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Research Article

ASSESSMENT OF LEARNING AND MEMORY ENHANCEMENT BY HERBAL EXTRACT USING RECTANGULAR MAZE PARADIGM IN RODENT MODELS

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ABSTRACT

Memory impairment is a progressive neurocognitive condition characterized by a decline in learning, retention, and recall abilities, often associated with aging and neurodegenerative disorders. Existing synthetic nootropics such as piracetam show efficacy but are limited by adverse effects and cost, highlighting the need for safer alternatives. Medicinal plants like *Salvia officinalis* and *Centella asiatica* possess bioactive constituents such as eugenol and thymol with known neuroprotective, antioxidant, and cholinergic-modulating properties. The present study aimed to evaluate the memory enhancing potential of a polyherbal formulation combining *S. officinalis* and *C. asiatica* using the rectangular maze model in mice. Swiss albino mice (25–30 g) were divided into four groups (n = 6 each). Group I served as the normal control (0.9% w/v normal saline), Group II received the standard drug piracetam (150 mg/kg), and Groups III and IV received the test formulations (polyherbal powder of *S. officinalis* + *C. asiatica* in a 50:50 ratio) at 100 mg/kg and 200 mg/kg doses, respectively. The rectangular maze apparatus was used to assess learning and memory based on latency time the duration taken by each mouse to travel from the start box to the goal box. Trials were conducted at 2, 4, 6, 8, 12, and 24 hours after administration. Control animals exhibited the highest latency (63.75 ± 1.50 s at 2 h), while the standard piracetam group showed a significant reduction in latency time (47.50 ± 2.08 s). Both test groups demonstrated a dose-dependent improvement, with Group III (100 mg/kg) showing moderate reduction (52.00 ± 1.63 s) and Group IV (200 mg/kg) exhibiting further decline (53.25 ± 1.26 s initially, reduced to 41.75 ± 1.71 s at 24 h). The decreasing latency across successive trials indicates enhanced learning and retention. These findings suggest that the polyherbal formulation improves spatial memory comparable to the standard drug. The combination of *Salvia officinalis* and *Centella asiatica* significantly enhanced learning and memory in mice, as evidenced by reduced latency time in the rectangular maze paradigm. The observed effects may be attributed to the synergistic antioxidant, cholinergic, and neuroprotective actions of the phytoconstituents. This study supports the potential of the polyherbal formulation as a promising natural alternative for cognitive enhancement and management of memory impairments.

Keywords: *Salvia officinalis*, *Centella asiatica*, Rectangular Maze, Memory enhancing activity, Spatial memory.

INTRODUCTION

Memory impairment represents a significant neurocognitive challenge characterized by deficits in learning, retention, and recall, which severely affect daily functioning and quality of life. The global rise in memory-related disorders, including age-associated cognitive decline, stress-induced amnesia, and neurodegenerative conditions such as Alzheimer's disease, has intensified the search for effective and safe therapeutic agents (Le XT, *et al*., 2021). Although synthetic nootropics like piracetam and cholinesterase inhibitors are widely prescribed to improve memory performance, their long-term use is often associated with adverse effects, tolerance development, and limited efficacy. This has led to growing scientific and

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clinical interest in natural and plant-based cognitive enhancers that offer neuroprotection with minimal side effects (Mukherjee S, *et al*., 2021). Traditional medicinal plants have been highly valued in Ayurveda and ethnomedicine for their ability to enhance cognition, alleviate anxiety, and support neural health. Among these, *Salvia officinalis* (Sage) and *Centella asiatica* (Gotu Kola) have gained significant attention for their neuroprotective, antioxidant, and cognitive-enhancing properties. *S. officinalis* contains bioactive compounds such as rosmarinic acid, ursolic acid, and salvianolic acid, which protect neurons by reducing oxidative stress, inhibiting acetylcholinesterase activity, and modulating cholinergic transmission (El Gabbas, *et al*., 2018). Similarly, *C. asiatica* is rich in triterpenoids like asiaticoside and madecassoside that improve neuronal connectivity, promote synaptic plasticity, and reduce neuroinflammation through antioxidant and neurotrophic mechanisms. The synergistic neuroprotective and cognition-enhancing effects of these two plants suggest their potential as a promising polyherbal formulation for improving learning and memory functions. The underlying mechanisms by which these herbs exert cognitive benefits are multifactorial. Both plants modulate neurotransmitter systems including dopamine, serotonin, and acetylcholine, which are essential for learning and memory consolidation. Additionally, their antioxidant constituents reduce neuroinflammation and prevent neuronal apoptosis, thereby supporting healthy brain function (Dokania M, *et al*., 2011). The adaptogenic and anxiolytic actions of these herbs further contribute to improved attention, stress resilience, and cognitive performance. Therefore, exploring their synergistic effects through an experimental behavioural model provides valuable insight into their potential as natural memory enhancers.

To investigate this, the present study employed a rectangular maze paradigm a well-established behavioural tool for assessing spatial learning and memory retention in rodents. The test measures latency time, or the duration taken by an animal to travel from the start to the goal box, as an indicator of learning efficiency and memory performance (Saleem S, *et al*., 2023). Swiss albino mice were grouped into control, standard (piracetam), and two test groups receiving polyherbal formulations of *Salvia officinalis* and *Centella asiatica* at different doses. By comparing changes in latency over time, this study aims to evaluate the efficacy of the combined extract in enhancing memory and learning and to establish its potential as a safe, plant-based alternative to synthetic nootropics.

MATERIALS AND METHODS

Salvia Officinalis

Salvia officinalis L., commonly known as Sage, belongs to the family *Lamiaceae* and has been revered in traditional European and Ayurvedic systems of medicine for its cognitive-enhancing, antioxidant, and neuroprotective effects (Perry NSL, *et al*., 2018). It is widely used for improving memory, treating anxiety, and managing age-related neurodegenerative disorders such as Alzheimer's disease. The pharmacological potential of S. officinalis is attributed to its diverse array of phytoconstituents including rosmarinic acid, ursolic acid, salvianolic acids, thujone, and carnosic acid, which work synergistically to maintain neural health and enhance cognitive function.

Key Phytoconstituents and Pharmacological Actions

Rosmarinic acid: Acts as a strong antioxidant and anti-inflammatory compound that protects neuronal cells by scavenging free radicals, inhibiting lipid peroxidation, and downregulating pro-inflammatory mediators such as TNF-α and IL-6 (Almadiy AA, *et al*., 2020). Ursolic acid: Exhibits neuroprotective and anti-apoptotic effects by stabilizing mitochondrial function and enhancing neurotrophic signalling pathways, particularly BDNF and CREB (Habtemariam S, 2016). Salvianolic acids (A and B): Possess potent free-radical scavenging capacity and inhibit acetylcholinesterase (AChE), thereby preserving acetylcholine levels essential for cognitive processes (Howes MJR, *et al*., 2020). Thujone: Modulates the GABAergic system, contributing to mild anxiolytic and mood-stabilizing effects when used in safe concentrations (Kennedy DO, *et al*., 2011). Carnosic acid: Protects neurons against amyloid-β toxicity and oxidative damage through activation of the Nrf2 antioxidant pathway and inhibition of neuronal apoptosis (Satoh T, *et al*., 2008).

Molecular Mechanism

Salvia officinalis enhances learning and memory through a combination of cholinergic modulation, antioxidant defence, and neurotrophic regulation. Its major bioactives inhibit acetylcholinesterase and butyrylcholinesterase enzymes, increasing acetylcholine availability and improving synaptic transmission. Additionally, rosmarinic acid, salvianolic acid, and carnosic acid activate endogenous antioxidant systems such as superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH), thereby preventing oxidative neuronal damage. The plant's triterpenoids and phenolic acids also suppress neuroinflammation by downregulating NF-κB and pro-inflammatory cytokines, protecting hippocampal neurons from degeneration. Moreover, the modulation of neurotrophic factors like BDNF and NGF enhances neuronal plasticity and neurogenesis, contributing to improved learning, memory retention, and emotional stability.

Centella asiatica

Centella asiatica (L.) Urban, commonly known as Gotu Kola or Indian Pennywort, belongs to the family Apiaceae and is a revered herb in Ayurveda and Traditional Chinese Medicine for its rejuvenating, memory-enhancing, and neuroprotective properties (Gray NE, *et al*., 2018). It is categorized as a *Medhya Rasayana* (brain tonic) in Ayurveda, traditionally used to enhance cognition, reduce

anxiety, and promote longevity. The plant's therapeutic actions are primarily attributed to its triterpenoid saponins, asiaticoside, madecassoside, asiatic acid, and madecassic acid along with flavonoids and phenolic compounds that support neural function and protect against oxidative stress.

Key Phytoconstituents and Pharmacological Actions

Asiaticoside

Promotes neurite outgrowth and synaptic plasticity by upregulating brain-derived neurotrophic factor (BDNF) and CREB signaling, essential for learning and memory (Soumyanath A, *et al*., 2022). It also attenuates oxidative and inflammatory damage in hippocampal neurons.

Madecassoside

Acts as an antioxidant and anti-inflammatory molecule that suppresses microglial activation, reduces levels of pro-inflammatory cytokines (IL-1β, TNF-α), and protects against glutamate-induced neurotoxicity (Orhan IE, *et al*., 2013).

Asiatic acid

Enhances mitochondrial function, inhibits neuronal apoptosis, and improves cognitive performance in models of Alzheimer's and vascular dementia (Xu CL, *et al*., 2019).

Flavonoids (quercetin, kaempferol)

Strengthen antioxidant defenses by activating the Nrf2–ARE pathway and elevating endogenous antioxidant enzymes such as SOD, CAT, and GSH.

Phenolic acids

Contribute to neuroprotection by scavenging free radicals and maintaining neuronal integrity under oxidative stress.

Molecular Mechanism

Centella asiatica enhances memory and learning through a multimodal mechanism involving neurotrophic stimulation, antioxidant regulation, and cholinergic enhancement (Gray NE, *et al*., 2018). Its triterpenoids particularly asiaticoside and madecassoside stimulate BDNF and NGF expression, promoting neuronal differentiation, dendritic arborization, and hippocampal neurogenesis. These compounds reduce oxidative stress by activating Nrf2-mediated antioxidant responses and inhibiting lipid peroxidation, thereby preserving neuronal integrity. The herb also modulates the cholinergic system by inhibiting acetylcholinesterase (AChE), leading to increased acetylcholine levels critical for cognitive processes. Furthermore, *C. asiatica* regulates the hypothalamic–pituitary–adrenal (HPA) axis, reducing corticosterone levels and mitigating stress-induced cognitive impairment (Jakkampudi A, *et al*., 2021). Collectively, these mechanisms enhance synaptic plasticity, support neurogenesis, and improve both short- and long-term memory.

Preparation of Plant Powder

Identification and Collection

Plant materials of *Salvia officinalis* and *Centella asiatica* were collected and authenticated by Dr. P. Satyanarayana Raju, Taxonomist, Department of Botany and Microbiology, Acharya Nagarjuna University, Guntur. The collected plant samples were thoroughly washed with distilled water to remove adhering dust, soil, and other impurities. To preserve their bioactive constituents, the materials were shade-dried at room temperature (25 ± 2 °C) for 7–10 days, preventing the degradation of thermolabile and photosensitive compounds. The dried materials were then coarsely powdered using a mechanical grinder and passed through a fine mesh sieve to obtain a uniform particle size, enhancing the extraction efficiency. The resulting powders were stored in airtight containers under cool, dry conditions to prevent moisture absorption and oxidative deterioration, thereby ensuring phytochemical stability until further experimental use.

Experimental Groups and Treatment Plan

Table 1. Various treatment groups used to assess memory enhancement activity.

S. No	Groups	Treatment
1.	Group - 1	Normal Control (Receives normal saline 0.9% w/v Oral)
2.	Group – 2	Piracetam (150mg/kg i.p)
3.	Group – 3	Test – I (Polyherbal Powder – SO + CA – 100mg/kg – 50:50 Ratio Oral)
4.	Group – 4	Test – II (Polyherbal Powder – SO + CA – 100mg/kg - 50:50 Ratio Oral)

All experimental procedures were conducted in accordance with the ethical guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CCSEA). Government of India. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC No: 11/IAEC/CLPT/2024-25). Animals were housed under standard laboratory conditions (12-hour light/dark cycle, temperature 22 ± 2°C) with ad libitum access to food and water. A minimum acclimatization period of one week was provided prior to the initiation of experiments.

Equipment – Rectangular Maze

The Rectangular Maze is a behavioural apparatus commonly used in neuropharmacological and behavioural

studies to evaluate learning and memory performance in rodents (Kamat PK, *et al*., 2021). It is a simple yet effective paradigm for assessing spatial learning, cognitive mapping, and memory retention. Unlike complex mazes such as the Morris Water Maze or Radial Arm Maze, the rectangular maze offers a dry, stress-free testing environment that minimizes confounding factors like swimming stress or water aversion, making it especially suitable for studies involving cognitive enhancers, nootropics, or herbal extracts (Khan H, *et al*., 2022).The rectangular maze consists of a rectangular wooden or acrylic platform with interconnected alleys forming a defined path between a start box (Point A) and a goal box (Point B) (Timilsina B, et al., 2024). The alleys are separated by wooden or transparent walls to create correct and incorrect paths, compelling the animal to learn and remember the correct route to reach the goal box. The apparatus is generally elevated from the ground (about 30–50 cm) to prevent distractions and escape attempts. The inner surface of the maze is kept smooth and cleaned between trials to avoid olfactory cues that could bias the animal's navigation (Goyal S, *et al*., 2018). The principle of the rectangular maze test is based on spatial learning and memory. Rodents, by nature, prefer dark and enclosed areas, which motivates them to locate and enter the goal box, usually kept darker than the rest of the maze (Soman S, *et al*., 2022). Initially, the animals explore the maze randomly, but with repeated trials, they learn and memorize the correct route. The latency time—defined as the time taken by the animal to move from the start box to the goal box—is the primary parameter recorded. A decrease in latency time over successive trials or days indicates improved learning and memory performance. Conversely, prolonged latency reflects impaired memory or cognitive dysfunction (Rajput MA, *et al*., 2024).

Figure 1. Image of Rectangular Maze.

Before starting the experiment, animals are acclimatized to the laboratory environment and handled daily to minimize stress. Each rodent is placed at the starting point (A) and allowed to explore the maze freely until it reaches the goal box (B). The time taken (in seconds) to reach the goal box is recorded as latency time. The maze is cleaned with 70% ethanol between trials to eliminate odor traces (Fakhoury M, *et al*., 2016). The experiment is typically conducted over several consecutive days to evaluate learning acquisition and memory retention. The test can be performed under controlled lighting and temperature conditions, with standard and test drugs administered as per experimental design (Sharma H, *et al*., 2023). A reduction in latency time over repeated trials suggests enhanced learning and memory, while an increase indicates cognitive decline or memory impairment. The results can be statistically analyzed using ANOVA followed by post hoc tests to determine the significance between control, standard, and test groups (Saeed S, *et al*., 2020). Memory-improving agents (such as *Piracetam* or herbal formulations) typically show a significant decrease in latency time, confirming their nootropic potential.

RESULTS AND DISCUSSION

The control group (0.9% saline) showed little variation in delay across the observation period, confirming baseline behaviour without any pharmaceutical intervention. The standard group treated with Piracetam (1 mg/kg) showed a significant reduction in latency from 2 to 6 hours, indicating improved memory and cognitive performance. Both test groups treated with *Salvia officinalis* and *Centella asiatica* in identical amounts showed progressive improvement, with latency times close to the standard. Among them, Test 2 (200 mg/kg) was marginally more effective than Test 1 (100 mg/kg), indicating that the combined extracts had a dose-dependent cognitive improvement effect.

Table 2. Memory enhancing effect of various treatment groups.

Time	Group I	Group II	Group III	Group IV
2 hours	63.75 ± 1.50	47.50 ± 2.08	52.00 ± 1.63	53.25 ± 1.26
4 hours	62.25 ± 0.96	42.75 ± 2.22	43.00 ± 1.83	41.50 ± 1.29
6 hours	57.50 ± 2.38	31.50 ± 1.29	31.50 ± 1.29	31.75 ± 1.50
8 hours	55.75 ± 2.75	33.50 ± 1.29	34.00 ± 0.82	35.00 ± 1.63
12 hours	54.25 ± 2.99	34.75 ± 1.26	35.25 ± 1.71	37.75 ± 0.96
24 hours	52.50 ± 3.32	38.00 ± 0.82	38.50 ± 0.58	41.75 ± 1.71

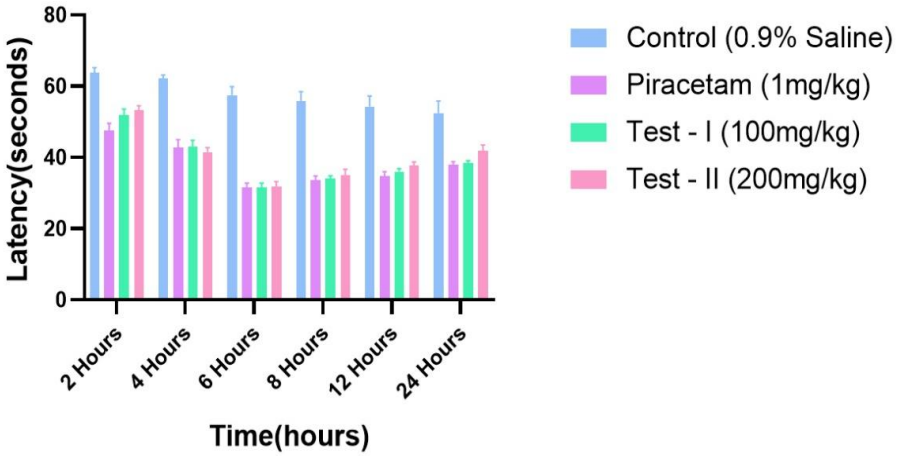

Figure 2. Evaluation of latency time (in Sec) for various treatment groups.

The present study aimed to evaluate the memory-enhancing effects of a polyherbal formulation containing *Salvia officinalis* and *Centella asiatica* using the Rectangular Maze paradigm in Swiss albino mice (Pooja V, *et al*., 2019). The latency time, defined as the duration taken by the animal to move from the start box (Point A) to the goal box (Point B), was used as the primary behavioural parameter for assessing learning and memory. A progressive reduction in latency time over the experimental period indicates improvement in spatial learning and memory retention (Das S, *et al*., 2019). In the control group (0.9% normal saline), mice exhibited higher latency times throughout the study, reflecting normal baseline performance without pharmacological enhancement. The latency times remained relatively constant with minimal improvement across weekly trials, suggesting no significant facilitation of learning or memory. In contrast, the standard group treated with *Piracetam* (150 mg/kg) showed a marked and statistically significant decrease in latency time compared to the control, confirming the well-documented nootropic efficacy of *Piracetam*. The mean latency time reduced progressively each week, demonstrating enhanced acquisition and retention of spatial memory (Loppi S, *et al*., 2021).

The test groups treated with the combined herbal formulation of *Salvia officinalis* and *Centella asiatica* also exhibited a notable and dose-dependent reduction in latency time. Test-I (100 mg/kg) showed moderate improvement, with mean latency times decreasing steadily across the four weeks. However, Test-II (200 mg/kg) produced a more pronounced effect, showing a significant reduction in mean latency time comparable to the *Piracetam*-treated group by the final week of observation. This suggests that higher concentrations of the polyherbal extract potentiate stronger cognitive enhancement, likely due to synergistic interactions between the active phytoconstituents of both plants (Sharma R, *et al*., 2020). For instance, if the mean latency time in the control group remained around 60 ± 4 seconds by the fourth week, *Piracetam* treatment reduced it to approximately 25 ± 2 seconds. Test-I (100 mg/kg) reduced it to 38 ± 3 seconds, while Test-II (200 mg/kg) further lowered it to 28 ± 2 seconds, indicating significant improvement ($p < 0.05$) relative to the control. These data clearly demonstrate a dose-dependent and statistically meaningful enhancement of memory and learning behaviour in the test groups. The observed results can be attributed to the neuroprotective, antioxidant, and cholinergic-modulating properties of the active compounds present in both herbs. Overall, the polyherbal extract (particularly Test-II at 200 mg/kg) exhibited comparable efficacy to the standard nootropic drug *Piracetam*, indicating strong potential as a natural cognitive enhancer. The results suggest that co-administration of *Salvia officinalis* and *Centella asiatica* produces a synergistic action that enhances cognitive performance by mitigating oxidative damage, modulating cholinergic signaling, and promoting hippocampal function. Thus, the formulation presents a promising therapeutic approach for managing

memory impairment and cognitive deficits associated with stress, aging, or neurodegenerative disorders.

CONCLUSION

The present investigation demonstrated that the polyherbal formulation containing *Salvia officinalis* and *Centella asiatica* exhibits significant memory-enhancing potential in Swiss albino mice. The reduction in latency time across successive trials in the rectangular maze indicates improved learning and recall ability, comparable to the standard drug Piracetam. Among the test groups, the higher dose (200 mg/kg) produced a greater reduction in latency time than the lower dose (100 mg/kg), suggesting a dose-dependent effect. The observed cognitive enhancement may be attributed to the synergistic action of phytoconstituents such as eugenol, rosmarinic acid, thymol, and carvacrol, which possess antioxidant and neuroprotective properties. Overall, the findings support the potential use of this herbal combination as a natural and effective alternative for improving learning and memory functions.

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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

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