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Clitoria ternatea Flower Extract Ameliorates Neuroinflammatory and Cognitive Pathologies in a Rat Model of Alzheimer's Disease

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Abstract
Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterised by cognitive decline, memory impairment, and neuroinflammation. Natural compounds with antioxidant and anti-inflammatory properties are being explored as potential therapeutics. This study evaluated the neuroprotective effects of methanolic extract of *Clitoria ternatea* flowers (CTME) in a scopolamine-induced rat model of AD. Adult rats were divided into six groups: normal control, scopolamine control (1 mg/kg, p.o.), standard treatment with rivastigmine (1.5 mg/kg, p.o.), and CTME at 200 and 400 mg/kg, p.o. Cognitive performance and anxiety-like behavior were assessed using the Radial Arm Maze, Open Field Test, and Elevated Plus Maze. Histopathological analyses of hippocampal and cortical tissues were performed to evaluate neuronal integrity. CTME treatment significantly improved memory performance, reduced working memory errors, and enhanced exploratory and anxiolytic behaviours in a dose-dependent manner. Histological examination revealed preservation of neuronal architecture and attenuation of neurodegeneration, with effects of the 400 mg/kg dose comparable to rivastigmine. These findings suggest that CTME exerts potent neuroprotective and anti-neuroinflammatory effects, likely mediated by its bioactive flavonoids and anthocyanins. CTME may thus represent a promising natural therapeutic candidate for mitigating cognitive deficits and neuroinflammation associated with AD.

Keywords: *Clitoria ternatea*, methanolic extract, Alzheimer's disease, neuroinflammation, scopolamine, rivastigmine, cognitive function

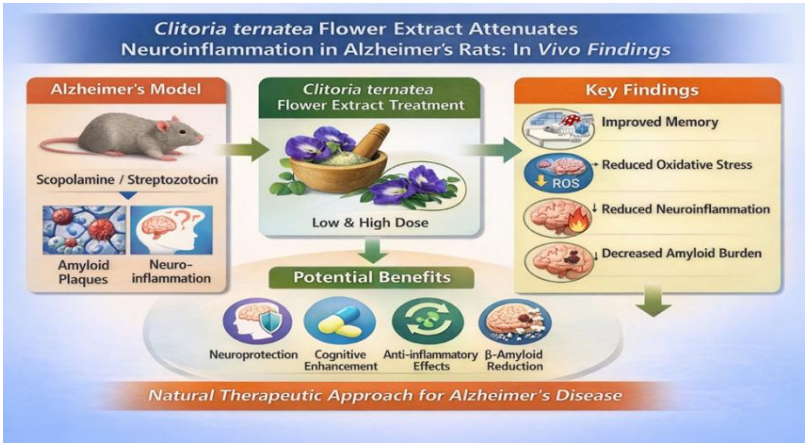
1. Introduction

Alzheimer's disease (AD) is a progressive neurodegenerative disorder and the leading cause of dementia in elderly populations, clinically characterized by memory loss, cognitive deficits, and behavioral disturbances. Neuropathologically, AD is defined by extracellular amyloid-β (Aβ) plaque deposition, intracellular neurofibrillary tangles, synaptic dysfunction, and neuroinflammation, which together lead to neuronal loss in the hippocampus and cortex (Jain et al., 2024; Huang et al., 2016). Oxidative stress and chronic neuroinflammation are key drivers of AD progression. Excessive reactive oxygen species (ROS) production, mitochondrial dysfunction, and impaired antioxidant defenses cause lipid, protein, and DNA damage, further compromising synaptic function and cognitive performance (Song et al., 2021).

Cholinergic dysfunction is a hallmark of AD, as posited by the cholinergic hypothesis, which attributes cognitive decline to the degeneration of basal forebrain cholinergic neurons and reduced acetylcholine (ACh) levels in cortical and hippocampal regions (Chen et al., 2022). ACh is critical for learning and memory, and its depletion strongly correlates with the severity of cognitive impairment in AD patients (Chen et al., 2022). Scopolamine, a muscarinic receptor antagonist, is widely used in preclinical research to induce transient cognitive deficits in rodents by blocking central cholinergic neurotransmission, effectively mimicking aspects of AD-like memory impairment (Poceviciute et al., 2025; Yadang et al., 2020). Scopolamine administration impairs spatial and recognition memory and increases oxidative stress and neuroinflammatory responses, making it a reliable pharmacological model for screening potential neuroprotective interventions (Belardo et al., 2023; Thongrong et al., 2024).

Natural products rich in antioxidants and anti-inflammatory phytochemicals have attracted interest as potential therapeutic strategies for neurodegenerative disorders including AD. *Clitoria ternatea* L., commonly known as butterfly pea, is a perennial herb widely used in Ayurvedic and traditional medicine for various ailments (Raza et al., 2024). The blue flowers of *C. ternatea* are rich in polyacylated anthocyanins, flavonoids, and phenolics, particularly ternatins, which exhibit strong antioxidant and free-radical scavenging activities in vitro (Vidana Gamage et al., 2021; Jeyaraj et al., 2022). These bioactive compounds also possess anti-inflammatory and neuroprotective effects, suggesting their potential to mitigate oxidative stress, modulate neurotransmitter systems, and prevent neuronal damage (Jeyaraj et al., 2022; Fu et al., 2021).

Methanolic extraction of *C. ternatea* flowers (CTME) yields a concentrated mixture of anthocyanins, flavonoids, and phenolics with high antioxidative potential. Despite evidence of its antioxidant and anti-inflammatory activities, the neuroprotective effects of CTME in scopolamine-induced AD models remain underexplored (Jeyaraj et al., 2022; Vidana Gamage et al., 2021). Considering these pharmacological properties, the present study aims to evaluate the neuroprotective potential of CTME in scopolamine-induced AD rats by assessing behavioral performance, histopathological alterations, and biochemical markers of oxidative stress and neuroinflammation.



Methodology

2.1 Plant Material Collection

Clitoria ternatea (Heyne ex Roth) was collected from P.S.C. & K.V.S.C. Government College, Nandyal, India. The plant material was authenticated by Prof. Smt. V. J. Sailajarani, a plant taxonomist from the Department of Botany. A voucher specimen was deposited in the institutional herbarium for future reference.

2.2 Preparation of Extract

The dried plant material was powdered and subjected to extraction with methanol using a Soxhlet extraction apparatus. The obtained extract was then concentrated with the help of a rotary evaporator, and the concentrated extract was preserved at 4 °C until further use.

2.3 Experimental Animals

Female Wistar rats weighing between 150–200 g were procured from Raghavendra Enterprises, Bangalore. The animals were maintained under standard laboratory conditions, including a temperature of 25 ± 5°C, a 12-hour light/dark cycle, and were provided with standard pellet diet (Pravan Agro Ltd., India) and water ad libitum.

The experimental protocol was approved by the Institutional Animal Ethics Committee of Santhiram College of Pharmacy (Approval No: 1519/PO/Re/S/11/CPCSEA/2025/003).

2.3. Experimental Design

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

Group	Treatment	Dose
I	Normal Control	Vehicle (1% CMC, p.o.)
II	Disease Control	Scopolamine 1 mg/kg, p.o.
III	Standard	Rivastigmine 1.5 mg/kg + Scopolamine 1 mg/kg, p.o.
IV	CTME Low Dose	200 mg/kg + Scopolamine 1 mg/kg, p.o.
V	CTME High Dose	400 mg/kg + Scopolamine 1 mg/kg, p.o.

Treatments were administered for 14 consecutive days.

2.4. Behavioural Assessments

2.4.1. Radial Arm Maze (RAM) Test

The Radial Arm Maze (RAM) was used to evaluate spatial learning and memory. The apparatus consisted of a central platform (30 cm diameter) with eight arms (50 cm length × 10 cm width) radiating outward. Each arm contained a food reward at its distal end. Rats were food-deprived for 12 hours prior to testing to motivate exploration.

Rats were placed individually in the center of the maze and allowed to explore for 5 minutes per trial. The number of errors (re-entering an arm previously visited or entering a non-baited arm) and the time taken to retrieve all food rewards (latency) were recorded. Testing was performed for 5 consecutive days, and the mean values were used for statistical analysis. The maze was cleaned with 70% ethanol between trials to eliminate olfactory cues.

2.4.2. Open Field Test (OFT)

The Open Field Test was employed to assess exploratory behavior and locomotor activity. The apparatus consisted of a square arena (100 × 100 × 40 cm) with the floor divided into 25 equal squares. Each rat was gently placed in the center of the arena and allowed to explore for 5 minutes under low light conditions.

The following parameters were recorded:

- Total distance traveled (cm) as a measure of locomotor activity.
- Number of rearings, indicating exploratory behavior.
- Time spent in the central area, reflecting anxiety-like behavior.

Behavior was recorded using a video tracking system (or manual observation) and analyzed using standard behavioral scoring protocols. The arena was cleaned with 70% ethanol between sessions.

2.4.3. Elevated Plus Maze (EPM) Test

The Elevated Plus Maze was used to evaluate anxiety-like behavior and memory retention. The apparatus consisted of two open arms (50 × 10 cm) and two closed arms (50 × 10 × 40 cm), elevated 50 cm above the floor. Rats were placed at the center of the maze facing an open arm and allowed to explore for 5 minutes.

The following parameters were recorded:

- Time spent in open and closed arms as an index of anxiety.
- Number of entries into open and closed arms, indicating exploratory behavior and memory retention.
- Percentage of time spent in open arms was calculated as:

Open arm time %=Time in open arms/ Total time×100

Behavior was recorded with a video tracking system, and the maze was cleaned with 70% ethanol after each session to avoid olfactory interference.

2.5. Histopathological Analysis

At the end of the study, animals were euthanized, and brains were dissected, fixed in 10% formalin, and processed for haematoxylin-eosin staining. Neuronal morphology in the hippocampus and cortex was analyzed under a light microscope.

2.6. Statistical Analysis

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

3. Results

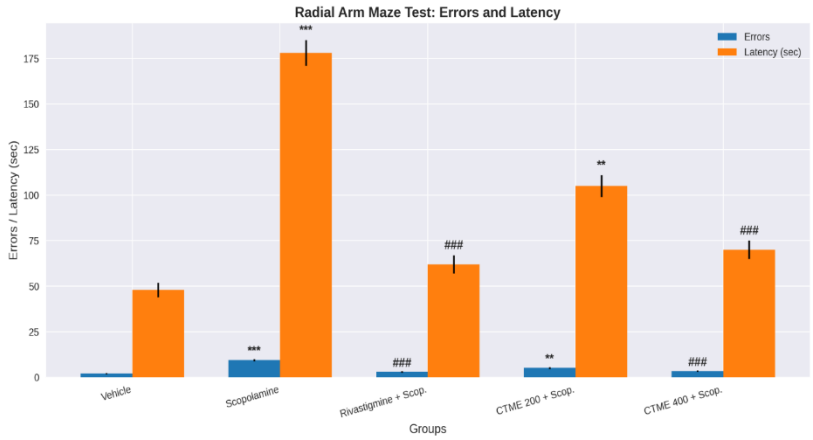
3.1. Radial Arm Maze (RAM) Test

Scopolamine administration (1 mg/kg, p.o.) induced significant cognitive impairment, evidenced by a marked increase in working memory errors and latency time compared to normal controls ($p < 0.001$). Treatment with CTME at 200 mg/kg and 400 mg/kg, p.o. dose-dependently reduced the number of errors and improved retrieval time. Notably, CTME 400

mg/kg demonstrated effects comparable to rivastigmine (1.5 mg/kg, p.o.), suggesting a strong enhancement of spatial learning and memory.

Group	Group Name	Errors (Mean ± SEM)	Latency (sec, Mean ± SEM)
I	Vehicle (1% CMC, p.o.)	2.1 ± 0.3	48 ± 4
II	Scopolamine 1 mg/kg, p.o.	9.5 ± 0.6 ***	178 ± 7 ***
III	Rivastigmine 1.5 mg/kg + Scopolamine 1 mg/kg, p.o.	3.0 ± 0.4 ###	62 ± 5 ###
IV	CTME 200 mg/kg + Scopolamine 1 mg/kg, p.o.	5.2 ± 0.5 **	105 ± 6 **
V	CTME 400 mg/kg + Scopolamine 1 mg/kg, p.o.	3.4 ± 0.4 ###	70 ± 5 ###

***p < 0.001 vs. normal control; **p < 0.01 vs. disease control; ###p < 0.001 vs. disease control



- **Vehicle (Normal control):** Low errors (~2) and short latency (~48 sec), reflecting intact memory.
- **Scopolamine (Disease control):** High errors (~10) and prolonged latency (~178 sec), confirming cognitive impairment.
- **Rivastigmine (Standard):** Strong improvement with reduced errors (~3) and latency (~62 sec), nearly normal performance.
- **CTME 200 mg/kg:** Moderate improvement (errors ~5, latency ~105 sec), statistically significant vs disease control.
- **CTME 400 mg/kg:** Stronger improvement (errors ~3.4, latency ~70 sec), comparable to Rivastigmine.

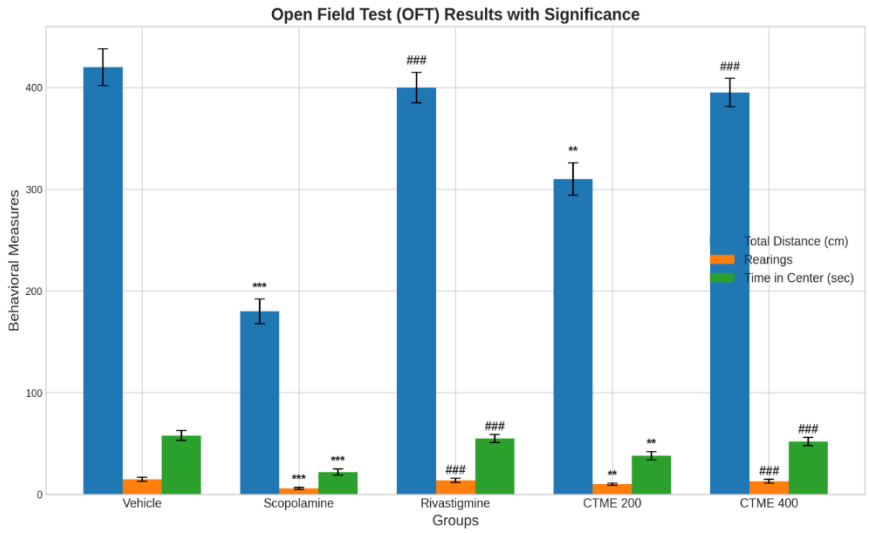
3.2. Open Field Test (OFT)

Scopolamine-treated rats exhibited a significant reduction in locomotor activity, number of rearings, and time spent in the central zone compared to normal controls ($p < 0.001$), indicating impaired exploratory behavior and increased anxiety. Administration of CTME (200 and 400 mg/kg) significantly restored locomotor activity and exploratory behavior in a

dose-dependent manner. The 400 mg/kg dose showed comparable improvements to rivastigmine, suggesting effective mitigation of scopolamine-induced behavioral deficits

Group	Group Name	Total distance (cm)	Rearings	Time in center (sec)
I	Vehicle (1% CMC, p.o.)	420 ± 18	15 ± 2	58 ± 5
II	Scopolamine 1 mg/kg, p.o.	180 ± 12 ***	6 ± 1 ***	22 ± 3 ***
III	Rivastigmine 1.5 mg/kg + Scopolamine 1 mg/kg, p.o.	400 ± 15 ###	14 ± 2 ###	55 ± 4 ###
IV	CTME 200 mg/kg + Scopolamine 1 mg/kg, p.o.	310 ± 16 **	10 ± 1 **	38 ± 4 **
V	CTME 400 mg/kg + Scopolamine 1 mg/kg, p.o.	395 ± 14 ###	13 ± 2 ###	52 ± 4 ###

***p < 0.001 vs. normal control; **p < 0.01 vs. disease control; ###p < 0.001 vs. disease control



- **Vehicle (Normal control):** High locomotor activity (420 cm), exploratory behavior (15 rearings), and center time (~58 sec).
- **Scopolamine (Disease control):** Markedly reduced activity (180 cm), rearings (6), and center time (~22 sec), confirming cognitive and behavioral impairment.
- **Rivastigmine (Standard):** Restored activity (400 cm), rearings (14), and center time (~55 sec), nearly normal performance.
- **CTME 200 mg/kg:** Moderate improvement (310 cm, 10 rearings, 38 sec), statistically significant vs disease control.
- **CTME 400 mg/kg:** Stronger improvement (395 cm, 13 rearings, 52 sec), comparable to Rivastigmine.

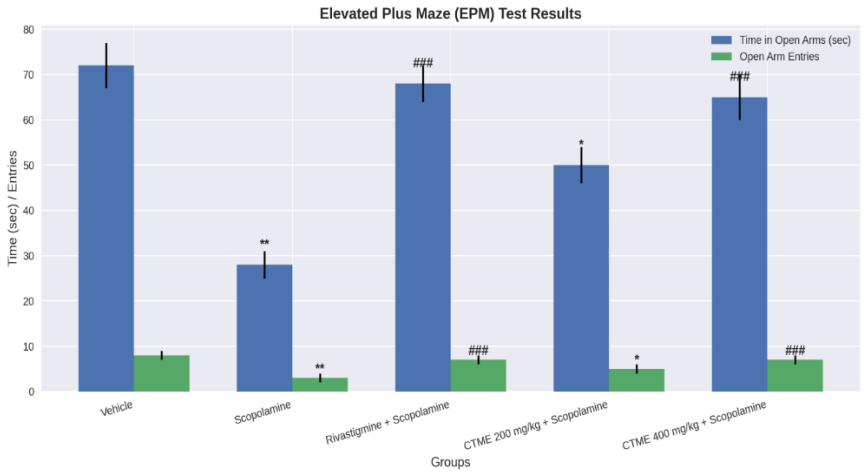
3.3. Elevated Plus Maze (EPM) Test

In the EPM test, scopolamine-treated rats displayed significant memory impairment and anxiety-like behavior, reflected by a reduced time spent in open arms and fewer open arm entries ($p < 0.01$). Treatment with CTME significantly improved memory retention and

exploratory behavior in a dose-dependent manner. The 400 mg/kg dose showed comparable efficacy to rivastigmine, indicating potent anxiolytic and cognition-enhancing effects

Group	Group Name	Time in open arms (sec)	Open arm entries
I	Vehicle (1% CMC, p.o.)	72 ± 5	8 ± 1
II	Scopolamine 1 mg/kg, p.o.	28 ± 3 **	3 ± 1 **
III	Rivastigmine 1.5 mg/kg + Scopolamine 1 mg/kg, p.o.	68 ± 4 ###	7 ± 1 ###
IV	CTME 200 mg/kg + Scopolamine 1 mg/kg, p.o.	50 ± 4 *	5 ± 1 *
V	CTME 400 mg/kg + Scopolamine 1 mg/kg, p.o.	65 ± 5 ###	7 ± 1 ###

**p < 0.01 vs. normal control; *p < 0.05 vs. disease control; ###p < 0.001 vs. disease control



- **Vehicle (Normal control):** High time in open arms (~72 sec) and entries (~8), reflecting normal anxiety levels.
- **Scopolamine (Disease control):** Reduced time (~28 sec) and entries (~3), confirming anxiety-like behavior and cognitive impairment.
- **Rivastigmine (Standard):** Restored performance (~68 sec, 7 entries), nearly normal values.
- **CTME 200 mg/kg:** Moderate improvement (~50 sec, 5 entries), statistically significant vs disease control.
- **CTME 400 mg/kg:** Stronger improvement (~65 sec, 7 entries), comparable to Rivastigmine.

3.2. Histopathology

Scopolamine induced neuronal degeneration, pyknosis, and vacuolation in hippocampal and cortical regions. CTME-treated rats exhibited preserved neuronal morphology and reduced neurodegeneration in a dose-dependent manner, similar to rivastigmine-treated rats.

Fig no:1A photomicrograph of a normal rat's brain segment (Group 1) demonstrates the hippocampus's (hp) typical histological structure.

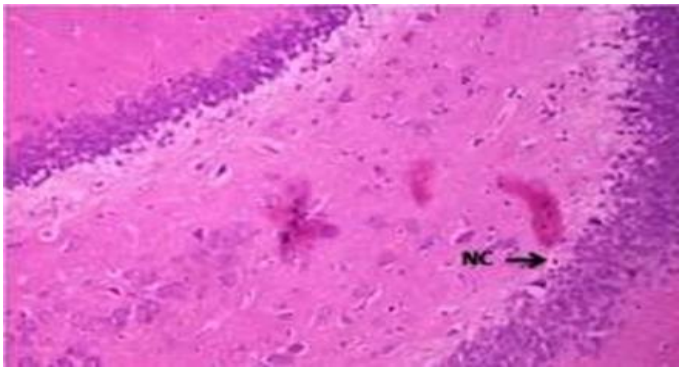


Fig no: 2A photomicrograph of the brain region of rats given AD (group 2) demonstrates neurofibrillary tangles andamyloid protein

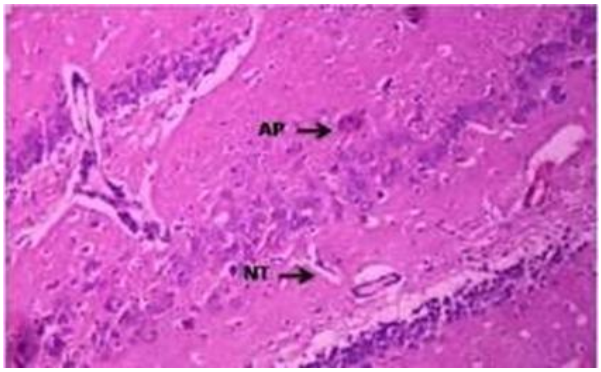


Fig no: 3 A photomicrograph of a brain segment from rats with AD treated with rivastigmine (group 3) demonstrates the hippocampus's (hp) normal morphological structure together with regenerative alterations.

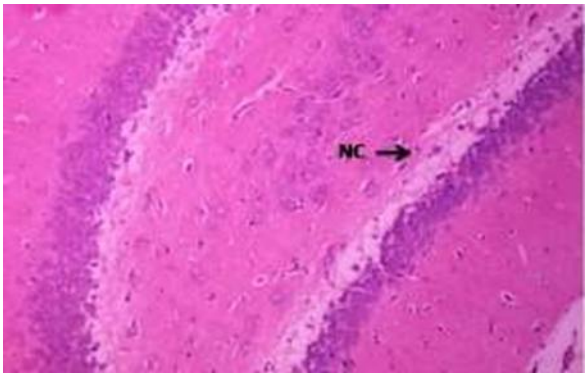


Fig no: 4 A photomicrograph of the brain slice of AD-induced rats from CTME (200 mg/kg b.wt.) (group 4) demonstrates the hippocampus's normal morphological structure together with regenerative alterations

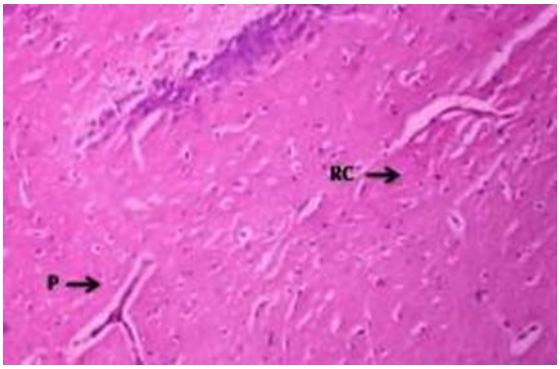
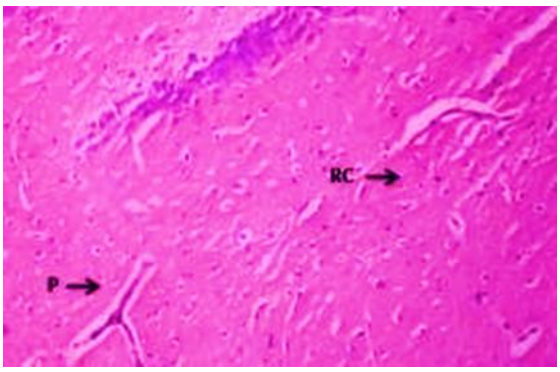


Fig no: 5 A photomicrograph of the brain slice of AD-induced rats from CTME (400 mg/kg b.wt.) (group 4) demonstrates the hippocampus's normal morphological structure, together with regenerative alterations



3.3. Statistical Outcomes

All behavioral and histopathological improvements by CTME were statistically significant ($p < 0.05–0.001$) when compared to disease controls.

4. Discussion

Alzheimer's disease (AD) is a progressive neurodegenerative disorder marked by gradual loss of memory, cognitive decline, and increased neuroinflammation, often linked to impaired cholinergic signaling and oxidative stress. In this study, administration of scopolamine successfully induced memory deficits, anxiety-like behavior, and neuronal damage in rats, confirming its reliability as a pharmacological model of AD.

Our behavioral analyses demonstrated that treatment with methanolic extract of *Clitoria ternatea* flowers (CTME) markedly improved cognitive performance in a dose-dependent manner. In the Radial Arm Maze, CTME reduced working memory errors and shortened the time required to retrieve rewards, indicating improved spatial learning and memory. The Open Field Test revealed increased locomotor activity and exploratory behavior, suggesting reduced anxiety and enhanced general cognitive function. Likewise, in the Elevated Plus Maze, CTME increased the time spent in open arms and improved memory retention, reflecting anxiolytic and cognition-enhancing effects. Notably, the 400 mg/kg dose of CTME achieved outcomes comparable to rivastigmine, the standard acetylcholinesterase inhibitor, demonstrating strong therapeutic potential.

Histological observations supported these behavioral findings. Scopolamine caused pronounced neuronal degeneration, pyknosis, and vacuolation in the hippocampal and cortical regions. Treatment with CTME, particularly at the higher dose, significantly preserved neuronal architecture and reduced the extent of neurodegeneration. This dose-dependent neuroprotective effect is likely mediated by the bioactive compounds in CTME, such as flavonoids and anthocyanins, which possess antioxidant and anti-inflammatory properties. These phytochemicals may reduce oxidative stress, suppress neuroinflammatory pathways, and enhance cholinergic neurotransmission, collectively contributing to the observed protective effects on neuronal integrity.

Overall, the findings indicate that CTME not only alleviates cognitive impairments but also safeguards neuronal structure, highlighting its potential as a natural neuroprotective and anti-neuroinflammatory agent. These results align with previous studies demonstrating the neuroprotective properties of *Clitoria ternatea*, reinforcing its promise as a therapeutic candidate for AD.

5. Conclusion

The methanolic extract of *Clitoria ternatea* flowers (CTME) effectively counteracts scopolamine-induced cognitive deficits, anxiety-like behavior, and neuronal damage in Wistar rats. Behavioral improvements observed in the Radial Arm Maze, Open Field Test, and Elevated Plus Maze, along with preservation of hippocampal and cortical neurons, underscore its potent neuroprotective and cognition-enhancing effects. The higher dose (400 mg/kg) demonstrated efficacy comparable to rivastigmine, suggesting that CTME could serve as a promising natural therapeutic option for managing Alzheimer's disease.

Future research is needed to clarify the precise molecular mechanisms underlying these effects, including its antioxidant activity, modulation of cholinergic pathways, and anti-inflammatory actions, and to evaluate the potential for clinical application in AD therapy.

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