placed just inside the arm and allowed to move freely through the apparatus for 6 min. In the second trial, the mice were placed in the maze and systematically explored arms A, B, and C. The ability to alternate requires that the mice know which arm they have visited. A total of 13 entries were recorded for each mouse, and the percentage of spontaneous alteration was calculated using the following formula:[18,19]

$$\% \text{ Alternations} = \frac{\text{Number of positive alternations}}{\text{Total arm entries} - 2} \times 100$$

2.8Estimation of acetylcholinesterase enzyme

Animals of same grouped in the Y-maze task after 15day, mice were decapitated, and the whole brains were removed quickly and stored in ice-cold saline. The frontal cortex, hippocampus, and septum were quickly dissected onto a chilled Petri dish containing crushed ice. The tissues were weighed and homogenized in 0.05 M phosphate buffer (pH 7.2). The homogenate (0.4 mL) was added to a test tube containing 2.6 mL phosphate buffer and 100 µL of 5,5-dithiobisnitrobenzoic acid, and the mixture was mixed thoroughly. The absorbance of the resulting mixture was measured at 412 nm using a spectrophotometer. The stable absorbance value was recorded. Acetylthiocholine iodide (20 µL) was added, and the change in absorbance per minute was determined . The mean change in absorbance was calculated using the following formula, and acetylcholinesterase activity was measured as µM/L/min/g of tissue [20,21].

$$R = (\delta \text{ OD volume of assay} / E) \times \text{mg of protein}$$

Where R is the Mice of enzyme activity in "n" mole of Ach iodide hydrolyzed per minute per mg of protein, δ OD is the change in absorbance per minute, and E is the extinction coefficient (1.36×10^4 $M^{-1}cm^{-1}$).

2.9Statistical analysis

Statistical analysis was performed using GraphPad Prism software version 6.0, and the results were compared using one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test . Statistical significance was set at $p < 0.05$.

3 RESULTS

3.1Preliminary phytochemical investigation

Results of preliminary phytochemical screening of the ethanolic leaf extract of *Neolamarckia cadamba* (ELENC). are given in Table 1. The ELENC extract tested positive for alkaloids, flavonoids, amino acids, glycosides, and saponins. Proteins, steroids, and phenols were not detected.

Table 1: Phytochemical analysis of the ethanolic leaf extract of *Neolamarckia cadamba* (ELENC).

S.N.	Chemical Test	Alcohol extract
1	Tests for steroids	+
2	Tests for saponin	+
3	Tests for Alkaloids	+
4	Tests for carbohydrates	-
5	Tests for Flavonoides	+
6	Tests for Tannins	+
7	Tests for Cardiac Glycosides	+
8	Tests for Proteins	+
9	Test for Amino acids	+

3.2Acute toxicity test

Because female mice are more sensitive to drugs than males, female mice were used in the acute oral toxicity study in accordance with OECD Guideline 420 [22]. ELENC did not show any toxic effects at an oral dose of 2000 mg/kg. Central nervous system (CNS) stimulation parameters, such as hyperactivity, irritability, tremors, and convulsions, were not observed in mice. CNS depressant parameters, such as hypoactivity, narcosis, and ataxia, were found to be negative in mice [23].

3.3Reduction of transfer latency in mice by ELENC using the Elevated Plus Maze.

In the Elevated Plus Maze, transfer latency is considered an index of exploratory drive and cognitive performance. Scopolamine administration significantly increased transfer latency (78.2 ± 4.14 on day 1 and 95.3 ± 3.856 on day 14, $p < 0.01$), confirming impairment of learning and memory. Donepezil treatment markedly reduced transfer latency (57.50 ± on day 1 and 40.67 ± 4.25 on day 14; $p < 0.01$), indicating significant protection against scopolamine-induced amnesia. The ELENC extract also demonstrated dose-dependent improvement in memory, as evidenced by a decrease in transfer latency (52.33 ± 3.46 at 100 mg/kg and 39.33 ± 1.99 at 400 mg/kg). The results were represented

Table 2: Effect of Ethanolic leaf extract of *Neolamarckia cadamba* (ELENC) and Donepezil on Transfer latency of mice using Elevated plus maze

Group	Transfer latency (Seconds) (Percent of memory protection)	
	DAY 14	DAY 15
Normal control	38.5 ± 2.28	39.00 ± 2.57
Disease control (Scopolamine 1. mg/kg)	78.2 ± 4.14#	95.3 ± 3.856#
Standard (Donepezil Hcl 10 mg/kg) + Scopolamine 1mg/kg	44.5 ± 2.95** (40.67)	40.67 ± 4.25** (57.50)
ELENC 100 mg/kg p.o+ Scopolamine 1mg/kg	65.8± 2.51* (52.33)	52.33±3.46* (45.08)
ELENC 400 mg/kg p.o+ Scopolamine 1mg/kg	46.2± 2.44** (39.33)	39.33 ±1.99** (58.7)

3.4Reduction of Working memory and reference memory errors in mice by ELENC using Radial Arm Maze.

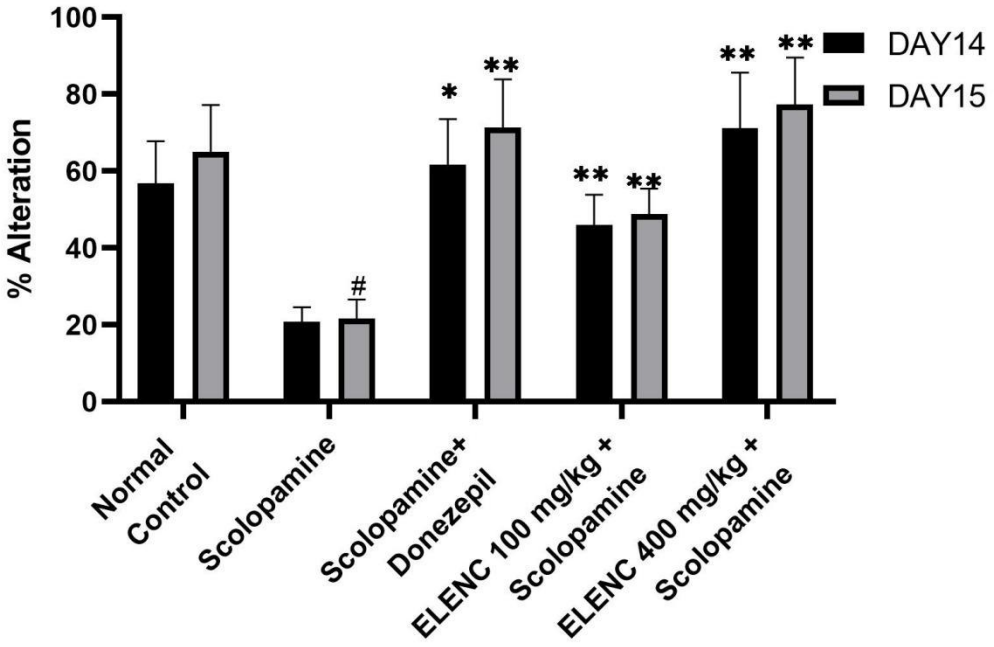
Scopolamine administration produced a pronounced increase in both reference and working memory errors during the acquisition and retention phases, confirming significant cognitive impairment in the mice. Specifically, reference memory errors increased to 5.00 ± 0.71 on day 14 and 5.40 ± 0.51 on day 15, whereas working memory errors increased to 4.00 ± 0.45 on day 14 and 4.8 ± 0.49 on day 15. Treatment with Donepezil markedly reduced these deficits, demonstrating the effective prevention of scopolamine-induced memory impairment. Similarly, ELENC produced dose-dependent protection on both days, significantly lowering memory errors and indicating improvements in both reference and working memory performance.

Table 3: Effect of ELENC on Working memory error and reference memory error in mice using the Radial Arm Maze.

Group	Working memory error - frequency (Number)		Reference memory error- frequency (Number)	
	DAY 14 (Acquisition)	DAY 15 (Retention)	DAY 14 (Acquisition)	DAY 15 (Retention)
Normal control	1.20 ± 0.37	1.0 ± 0.45	2.20 ± 0.37	1.80 ± 0.37
Disease control (Scopolamine 1mg/kg)	4.00 ± 0.45#	4.8 ± 0.49#	5.00 ± 0.71 #	5.40 ±0.51#
Standard (Donezepil Hcl 10 mg/kg)	2.00 ± 0.32**	1.8 ± 0.37**	2.00 ± 0.32**	2.20± 0.37**
ELENC 100 mg/kg p.o + Scopolamine 1mg/kg	1.60 ± 0.25^{ns}	2.0 ± 0.32*	2.20 ± 0.37*	2.40±0.25*
ELENC 400 mg/kg p.o + Scopolamine 1mg/kg	1.40 ± 0.245**	1.6 ± 0.24**	2.00 ± 0.32**	2.20± 0.20*

3.5*Reduction in* Alteration in mice Y-maze *task*

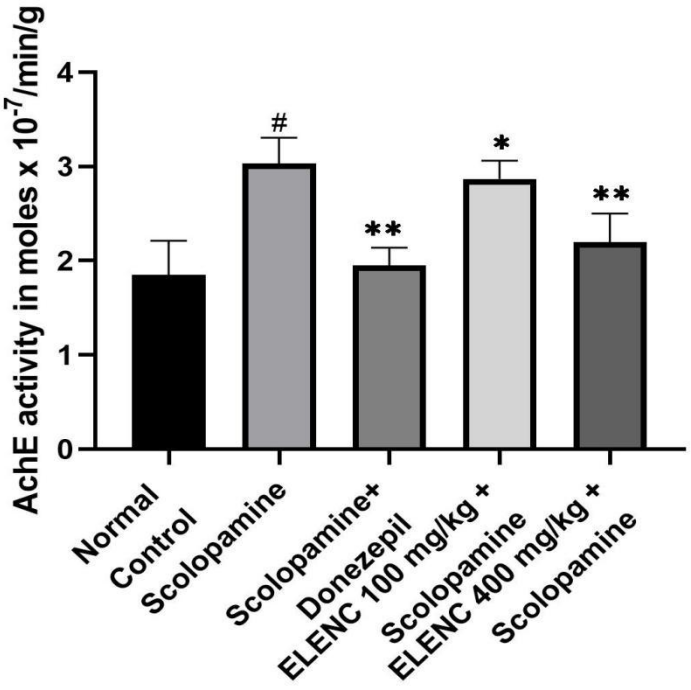
The Y-maze model is a sensitive measure of spatial recognition memory. In the Y-maze, scopolamine markedly decreased the percentage alternation alteration compared to the normal control on both Day 14 (20.8± 1.56) and Day 15 (21.7 ± 2.00), indicating reduced short-term spatial working memory. Donepezil significantly improved the number of squares crossed on both days of the test. ELENC also produced a dose-dependent increase in alteration activity in scopolamine-treated mice on days 14 and 15. The results are shown in Graph 1.



Graph 1: Effect of ELENC on the % Alteration by Y maze

3.6Reduction of Aetylcholinesterase (AChE) enzyme by EELC

The results for AChE levels are shown in Graph 2. In the normal control group, scopolamine administration significantly increased brain AChE activity in mice compared to the control group. Donepezil treatment markedly decreased AChE activity (1.95 ± 0.08) compared to the disease control group. Similarly, ELENC produced a dose-dependent, significant reduction in AChE activity compared with the disease control group.



Graph 2: Effect of EELC and donepezil on acetylcholinesterase (AChE) levels challenged with scopolamine.

4 DISCUSSION

Nootropics are drugs, also known as smart drugs, cognitive enhancers, supplements, functional foods, or nutraceuticals, that improve mental functions such as attention, concentration, motivation, intelligence, and memory. The other Nootropic agents are: Aniracetam, Fosracetam, Nefiracetam, Pramiracetam, Nebracetam and Oxiracetam, Deprenyl, Modafinil, Piracetam, Centrophenoxine, and Human growth hormone.

The current study aimed to explore the nootropic effect of the ethanolic leaf extract of *Neolamarckia cadamba* (ELENC) using different screening methods. To explore the nootropic activity, amnesia was induced by scopolamine at a dose of 1 mg/kg body weight to produce cognitive and memory changes [7,14]. First, the animals were trained for 1week for the elevated plus maze test, radial arm maze test, and Y maze test.

In the Elevated Plus Maze test, mice received ELENC (100 or 400 mg/kg, p.o.) or vehicle 30 min before placement in the open arm, and the transfer latency (time taken to enter a closed arm) was recorded. Shorter transfer latency reflects improved memory. Scopolamine produced a marked increase in transfer latency compared to the normal control, confirming its memory-impairing action via cholinergic blockade. In contrast, ELENC treatment significantly reduced transfer latency in a dose-dependent manner on both days 14 and 15, indicating protection of memory retention against scopolamine, with effects comparable to those of donepezil.

In the Radial Arm Maze test, animals were administered the standard drug donepezil for 14 consecutive days, and 24 h after the final dose (Day 15), amnesia was induced with scopolamine (1.4 mg/kg, i.p.). Reference Memory Errors (RME) were recorded when the animal entered an arm that never contained food, while Working Memory Errors (WME) were counted when the animal revisited an already explored arm within the same session[15,17]. These parameters reflect long- and short-term memory performance, respectively. After 60 minutes of scopolamine administration, each animal was exposed to the maze. Scopolamine markedly increased both RME and WME, confirming the induction of amnesia [15]. Treatment with ELENC reduced these errors in a dose-dependent manner on both days 14 and 15, demonstrating memory protection. This improvement was comparable to that observed with donepezil.

In the Y-maze test, the total number of arm entries and the number of positive alternations were recorded. Scopolamine markedly reduced spontaneous alternation behavior, indicating impaired spatial awareness and cognitive performance[15]. Treatment with ELENC (100 and 400 mg/kg) and donepezil significantly increased the percentage of alternations compared to the disease control group, demonstrating protection against scopolamine-induced memory deficits and improvement in short-term spatial memory.

Dose-dependent cognitive improvement with ELENC was consistently observed in the Elevated Plus Maze, radial arm, and Y-maze assessments on both days 14 and 15, suggesting sustained memory-enhancing effects. These behavioral findings were further supported by the significant inhibition of AChE activity in the mouse brain tissue. The cognitive enhancement produced by ELENC may be attributed to the diverse bioactive constituents present in *Neolamarckia cadamba* leaves, including alkaloids, flavonoids, glycosides, polyacetylenes, triterpenoids, phenolic acids, saponins, sterols, and sesquiterpene lactones

5 CONCLUSION:

The present study demonstrated that the ethanolic leaf extract of *Neolamarckia cadamba* exhibits significant nootropic activity in a scopolamine-induced cognitive dysfunction model. Scopolamine caused pronounced impairments in learning, memory acquisition, retention, and consolidation, whereas treatment with the extract markedly improved cognitive performance in a dose-dependent way. These results indicate that the memory-enhancing effects of the extract may be linked to cholinergic modulation. The observed neuroprotective and cognitive benefits are likely associated with its diverse phytoconstituents, including alkaloids, flavonoids, glycosides, polyacetylenes, triterpenoids, and phenolic acids. Although behavioral and biochemical evaluations were performed in this study, further research is needed to isolate and characterize the active constituents of *Neolamarckia cadamba* and to confirm its therapeutic potential in dementia and related neurocognitive disorders.

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Conflict of Interest: The authors attest that they have no conflict of interest in this study.

Author's contributions: SM conceived and designed the study, supervised the experiments, analyzed the data, and drafted the manuscript. SA performed the experimental work and assisted in data analysis and manuscript preparation. SN and DK contributed to data interpretation and manuscript writing. EK provided scientific guidance and revised the manuscript. All authors read and approved the final manuscript.

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