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Unlocking the Nootropic Effect of *Trachyspermum ammi* Seeds against Scopolamine-induced Memory Deficits in Rodents

Muhammad Ali Rajput[1], Mohammad Mesbahuzzaman[1], Pallav Sengupta[1], Azra Batool[2], Saeed Ahmed Lakho[3], Salman Ahmed[3], Zeeshan Ahmed[4]

[1]*Department of Biomedical Sciences, College of Medicine, Gulf Medical University, Ajman, UAE*
[2]*Faculty of Pharmacy, Hamdard University Islamabad Campus, Islamabad, Pakistan*
[3]*Faculty of Pharmacy, University of Sindh, Jamshoro, Sindh, Pakistan*
[4]*Faculty of Pharmacy, IQRA University, Karachi, Sindh, Pakistan*
Correspondence: *drmuhammadali2016@gmail.com*
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ABSTRACT

OBJECTIVE: To evaluate the potential of TASE in improving memory and cognitive function using animal models.
METHODOLOGY: These experimental trials were conducted in the Pharmacology Laboratory, University of Karachi, from January - November 2021. Thirty-five male Wistar Rats and thirty-five male mice weighing 200-230g and 20-40g, respectively, were used for Morris water maze (MWM) and passive avoidance response (PAR) tests. The subjects were distributed equally into five groups. Group 1 (control) received 2% gum tragacanth, Groups 2, 3, and 4 received TASE at 50, 100, and 250mg/kg, respectively, and Group 5 received Piracetam at 200mg/kg. Scopolamine at 0.4mg/kg was administered intraperitoneally to induce amnesia in rodents.
RESULTS: In the MWM test, TASE at 250 and 100mg/kg significantly decreased the escape latency in rats compared to the control group. Similarly, in the PAR test, TASE at 250 and 100mg/kg significantly delayed the compartment change time in mice compared to the control group. The outcomes of both experiments were comparable to the reference drug Piracetam, indicating that TASE improves cognition and has substantial potential as an anti-amnesic agent. This potential may be due to the presence of significant phytochemicals in TASE, such as Thymol, saponins, flavonoids, α- and β-pinene, and γ-terpinene, which improve cholinergic neurotransmission and exert antioxidant effects.
CONCLUSION: TASE improves memory and cognitive function, likely due to its significant phytochemical content. Identifying these nutritional elements is crucial for researching and developing innovative and potent medications.

KEYWORDS*: Trachyspermum ammi;* Alzheimer's disease; Morri's water maze; Antioxidant; Memory enhancement; Cognitive function; Phytochemicals

INTRODUCTION

Nootropics are intelligent agents and are a miscellaneous set of medications, which improve cognition, particularly in situations where these functions are depressed. Learning is the capacity of a person to acquire new information, while memory represents the method through which the brain retains and recalls this information as needed [1].

Gradual decline in retrieving past events or dementia is common in older adults. Alzheimer's disease (AD), in this context, is a significant cause of dementia [2]. AD is a progressive neurodegenerative disorder marked by the gradual decline of cognitive functions. This condition is associated with the degeneration of cholinergic neurons, critical for mental processes, within the brain's cerebral cortex and hippocampus regions; this leads to a reduction in the activity of the enzyme acetylcholinesterase. Hence, acetylcholinesterase blockers were synthesized to treat memory losses associated with AD [3,4].

Analogously, scopolamine is an antimuscarinic drug which declines cognition in humans and rodents[5]. It is an extensively used and recognized technique to assess the anti-amnesic properties of natural medicines [6]. Impediment and retardment in the inception of memory deficits will significantly influence people and governments by decreasing the period of sickness and expenses associated with it [7].

Traditional medicines are becoming famous for their holistic approach to ailments [8]. Several plants categorized as the 'Medhya' are testified to facilitate the sequence of cognition [9]. Researchers have collected various facts about dozens of plant species related to their influence on cognition, specially memory-enhancing activities of plants, which are established and documented [10]. Extensive research is essential to explore the scientific properties of medicinal plants, which could lead to the identification of novel compounds within these natural remedies, thereby legitimizing their application for the advancement of human health.

Trachyspermum ammi (*T. ammi*), Carum copticum, 'Ajwain', from the family *Umbelliferae,* is a well-known spice, frequently used by people from Asian countries for many purposes such as culinary and medicinal. The fruit of *T. ammi* is diminutive and oval, bearing a gray hue akin to the seeds of a bishop's weed, leading to the misnomer of referring to these fruits as seeds. Phytochemical analyses of the so-called *T. ammi* seeds have identified various constituents, prominently featuring a volatile essential oil rich in Thymol, which constitutes 60-90% of the oil. Additionally, the fruit contains a spectrum of secondary metabolites, including saponins, flavonoids such as Quercetin, and other compounds like terpinene paracymene and α and β-pinene, carvacrol, and various phenolic compounds. Carbohydrates and glycosides are also found in seeds [11,12].

T. ammi seeds are widely used as traditional medicine to prevent and cure various allergic conditions, such as cough, pain, and dysentery. Recently conducted research works have confirmed its traditional uses and have also revealed its significant impacts on behavioral and blood disorders such as anxiety, epilepsy and thrombosis. As very inadequate scientific evidence was available regarding the effects of *T. ammi* on memory, thus the present study was dedicated to assessing the influences of *T. ammi* seeds on cognition in rodents.

METHODOLOGY

These experimental trials were conducted in the Pharmacology Laboratory, University of Karachi, from January - November 2021 after attaining endorsement from the University's Departmental Research Committee and Board of Advance Studies and Research.

Plant authentication and grounding of extract

Trachyspermum ammi seeds were sourced from a market in Hyderabad, Pakistan, and subsequently authenticated by the Herbarium and Botanic Garden at the University of Karachi, from which a voucher specimen (TA-10-12) was also secured via the Department of Pharmacognosy. A cold extraction method [13], was employed to produce the crude *T. ammi* seed extract (TASE). Initially, the seeds were manually purified to remove any contaminants. Subsequently, 1 kilogram of these seeds were immersed in 2000 milliliters of 80% methanol. This mixture was intermittently agitated and blended over two weeks until it acquired a green hue. The mixture was then filtered using Whatman No. 1 filter paper. The final solution was subjected to concentration under a vacuum with the help of a rotary evaporator, where the temperatures were maintained between 40°C to 45°C. Subsequently, it was freeze-dried at a temperature of -30°C. The finished TASE was then stored at -20°C. The process yielded 75 grams of dry-weight TASE. For 15 days, TASE was orally administered at 50, 100, and 250mg/kg body weight daily in both experiments.

Preparation of drugs

At a concentration of 2%, Gum tragacanth powder was sourced from Merck to prepare suspension formulations for a control group and three varying dosages for a test group, precisely 50, 100, and 200 mg/kg TASE. The control group received this as a placebo via oral administration at a volume of 10ml/kg. 2 grams of the gum tragacanth powder was dissolved in 100 ml of warm distilled water to create the 2% suspension. Each dosing utilized suspensions that were prepared freshly. Furthermore, piracetam tablets, each containing 0.8 grams, were procured from a pharmacy in Karachi. These tablets were ground into a powder, suspended in distilled water, and administered orally to the rodent subjects at 200mg/kg [14].

Scopolamine hydrobromide trihydrate powder was attained from Merck, and its dilution was made in 0.9% normal saline and administered at a dose of 0.4 mg/kg intraperitoneally (IP) for the induction of amnesia in rodents 40 min after the last dose on 15th day to induce amnesia in rats belongs to treated groups and reference group.

Animals and grouping

To evaluate the impact of TASE on the Morris Water Maze (MWM) task, a cohort of 35 male Wistar rats, each weighing between 200–220 grams, was utilized, and for the Passive Avoidance Response (PAR) test, 35 male mice weighing between 20-40 grams were employed. These animals were systematically allocated into five distinct groups. Group 1 functioned as the control group and was administered a 10 ml/kg dosage of a non-active substance (tragacanth gum). Groups 2, 3, and 4 were the experimental groups and received varying dosages of TASE—50, 100, and 250 mg/kg, respectively, while Group 5 acted as the benchmark and was treated with 200 mg/kg of Piracetam. All substances were administered orally daily for 15 days using an orogastric tube. On the 15th day, precisely 40 minutes after the final dose of TASE and Piracetam, the animals were intraperitoneally injected with scopolamine at a dosage of 0.4 mg/kg to induce cognitive impairment. Subsequently, the rats were subjected to the MWM and the mice

to the PAR test. The Faculty of Pharmacy at the University of Karachi validated animal experimentation by the guidelines set by the National Advisory Committee for Laboratory Animal Research (NACLAR, 2004) and the National Institutes of Health (*NIH*, 2011) for the ethical care and use of laboratory animals.

Animal housing

The rats and mice were each placed in separate plastic enclosures. The temperature of their environment was consistently maintained at 23±2°C. The humidity levels were kept within a range of 50 to 60%. The lighting conditions in the housing facility followed a regular cycle, alternating between light and darkness over 12-hour periods. The animals had continuous access to a standard diet and water. Each day, the animals were moved from their housing facility to the laboratory about an hour before experiments began, ensuring that all experimental procedures took place during the day. Before dosing, a thorough health evaluation of the rodents was performed throughout a week-long acclimatization period, focusing on signs such as edema, diarrhea, lethargy, muscle tone, and ulcers.

Morris water maze (MWM) TASK

MWM task is the most effective test widely accepted today for evaluating hippocampal functioning, such as memory and learning. It has been more extensively used as a test rather than its precursors (radial-arm maze, T. mazes and their variations) since its creation 40 years ago because the effects recorded through it are easier to reproduce than its precursors. The equipment is exceptionally straightforward to organize [16]. On the fifteenth day of the experimental period, an intraperitoneal injection of Scopolamine hydrobromide trihydrate at 0.4 mg/kg was given to animals from all groups, 40 minutes after the drug administration to induce amnesia. Subsequently, the subjects were subjected to the Morris Water Maze (MWM) task. Before this, an initial trial of the TASE extract at a dosage of 20 mg/kg was conducted over 15 days, which yielded no notable outcomes.

Procedure

The Morris Water Maze (MWM) test was carried out in a circular tank with a diameter of 60 centimetres and a height of 25 centimetres filled with water. The water temperature was consistently held at 26 ±1°C. The water was rendered opaque with non-toxic milk to prevent visibility. The tank was segmented into four equal sections using two strings crossing each other at right angles at the tank's edge. A white-topped submerged platform was placed in the specified target section (C4 for this study), positioned 1 cm above the water level during the training phase. The platform remained in the exact location throughout this phase. Each rat underwent four consecutive trials daily, with a 5-minute interval between trials, where they were allowed to find and remain on the concealed platform for 20 seconds. For the test phase, the rats were introduced into the water at the boundary of the sections, facing away from the centre, with varying starting positions for each trial. They had 120 seconds to locate the platform, which was now submerged 1 cm below the water surface. Rats failing to find the platform within the allotted time were guided to it and allowed a 20-second stay. The measured outcome was the escape latency (EL), denoting the duration it took for the rats to locate and climb onto the hidden platform.

Passive avoidance response test

The passive avoidance response is a behavior motivated by fear that serves as a traditional method for assessing both short-term and long-term memory in rodent species. It is characterized by suppressing a rodent's natural inclination to seek out the darker areas within the passive avoidance apparatus, as noted by Ogren and Oliver in 2010 [17]. Rodents demonstrate learning of passive avoidance in just one trial, which manifests as a significant prolongation in the time taken to move to the other side. This response involves connecting a typically non-threatening setting and an unpleasant stimulus and relies on the proper hippocampus functioning.

Conditioning phase

Before the testing phase, the animals underwent a 24-hour exploratory period. During this stage, mice were placed in an illuminated white compartment, where they were given a brief period to familiarize themselves with the environment, with the guillotine gate remaining shut. After this familiarization period, the gate was opened, and the latency period, defined as the time taken by the mice to enter the dark chamber, was measured. If the second mouse did not move into the dark compartment within the predetermined latency threshold of 90 seconds, the trial was discontinued, and the mouse was excluded from the study. The process involved the mouse entering a shaded section, which prompted the descent of a guillotine-like barrier. Subsequently, a predetermined electric shock was administered through the floor's grid. Right after this event, the mouse was removed from the dark area and returned to its familiar enclosure. The apparatus was meticulously cleaned after each session. Test phase

The assessment was conducted 24 hours post-conditioning to evaluate long-term memory retention on the fifteenth day. This timing was explicitly chosen to coincide with 40 minutes after administering TASE and Piracetam and 10 minutes after the scopolamine dose. The procedure involved placing the mouse within a brightly lit white area for several minutes. The critical measure was the time it took for the mouse to move from the door opening into the darker compartment, referred to as the 'compartment change time.' An indication of enhanced memory function was reflected by a more significant delay in entering the dark compartment during the testing phase; typically, this delay is around 180 seconds for mice and 280 seconds for rats. The Passive Avoidance Response (PAR) testing device used in this experiment was supplied by Panlab, S.L, in Barcelona, Spain. It features a compact, dark, black, separated by a guillotine-style door from a larger, illuminated and painted white compartment. A computer interfaced with the SHUTAVOID-01 software manages the operations of this apparatus.

Statistical evaluation

The data were analyzed utilizing SPSS version 23, where the mean and the standard error of the mean were computed through a two-sample Student's t-test. Statistical significance was established at p-values less than 0.05, while p-values less than 0.005 were considered highly significant.

RESULTS

Effect of TASE on memory deficits induced by scopolamine in rodents

Table I shows the effect of TASE and Piracetam on EL of rats administered scopolamine to induce memory deficits in the MWM test. TASE 250 mg/kg very notably reduced the escape latency in amnestic rats at a dose of 100 mg/kg; the TASE notably reduced the escape latency about control. TASE did not reveal any notable effect at a dose of 50 mg/kg; however, Piracetam at a dose of 200 mg/kg notably reduced the escape latency in amnestic rats in relation to control.

Table I: Influence of Piracetam and TASE on Escape Latency of in MWM Test

Groups	Dose	Escape-Latency (EL)
Control	2% Gum tragacanth	108±4.6
Group 1	TASE 50 mg/kg	100.7±2.3
Group 2	TASE 100 mg/kg	95±3.2*
Group 3	TASE 250 mg/kg	79.3±4.2**
Standard	Piracetam 200 mg/kg	72.7±3.4**

*Values were measured as Mean ± S.E.M, *p ≤ 0.05 Notable in relation to control, **p ≤ 0.005 Very Notable in relation to control, n =7*

Table II revealed the ameliorating effects of TASE and Piracetam on memory measured through the PAR test after continuous administration of TASE and Piracetam in specified doses for 15 days. The duration between the opening of the door and the entry of the mice into the dark compartment was meticulously measured. When compared to the control group, there was a significant decrease in the time it took for animals treated with TASE at doses of 250 mg/kg and 100 mg/kg to switch compartments. This reduction was comparable to the cognitive enhancement effects observed with Piracetam.

Table II: Influence of TASE and Piracetam on Escape Latency in PAR Test

Groups	Dose	Escape-Latency (EL)
Control	2% Gum tragacanth	182.9±11
Group 1	TASE 50 mg/kg	170.9±11
Group 2	TASE 100 mg/kg	121±3.2*
Group 3	TASE 250 mg/kg	76.7±14**
Standard	Piracetam 200 mg/kg	74±13**

*Values were measured as mean±sem, *p<0.05 notable in relation to control, **p<0.005 very notable in relation to the control group, n=7*

DISCUSSION

Current research has highlighted the significant effects of TASE on escape latency (EL) in rodents experiencing scopolamine-induced memory impairments, as assessed using the Morris Water Maze (MWM) test. The administration of TASE at a concentration of 250 mg/kg markedly reduced the time it took for the amnesic rats to escape the maze compared to the control group, indicating an improvement in spatial memory. Additionally, a lower dose of TASE at 100 mg/kg significantly shortened the escape latency relative to the control group, suggesting that even at reduced dosages, TASE exerts beneficial effects on memory performance.

Furthermore, the study examined the anti-amnestic effects of TASE and Piracetam on long-term memory using the Passive Avoidance Reaction (PAR) test. Mice were administered specified doses of TASE and Piracetam over 15 days. The results showed a significant delay in the compartment change time for TASE-treated animals at 250 mg/kg and 100 mg/kg doses compared to the control group. This delay indicates enhanced memory retention and the potential of TASE to counteract memory impairments.

In both the MWM and PAR tests, TASE's nootropic effects were highly comparable to those of Piracetam, a well-known cognitive enhancer. The ability of TASE to improve memory performance in these rodent models underscores its potential as a cognitive enhancer and its efficacy in mitigating memory impairments. This research suggests that TASE could be a promising candidate for developing treatments for memory-related disorders, offering a natural alternative or complement to existing pharmacological options like Piracetam.

Overall, these findings provide compelling evidence for the cognitive benefits of TASE, particularly in improving memory performance and reducing memory deficits in rodent models, paving the way for future studies to explore its potential applications in human cognitive health.

Amnesia provoked by scopolamine administration is a well-known and documented model that simulates dementia. Administering scopolamine can cause short-term memory impairment when applied just before a test. The capacity of different cholinergic agents to counteract scopolamine's memory-disrupting effects is now well-documented in animal and human studies[18]. Additionally, recent research has highlighted the potential of various flavonoids to bind to Muscarinic (M1) acetylcholine receptors, suggesting they may be beneficial in treating AD [19].

Past research has shown a protective correlation between the consumption of flavonoids and the risk of several diseases. These investigations have indicated that taking flavonoids as dietary supplements can enhance cognitive functions and memory by improving cerebral blood flow [20].

Current research focuses on how flavonoids may influence synaptic changes that affect learning and memory, given their direct involvement in cellular signaling pathways regulating cAMP, CREB, and PI3K, PKC, AKT, BDNF [21]. They also reduce neuronal inflammation, oxidative stress, improve synaptic plasticity and reverse symptoms associated with AD. All these actions indicate neuroprotective solid effects and are reversibly proportional to cognitive improvement, especially in those suffering from brain disorders [21].

A recent investigation has revealed that Thymol, present in TASE, demonstrates protective benefits for neural health and enhances memory in a mouse model of Alzheimer's disease induced by scopolamine [22]. This protection is evident from the significant decrease in oxidative stress markers, including hydrogen peroxide, malondialdehyde, and brain glutathione, as measured in the homogenates of the entire brain of the mice. Additionally, TNF alpha, a pro-inflammatory cytokine, was notably suppressed. Concurrently, there was an elevation in the

levels of Brain-Derived Neurotrophic Factor (BDNF) and the phosphorylated form of glycogen synthase kinase-3 beta, which are associated with the observed improvement in memory in the mice treated with TASE [23].

In another recently conducted study, *Eleutherococcus senticosus* revealed its saponin fraction's neuroprotective and memory-enhancing effects. Similarly, phytochemicals such as α and β pinene and cymene, which are also found in significant amounts in TASE, have demonstrated antioxidant effects, reversal of scopolamine-induced memory deficits, and anticholinesterase inhibitory effects, which are substantial for eliciting anti-amnesia effects [24,25].

TASE contains an abundance of Thymol alongside a diverse array of secondary compounds, including flavonoids (notably Quercetin), saponins, alpha and beta-pinene, and cymene. Research has consistently demonstrated that these substances enhance memory. Thus, it can be inferred that the mitigating impact on memory deficits observed in rodent models with scopolamine-induced amnesia can be attributed to the combined effects of these bioactive elements. Thymol is known for its antioxidant and anti-inflammatory properties, which may protect neural cells from damage. Quercetin, a well-studied flavonoid, improves cognitive function by modulating signaling pathways associated with memory and learning [26]. Saponins are recognized for their neuroprotective effects, potentially contributing to preventing memory loss. Alpha and beta-pinene, found in various essential oils, are associated with cognitive enhancement and improved focus [26]. Cymene, another key compound, is known for its antioxidant properties, which may help reduce oxidative stress in the brain. The synergistic effects of these compounds likely contribute to the observed improvement in memory performance in rodent models treated with TASE. Therefore, the presence of these bioactive elements in TASE underscores its potential as a natural cognitive enhancer and provides a scientific basis for its use in mitigating memory impairments; this highlights the importance of further research to explore the potential applications of TASE in human cognitive health.

CONCLUSION

TASE has revealed memory-enhancing effects, possibly because of the presence of various significant constituents in it, such as Thymol, saponins, flavonoids, α and β pinene and cymene, which can significantly potentiate cholinergic activity and exerts neuroprotective effects and thus have immense potential in the management of memory disorders; still further studies on large and higher groups of animals and in humans are obligatory to approve these conclusions.

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Data Sharing Statement: The corresponding author can provide the data proving the findings of this study on request. Privacy or ethical restrictions bound us from sharing the data publicly.

AUTHOR CONTRIBUTION

Rajput MA:	Conceived and designed the study, conducted research, data collection, data analysis and interpretation and initial and final draft of the article.
Mesbahuzzaman M:	Conceived and designed the study, conducted research, data collection, data analysis and interpretation and initial and final draft of the article.
Sengupta P:	Conceived and designed the study, conducted research, data collection, data analysis and interpretation and initial and final draft of the article.
Batool A:	Data analysis and final drafting.
Lakho SA:	Data analysis and final drafting.
Ahmed S:	Data analysis and final drafting.
Ahmed Z:	Data analysis and final drafting.

All authors have critically reviewed and approved the final draft and are responsible for the manuscript's content.

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