ORIGINAL ARTICLE

Cognitive enhancing, anti-acetylcholinesterase, and antioxidant properties of *Tagetes patula* on scopolamine-induced amnesia in mice

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Background: Alzheimer's disease is a progressive neurodegenerative disorder characterized by a gradual decline in memory associated with shrinkage of brain tissue and loss of neurons with a diminished level of the central cholinergic neurotransmitter acetylcholine. **Objective:** The present study was performed to examine the effect of ethanolic extract of *Tagetes patula* (EETP) on cognitive impairment induced by scopolamine, a muscarinic antagonist, in mice. **Materials and Methods:** Rats were treated with EETP and donepezil for 15 successive days followed by treatment with scopolamine (1 mg/kg) for 3 days. The changes in behavioral, biochemical, and neurotransmitters were assessed in rats. Cognitive functions were assessed using step-through latency on a passive avoidance apparatus and Morris water maze test. Antioxidants parametes such as superoxide dismutase (SOD), glutathione reductase (GR), lipid peroxidation (LPO), and nitrates were assessed. Neurotransmitters including acetylcholinesterase (AChE), dopamine (DA), and serotonin were also assessed, and neuronal damage was also analyzed. **Results:** Scopolamine-treated rats showed impaired learning and memory, increased activity of AChE, LPO and decreased levels of SOD, reduced glutathione, nitrates, serotonin, and DA. The EETP significantly reversed the scopolamine-induced cognitive impairment in mice was measured by the passive avoidance test. In addition, EETP decreased escape latency in the Morris water maze. In probe trail session, EETP increased the latency time in the target quadrant. *Ex vivo* EETP inhibited AChE activity in the mice brain. EETP treated mice significantly increased the SOD, GR, nitrates, DA, and serotonin levels, and decreased the level of LPO when compared with scopolamine-treated mice. **Conclusion:** These results indicate that EETP may exert anti-amnesic effect through both by anti-AChE and antioxidant mechanisms.

Key words: Cognition, Morris water maze, passive avoidance, scopolamine, *Tagetes patula*, transfer latency

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative disorder common in people over 65 years of age. Characteristic pathological features of the central nervous system (CNS) in AD are senile plaques, neurofibrillary tangles formation, aberrant oxidative and inflammatory processes, and neurotransmitter disturbances. Neuronal damage or loss in the basal forebrain particularly within the hippocampal acetylcholinergic systems involved in learning and memory processes constitutes a pathological hallmark of AD. Although the exact biochemical basis of AD is not well-understood, it is known that deficiencies of the brain cholinergic system and other neurotransmitters are present.[1] Treatment for AD is still the using of acetylcholinesterase (AChE) inhibitors.[2] Moreover, AChE inhibitors, such as physostigmine, tacrine, and donepezil, have limited therapeutic success as they only improve memory in mild dementia and cannot stop the process of neurodegeneration.[3-5] However, AChE inhibitors present some limitations such as their short half-lives and severe side effects such as hepatotoxicity which is important side effects of this treatment. Complimentary medicines are essential to develop the novel anti-dementia drugs.[6]

Tagetes patula is an aromatic annual herb belongs to the family *Asteraceae*. The main aromatic hydrocarbon component present in it is terpenes. *T. patula* exhibits anti-inflammatory, antibacterial, antifungal insecticidal, astringent, diuretic, skin disorders, and hepatic disorder activity, in addition to antioxidant activities associated

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with free radical scavenging.[7] Generation of free radicals associated with enhanced oxidative stress has been found to be attributed in the pathogenesis of AD resulting in learning and memory impairment. Limonene is the one of the chemical constituents present in the *T. patula* and D-isomer of an aromatic hydrocarbon of terpenes acts on CNS via blood brain barrier either in its intact form or as a metabolite. Limonene has anti-stress effects through its anti-oxidant potential and increases the levels of serotonin, dopamine (DA), gamma-aminobutyric acid (GABA), and neurotransmission conductions in the rat brain suggesting that limonene could inhibit physical stress through GABA receptor.[8] However, no studies have addressed the anti-amnesic effect of *T. patula* on learning and memory in mice.

Scopolamine, a nonselective muscarinic receptor antagonist, causes impairment in learning and memory by reducing cholinergic activity.[9] An intraperitoneal (i.p.) administration of scopolamine, the cholinergic neurotransmission was blocked, leading to cholinergic dysfunction and impaired cognition in mice.[10] Recently, it has been reported that memory impairment induced by scopolamine in mice is associated with altered brain oxidative stress status and neuronal damage.[11] Oxidative stress is also one of the affecting factors in AD, so several antioxidants have been studied for the reduction of oxidative stress occurring during AD.[12,13] Therefore, mice with scopolamine-induced memory deficits were used as an animal model for screening anti-dementia activity.

We investigate whether the neuroprotective effect of ethanolic extract of *T. patula* (EETP) attenuated learning and memory impairment by a muscarinic antagonist scopolamine in mice. We evaluated the effect of EETP on scopolamine-induced learning and memory impairment in the passive avoidance test and Morris water maze tests. In addition, we investigated the effect of EETP on neurotransmitters such as AChE, DA, and serotonin, and antioxidants including superoxide dismutase (SOD), lipid peroxidation (LPO), glutathione reductase (GR), and nicotinamide adenine dinucleotide phosphate-oxidase (NADPH) oxidase and neuronal damage were assessed.

MATERIALS AND METHODS

Authentication of Plant Material and Preparation of Ethanolic Extract of *Tagetes patula*

The flower part of the plant *T. patula* (*Asteraceae*) was collected from Andhra Pradesh. The plant material was identified and authenticated by Dr. D. Narasimhan, Associate Professor, Centre for Floristic Research, Madras Christian College, Tambaram, Chennai. The fresh flower was collected and washed, shade dried at room temperature the dried plant material was made into coarse powder. The powder was extracted with ethanol (68–78°C) in Soxhlet apparatus for 24 h. The extracts were concentrated on water bath (50°C). After concentrated preparation, the dried powder extract was stored at room temperature. The yields of the ethanolic extract were found to be 1.0% (w/w), respectively.

Animals

Male Swiss mice (25–30 g: 30 mice) were procured from CL Baid Metha College of Pharmacy, Chennai and divided into five groups of six animals each. The mice were housed in colony cages at an aberrant temperature of 25°C ± 2°C with a 12 h light/dark cycle. The animals had free access to standard pellet diet and drinking water. Behavioral studies were carried out in a quiet room between 9.00 am and 11.00 am to avoid circadian variation. The study was approved by Institutional Animal Ethical Committee, and work was carried out as per CPCSEA Guidelines, New Delhi.

Drugs and Chemicals

Scopolamine (Tokyo Chemical Industry Ltd., Japan), Donepezil (Eisai Pharmaceuticals Ltd., Mumbai), Thiobarbituric acid (S. D. Fine Chemicals Ltd., Mumbai), and SOD (HiMedia Research Laboratory, Mumbai) were used. All other chemicals and reagents unless specified were of analytical grade.

Experimental Protocol and Treatment Schedule

To observe the effect of EETP on scopolamine-induced learning and memory impairment in 30 mice (6 in each group were divided into 5 groups): The first group (control) received normal saline (0.9% NaCl) once daily for 18 days. The second group (saline and scopolamine (1 mg/kg i.p.) received normal saline for 15 days followed by scopolamine for 3 days. The third group received donepezil (5 mg/kg p.o.) for 15 days followed by scopolamine (1 mg/kg i.p.) for 3 days. The fourth group received EETP (200 mg/kg p.o.) for 15 days followed by scopolamine (1 mg/kg i.p.) for 3 days. The fifth group received EETP (400 mg/kg p.o.) for 15 days followed by scopolamine (1 mg/kg i.p.) for 3 days. Scopolamine was dissolved in sterile water for injection. Donepezil were dissolved 0.1% critical micellar concentration solution. EETP were dissolved in distilled water. All drugs were prepared freshly and given once daily in the morning and followed the same regimen. The learning and memory parameters were performed 120 min after drug administration. Doses were given according to the respective mice weights [Table 1].

Learning and Memory Evaluations

Step-through Passive Avoidance Test

The step-through passive avoidance apparatus consisted of an illuminated chamber (11.5 cm Â 9.5 cm Â 11 cm)

Table 1: Experimental design

Groups	Treatment
Group-I	Normal saline (0.9% NaCl i.p.)
Group-II	(0.9% NaCl i.p.)+ scopolamine (1 mg/kg i.p.)
Group-III	Donepezil (5 mg/kg p.o.)+ scopolamine (1 mg/kg i.p.)
Group-IV	EETP (200 mg/kg p.o.)+ scopolamine (1 mg/kg i.p.)
Group-V	EETP (400 mg/kg p.o.)+ scopolamine (1 mg/kg i.p.)

EETP – Ethanolic extract of *Tagetes patula*

attached to a darkened chamber (23.5 cm Â 9.5 cm Â 11 cm) containing a metal floor that could deliver foot shocks. The two compartments were separated by a guillotine door. The illuminated chamber was lit with a 25 W lamp. Briefly, mice were placed in the dimly lit room containing the apparatus 0.5 h before training to acclimatize to the new environment. Each mouse was then placed individually into the illuminated chamber, facing away from the door to the dark chamber, and allowed to acclimatize for 1 min. As soon as the mouse entered the dark chamber, the door was slid back into place, triggering a mild foot shock (0.3 mA, 50 Hz, 5 s). The mouse was then immediately removed from the chamber and returned to its home cage. The latency (time used to change compartment) was recorded. The retention test was conducted 24 h later with the mouse again being placed in the illuminated chamber and subjected to the same protocol in the absence of foot shock. The upper time limit was set at 300 s.[14]

Morris Water Maze Test

Spatial learning and memory were evaluated by the Morris water maze. The procedure included two steps. The first step was the place navigation test from day 1 to 4, in which the escape latency (EL) (the time required to escape onto the hidden platform) was used to evaluate learning and memory function. Mice that found the platform were allowed to remain on the platform for 20 s and were then returned to the home cage. If mice did not reach the platform within 120 s, it was gently guided to the platform by the experimenter, where it remained for 20 s. The last trial was regarded as the probe test. The second step was the spatial probe test on day 5 after removal of the platform and after the space navigation test, which was performed to test the ability of mice to find the removed platform by memory.[15]

Biochemical Estimation

Mice were euthanized 60 min after treatment, and the brain were removed following the Morris water maze test. The harvested brains were used for biochemical assessment.

Antioxidant Estimation

Estimation of Superoxide Dismutase

The supernatant (500 µL) was added to 0.8 ml of carbonate buffer (100 mM, pH 10.2) and 100 µL of epinephrine (3 mM). The change in absorbance of each sample was then recorded at 480 nm in spectrophotometer for 2 min at an interval of 15 s. Parallel blank and standard were run for determination of SOD activity. One unit of SOD is defined as the amount of enzyme required to produce 50% inhibition of epinephrine auto-oxidation. The reaction mixtures are diluted 1/10 just before taking the readings in the spectrophotometer.[16]

Estimation of Lipid Peroxidation

LPO was evaluated by measuring the thiobarbituric acid reactive substances (TBARS) content. To 0.2 mL of brain homogenate, 0.2 mL of sodium dodecyl sulfate, 1.5 mL of acetic acid, and 1.5 mL of thiobarbituric acid were added. The mixture was made up to 4 mL with water and then heated in a water bath at 95°C for 60 min. After cooling, 1 mL of water and 5 mL of n-butanol/pyridine mixture were added and shaken vigorously. After centrifugation at 4000 rpm for 10 min, the organic layer was taken, and its absorbance was read at 532 nm. The levels of TBARS were expressed as nmoles of malondialdehyde/min/mg protein in brain homogenate.[17]

Estimation of Glutathione Reductase

The reaction mixture containing 1 mL of phosphate buffer, 0.5 mL of ethylenediamine tetraacetic acid (EDTA), 0.5 mL of oxidized glutathione and 0.2 mL of NADPH was made up to 3 mL with distilled water. After the addition of 0.1 mL of tissue homogenate, the change in optical density at 340 nm was monitored for 2 min at 30 s intervals. One unit of the enzyme activity was expressed as nmoles of NADPH oxidized/min/mg protein.[18]

Estimation of Nitrates

One hundred microgram of sample was mixed with 100 µL of freshly prepared Griess reagent (mixture of 0.1% N-1-napthylethylenediamine in water and 1% sulfanilamide in 5% phosphoric acid) and absorbance was observed at 450 nm using Bio-rad enzyme-linked immunosorbent assay reader. The levels were expressed as µg/mL of tissue.[19]

Neurotransmitters Estimation

Estimation of Acetylcholinesterase Enzyme

Twenty milligram of brain tissue/mL of phosphate buffer (pH 8, 0.1 M) was homogenized in a Potter-Elvehjem homogenizer. A 0.4 mL of brain tissue was added containing 206 mL of 0.1 M phosphate buffer. One hundred microliter of the DTNB reagent was added to the photocell. The absorbance was measured at 412 nm then acetylthiocholine iodide was added. Changes in the absorbance were recorded and change in absorbance per minute was calculated. The enzyme activity is expressed as µmol/min/mg tissue.[20]

Estimation of Dopamine

To the 0.2 mL of aqueous phase, 0.05 mL of 0.4 M HCl and 0.1 mL of EDTA/sodium acetate buffer (pH 6.9) were added, followed by 0.1 mL of iodine solution (0.1 mL in ethanol)

for oxidation. The reaction was stopped after 2 min by addition of 0.1 mL Na_2SO_3 solution. To 0.1 mL, acetic acid is added after 1.5 min. The solution was then heated to 100°C for 6 min when the tissue homogenate reaches room temperature, excitation and emission spectra were read from the spectrofluorimeter. The readings were taken at 330–375 nm for DA.[21]

Estimation of Serotonin

Three millilitre of brain homogenate in 0.1mL of hydrochloric acid-nbutanol for one minute in glass homogenizer. The sample was centrifuged for 10 min at 2000 rpm. Supernatant phase was removed and added to Eppendorf reagent tubes containing 0.2 mL of heptane and 0.025 mL of HCl 0.1 mL. After 10 min of vigorous shaking, the tubes were centrifuged under the same condition as above to separate the two phases. The aqueous phase was taken, and phthaldialdehyde was added. The fluorophore was developed by heating to 100°C for 10 min; after the sample reached the equilibrium, the intensity was measured at 360–470 nm in the spectrofluorimeter.[22]

Histopathological Examination

The brain was removed without any injury after opening the skull. The collected sample were washed with normal saline and fixed in 10% neutral formalin for 48 h for further histological observations. Paraffin section was taken at 5 µm thickness processed in alcohol xylene series and was stained with hematoxylin and eosin dye. The brain sections were examined microscopically for histopathological changes.[23]

Statistical Analysis

The results are reported as the mean ± standard error of the mean analysis of variance followed by the Tukey multiple comparison test was used for comparison. Differences were considered significantly at $P < 0.05$.

RESULTS

Effect of Ethanolic Extract of *Tagetes patula* on Passive Avoidance Test

The effect of EETP on long-term memory was investigated in the step-through passive avoidance test. During the training session (day 1) of the step-through passive avoidance task, there were no significant differences between any groups [Figure 1a]. However, there was a significant difference between groups in the retention test. Scopolamine-treated mice showed a significantly ($P < 0.01$) lower latency compare to control mice in the retention test, which was performed 24 h after the training test. The reduced retention latency indicates learning and memory impairment in mice. The effect of scopolamine was reversed by 200 and 400 mg/kg of EETP ($P < 0.001$) treated mice. Donepezil-treated mice significantly ($P < 0.001$) increased latency during retention test when compared to scopolamine-treated mice. EETP 200 mg/kg treated mice significantly ($P < 0.001$) decreased latency when compared with the donepezil-treated mice. There were no significant differences between the EETP 400 mg/kg and donepezil-treated mice. Results are shown in Figure 1b.

Effect of Ethanolic Extract of *Tagetes patula* on Morris Water Maze Test

Effect of EETP on spatial memory was investigated in the Morris water maze test. There was no significant difference in the mean EL between any groups on days 1 and 2. Significant differences were observed in the mean EL among the groups on days 3 and 4. Scopolamine-treated mice show significant delay EL compared to control mice on days 3 and 4 ($P < 0.001$). Moreover, the administration of EETP (200 and

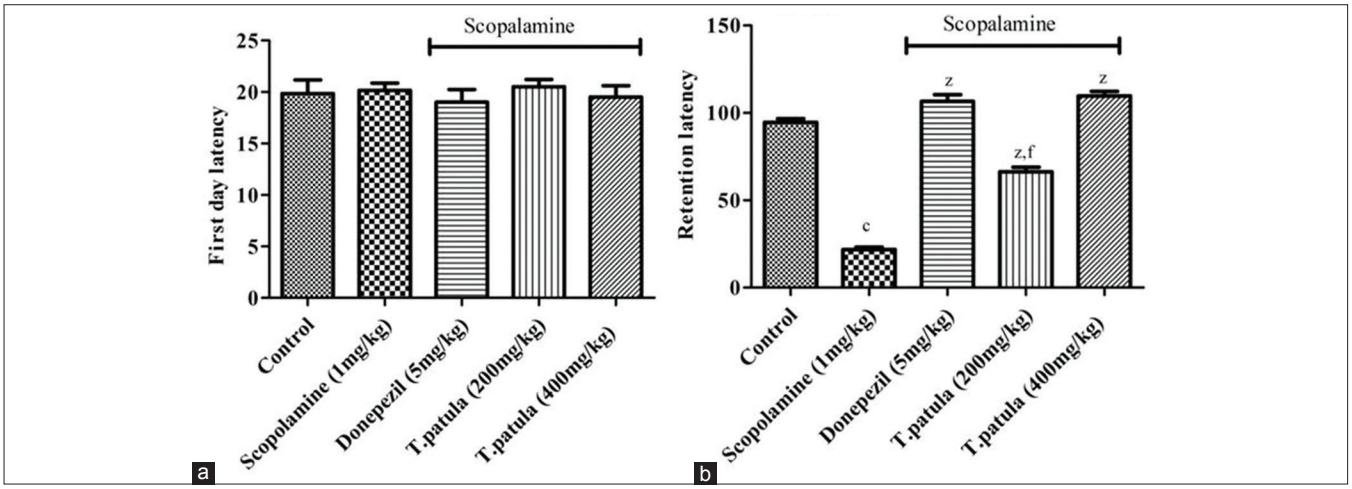

Figure 1: (a) The effects of ethanolic extract of *Tagetes patula* (200 and 400 mg/kg) and donepezil (5mg/kg) on first day latency in the passive avoidance test on scopolamine-induced amnesic mice. (b) Ethanolic extract of *Tagetes patula* (200 and 400 mg/kg) and donepezil (5 mg/kg) on retention latency in the passive avoidance test on scopolamine-induced amnesic mice. c: $P < 0.001$ compared to control mice. z: $P < 001$ compared to scopolamine-treated mice. f: $P < 0.001$ compared to donepezil-treated mice. The data are expressed as mean ± standard error of the mean values

400 mg/kg) significantly decreased EL on days 3 and 4 ($P < 0.001$) when compared to scopolamine-treated mice. Donepezil-treated mice significantly decreased EL on days 3 and 4 ($P < 0.001$) when compared to scopolamine-treated mice. In the probe trial followed by last training session, scopolamine-treated mice decreased the time spent in the target quadrant after the platform was removed but did not produce significant differences when compared to control mice. EETP (200 and 400 mg/kg) treated mice increased the time spent in the target quadrant after the platform was removed ($P < 0.01$) when compared to scopolamine-treated mice. Donepezil (5 mg/kg) treated mice increased the time spent in the target quadrant after the platform was removed ($P < 0.01$) when compared to scopolamine-treated mice. Scopolamine-treated mice significantly ($P < 0.01$) decreased the swimming speed in the target quadrant when compared to control mice. EETP (200 and 400 mg/kg) and donepezil (5 mg/kg) treated mice significantly ($P < 0.05$, $P < 0.001$, $P < 0.01$) increased the swimming speed in the target quadrant when compared to scopolamine-treated mice. Results are shown in Figure 2a-c.

Effect of Ethanolic Extract of *Tagetes patula* on Superoxide Dismutase Level

Scopolamine-treated mice significantly ($P < 0.01$) decreased the level of SOD when compared to control mice. Donepezil-treated mice significantly ($P < 0.01$) increased the level of SOD when compared to scopolamine-treated mice. EETP (200 and 400 mg/kg) treated mice increased the level of SOD but did not produce significant differences when compared to scopolamine-treated mice. EETP (200 and 400 mg/kg) treated mice decreased the level of SOD but did not produce significant differences when compared to donepezil-treated mice. Results are shown in Table 2.

Effect of Ethanolic Extract of *Tagetes patula* on Lipid Peroxidation Level

Scopolamine-treated mice significantly ($P < 0.001$) increased the level of LPO when compared to the control mice. Donepezil-treated mice increased the level of LPO but did not produce significant differences when compared to control mice. EETP (200 and 400 mg/kg) treated mice decreased the level of LPO but did not produce significant differences when compared to control mice. Donepezil-treated mice produced significantly ($P < 0.001$) decreased the level of LPO when compared to the scopolamine-treated mice. EETP (200 and 400 mg/kg) treated mice produced significantly ($P < 0.001$) decreased the level of LPO when compared to the scopolamine-treated mice. EETP (200 and 400 mg/kg) treated mice increased the level of LPO but did not produce significant differences when compared to the donepezil-treated mice. Results are shown in Table 2.

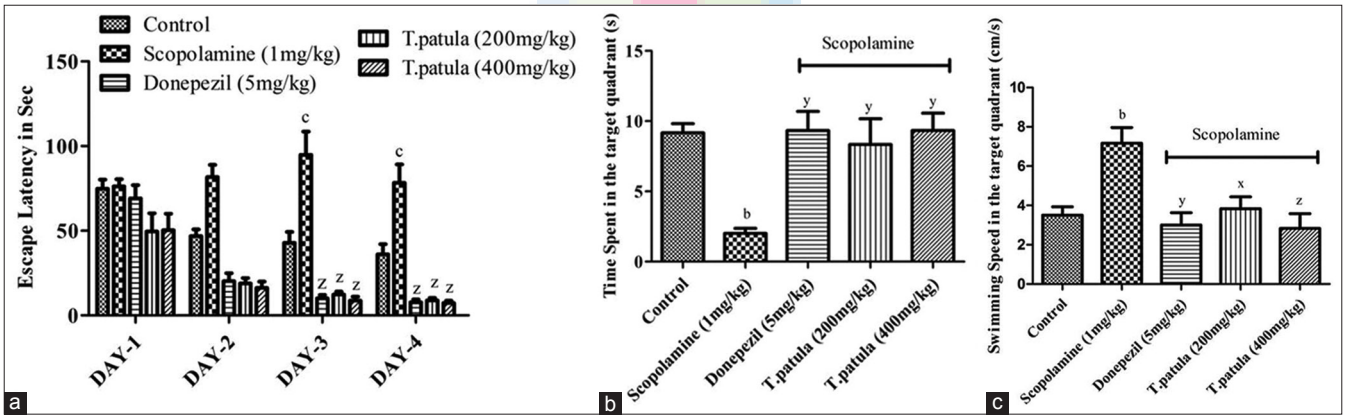

Figure 2: Effect of ethanolic extract of *Tagetes patula* on average latency time (a) time spent, (b) and swimming speed, (c) in trial sessions of the Morris water maze test. Ethanolic extract of *Tagetes patula* (200 and 400 mg/kg) and donepezil (5 mg/kg) were administered to the mice. The mice were treated with scopolamine (1 mg/kg) and tested in the Morris water maze test. Probe trial sessions were performed for 60 s. The data represent mean ± standard error of mean b: $P < 0.01$ and c: $P < 0.001$ compared to control mice. x: $P < 0.05$, y: $P < 0.01$, and z: $P < 0.001$ compared to scopolamine-treated mice

Table 2: Effect of EETP on scopolamine-induced alteration in oxidative stress parameters such as SOD, LPO, GR, and nitrites levels of mice brain tissue

Groups	SOD (units/ mg protein)	LPO (nmoles of MDA/min/mg protein)	GR (units/min/ mg protein)	NOx (µg/ mL tissue)
Control	8.63±0.34	2.83±0.17	35.67±3.48	31.00±3.46
Scopolamine	5.16±0.66^{b}	10.30±0.90^{c}	24.00±2.88	16.33±2.60^{a}
Donepezil+scopolamine	8.40±0.62^{y}	3.63±0.46^{z}	33.00±2.88	27.33±3.18
EETP (200 mg/kg)+scopolamine	6.86±0.40	5.00±0.11^{z}	29.33±2.60	23.00±2.64
EETP (400 mg/kg)+scopolamine	7.23±0.43	3.96±0.29^{z}	32.33±3.52	27.33±3.28

All the values are expressed as mean±SEM. $^{a}P<0.05$, $^{b}P<0.01$, $^{c}P<0.001$ as compared to control mice. $^{y}P<0.01$, and $^{z}P<0.001$ as compared to scopolamine-treated mice (one-way ANOVA followed by Tukey test). ANOVA – Analysis of variance; SEM – Standard error of the mean; EETP – Ethanolic extract of *Tagetes patula*; SOD – Superoxide dismutase; LPO – Lipid peroxidation; GR – Glutathione reductase; NOx – NADPH oxidase; NADPH – Nicotinamide adenine dinucleotide phosphate-oxidase

Effect of Ethanolic Extract of *Tagetes patula* on Glutathione Reductase Level

Scopolamine-treated mice decreased in the level of GR but did not produce significant differences when compared to control mice. Donepezil-treated mice increased the level of GR but did not produce significant differences when compared to scopolamine-treated mice. EETP (200 and 400 mg/kg) treated mice increased the level of GR but did not produce significant differences when compared to scopolamine-treated mice. EETP (200 and 400 mg/kg) treated mice decreased the level of GR but did not produce significant differences when compared to Donepezil-treated mice. Results are shown in Table 2.

Effect of Ethanolic Extract of *Tagetes patula* on Nitrates Level

Scopolamine-treated mice significantly ($P < 0.05$) decreased the level of nitrates when compared to control mice. Donepezil-treated mice decreased the level of nitrates but did not produce significant differences when compared to control mice. EETP (200 and 400 mg/kg) treated mice decreased the level of nitrates but did not produce significant differences when compared to control mice. Donepezil-treated mice increased the level of nitrates but did not produce significant differences when compared to scopolamine-treated mice. EETP (200 and 400 mg/kg) treated mice increased the level of nitrates but did not produce significant differences when compared to scopolamine-treated mice. EETP (200 and 400 mg/kg) treated mice decreased in the level of nitrates but did not produce significant differences when compared to donepezil-treated mice. Results are shown in Table 2.

Effect of Ethanolic Extract of *Tagetes patula* on Acetylcholinesterase Enzyme Level

Scopolamine-treated mice increased the level of AChE level but did not produce significance differences when compared to control mice. Donepezil-treated mice and EETP (200 and 400 mg/kg) treated mice decreased the level of AChE enzyme but did not produce significant differences when compared to scopolamine-treated mice. EETP (200 and 400 mg/kg) treated mice increased the level of AChE but did not produce significant differences when compared to donepezil-treated mice. Results are shown in Table 3.

Effect of Ethanolic Extract of *Tagetes patula* on Dopamine Level

Scopolamine-treated mice significantly ($P < 0.001$) decreased DA level when compared to control mice. Donepezil-treated mice significantly ($P < 0.01$) increased the DA level when compared to control mice. EETP (200 and 400 mg/kg) significantly ($P < 0.001$) increased the DA level when compared to control mice. Donepezil-treated mice significantly ($P < 0.001$) increased the level of DA when compared to scopolamine-treated mice. EETP 200 mg/kg treated mice significantly ($P < 0.001$) increased the level of DA when compared to scopolamine-treated mice. EETP 400 mg/kg treated mice significantly ($P < 0.01$) increased the DA level when compared to scopolamine-treated mice. EETP (200 and 400 mg/kg) treated mice significantly ($P < 0.05$) decreased the level of DA when compared to donepezil-treated mice. Results are shown in Table 3.

Effect of Ethanolic Extract of *Tagetes patula* on Serotonin Level

Scopolamine-treated mice significantly ($P < 0.01$) decreased the level of serotonin when compared to control mice. Donepezil-treated mice decreased the level of serotonin but did not produce significant differences when compared to control mice. EETP (200 and 400 mg/kg) treated mice decreased the level of serotonin but did not produce significant differences when compared to control mice. Donepezil-treated mice increased the level of serotonin but did not produce significant differences when compared to scopolamine-treated mice. EETP (200 and 400 mg/kg) treated mice increased the level of serotonin but did not produce significant differences when compared to scopolamine-treated mice. EETP (200 and 400 mg/kg) treated mice decreased the level of serotonin but did not produce significant differences when compared to donepezil-treated mice. Results are shown in Table 3.

HISTOPATHOLOGY ASSESSMENT

There was an increase in neuronal degeneration and decrease in the number of neuronal cells in brain tissue in scopolamine-treated mice when compared to control mice. Pretreated with EETP 200 mg/kg mice showed partially

Table 3: Effect of EETP on scopolamine-induced neurotransmitter alterations such as AChE, DA, and serotonin levels in mice brain tissue

Groups	AChE (μ moles/min/mg protein)	DA (η/mg protein)	Serotonin (η/mg protein)
Control	17.33±1.45	552.70±3.71	323.30±8.81
Scopolamine	30.00±2.64	311.70±6.00^{c,z}	213.30±14.53^{b}
Donepezil+scopolamine	18.33±2.02	427.70±8.96^{b}	280.00±15.28
EETP (200 mg/kg)+scopolamine	25.33±3.83	415.70±17.29^{c,z,d}	271.00±10.69
EETP (400 mg/kg)+scopolamine	22.67±3.71	406.00±14.00^{c,y,d}	261.00±30.57

All the values are expressed as mean±SEM $^{b}P<0.01$, and $^{c}P<0.001$ as compared to control mice. $^{y}P<0.01$, and $^{z}P<0.001$ as compared to scopolamine-treated mice. $^{d}P<0.05$ as compared to donepezil-treated mice (one-way ANOVA followed by Tukey test). ANOVA – Analysis of variance; SEM – Standard error of the mean; EETP – Ethanolic extract of *Tagetes patula*; AChE – Acetylcholinesterase; DA – Dopamine

recovered from the neuronal degeneration and cell edema when compared to scopolamine-treated mice. Pretreated with EETP 400 mg/kg mice significantly recovered the neuronal degeneration, cell edema, and neuronal congestion when compared to scopolamine-treated mice. Donepezil (5 mg/kg) treated mice significantly attenuated the increase the neuronal degeneration and decreased in the number of neuronal cells when compared to scopolamine-treated mice. Histopathological figures are shown in Figure 3a-e.

DISCUSSION

The present study was designed to assess the cognitive enhancing activities of EETP on amnesic mice using the passive avoidance and Morris water maze tests. Scopolamine interferes with memory and cognitive function, and subsequently causes impairment of reference and working memory.

The passive avoidance and Morris water maze tests are useful methods for the determination of standard learning and memory. Here, EETP (200 and 400 mg/kg) administration inhibited reduction in step-through latency induced by i.p. treatment of scopolamine (1 mg/kg) in the retention trail but did not change latencies during the training trails. Previous reports supports for our present study dose of scopolamine have reported to have no effect on training trails or swimming ability and appears to be dissociated from drug-induced hyperactivity.[3] In our Morris water maze test, increased the EL on days 3 and 4 and reduction in time spent and swimming speed in the target quadrant during the probe trial was observed in scopolamine-treated mice compared to control mice, it can reveal that scopolamine-induced memory impairment. EETP (200 and 400 mg/kg) was reversed the scopolamine-induced memory impairment in the Morris water maze test by increasing the EL by days 3 and 4 and increased the time spent and swimming speed in the probe test by dose-dependently. Accordingly to these results suggest that EETP attenuates long-term and reference memory impairment induced by scopolamine through the rescue by antioxidant mechanisms and acetylcholine system.

In order to elucidate the underlying mechanism of action of EETP and oxidative damage in scopolamine-induced learning and memory deficient mice brains. We assessed the effect of EETP on oxidative damage on the scopolamine-induced learning and memory deficits mice brain. Many clinical studies reported strong evidence that oxidative stress reported involved in the pathogenesis of AD.[24] Antioxidants can delay the development of AD.[25] *T. patula* possess antioxidant activities associated with free radical scavenging. On the other hand, scopolamine additionally triggers reactive oxygen species inducing free radical injury. In our present study, scopolamine-treated mice exhibit increased LPO levels and decreased the levels of GR, SOD and nitrates when compared to control mice. EETP (200 and 400 mg/kg) treated mice ameliorates the scopolamine-induced oxidative damage such as decreased LPO level and increased levels of GR, SOD, and nitrates.

Cholinergic deficits are neuropathological occurrence that are consistently associated with memory loss and are corrected with the severity of AD.[26] Cholinergic transmission is terminated mainly by acetylcholine hydrolysis via the enzyme AChE. This enzyme is essential in maintaining the normal function of the nervous system since it rapidly terminates the action of acetylcholine released into the synapse.Treatment of AD patients and rational target to restore the cholinergic function. The effect of EETP (200 and 400 mg/kg) inhibited acetylcholinesterase enzyme level in the brain and restored the cholinergic function as compared to scopolamine treated mice. The memory improving action of EETP in

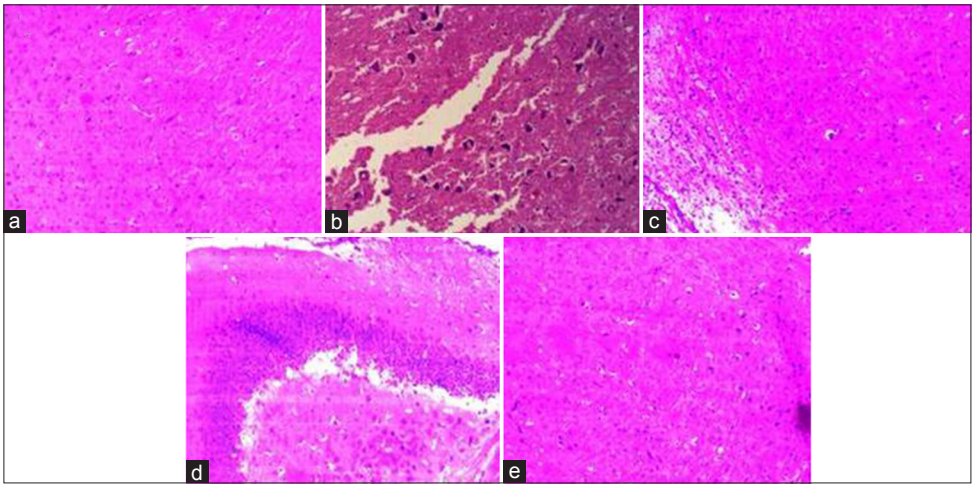

Figure 3: (a) Control, (b) scopolamine (1 mg/kg), (c) donepezil (5 mg/kg), (d) *Tagetes patula* (200 mg/kg), (e) *Tagetes patula* (400 mg/kg)

scopolamine-induced amnesia could be explained, in part, by neurochemical changes in the brain. EETP (200 and 400 mg/kg) significantly increased the level of DA and serotonin in the mice brain. In conclusion, EETP showed potent cognitive enhancing activities by the inhibition of AChE and oxidative damage mechanism. EETP might offer a useful therapeutic choice in either the prevention or the treatment of AD.

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Conflicts of Interest

There are no conflicts of interest.

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