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EFFECT OF ETHANOLIC EXTRACT OF *TECOMA STANS* ON LEARNING AND MEMORY IN NORMAL AND MEMORY DEFICIT ANIMALS

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ABSTRACT

Objective to investigate the possible effect of ethanolic extract of *Tecoma stans* (EETS) on learning and memory in normal and memory deficit animals in two different age groups of mice. The effect of EETS on learning and memory was evaluated using Elevated plus maze and Rectangular plus maze screening methods on young and adult mice. Three doses of *EETS* 5.4, 10.8 and 21.6 mg/kg, p. o were administered for 7 days and 15 days in experiments involving Elevated plus maze and Rectangular plus maze respectively in the separate group of animals. As a response to Elevated plus maze method adult and young mice showed the marked decrease in transfer latency ($p<0.001$) on 8th day when compared to negative control diazepam (1 mg/kg, i. P) indicates learning and retention of the learned task or memory in mice. Furthermore, in Rectangular plus maze the time taken by the mice to reach the reward chamber 'B' from the entry chamber 'A' in *EETS* treated animals was reduced, at 5.4 mg/kg did not show any significant impact on memory of young and old mice. Whereas extract at the dose of 10.8 and 21.6 mg/kg, p.o proved to improve the memory in young as well as old mice. This study shows that *EETS* possesses learning and memory improving activity by inhibiting phospholipase A2 in mice models.

Key words: Alzheimer's disease, *Tecoma stans*, Phospholipase A2, Elevated Plus Maze, Rectangular Plus Maze.

INTRODUCTION

Alzheimer's disease is a progressive neurodegenerative disorder characterized by a gradual decline in memory [1,2]. It is the most common form of dementia in aging. In addition to age, various pathological events have been found to precede Alzheimer's disease. Arachidonic acid and specific isoforms of phospholipase A2 appear to be critical mediators in amyloid-beta induced pathogenesis leading to learning, memory and behavioral impairments in mouse models of Alzheimer's disease [3]. It is also reported that elevated plasma triglyceride levels precede amyloid deposition in Alzheimer disease mouse models [4-6]. *Tecoma stans* (common name yellow bell) also known as yellow trumpet bush belongs to the family bignoniaceae. It is an ornamental plant. It is an erect, branched, sparingly hairy or nearly smooth shrub two to four meters in height. The leaves are opposite, odd-pinnate, Up to 20 centimeters in length with 5 to 7 leaflets. The leaflets are lanceolate to oblong- lanceolate, 6 to 13 centimeters long, pointed at both ends and toothed on the margins. Trumpet shaped flowers are yellow faintly scented and borne in short, dense, terminal clusters. The calys is green. 5 to 7 millimeters long and 5 toothed. Flowering can begin as early as April and continue in to fall. The flowers are followed by 6 inch long, tan pods that are filled with small, papery winged seeds [7]. Leaves of *Tecoma stans* contain the alkaloids tecomin and tecostamine are potent hypoglycaemic agent when given intravenously. Anthranilic acid is responsible for the anti-diabetic activity. Roots are powerful diuretic and vermifuge [8].

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Tecoma is not a toxic because this plant is used in latine America as a remedy for diabetes and moreover for feeding cattle and goats in mexico [9]. The preliminary phytochemical screening of methanolic extract of flower extract of *Tecoma stans* showed the presence of flavaniods, phenol, alkaloids, tannins, steroids, triterpenes, anthraqunones and saponins etc.

Various studies have put forth essential information regarding the role of phospholipase A2 in the development of Alzheimer disease. Furthermore, recent studies have shown the link between phospholipase A2 and their impact on cognitive functions. The literature survey has helped us in understanding that, inhibition of phospholipase A2 plays a major role in treating Alzheimer state. In conclusion, in the current study, the experiments are designed to evaluate the effect of EETS as phospholipase A2 inhibitory drug, which will have an impact on learning and memory in normal and memory deficit animals. The present study will help as in decoding one of the important mechanisms behind the treatment of Alzheimer and pave the way for other researchers to develop drugs acting by this mechanism of action in future.

MATERIALS AND METHODS

Dose calculation [10]

Rat dose (mg/200g body weight) = Human therapeutic dose × CF (0.018)
= 120 mg × CF (0.018)
= 2.16 mg /200 g

Dose	Dose (mg/kg)	Mice [30 g] (mg/30g)
½ Therapeutic Dose	5.4	0.16
Therapeutic Dose	10.8	0.32
2 Therapeutic Dose	21.6	0.6

Animals

Swiss albino mice of both sexes weighing around 25-30 g were selected in the present study. They were acclimatized to the laboratory conditions for 5 d before doing the experiment. The animals were provided with alternate light and dark cycles of 12 h each. All experiments were carried out in the day time during 09:00 to 16:00 h.

Collection and extraction of plant

The flowers of *Tecoma stans* were collected in the month of May 2011 from Rasipuram [Namakkal District] Tamil Nadu. A herbarium specimen of the plant was deposited in the Department of Pharmacognosy. The plant was identified by Dr.G.V.S.Murthy, Joint Director of the Botanical Survey of India, Southern circle, TNAU Campus, Coimbatore, who authenticated the plant from information available in the literature. The flower petals were dried in the shade for 10–12 days. After complete drying, the flower petals were pulverized to a coarse powder of 40 mesh size in a mechanical grinder.

The powdered material was subjected to sohxlet extraction for 18 h at 50–55^{o}C. The extract was thereafter concentrated under vacuum and air-dried. EETS being insoluble in water was administered orally by suspending in 5% acacia. Diazepam, Piracetam, Gum acacia are also used in the study.

Groups of animals

In the present study, the young and the adult animals were divided it to 6 groups. Each consists of 5 animals. Group I served as control 5% Gum acacia suspension. Group II - Diazepam (1 mg/kg, i. p). Group III - Diazepam (1 mg/kg, i. p) and Piracetam (400 mg/kg, i. p). Group IV - Diazepam (1 mg/kg, i. p) and EETS acacia suspension (5.4 mg/kg, p. o). Group V - Diazepam (1 mg/kg, i. p) and EETS acacia suspension (10.8 mg/kg, p. o). Group VI - Diazepam (1 mg/kg, i. p) and EETS acacia suspension (21.6 mg/kg, p. o).

Laboratory models for testing learning and memory Elevated plus maze

The elevated plus maze consist of two open arms (0.16×0.05 m) and two covered arms (0.16×0.05×0.12 m) extended from the central platform (0.05×0.05 m) and the maze was elevated to a height of 0.25 m from the floor [1].

Rectangular plus maze

The Hebb's William Maze (Rectangular Maze) consists of completely enclosed rectangular box with an entry and a reward chamber appended at opposite ends. The box is partitioned with wooden slats into blind passages leaving just one twisting corridor leading from the entry to the reward chamber. The maze is divided into chamber A, in which the mice is placed. Chamber B, at the other end of the maze in which the reward is kept. The middle chamber is C. All the three divisions of the maze are covered by hinged separate top-lids so as to maintain a uniform environment inside the maze and prevent any kind of outside stimulus or clue to be delivered to the animal. The 'A' light will go out as soon as the animal leaves the chamber and moves into the maze. Simultaneously the 'C' light will start to glow, and then the timer starts as soon as the light 'C' glows. The 'C light will go out as soon as the animal enters the end compartment i. e. chamber B**,** and the 'B' light will begin to glow. This electrical system provides indication enabling the reaction time to be noted without observing the animal. A four digit timer records the time taken by the animal in exploring the maze.

Protocol Elevated plus maze

In this method the test drug (EETS) and the standard drug (Piracetam) was administered for seven successive days to mice. Amnesia inducing drug Diazepam was administered 60 min after the last dose of test and standard drug. The animals were exposed to the training session after 45 min of diazepam injection. On the 7th d of the drug treatment each mouse was placed at the end of the

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open arm, facing away from the central platform. Transfer latency were taken as the time (in s) taken by the animal to move from open arm into the one of the covered arms with all its four legs. Transfer latency was recorded on the 7th d for each animal. The mouse was allowed to explore the maze for another 2 min and then returned to its home. Retention of its learned task (memory) was examined 24 h (8th d) after last dose. Significant reduction in the Transfer latency value of retention indicated improvement in memory [1,12].

Rectangular plus maze

On the 16th d all the mice were familiarized with the rectangular maze for a period of 10 min. From 17th to 20th d, the mice received four consecutive trials of training per day in the maze. In each trial the mice were placed in the entry chamber A, the 'A' light will begin to glow. Top-lid of all the three compartments were closed and left the apparatus as such to let the animal acclimatize to the environment inside the maze. After allowing sufficient time to the animal to get used to the environment the slide door is opened. The time taken for the mice to reach the reward chamber was taken as the learning score of the trial. This indicates the end of the experiment and the time is noted. The reading recorded in the timer will be the total time taken in seconds. The average of the four trials was taken as the learning score for the day. Lower scores of assessment indicate efficient learning while higher score indicate poor learning in animals. During learning assessment, the animals were exposed to food and water only after 1 hour of maze exposure [13].

Statistical analysis

Results were expressed as mean ± SEM. The results were analyzed statistically by means of the Student's *t* test $p<0.001$ was taken as the criterion for significance.

RESULTS

Elevated plus maze

Transfer latency on 7th d was compared with the transfer latency recorded on 8th d trial in adult and young mice. The animal groups showed shortened transfer latency on 8th d trail which shows acquisition and retention of learned task or memory. Piracetam (used as positive control) at the dose of 400 mg/kg, i. p was treated for seven days decreased transfer latency values on the 7th and 8th d as compared to the control group, indicating improvement in both learning and memory ($p<0.001$) of both young and adult mice. Diazepam (1 mg/kg, i. p) was administered 45 min before the training session. Diazepam treated animals showed higher transfer latency values on 7th and 8th d, indicating impairment in learning and memory (amnesia). EETS being insoluble water was administered orally by suspending in 5% acacia. The test drug EETS was administered for seven days in three different concentrations 5.4, 10.8 and 21.6 mg/kg orally for both age groups of animals. The results showed marked decrease in transfer latency ($p<0.001$) on 8th d compared to 7th d trial in young mice at all dose levels proving significant improvement in the learning and retention of learned task reversing the amnesia induced by Diazepam when compared to control group (table 1).

Rectangular maze

The time taken by the animals to reach the reward chamber 'B' from the entry chamber 'A' was recorded in various groups of animals. The time taken for the mice to reach the reward chamber was taken as the learning score of the trial. The reading recorded in the timer will be the total time taken in seconds. The average of the four trials was taken as the learning score for the day. Lower scores of an assessment indicate efficient learning and memory retention while higher score indicates poor learning and amnesia in animals. The test was carried out for four consecutive days. Diazepam (used as negative control) at the dose of 1 mg/kg, i. p was treated group showed the higher score indicating impairment in learning and learned task or memory retention. Piracetam at the dose 400 mg/kg, i. p was used as positive control in the experiment. Piracetam treated group took less time to reach the reward chamber 'B' from the entry chamber 'A' indicating improvement in learning and memory in both young and adult mice. The test drug EETS was administered in three different concentrations 5.4, 10.8 and 21.6 mg/kg orally for both age groups of animals. EETS administered orally by suspending in 5% acacia. The results showed that extract at dose 5.4 mg/kg, p. o do not produce any significant change in the memory ($p<0.5$) of the young mice. Furthermore, the response of the mice recorded for EETS (10.8 mg/kg, p. o) dose showed a response of ($p<0.05$) for all the four days of the test by lowering the time taken to reach the reward chamber which is indication of learning and retention of memory in this group of mice.

Mice treated with EETS at the dose of 21.6 mg/kg, p. o showed significant response ($p<0.001$) in the 19th day of the experiment (table 3). Similarly, the experiments were carried out for the adult mice and the time taken to reach the reward chamber 'B' from entry chamber 'A' was recorded as the parameter to test the acquisition and memory retention. The adult mice groups underwent the same drug treatment as young ones. Using student's *t* test EETS at the dose levels of 10.8 and 21.6 mg/kg, p. o showed Significant response ($p<0.001$) for 17th, 18th and 19th d of the experiment indicating acquisition and retention of learned task or memory. On the other hand, EETS (5.4 mg/kg, p. o) produce $p<0.05$ on 17th day and $p<0.01$on 18th and 20th d (table 4) showing poor response at this dose level. Hence, it is evident that higher doses of EETS 10.8 and 21.6 mg/kg, p. o show the possible learning and memory retention qualities as the animal groups were able to successfully reverse the amnesia induced by Diazepam resulting in learning and retention of memory.

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Table 1. Effect of EETS on the transfer latency by using Elevated plus maze on young mice

Groups	Dose	Transfer latency (in s)	
		7th d	**After 24 h 8th** d
Control 5% Gum acacia suspension	0.3 ml	67 ± 7.4	70 ± 0.46
Diazepam	1 mg/kg	68 ± 1.47	88 ± 2.24
Piracetam + Diazepam	400 mg/kg+ 1 mg/kg	19 ± 0.14*	3 ± 0.47*
Diazepam + ½ EETS	1 mg/kg+ 5.4 mg/kg	26± 2.41*	6.8 ± 0.26*
Diazepam + EETS	1 mg/kg+ 10.8 mg/kg	11 ± 0.24*	6.6 ± 1.18*
Diazepam + 2 EETS	1 mg/kg+ 21.6 mg/kg	15 ± 1.34*	5.6 ± 0.47*

Using student's t test all groups showed significant result p<0.001 Vs negative control (diazepam); where n=5

Similarly, the experiment carried out on adult mice showed improved memory (p<0.001) on the 7th and 8th day of the experiment (table 2) and provide an evidence for acquisition and memory retention in adult mice as well. Using student's t test different doses of EETS showed significant result p<0.001 Vs negative control (diazepam).

Table 2. Effect of EETS on the transfer latency by using Elevated plus maze on old mice

Groups	Dose	Transfer latency (in s)	
		7th d	**After 24 h 8th** d
Control 5% Gum acacia suspension	0.3 ml	94 ± 6.14	100 ± 0.54
Diazepam	1 mg/kg	66.9 ± 1.21	95 ± 2.24
Piracetam + Diazepam	400 mg/kg + 1 mg/kg	16.4 ± 3.47	13.4 ± 0.24
Diazepam + ½ EETS	1 mg/kg + 5.4 mg/kg	25.4 ± 2.4*	105 ± 4.1*
Diazepam + EETS	1 mg/kg + 10.8 mg/kg	10.5 ± 0.47*	9 ± 0.1*
Diazepam + 2 EETS	1 mg/kg + 21.6 mg/kg	12.24 ± 0.64*	10.65 ± 0.14*

Using student's t test all groups showed significant result *p<0.001 Vs negative control (diazepam); where n=5.

Table 3. Effect of EETS on the learning memory of young mice by Rectangular plus maze method

Groups	Dose	Learning scores (time in s)			
		Day1 17th d	**Day 2 18th** d	**Day 3 19th** d	**Day 4 20th** d
Control 5% Gum acacia suspension	0.3 ml	69.5 ± 0.47	53.5 ± 6.48	38.8 ± 0.06	49.8 ± 8.49
Diazepam	1 mg/kg	77.6 ± 0.12	55.4 ± 0.4	128.4 ± 0.14	74.5 ± 1.4
Piracetam+ Diazepam	400 mg/kg+1 mg/kg	16.1 ± 1.5	26.4 ± 1.24	27.5 ± 2.14	28.5 ± 2.42
Diazepam+ ½ EETS	1 mg/kg+5.4 mg/kg	30. 4± 5.21*	41.3 ± 3.24*	49.5 ± 9.21	35 ± 5.14*
Diazepam+ EETS	1 mg/kg+10.8 mg/kg	14.4 ± 2.14**	10.6 ± 2.1**	34.5 ± 6.01**	8.4 ± 1.4**
Diazepam+ 2 EETS	1 mg/kg+21.6 mg/kg	12 ± 0.14**	29.4 ± 4.24#	28.6 ±7.21***	24.4 ± 4.14*

Using student's t test all groups showed significant result *** p<0.001, *p<0.05 **p<0.01 Vs negative control (diazepam) and #p<0.5 vs negative control shows not significant result; where n=5

Table 4. Effect of EETS on the learning memory of old mice by rectangular plus maze method

Groups	Dose	Learning scores (time in s)			
		Day1 17th d	**Day 2 18th** d	**Day 3 19th** d	**Day 4 20th** d
Control 5% Gum acacia suspension	0.3 ml	102.4 ± 3.41	108 ± 2.72	105.5 ± 2.47	115.5 ± 4.45
Diazepam	1 mg/kg	112.1 ± 0.13	155.5 ± 1.45	158.4 ± 1.21	134.4 ± 2.54
Piracetam+ Diazepam	400 mg/kg+1 mg/kg	10.5 ± 1.2	23.4 ± 0.45	33.4 ± 0.14	43.7 ± 0.67
Diazepam+ ½ EETS	1 mg/kg+5.4 mg/kg	80.4 ± 6.41*	107.2 ± 7.24**	151.4 ± 1.41#	69.4 ± 4.24***
Diazepam+ EETS	1 mg/kg+10.8 mg/kg	14.5± 2.14***	19.6 ± 2.14***	46.4 ± 5.24***	84.4 ± 1.12**
Diazepam+ 2 EETS	1 mg/kg+21.6 mg/kg	15.5 ±2.14***	22.8 ±4.14***	63.4 ± 2.6***	74.6 ± 7.6**

Using student's t test all groups showed significant result *** p<0.001, **p<0.05 #p<0.01 Vs negative control (diazepam) and *p<0.5 Vs negative control shows not significant result; where n=5

DISCUSSION

Alzheimer's disease is most commonly a disease of late life that derives from pathogenic processes underlying abnormal accumulation of amyloid-β peptides and hyperphosphorylation of *tau* in certain regions of

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cerebrum [12]. Amyloid plaques are found in the tissue between the nerve cells. They are unusual clumps of protein called β amyloid along with degenerating bits of neurons and other cells. It is progressive neurodegenerative disorder characterized by a gradual decline in memory [1, 2]. Extensive studies have been carried out put forth the reasons that lead to Alzheimer's pathology and drugs to combat the memory retention issues. Studies show that arachidonic acid and specific isoforms of phospholipase A2 appear to be critical mediators in amyloid-beta induced pathogenesis, leading to learning, memory, and behavioral impairments in mouse models of Alzheimer's disease [3]. Phospholipase A2 provides precursor for the production of eicosanoids and platelet activating factor. These lipid mediators play critical roles in the initiation and modulation of inflammation and oxidative stress [14]. Oxygen free radicals, the harmful byproducts of oxidative metabolism are known to cause organic damage to the living system. They are implicated in various pathological events such as mutagenesis and neurodegenerative disorders [15]. EETS inhibits phospholipase A2 action which intern can inhibits the generation of Arachidonic acid. Furthermore, the elevation of phospholipid degradation metabolites such as phosphormonoesters and phosphodiesters, in Alzheimer's disease brain supports the finding of increased phospholipase A2 activities. The increase in phosphormonoesters and phosphodiesters correlates with pathological markers of Alzheimer's disease, such as neurofibrillary tangles and senile plaques [13]. The ability of EETS to potently inhibit phospholipase A2, lipoprotein lipase [16].

Monoacylglycerol lipase and diacylglycerol lipase which are also involved in Alzheimer's disease causation is employed as a tool to assess the memory enhancement activity [17-20]. Series of experiments involving Elevated plus maze and Rectangular plus maze were carried out on two different age group of mice. In Elevated Plus maze procedure, the end results obtained, showcased the positive response. Considerable decrease in transfer latency was seen in all dose levels and on both the age group of mice. This is evident from the experiment that there is an impact on learning and memory due to EETS dose. Results obtained in Rectangular plus maze procedure showed minimal response of both age groups of mice in ½ therapeutic dose when compared to the impacts produced by therapeutic and double therapeutic dose levels on both age groups of mice. These data obtained during experiments relate to the possible effect of EETS on learning and memory retention.

CONCLUSION

In conclusion the *EETS* possesses the significant learning and memory improving potency; this may be due to the presence of various active phytoconstituent. They may be act by inhibiting phospholipase A2 in mice models. Further work can be proceeded by isolating the active constituent responsible for the activity.

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CONFLICT OF INTEREST:

The authors declare that they have no conflict of interest.

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