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Research Article

MAZE LEARNING MEETS AI: SMART ANALYSIS OF MEMORY ENHANCEMENT IN RODENTS USING 8 ARM RADIAL MAZE

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ABSTRACT

Memory and learning are crucial cognitive functions, often compromised in neurodegenerative disorders such as Alzheimer's disease, dementia, and age-related cognitive decline. There is increasing interest in herbal nootropic agents due to their safety and reduced side-effect profile compared to synthetic drugs. *Ocimum sanctum* (Tulasi) and *Trachyspermum ammi* (Ajwain) are two medicinal plants traditionally used in Ayurveda for their neuroprotective and adaptogenic properties. This study investigates the memory-enhancing effects of a combination of *Ocimum sanctum* and *Trachyspermum ammi* powders at two different concentrations (100 mg/kg and 200 mg/kg), compared with the standard nootropic drug Piracetam (1 mg/kg), using the 8-Arm Radial Maze model in mice. Male mice were randomly divided into four groups: Group A (Control, 0.9% saline), Group B (Piracetam 1 mg/kg), Group C (Test 1: 100 mg/kg of Tulasi-Ajwain combination in 1:1 ratio), and Group D (Test 2: 200 mg/kg of the same combination). Each group contained four animals (n = 4). The 8-Arm Radial Maze was used to assess spatial memory by recording the number of correct arm entries (out of 8) at 2, 4, 6, 8, 12, and 24 hours post-administration. Higher numbers of correct entries indicate better memory performance. The control group (Group A) showed a gradual increase in correct entries over time, reaching an average of ~11 at 24 hours. Group B (Piracetam) consistently demonstrated improved performance, with an average of 15 correct entries at 24 hours, indicating strong nootropic activity. Test groups C and D, receiving the herbal combination at 100 mg/kg and 200 mg/kg respectively, also showed significant improvement in correct entries over time. Group D (200 mg/kg) exhibited superior performance among the test groups, with correct entries reaching 12–17 by 24 hours, closely approaching Piracetam's efficacy. Group C (100 mg/kg) showed moderate but consistent improvement, with 11–15 correct entries at 24 hours. These results suggest a dose-dependent enhancement of spatial memory by the herbal combinations. The present study demonstrates that the combination of *Ocimum sanctum* (Tulasi) and *Trachyspermum ammi* (Ajwain) exerts significant memory-enhancing effects in mice, as evidenced by improved performance in the 8-Arm Radial Maze test. Both test doses (100 mg/kg and 200 mg/kg) showed progressive enhancement in spatial memory, with the higher dose (200 mg/kg) producing results comparable to the standard nootropic drug Piracetam. These findings suggest a dose-dependent nootropic potential of the Tulasi–Ajwain combination, supporting its traditional use in cognitive enhancement and highlighting its promise as a safe, herbal alternative for managing cognitive decline and neurodegenerative disorders.

Keywords: *Ocimum sanctum*, *Trachyspermum ammi*, Nootropic, Memory enhancement, Spatial memory.

INTRODUCTION

Memory and learning have led to increasing interest in herbal and natural nootropic substances, which possess Memory and learning are essential cognitive processes that form the basis of intelligence and behavior. Impairment in these functions is a characteristic feature of several neurological disorders such as Alzheimer's disease, dementia, and age-related cognitive decline. The search for safe and effective agents that can enhance fewer side effects compared to synthetic drugs. The 8-Arm Radial Maze is one of the most widely used behavioral models for evaluating spatial learning and memory in laboratory animals such as mice and rats. The maze consists of a central platform from which eight arms radiate outward,

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each arm potentially containing a food reward. During the training and testing sessions, animals learn to remember which arms they have already visited and which ones contain rewards. The number of errors made and the time taken to complete the task serve as indicators of memory and learning performance. Hence, this model is highly reliable for screening potential memory-enhancing (nootropic) agents. In the present study, Piracetam is used as the standard drug, as it is a well-established nootropic that improves memory and cognitive function. Piracetam acts by enhancing neuronal communication, improving cerebral blood flow, and modulating neurotransmitter functions, particularly those related to acetylcholine, which is vital for learning and memory processes.

Trachyspermum ammi L. (Ajwain / Carom Seeds), belonging to the family Apiaceae, is an annual aromatic herb native to Egypt and the Eastern Mediterranean (Timalsina *et al*., 2023), now widely cultivated across India. The plant grows up to 60–90 cm tall, with feathery leaves, small white umbels of flowers, and oval brownish fruits possessing a distinct thyme-like aroma due to thymol-rich essential oil (Sharma *et al*., 2023). Its phytochemical profile includes thymol, carvacrol, γ-terpinene, p-cymene, flavonoids, saponins, and tannins, which contribute to its diverse pharmacological activities such as (Timalsina *et al*.,2023), antioxidant, antimicrobial, anti-inflammatory, hepatoprotective, (Ghasemi *et al*.,2023), and neuroprotective effects. Traditionally, Ajwain is used in Ayurveda and Unani systems as a digestive aid, (Musa *et al*.,2024) carminative, and remedy for colds, coughs, and abdominal discomfort.

Ajwain exhibits notable memory-enhancing properties attributed to its key compounds, (Hejazian *et al*.,2014), thymol and carvacrol, which improve cholinergic transmission and reduce oxidative stress in the brain. These bioactives act as acetylcholinesterase inhibitors, increasing acetylcholine levels and enhancing learning and memory. They also provide neuroprotection by scavenging free radicals, (Bairwa *et al*., 2012) suppressing neuroinflammation (via NF-κB inhibition), and supporting mitochondrial function. Flavonoids and other polyphenols further protect neurons by preventing lipid peroxidation and promoting synaptic plasticity. Collectively, these mechanisms help maintain neuronal integrity, regulate neurotransmitters like acetylcholine, dopamine, and GABA, and enhance cognitive performance under oxidative or inflammatory stress.

Ocimum sanctum L. (Holy Basil / Tulsi), belonging to the family Lamiaceae, (Arya *et al*.,2024) is a sacred aromatic herb widely cultivated across India and Southeast Asia. It is a small, (Rodrigues *et al*.,2022) branched perennial plant with fragrant leaves, purplish flowers, and oval fruits. The plant contains essential oils rich in eugenol, ursolic acid, carvacrol, linalool, and flavonoids such as orientin and vicenin. Traditionally, (Pushpangadan *et al*.,2012) Tulsi has been revered in Ayurveda for its adaptogenic, antimicrobial, antidiabetic, (Joshi *et al*.,2006) and anti-inflammatory properties and is used to treat respiratory ailments, digestive disorders, and stress-related conditions. Tulsi demonstrates strong neuroprotective (Stockburger *et al*., 2016) and memory-enhancing effects due to its antioxidant and cholinergic-modulating properties. Compounds like eugenol and ursolic acid scavenge free radicals, reduce lipid peroxidation, and protect neurons from oxidative and inflammatory damage. Flavonoids such as orientin and vicenin enhance cognitive performance by improving synaptic transmission and reducing stress-induced neurodegeneration. Tulsi also regulates neurotransmitters like dopamine, serotonin, and acetylcholine, improving learning, memory, and focus. Through its combined antioxidant, anti-inflammatory, and adaptogenic mechanisms, Ocimum sanctum supports brain health and prevents cognitive decline associated with aging and oxidative stress.

MATERIALS AND METHODS

Preparation of plant powder

The collected plants of *Trachyspermum ammi* (Ajwain), (Rahman *et al*., 2018), and *Ocimum sanctum* (Tulsi), (Le *et al*.,2019), were shade-dried and coarsely powdered. The powders were mixed in an equal ratio (1:1 w/w) to prepare the test formulation (Hening *et al*.,2018). For administration, (Sarker *et al*.,2006), the mixed powder was suspended in distilled water to achieve the desired concentrations corresponding to two dose levels: Test I (100 mg/kg) and Test II (200 mg/kg). The required dose for each mouse was calculated based on its body weight, and the suspension was freshly prepared each day to ensure stability. The formulation was administered orally by mixing with the animals' drinking water, maintaining a calculated intake volume of 4 mL per animal. The suspension was continuously stirred to ensure uniform dispersion of the powder throughout administration.

Experimental Groups

The experimental animals were divided into four groups, each consisting of 6 number of mice: Group I – Control: Received 0.9% v/v normal saline orally, serving as the baseline control group. Group II – Standard: Administered Piracetam at a dose of 150 mg/kg/day orally, serving as the reference standard for cognitive enhancement. Group III – Test I: Received the polyherbal formulation of *Trachyspermum ammi* and *Ocimum sanctum* (1:1 ratio) at a dose of 100 mg/kg/day orally. Group IV – Test II: Received the same polyherbal formulation at a higher dose of 200 mg/kg/day orally. All treatments were given once daily, and the formulations were freshly prepared and administered throughout the study period to ensure consistency and accuracy.

Ethical Concerns

Healthy adult Swiss albino mice of either sex, weighing between 20–30 g, were used for the study. The animals were housed in clean, properly ventilated polypropylene

cages under standard laboratory conditions, maintained at a temperature of 22 ± 2°C, relative humidity of 50–60%, and a 12-hour light/dark cycle. They were provided with a standard pellet diet and water ad libitum throughout the experimental period. The animals were allowed to acclimatize to the laboratory environment for at least seven days before the commencement of the experiment to minimize stress and ensure uniform physiological conditions. All animal handling and experimental procedures were conducted in accordance with the guidelines of the Committee for Control and Supervision of Experiments on Animals (CCSEA), Government of India. The study protocol was reviewed and approved by the Institutional Animal Ethics Committee (IAEC) of the respective institute, and due care was taken to minimize pain or discomfort to the animals during the study (IAEC No: 11/IAEC/CLPT/2024-25).

Equipment – 8 Arm Radial Maze

The 8-Arm Radial Maze is a behavioral apparatus designed to assess learning and memory in rodents. It consists of a central platform with eight arms radiating outward, resembling the spokes of a wheel, with a food reward (such as a pellet) placed at the end of each arm. The task requires the animal to use spatial memory (Kohler *et al*.,2022) to remember which arms have already been visited and which still contain rewards. This model evaluates two types of memory: working memory, which involves recalling the arms (Waegemans *et al*., 2002) visited within a single trial to avoid revisits, and reference memory, which involves remembering across multiple trials which specific arms are consistently baited or unbaited. The 8-Arm Radial Maze is widely used in neuropharmacological research to assess cognitive enhancers and memory-improving drugs, including cholinergic agents and nootropics, and serves as a valuable model for studying Alzheimer's disease and dementia. It offers advantages such as high sensitivity to both working and reference memory, reliability, reproducibility, and non-invasiveness, although it requires slight food deprivation to maintain the animals' motivation for the task.

Procedure

The 8-arm radial maze test was conducted to assess spatial learning (Dubreuil *et al*.,2003) and reference memory in mice. After a 7-day acclimatization period, animals were mildly food-restricted to 85–90% of their normal body weight to enhance motivation for the food reward. The maze consisted of a central platform with eight arms radiating outward, and visual cues were placed around the room for spatial orientation. During the habituation phase, all arms were open, and food pellets were scattered to allow free exploration for 5–10 minutes. In the training phase, (Leuner *et al*.,2010), a fixed set of arms (two to four) were consistently baited across sessions. Each animal was placed on the central platform, and the session continued until all baits were collected or 10 minutes elapsed. Entries into unbaited arms were recorded as reference memory errors, while re-entries into previously visited arms were noted as working memory errors. The number of correct choices, total errors, and latency to complete the task were recorded daily. Trials were conducted for consecutive days until performance stabilized, with a decrease in reference memory errors indicating learning (Olney *et al*.,2015) and memory retention. The maze was cleaned between trials to eliminate scent cues, and data were analyzed statistically to compare treatment effects.

Figure 1. Design of 8 Arm Radial Maze.

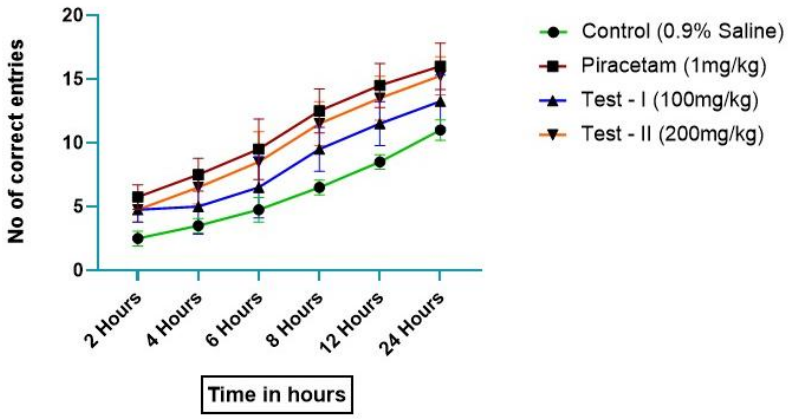
RESULTS AND DISCUSSION

The results of the 8-Arm Radial Maze test demonstrated a clear improvement in learning and memory performance in mice treated with the standard drug (Piracetam), (Kurz *et al*.,2010), and the polyherbal formulations (*Trachyspermum ammi* and *Ocimum sanctum*) compared to the control group. The average number of correct arm entries progressively increased over time across all groups, indicating learning with repeated exposure to the maze. However, the rate and extent of improvement varied notably among the groups. The control group (0.9% saline) showed a gradual but limited increase in correct choices from 2.5 at 2 hours to 11.0 at 24 hours, reflecting normal learning through repetition without pharmacological enhancement. In contrast, the standard Piracetam group exhibited a substantial improvement, (Costa *et al*., 2013), with correct entries rising from 5.75 at 2 hours to 16.0 at 24 hours, confirming its well-established nootropic effect through enhancement of cholinergic transmission and facilitation of synaptic plasticity (Table 1).

Both Test I (100 mg/kg) and Test II (200 mg/kg) groups treated with the polyherbal formulation also showed marked improvements compared to the control. Test I animals increased from 4.75 at 2 hours to 14.0 at 24 hours, while Test II animals rose from 4.75 at 2 hours to 15.5 at 24 hours. This dose-dependent enhancement suggests that the combination of *Trachyspermum ammi* and *Ocimum sanctum* effectively improved spatial reference memory and learning ability. The higher dose (200 mg/kg) demonstrated slightly better performance, likely due to synergistic effects of active constituents such as thymol, (Timalsina *et al*.,2023) carvacrol, eugenol, and flavonoids, which are known for their antioxidant, anti-inflammatory, and cholinergic-modulating properties. Overall, the findings indicate that the polyherbal combination exhibits significant cognitive-enhancing (Malik *et al*.,2022), effects comparable to Piracetam. The observed improvements in maze performance may be attributed to reduced oxidative stress, inhibition of acetylcholinesterase, and neuroprotective actions within the hippocampus. Thus, the formulation shows potential as a natural nootropic agent for improving learning and memory functions.

Table 1. Evaluation of memory enhancement activity for various treatment groups.

Time	Group A (Control)	Group B (Piracetam 150 mg/kg)	Group C (Test I 100 mg/kg)	Group D (Test II 200 mg/kg)
2 Hours	2.50	5.75	4.75	4.75
4 Hours	3.50	7.50	5.00	6.50
6 Hours	4.75	9.50	6.50	8.50
8 Hours	6.50	12.50	9.50	11.50
12 Hours	8.50	14.50	13.00	14.00
24 Hours	11.00	16.00	14.00	15.50



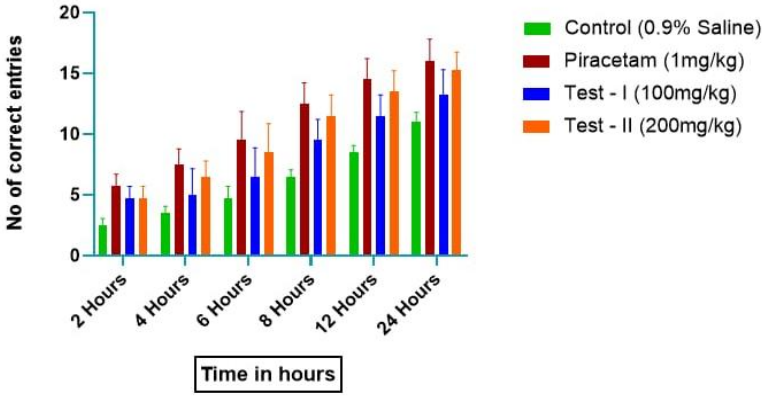


Figure 2. Graphical representation memory enhancement activity for various treatment groups.

CONCLUSION

The present study demonstrates that the polyherbal formulation containing *Trachyspermum ammi* (Ajwain) and *Ocimum sanctum* (Tulsi) exerts significant cognitive-enhancing effects in mice, as evidenced by improved performance in the 8-Arm Radial Maze test. Both test doses (100 mg/kg and 200 mg/kg) produced a dose-dependent increase in correct arm entries, indicating enhanced spatial learning and reference memory compared to the control group. The higher dose (200 mg/kg) showed performance comparable to the standard nootropic drug, Piracetam (150 mg/kg), suggesting potent memory-facilitating activity. These effects may be attributed to the synergistic action of bioactive compounds such as thymol, carvacrol, and eugenol, which enhance cholinergic transmission and protect neuronal cells from oxidative and inflammatory damage. Overall, the results support the potential of this polyherbal combination as a safe and effective natural alternative for improving memory and cognitive function.

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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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