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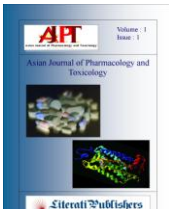

RESEARCH ARTICLE

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Investigation on Essential oil of *Cymbopogon citratus* in Treatment of Alzheimer's disease

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ABSTRACT

Nootropic acitivity of essential oil of *Cymbopogon Citratus* (EOCC) was studied in mice. Morries water maze employed to evaluate learning and memory parameters. Scopolamine (0.4 mg/kg, i.p.) was used to induce amnesia in mice. The essential oil (1gm/kg) significantly attenuated amnestic deficits induced by scopolamine (0.4 mg/kg, i.p.) and natural aging, also exhibited decreased escape latencies time (ELT) and increased Time Spend in Target Quadrant (TSTQ) significantly in the aged mice and scopolamine induced amnesic mice as compared with Piracetam (200 mg/kg, i.p.). To delineate the possible mechanism through which *Cymbopogon Citratus* elicits the anti-amnesic effects, we studied its influence on central cholinergic activity by estimating the whole brain acetylcholinesterase activity. *Cymbopogon Citratus* significantly decreased acetyl cholinesterase activity in mice. The results indicate that essential oil of *Cymbopogon Citratus* might prove to be a useful memory restorative agent in the treatment of dementia seen in elderly. The underlying mechanism of action can be attributed to its anti acetylcholinesterase property.

Keywords: Nootropic activity, *Cymbopogon Citratus*, Memory, anti acetylcholinesterase

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1. INTRODUCTION

Age, stress, emotions are conditions that may lead to anxiety, high BP and dementia or to more ominous threats like schizophrenia and Alzheimer's disease (AD)[1]. Nootropic agents such as piracetam, pramiracetam, aniracetam and choline esterase inhibitors like Donepezil are presently used for improving memory, mood and behavior. Allopathic psychoactive drugs have been the main stay of treating mental illness in India and worldwide. Some nootropic agents (Piracetam) are widely used but the resulting chemophobia associated with them and other similar agents has made their use limited [2]. During last few years there has been increase in usage of alternative medicines by the patients for such ailments, many herbal medicines have been accepted in our country for treating anxiety disorders and cognitive dysfunctions.

Cymbopogon citratus, commonly known as lemongrass, is a tropical perennial herb belonging to the family Poaceae (true grasses), commonly used in traditional Indian, Chinese, and Brazilian medicines. *Cymbopogon citratus* has been shown to be effective in the treatment of fever and infection, headaches, stomach aches, and rheumatic pain, also reported as sedative, antispasmodic, analgesic, anti-inflammatory and antihypertensive agents[3-4].

2. MATERIALS AND METHODS

Plant Materials: *Cymbopogon Citratus* were collected from local areas of Udupi Karnataka and were identified by Dr. Kempegowda HOD Botany Department of Bangalore University Karnataka. Voucher specimens (KCP/BG-11) of the collected samples were deposited in the Departmental laboratory.

Isolation of the essential oil:

Plant material was subjected to hydrodistillation for 3 h using a Clevenger type apparatus. The oil was dried over anhydrous Na2SO4 and preserved in a sealed vial at 4 ^{0}C until further analysis All the doses were prepared in distilled water using 5% Tween 80 solution as suspending agent and administered orally. In all cases, the concentrations were prepared in 1 ml/100g of body weight. The test substances were administered in a single dose using a gastric intubation tube after fasting for 3 to 4 h.

Drugs and Chemicals: Scopolamine Hydrobromide IP (Cadila health care Ltd Goa), Piracetam (Normabrain® Torent Pharmaceuticals LTD, vill, India), DTNB (5,5-dithiobis-2- nitro benzoic acid), Acetylcholine iodide, Sodium dihydrogen phosphate, Dipotasium hydrogen phosphate (Hi-Media, India). Scopolamine hydro bromide was dissolved separately in normal saline and injected i.p., volume of i.p. injection was 1 ml/100 g of mouse.

Acute Toxicity Studies*: Cymbopogon Citratus* at different doses (50-2000 mg/kg) was administered orally to mice with the help of a specially designed oral needle connected to a polythene tube. ECCO was administered at the same time on each day. During the first four hours after the drug administration, the animals were observed for gross behavioral changes if any, for 7 days. Parameters such as hyperactivity, grooming, convulsions, sedation served. The dose 1gm/kg/day was selected.

Animals*:* Swiss mice of either sex weighing around 18 g (younger ones, aged 8 weeks) and 25 g (older ones, aged 28 weeks) were used in the present study. Animals were procured from disease free. They were acclimatized to the laboratory conditions for 5 days before behavioral studies. The animals had free access to food and water and were maintained under 12:12 h light and dark cycles. Institutional Animals Ethics Committee (IAEC) had approved the experimental design and care was taken as per guidelines of CPCSEA, Dept. Govt. of India.

BEHAVIORAL MODELS[5-8]:

MORRIS WATER MAZE (MWM):

It represents a more specific test of spatial memory. The technique of using escape from water to motivate learning has been used traditionally. The simplicity and versatility of the tank makes it the most widely acceptable experimental model for the assessment of cognitive skills in the animals. The essential feature of the technique is that mice are placed in to a large circular pool of water and can escape onto a hidden platform (opaque water). Thus the platform offers no local cues to guide the escape behavior. The mice can escape from swimming by climbing onto the platform and over time the mice apparently learns the spatial location of the platform from any starting position at the circumference of the pool. The only spatial cues are those outside of the water tank and are primarily visual cues.

Construction: The apparatus used is a circular water tank (100 cm in diameter) filled to a depth of 30 cm with water (25^{o}C). Four points equally distributed along the perimeter of the tank served as starting locations. The tank was divided arbitrarily into four equal quadrants and a small platform (5 cm width) was located in the center of one of the quadrants. The platform remained in the same position during the training days.

PROCEDURE:

The mice were released into the water and allowed 90 s to find the platform. Animals received 4 trials per day with 5 min inter-trial interval for 8 days until the performance was stable and the latency to find the platform was low (<10 sec). The test formulations were administered 30 min prior to the first trial daily. Time to find the hidden platform is considered as escape latency (EL). The platform in the water maze was kept at the same position throughout the test to assess the effect of EOCC on spatial reference memory.

GROUPING OF ANIMALS:

Group I: Control (normal saline was administered orally for 8 days. ELT (Escape latency Time) and TSTQ (Time (in second) Spend in Target Quadrant) was noted after 45 min of administration on the 8th day, again on the 12th day i.e. on 4th day.)

Group II: Scopolamine (0.4 mg/kg, i.p.) Was injected intraperitonially and ELT (Escape latency Time) and TSTQ (Time (in second) Spend in Target Quadrant) was noted after 45 min of administration on the 8th day, again on the 12th day i.e. on 4th day.

Group III: Piracetam (200 mg/kg, p.o.) + Scopolamine (0.4 mg/kg, i.p.): Piracetam was administered orally for 8 days. ELT (Escape latency Time) and TSTQ (Time (in second) Spend in Target Quadrant) was noted after 45 min of administration on the 8th day, after 45 min injected Scopolamine intraperitonially for 1 days. ELT (Escape latency Time) and TSTQ (Time (in second) Spend in Target Quadrant) was noted after 45 min of administration on the 8th day, again on the 12th day i.e. on 4th day.

Group IV: EOCC (1gm/kg) + Scopolamine (0.4 mg/kg, i.p.): EOCC(1gm/kg) was administered orally for 8 days. ELT (Escape latency Time) and TSTQ (Time (in second) Spend in Target Quadrant) was noted after 45 min of administration on the 8th day, after 45 min injected Scopolamine intraperitonially for 1 days. ELT (Escape latency Time) and TSTQ (Time (in second) Spend in Target Quadrant) was noted after 45 min of administration on the 8th day, again on the 12th day i.e. on 4th day.

Group V: Only EOCC (1gm/kg) was administered orally for 8 days. ELT (Escape latency Time) and TSTQ (Time (in second) Spend in Target Quadrant) was noted after 45 min of administration on the 8th day, again on the 12th day i.e. on 4th day.

Group VI: Only Piracetam (200 mg/kg, p.o.) was administered orally for 8 days. ELT (Escape latency Time) and TSTQ (Time (in second) Spent in Target Quadrant) was noted after 45 min of administration on the 8th day, again on the 12th day i.e. on 4th day.

Aged groups:

Group I: Control (normal saline was administered orally for 8 days. ELT (Escape latency Time) and TSTQ (Time (in second) Spend in Target Quadrant) was noted after 45 min of administration on the 8th day, again on the 12th day i.e. on 4th day.

Group II: Piracetam (200 mg/kg, p.o.) was administered orally for 8 days. ELT (Escape latency Time) and TSTQ (Time (in second) Spend in Target Quadrant) was noted after 45 min of administration on the 8th day, again on the 12th day i.e. on 4th day.

Group III: EOCC (1gm/kg) was administered orally for 8 days. ELT (Escape latency Time) and TSTQ (Time (in second) Spent in Target Quadrant) was noted after 45 min of administration on the 8th day, again on the 12th day i.e. on 4th day.

Estimation of Brain Acetyl Cholinesterase (AChE) Activity[9,10] :

On the 9th day the animals were killed by cervical dislocation carefully to avoid any injuries to the tissue. Mice brains were isolated quickly and placed in ice-cold saline. The tissues were weighed and homogenized in 0.1 M Phosphate buffer (pH 8). 0.4ml aliquot of the homogenate was added to a cuvette containing 2.6 ml Phosphate buffer (0.1M, ph 8) and 100µl of DTNB. The contents of the cuvette were mixed thoroughly by bubbling air and absorbance was measured at 412 nm in a UV spectrophotometer. When absorbance reaches a stable value, it was recorded as the basal reading. 20µl of substrate i.e acetylthiocholine was added and change in absorbance is recorded for a period of 10 min at interval of 2 min. Change in the absorbance per minute is thus determined. Acetyl cholinesterase (AChE) activity was determined on 9th day, and calculated using following formula.

$R = 5.74 \times 10^{-4} \times A / CO$

Where,

R = Rate in moles of substrate hydrolyzed / minute / gm tissue

A = Change in absorbance / min

CO = Original concentration of the tissue (mg / ml).

Statistical Analysis

All the results were expressed as mean ± Standard error. The data was analyzed using one-way ANOVA followed by multiple range tests was used for the analysis of non-normally distributed data. $p < 0.05$ was considered as significant.

3. RESULTS:

Acute Toxicity Study: No mortality was observed following oral administration of EOCC even with the highest dose (2000 mg/kg). However HS at doses more than 2000 mg/kg produced profuse watery stools in

animals. Both the doses of EOCC had no toxic effect on the normal behavior of the rats.

Effect of *Essential oil of Cymbopogon Citratus* on ELT in young mice (Day1):

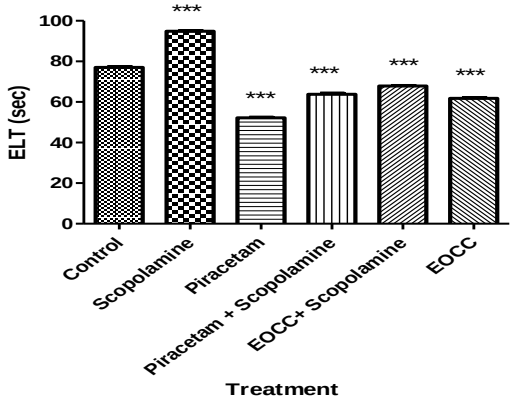
The young animals treated with *Essential oil of Cymbopogon Citratus* (1 gm /kg, p.o.) showed significant($p<0.01$) reduction in ELT of both learning and memory task as compared to normal control of young mice. EOCP+ Scopalamine group showed significant ($p<0.001$) reduction in ELT of both learning and memory task as compared to scopolamine induced group. Piracetam+ Scopalamine group also showed significant similar effect. Piracetam (200 mg/kg, i.p.) signinficantly ($p<0.001$) improved ELT in learning and memory, as compared with normal control group of young mice. (Table & graph:1)

Group No.	Treatment	Dose	ELT (sec) mean ± S.E.M [n=6]
			Day I
I	Control	10 ml/kg	77.00 ± 0.3651
II	Scopolamine	0.4 mg/kg	94.83 ± 0.3073***
III	Piracetamp	200 mg/kg	52.17 ± 0.3073***
IV	Piracetam + Scopolamine	200 mg/kg + 0.4 mg/kg	63.83 ± 0.4773***
V	EOCC + Scopolamine	1 gm/kg + 0.4 mg/kg	67.83 ± 0.3073***
VI	EOCC	1 gm/kg	61.83 ± 0.3073***

n=6 in each group. Data expressed as mean ± S.E.M.. statistical analysis were performed using one-way ANOVA followed by Dunnett's test. *P < 0.05, **P < 0.01, ***P < 0.001 vs. control

Table No.1. Effect of EOCC on Escape Latency in Morries Water Maze : (Young)

Young Mice - Day I – EOCC :



Graph No.1. Effect of EOCC on Escape Latency in Morries Water Maze : (Young)

(B) Effect of *Essential oil of Cymbopogon Citratus* on ELT in young mice (Day4):

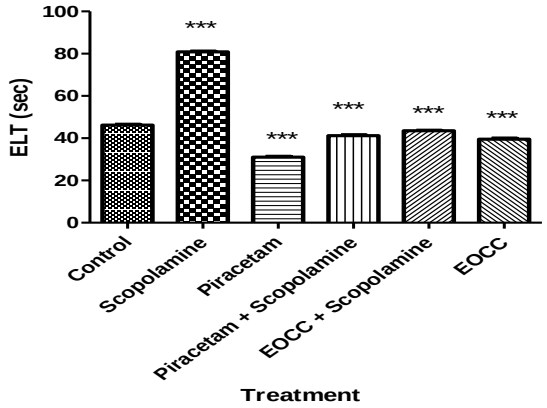
The young animals treated with *Essential oil of Cymbopogon Citratus* (1gm/kg, p.o.) showed significant ($p<0.01$) reduction in ELT of both learning and memory task as compared to normal control of young mice. EOCC+Scopalamine group showed significant ($p<0.001$) reduction in ELT of both learning and memory task as compared to scopolamine induced group. Piracetam+Scopalamine group showed significant ($p<0.001$) reduction in ELT of both learning and memory task as compared to scopolamine induced group. Piracetam (200 mg/kg, p.o.) signinficantly ($p<0.001$) improved ELT in learning and memory, as compared with normal control group of young mice. (Table & graph: 2)

Group No.	Treatment	Dose	ELT (sec) mean ± S.E.M [n=6]
			Day IV
I	Control	10 ml/kg	46.17 ± 0.4014
II	Scopolamine	0.4 mg/kg	80.83 ± 0.3073***
III	Piracetam	200 mg/kg	31.00 ± 0.3651***
IV	Piracetam + Scopolamine	200 mg/kg + 0.4 mg/kg	41.17 ± 0.3073***
V	EOCC + Scopolamine	1 gm/kg + 0.4 mg/kg	43.50 ± 0.2236***
VI	EOCC	1 gm/kg	39.50 ± 0.4282***

n=6 in each group. Data expressed as mean ± S.E.M.. Statistical analysis were performed using one-way ANOVA followed by Dunnett's test. *P < 0.05, **P < 0.01, ***P < 0.001 vs. control

Table No.2. Effect of EOCC on Escape Latency in Morries Water Maze on day 4: (Young)

Young Mice – Day IV - EOCC :



Graph No.2. Effect of EOCC on Escape Latency in Morries Water Maze: (Young)

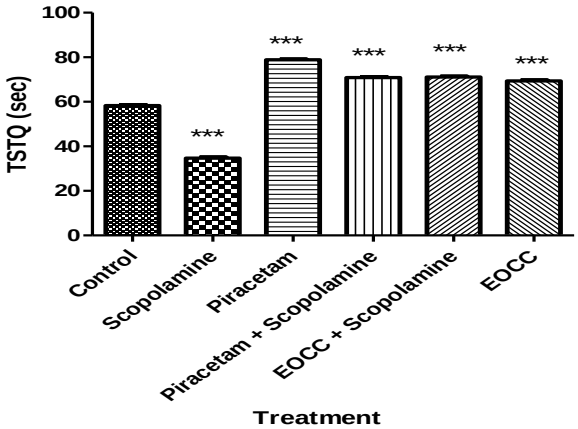
(C) Effect of *Essential oil of Cymbopogon Citratus* on TSTQ in young mice:

The young animals treated with *Essential oil of Cymbopogon Citratus* (1gm/kg, p.o.) showed significant($p<0.01$) improvement in TSTQ values of both learning and memory task as compared to normal control group of young mice. EOCC+Scopolamine group showed significant ($p<0.001$) improvement in TSTQ value of both learning and memory task as compared to scopolamine induced group. Piracetam+Scopolamine group showed significant ($p<0.001$) improvement in TSTQ value of both learning and memory task as compared to scopolamine induced group. Piracetam (200 mg/kg, i.p.) signinficantly ($p<0.001$) increased in TSTQ values in learning and memory, as compared with control group of young mice. (Table & graph: 3)

Group No.	Treatment	Dose	TSTQ (sec) mean ± S.E.M [n=6]
I	Control	10 ml/kg	58.33 ± 0.3333
II	Scopolamine	0.4 mg/kg	34.67 ± 0.4216***
III	Piracetam	200 mg/kg	78.83 ± 0.3073***
IV	Piracetam + Scopolamine	200 mg/kg + 0.4 mg/kg	70.83 ± 0.4014***
V	EOCC + Scopolamine	1 gm/kg + 0.4 mg/kg	71.17 ± 0.3073***
VI	EOCC	1 gm/kg	69.33 ± 0.3333***

n=6 in each group. Data expressed as mean ± S.E.M.. statistical analysis were performed using one-way ANOVA followed by Dunnett's test. *P < 0.05, **P < 0.01, ***P < 0.001 vs. control

Table No.3. Effect of EOCC on TSTQ in Morries Water Maze : (Young)



Graph No.3. Effect of EOCC on TSTQ in Morries Water Maze : (Young)

(D) Effect of *Essential oil of Cymbopogon Citratus* on ELT in aged mice (Day1):

The aged animals treated with *Essential oil of Cymbopogon Citratus* (1gm/kg, p.o.) showed significant ($p<0.01$) reduction in ELT of both learning and memory task as compared to control group of aged

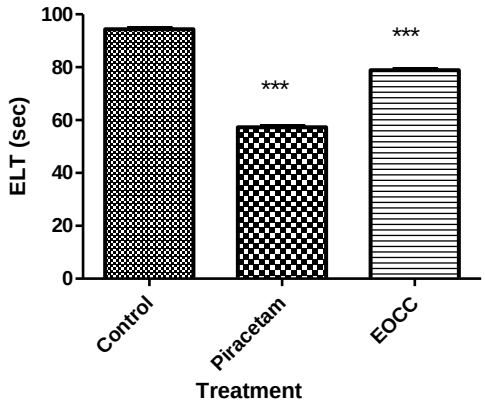
mice. Piracetam (200 mg/kg, p.o.) signinficantly (p<0.001) decreased ELT in learning and memory, as compared with normal control group of aged mice. (Table & graph:4)

Group No.	Treatment	Dose	ELT (sec) mean ± S.E.M [n=6] Day I
I	Control	10 ml/kg	89.33 v 0.333
II	Piracetam	200 mg/kg	56.17 ± 0.4014***
III	EOCC	1 gm/kg	75.17 ± 0.3073***

n=6 in each group. Data expressed as mean ± S.E.M.. statistical analysis were performed using one-way ANOVA followed by Dunnett's test. *P < 0.05, **P < 0.01, ***P < 0.001 vs. control

Table No.4. Effect of EOCC on Escape Latency in Morries Water Maze : (Aged)

Aged Mice – Day I – EOCC :



Graph No.4. Effect of EOCC on Escape Latency in Morries Water Maze : (Aged)

(E) Effect of *Essential oil of Cymbopogon Citratus* on ELT in aged mice (Day4):

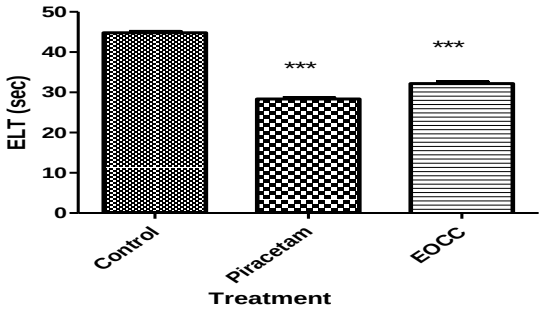
The aged animals treated with Essential oil of Cymbopogon Citratus (1gm/kg, p.o.) showed significant(p<0.01) reduction in ELT of both learning and memory task as compared to control group of aged mice. Piracetam (200 mg/kg, p.o.) signinficantly (p<0.001) decreased ELT in learning and memory, as compared with normal control group of aged mice. (Table & graph:5)

Group No.	Treatment	Dose	ELT (sec) mean ± S.E.M [n=6] Day IV
I	Control	10 ml/kg	42.33 ± 0.3333
II	Piracetam	200 mg/kg	26.83 ± 0.3073***
III	EOCC	1 gm/kg	30.67 ± 0.3333***

n=6 in each group. Data expressed as mean ± S.E.M.. statistical analysis were performed using one-way ANOVA followed by Dunnett's test. *P < 0.05, **P < 0.01, ***P < 0.001 vs. control

Table No.5. Effect of EOCC on Escape Latency in Morries Water Maze : (Aged)

Aged Mice – Day IV – EOCC :



Graph No.5. Effect of EOCC on Escape Latency in Morries Water Maze : (Aged)

(F) Effect of *Essential oil of Cymbopogon Citratus* on TSTQ in aged mice :

The aged animals treated with *Essential oil of Cymbopogon Citratus* (1gm/kg, p.o.) showed significant(p<0.01) improvement in TSTQ values of both learning and memory task as compared to normal

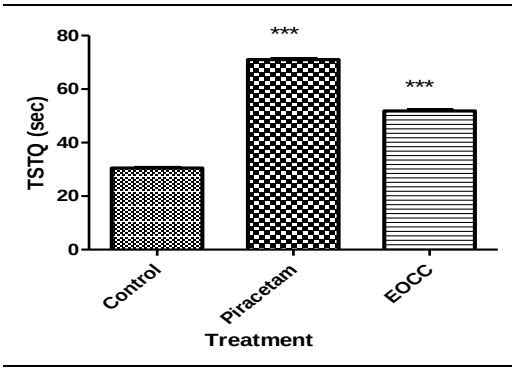
control group of aged mice. Piracetam (200 mg/kg, p.o.) signinficantly (p<0.001) increased in TSTQ values in learning and memory, as compared with normal control group of aged mice. (Table & graph: 6)

Group No.	Treatment	Dose	TSTQ (sec) mean ± S.E.M [n=6]
I	Control	10 ml/kg	30.50 ± 0.2236
II	Piracetam	200 mg/kg	71.00 ± 0.3651***
III	EOCC	1 gm/kg	51.83 ± 0.3073***

n=6 in each group. Data expressed as mean ± S.E.M.. statistical analysis were performed using one-way ANOVA followed by Dunnett's test. *P < 0.05, **P < 0.01, ***P < 0.001 vs. control

Table No.6. Effect of EOCC on TSTQ in Morries Water Maze : (Aged)

Old Mice – EOCC :



Graph No.6. Effect of EOCC on TSTQ in Morries Water Maze : (Aged)

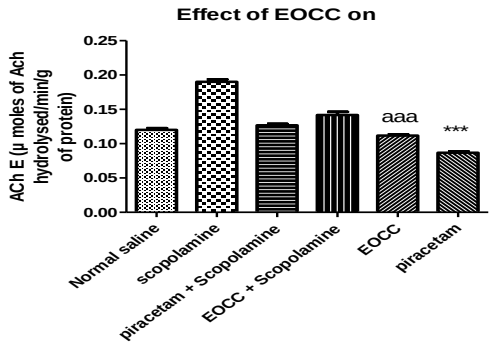
Effect on Acetylcholinesterase Activity:

The acetylcholiesterase activity of whole brain was markedly elevated (p < 0.05) after scopolamine (0-4 mg/kg, p.o.) treatment. Piracetam (200 mg/kg, p.o.) and EOCC (1gm/kg, p.o.) significantly lowered AChE activity (Fig 7).

Group	Treatment	Dose	AChE (µM)
I	Normal control	10 ml/kg(p.o.)	0.12±0.0025
II	Scopolamine	0.4 mg/kg(i.p.)	0.18±0.0047***
III	Piracetam+Scopolamine	200 + 0.4(mg/kg)	0.12±0.0028###
IV	EOCC+Scopolamine	30 + 0.4(mg/kg)	0.14±0.0021 ###
V	EOCC	30 mg/kg (p.o.)	0.11±0.0022 aaa
VI	Piracetam	200 (p.o.)	0.09±0.0033***

Each group consists of 6 animals. Values are mean ±S.E.M. ***P < 0.001 compared to normal control group. aaaP< 0.001 compared to scopolamine treated group. ###P < 0.001 compared to scopolamine treated group.

Table7: Effect of *EOCC* on whole brain acetyl cholinesterase activity on young mice using Elevated plus maze

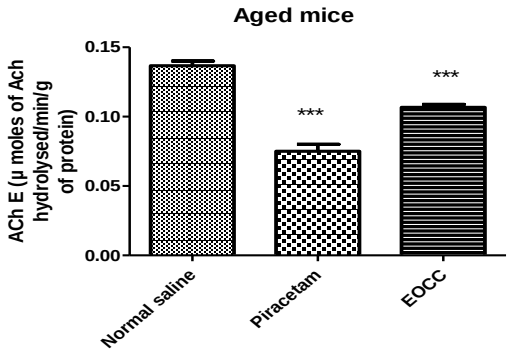


graph:7 Effect of *EOCC* (1gm/kg, p.o.) On Whole brain acetyl cholinesterase activity of young mice .

Group	Treatment	Dose	AChE (µM)
I	Normal control	10 ml/kg	0.13±0.0033
II	Piracetam	200 mg/kg (p.o.)	0.07±0.0050***
III	EOCC	30 mg/kg (p.o.)	0.11±0.0021***

Each group consists of 6 animals. Values are mean ±S.E.M, (n=6). One way ANOVA followed by Dunnett's test . ***indicates p< 0.001 as compared to normal control group of aged mice. ** indicates p< 0.01 as compared to normal control group of aged mice. * indicates p< 0.05 as compared to normal control group of aged mice

Table8: Effect of *EOCC* on whole brain acetyl cholinesterase activity on aged mice using Elevated plus maze



Graph:8 Effect of *EOCC* (1gm/kg, p.o.) on Whole brain acetyl cholinesterase activity of aged mice

Values are mean ± SEM, AChE- whole brain AChE activity, [a] indicates $p<0.05$ Vs control, H = 16.67; df = 5; $p < 0.05$.

4. DISCUSSION

Aromatic plants had been used since ancient times for their preservative and medicinal properties, and to impart aroma and flavor to food. Hippocrates, sometimes referred to as the 'father of medicine', prescribed perfume fumigations. The pharmaceutical properties of aromatic plants are partially attributed to essential oils. The term 'essential oil' was used for the first time in the 16th century by Paracelsus von Hohenheim, who named the effective component of a drug, 'Quinta essential'[11]

Dementia of the Alzheimer type (DAT) is a common disease with important consequences to the patient's life quality.[2] Inhibition of the enzyme acetylcholinesterase (AChE) is the basis of most drugs used clinically for symptomatic relief of the early stages of AD. Inhibition of AChE (i.e., reduction of the enzyme responsible for breaking down ACh) results in elevated levels of ACh in the brain, which is associated with improvement of cognitive function including memory[12].

The ability of cholinesterase inhibitory activity in cyclic monoterpenes was identified as the most active compounds. These include Camphor, 1, 8-Cineole, Beta-pinene, Alpha-pinene, together with their inhibitory activities. It can be seen that, of the active components, 1,8-cineole is likely to contribute most to the activity of the oil since it is present in the greatest concentration.[13-14].

The cholinesterase inhibitory properties of these 1,8-cineole monoterpenes were only recently reported. Antioxidant effects were noted with 1,8-cineole, alpha-pinene and beta-pinene, but a pro-oxidant effect was produced by camphor, a relatively major component of the oil. It is likely that the pro-oxidant activity of camphor is eclipsed by the antioxidant compounds so that the total oil would have an overall antioxidant effect[15].

EOCC also reversed the scopolamine-induced impairment in learning and memory, when assessed on MWM. Piracetam, the first representation of a class of nootropic agents, has been shown to improve memory deficits in geriatric individuals. Repeated injections of piracetam had improved learning abilities and memory capacities of laboratory animals[16-18]. Both piracetam and *Cymopogon citratus* meet major criteria for nootropic activity, namely improvement of memory in absence of cognitive deficit[19,20].

In the present study, *Cymopogon citratus* significantly inhibited the AChE activity in the mice whole brain homogenate, indicating its potential in the attenuation of symptoms of cognitive deficits. Hence, the memory improving activity of *EOCC* may be attributed to its antioxidant, neuropro-tective, pro-cholinergic and anti-acetylcholinesterase properties and can be of enormous use in delaying the onset and reducing the severity of Alzheimer's disease. Further investigations

using more experimental paradigms are required for further confirmation of nootropic potential of essential oil of *Cymopogon citratus* in the treatment of various cognitive disorders.

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5. ACKNOWLEDGEMENTS

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