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Nootropic Activity of *Zingiber Officinale* in Albino Mice: A Behavioral and Neurochemical Approach

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ABSTRACT

Acetone extract of *Z. oficinale (AEZ)* possessing anti-5HT effect which can be related to antagonism of $5HT_3$ receptors. $5HT_3$ receptor antagonists improve basal performance in rhodent and primate tests of cognition and inhibit the cognitive impairments.Material & methods: Spontaneous alteration task paradigm using Y-maze, transfer latency on elevated plus maze, estimation of brain acetylcholinoesterase activity, passive avoidance paradigm and transfer latency on rectangular maze were used as models. Piracetam 200mg/kg p.o. was used as standard. Results: *(AEZ)* (200mg/kg p.o.) significantly reversed scopolamine induced reduction in spontaneous alteration in behaviour (SAB) and step down latency (SDL) and increase in transfer latency, acetylcholinoesterase (AChE) activity , stepdown error(SDE) and time spent in shock zone(TSZ).Discussion: Central cholinergic system plays an important role in learning and memory. $5HT_3$ receptor mediates inhibition of acetyl choline release in cortical tissue. *(AEZ)* is a $5HT_3$ receptor antagonist. So, it might increase the release of acetylcholine to act as memory enhancer.Conclusion: AEZ is found to be effective in improving both spatial working memory and long term memory.

Key words: 5-HT3 R, Y Maze, EPM, HWM, Acetylcholine esterase

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INTRODUCTION

It has been reported that acetone extract of ginger and its fraction have anti-5HT effects which can be related to antagonism of $5HT_3$ receptors. (Huang et al., 1991).

5-HT_3 receptor antagonists like Ondansetron potently improves basal performance in rhodent and primate tests of cognition.[1, 2] and inhibits the cognitive impairments caused by cholinergic deficits and glutamatergic hypofunction .[3]

So the present study was undertaken to investigate the effect of *AEZ* on learning and memory in mice. Both working memory and long term memory were evaluated.

MATERIAL AND METHOD

Animals

Swiss albino mice of either sex (25-30g) procured from Animal house of School of Pharmaceutical sciences, Siksha O Anusandhan University were used. They were acclimatized to the laboratory conditions one week prior to studies. The animals had free access to food and water and maintained under 12:12hr light and dark cycles.

All experiments were carried out during day time from 9.00 to 14.00 hrs, after due approval from institutional animal ethical committee.

Drug

Acetone extract of Ginger (*Zingiber officinale*) (AEZ) was purchased from Indian Herbs Research & Supply Co Ltd, Shaharanpur.

A pilot study is initially conducted to select dose of *AEZ*. *AEZ 200mg*/kg was administered 1hr prior to each observation. Control group were administered saline (0.9%w/v NaCl) 2ml/kg body weight. All studies were done for 7 days and drugs were administered between 10-12AM every day. All observations were made on day 8 days after 1hr of *(AEZ)* administration.

Piracetam: Piracetam (Nootropil syrup, UCB) was given per oral dose of 200mg/kg prior to each experiment.

Scopolamine: Scopolamine hydrobromide (Sigma Aldrich, USA) was used in a dose of 0.4 mg/kg i.p.

Effect on Cognition dysfunction

(i) Spontaneous alteration task paradism using Y-maze

The spatial working memory was measured through the spontaneous alteration of behaviour in Y-maze (INCO). The Y-maze consists of three identical removal sun mica lined chambers arranged in Y-shape connected to the central chamber. Each arm has a working dimension of approx. 30 ×15× 15 cm with mouse presence indicator and hinged top. Each mouse is placed in the central chamber and allowed to move freely through the maze during an 8-minute session. The mouse trend to explore the maze systematically, entering each arm in turn. When mouse enters one arm the rat presence indicator glows. The series of arm entries was recorded. Alteration is defined as the number of successive entries in to the three arms on overlapping triplet sets. The percentage alteration was calculated as the ratio of actual to possible alterations (defined as the total no. of arm entries minus two), multiplied by 100 .[4]

(ii) Transfer latency on elevated plus maze

The spatial long term memory was assessed by using elevated plus maze. The elevated plus maze consisted of two open arms (50×10×40 cm) with an open roof. The maze was elevated to a height of 50cm from the floor. Transfer latency (TL) was used as an index of learning and memory. TL is the time in which animal moves from the open arm to the closed arm. Animal not entering the closed arm within 180 sec were assigned the transfer latency of 180s.The animals were trained 24hr prior to testing.[5]

(iii) Estimation of brain acetyl cholinesterase (AChE) activity

Ellmen *et al* method was used to estimate whole brain AChE within one hour of sampling in all mice. Brain was weighted and homogenized in 0.1M phosphate buffer (P^H8.0) at a concentration of 5mg tissue/ml of buffer. AChE activity was determined spectrophotometrically at 412nm with 0.01M dithio-bis-nitrobenzoic acid and 0.075M acetylthiocholine iodide as substrate at 25^0C[6].

(iv) Passive avoidance paradism

This method uses the principle of negative reinforcement to examine the long term memory. Step down latency (SDL) was recorded and used as the index to measure the passive avoidance paradigm in a Esho memory evaluator. SDL is the time taken in seconds by the mouse to step down from wooden platform to grid floor with all the four paws on the grid floor [7-9].

(v) Learning, memory and reasoning evaluation using Hebb's William maze (Rectangular maze)

Rectangular maze (INCO) is used for studying learning, memory and reasoning in animals. The clever the mouse, the more quickly it is able to make use of past experience and therefore more quickly it learns its way out in the maze. The rectangular maze is divided into chamber A, in which the mouse is placed and has a sliding door that is opened to allow the mouse to enter the maze; chamber C. Animal has to explore chamber B. At the other end of maze in which the reward is kept.

An electrical system provides indication when the mouse is placed in chamber A, when it leaves it to enter the maze i.e. chamber C and when enters chamber B, thus enabling the reaction time to be noted without observing the animal. A four digit timer records the time taken by animal in exploring the maze. [10]

RESULTS

(i) Effect on spontaneous alteration behaviour (SAB) using Y-maze

The effect of *AEZ*, Piracetam on SAB in normal and scopolamine treated animals is given in **Table 1**. Scopolamine (0.4mg/kg i.p) significantly ($p<0.05$) reduced the spontaneous alteration behaviour (SAB) in mice as compared to control. Both Piracetam (200mg/kg p.o) and *AEZ* (200mg/kg p.o) significantly ($p<0.05$) improved the scopolamine induced reduction in SAB.

(ii) Effect on transfer latency using Elevated plus maze

The effect of *AEZ*, Piracetam on transfer latency in normal and scopolamine treated animals is given in **Table 2**. Piracetam (200mg/kg p.o.) significantly ($p<0.05$) reduced the transfer latency as compared to control but this reduction is not significant in case of AEZ. Scopolamine as compared to control significantly increased the transfer latency which was significantly reduced by both Piracetam (200mg/kg p.o) and *AEZ* (200mg/kg p.o).

(iii) Effect of transfer latency using Rectangular maze

The effect of *AEZ*, Piracetam on transfer latency in normal and scopolamine treated animals is given in **Table 3**. Scopolamine (0.4mg/kg i.p) significantly increased the transfer latency as compared to control in a rectangular maze. This was significantly ($p<0.05$) reduced by both Piracetam (200mg/kg p.o) and *AEZ* (200mg/kg p.o).

(iv) Effect of Acetylcholine esterase (AChE) activity

The acetylcholine esterase activity was significantly increased by scopolamine (0.4mg/kg i.p) as compared to control (**Table 4**). The increase in AChE activity by scopolamine was significantly reduced by both Piracetam (200mg/kg p.o) and *AEZ* (200mg/kg p.o).

(v) Effect of SDL, SDE and TSZ

Both Piracetam (200mg/kg p.o) and *AEZ* (200mg/kg p.o) significantly reversed the decrese in SDL and increase in SDE and TSZ induced by scopolamine (0.4mg/kg i.p). The results are given in **Table 5**.

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Table 1: Effect of *(AEZ)* 200mg/kg on spontaneous alteration behaviour in mice using a Y-maze.

Group	Treatment	Dose	% alteration(3 out of 3)
I	Control	2ml/kg i.p	75.83± 2.13
II	Piracetam	200 mg/kg p.o	72.50 ± 1.78
III	*(AEZ)*	200 mg/kg p.o	71.8± 2.03
IV	Scopolamine	0.4mg/kg i.p	50.66±1.76*
V	Scopolamine + Piracetam	0.4mg/kg i.p 200 mg/kg p.o.	67.66± 0.91*
VI	Scopolamine+ *(AEZ)*	0.4 mg/kg i.p 200 mg/kg p.o	65.33±2.75*
$F_{(5,30)}$			22.08*

*P<0.05

One way ANOVA followed by Dunnet's t-test. Gr II, III, IV are compared with Gr I (Control) and Gr V, VI are compared with Gr IV (Scopolamine)

Table 2: Effect of *(AEZ)* 200mg/kg on transfer latency in mice using a plus-maze.

Group	Treatment	Dose	Transfer latency(in sec) (Plus maze)
I	Control	2ml/kg i.p	5.83 ± 0.60
II	Piracetam	200 mg/kg p.o	3.67 ± 0.33*
III	*(AEZ)*	200 mg/kg p.o	4.16± 0.30
IV	Scopolamine	0.4mg/kg i.p	15.33 ± 1.65*
V	Scopolamine + Piracetam	0.4mg/kg i.p 200 mg/kg p.o.	4.5± 0.42*
VI	Scopolamine+ *(AEZ)*	0.4mg/kg i.p 200 mg/kg p.o	6.50±0.42*
$F_{(5,30)}$			56.21*

*P<0.05

One way ANOVA followed by Dunnet's t-test.Gr II, III, IV are compared with Gr I(Control) and Gr V, VI are compared with Gr IV(Scopolamine)

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Table 3: Effect of *(AEZ)* 200mg/kg on transfer latency in mice using a Rectangular maze.

Group	Treatment	Dose	Transfer latency (in sec) (Rectangular maze)
I	Control	2ml/kg i.p	74.50 ± 2.68
II	Piracetam	200 mg/kg p.o	67.16 ± 3.51
III	*(AEZ)*	200 mg/kg p.o	68.50 ± 2.56
IV	Scopolamine	0.4mg/kg i.p	119.16±4.36 *
V	Scopolamine + Piracetam	0.4mg/kg i.p 200 mg/kg p.o.	79.16 ± 4.36 *
VI	Scopolamine+ *(AEZ)*	0.4mg/kg i.p 200 mg/kg p.o	84.33±3.52*
$F_{(5,30)}$			30.54*

*P<0.05

One way ANOVA followed by Dunnet's t-test.Gr II, III, IV are compared with Gr I (Control) and Gr V, VI are compared with Gr IV (Scopolamine)

Table 4: Effect of *(AEZ)* 200mg/kg on AChE activity in mice.

Group	Treatment	Dose	AChE activity(µmoles)
I	Control	2ml/kg i.p	132.50± 11.67
II	Piracetam	200 mg/kg p.o	118.33± 10.38
III	*(AEZ)*	200 mg/kg p.o	127.5± 9.46
IV	Scopolamine	0.4mg/kg i.p	253.33 17.11*
V	Scopolamine + Piracetam	0.4mg/kg i.p 200 mg/kg p.o	136.66 ± 14.47*
VI	Scopolamine+ *(AEZ)*	0.4mg/kg i.p 200 mg/kg p.o	143.33±9.63*
$F_{(5,30)}$			16.40*

*P<0.05

One way ANOVA followed by Dunnet's t-test. Gr II, III, IV are compared with Gr I (Control) and Gr V, VI are compared with Gr IV (Scopolamine)

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Table 5: Effect of *(AEZ)* 200mg/kg on step down latency (SDL), step down error (SDE) and time spent by animal in shock zone (TSZ) in mice using passive shock avoidance paradism..

Group	Treatment	Dose	SDL (in sec)	SDE(in sec)	TSZ(in sec)
I	Control	2ml/kg i.p	3.17±0.54	7.66±0.33	134.16±3.69
II	Piracetam	200 mg/kg p.o	3.67±0.42	7.16±0.48	126.83±2.71
III	*(AEZ)*	200 mg/kg p.o	2.83±0.40	7.33±0.33	130.83±4.72
IV	Scopolamine	0.4mg/kg i.p	1.33 ±0.40 *	9.83 ±0.60*	235.83±5.35*
V	Scopolamine + Piracetam	0.4mg/kg i.p 200 mg/kg p.o.	4.16±0.03 *	7.55±0.42 *	143.3±4.77*
VI	Scopolamine+ *(AEZ)*	0.4 mg/kg i.p 200 mg/kg p.o	3.83±0.48 *	7.83±0.30 *	153.33±6.00*
	$F_{(5,30)}$		6.20 *	5.30 *	80.52*

*P<0.05

One way ANOVA followed by Dunnet's t-test.Gr II, III, IV are compared with Gr I (Control) and Gr V, VI are compared with Gr IV(Scopolamine)

Figure 1: Effect of AEZ on SAB in mice using a Y-maze.

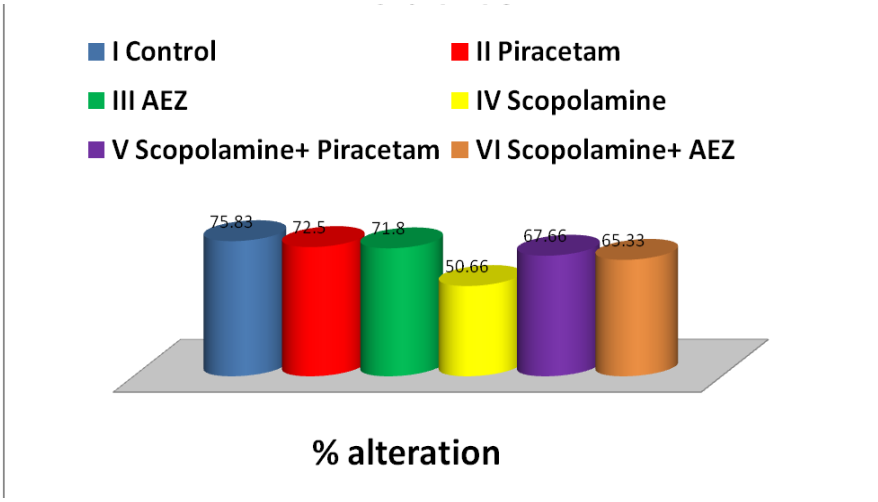

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Figure2:Effect of *AEZ* on TL in mice using EPM

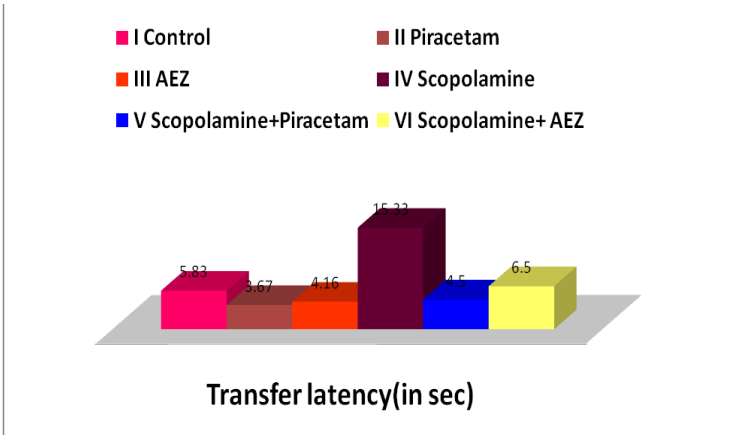


Figure 3:Effect of *AEZ* on transfer latency in mice using a Rectangular maze.

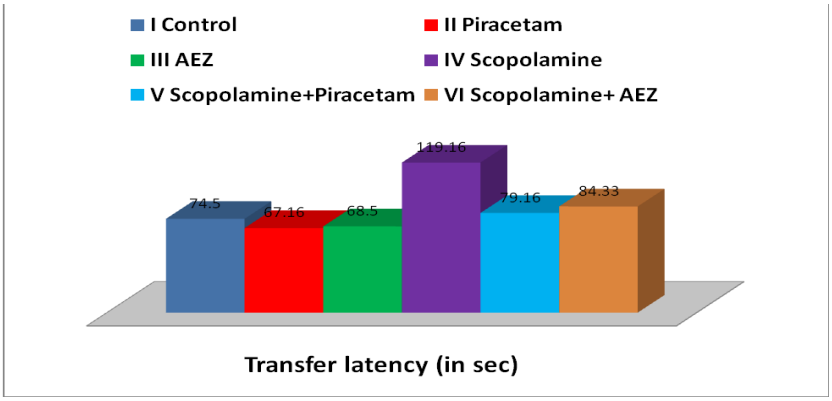
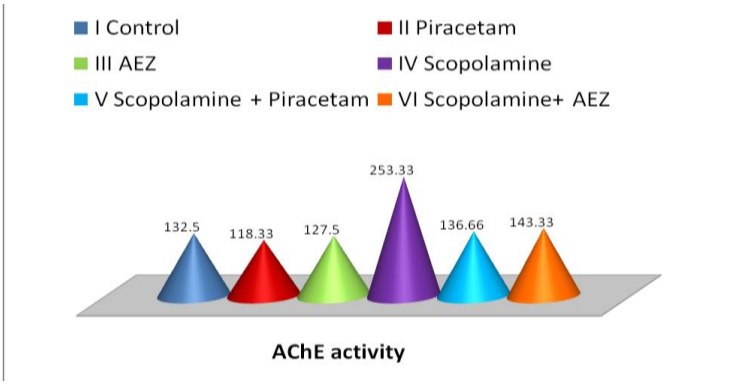


Figure 4:Effect of *AEZ* on AChE activity in mice



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DISCUSSION

The present study aims at evaluating the memory enhancing effect of *AEZ*. Both spatial long term memory and working memory were evaluated. Model like Y-maze, rectangular maze, elevated plus maze, passive shock avoidance paradigm and AchE activity were used. Working memory allows animals to remember information that is useful for subsequent sessions. It is a form of short term memory with limited capacity and an extremely rapid decay. Its importance is more depictive of memory disorder in the Alzheimer's dementia.[11]
Scopolamine is used in models of passive avoidance task, spatial memory deficits, and working memory impairment in radial arm maze .[12- 14]

Spatial orientation and memory performance in the Y-maze is at least partly dependant on the hippocampus a brain area often affected by aging.[15,16] *AEZ* improves SAB in Y-maze. So, it may have action on hippocampus.

Passive avoidance response (PAR) is extensively used for screening of drugs affecting learning and memory. Parameter like step down latency (SDL), step down error (SDE) and time spent by animal in shock zone (TSZ) were evaluated. Scopolamine significantly reduced SDL and increased SDE and TSL. *AEZ* (200mg/kg, p.o) significantly ($p<0.05$) reverse the amnesia produced by scopolamine. [17- 19]

AEZ reduced the transfer latency in elevated plus maze. It measures spatial long term memory. Acquisition and retention of memory can be evaluated.
The Hebb's William maze or rectangular maze is an incentive-based exteroceptive behavioural model useful for measuring the natural working memory of rats. [20, 5, 21] *AEZ* significantly reduced the transfer latency in rectangular maze. So, it may be improving the working memory.
Central cholinergic system plays an important role in learning and memory. *Z. officinale* is a potential anti-cholinesterase agent. In addition to that its anti-inflammatory, antioxidant and neuroprotective effect also contribute towards nootropic activity of *Z. officinale*. In our study *AEZ* decreases AChE activity.

Z. officinale shows antagonism of 5-HT_3 receptors. Several studies describe that 5-HT_3 receptor antagonist can act as cognitive enhancer.[8]
Various studies have demonstrated that $5HT_3$ receptor antagonists inhibit impairments in cognition caused by cholinergic deficits.[22] $5HT_3$ receptor antagonism facilitates the cognitive performance through induction of long term potentiation, possibly through acetylcholine release. [23, 24] Antagonists at $5HT_3$ receptors have demonstrated to reverse scopolamine induced impaired task in passive avoidance paradigm and morris water maze . [25, 26] In another study, ondansetron but not tropisetron reversed the memory deficits due to scopolamine treatment in the step through passive avoidance task. Contrariwise, spatial navigation impairments induced by scopolamine successfully antagonized by tropisetron but not ondansetron in morris water maze. The release of cerebral acetylcholine from terminals in cerebral cortex has been shown to be regulated by 5-hydroxy triptamine (5-HT). There is evidence that 5-HT_3 receptors mediate inhibition of acetylcholine release in cortical tissue

(Barnes et al., 1989). So, *(AEZ)* being a 5-HT_3 receptor antagonist might be increasing the release of ACh.

CONCLUSION

AEZ is found to be effective not only in improving spatial working memory but also long term memory. This memory enhancing effect of *AEZ* may be attributed to its $5HT_3$ antagonistic activity which in turn might be increasing the release of acetylcholine in cerebral cortex.

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