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SMILAX ZEYLANICA ETHANOLIC EXTRACT IN COGNITIVE DYSFUNCTION AGAINST ALUMINIUM CHLORIDE INDUCED RAT MODEL OF ALZHEIMER'S DISEASE

B. Sabari Senthil[1]*, V.K. Kalaichelvan[1] and S. Vigil Anbiah[2]

[1]Department of Pharmacy, Annamalai University, Annamalai Nagar - 608 002. Tamil Nadu, India.
[2]Central Animal House, RMMC, Annamalai University, Annamalai Nagar - 608 002. Tamil Nadu, India.

***Corresponding Author Email: sabarisenthilcology@gmail.com**

ABSTRACT

The objective of the present study is to investigate the effects of roots of ethanolic extract of Smilax zeylanica (EESZ) on neuroprotective effect in cognitive induced by aluminium chloride in rats. The ethanolic extract from the roots of Smilax zivania by hot continuous percolation method. The rats were divided into 5 groups and each group consists of 6 animals. Rats were treated with EESZ for 150 and 300 mg/ kg of body weight and piracetam, 0.5 mg/ kg of body weight for 14 successive days after inducing behavioural and cognitive dysfunction with aluminium chloride (100 mg/ kg of body weight) for 60 days. The changes in behavioral and neurotransmitters were measured in rats. Cognitive abilities were evaluated through latency on a passive avoidance test and Morris water maze test. $AlCl_3$ induced rats showed decreased learning and memory activity and increased activity of acetylcholine esterase (AChE). The ethanolic extract from the roots of Smilax zeylanica at higher dose 300 mg/ kg of body weight significantly ($P < 0.001$) reversed the $AlCl_3$ induced impairment of cognitive activity in rats was estimated by the passive avoidance test and when compared with the control group. Ethanolic extract from the roots of Smilax zeylanica higher dose reduced escape latency in the Morris water maze significantly ($P < 0.001$) when compared with the control group. Latency time was increased by EESZ, which is revealed from EPM test significantly ($P < 0.001$) when compared with the control group. EESZ reserved AChE activity in the rats brain significantly ($P < 0.001$) when compared with the control group. Findings of the present study revealed that ethanolic extract from the roots of Smilax zeylanica may exert anti-alzheimer's disease effect through behavioural, cognitive and anti-AChE activities.

KEY WORDS

Alzheimer's disease, ethanolic extract, Smilax zeylanica , AChE activity.

INTRODUCTION

Alzheimer's disease (AD) affects millions of people and it has become a major medical and social burden globally[1]. During the course of the disease, the senile plaques (SPs) of amyloid beta (A-beta) peptides and the neurofibrillary tangles (NFTs) of the tau protein develop in the specific regions of brain, leading to the death of neuronal cells[2]. Cholinergic system in the brain, especially the basal forebrain projections to hippocampus and cortex, is responsible for memory and learning and known to be affected in Alzheimer's disease[3]. Alzheimer's brain have low levels of acetyl choline which can arise from the accumulation of A-beta protein fragments, which forms hard plaques that can interfere with the ability of acetyl choline to effect synaptic transmission. Some of the isolated compounds like flavonoids, Gingko biloba, Ginseng[4,5] have reported for effective treatment for AD. Recently, many

experiments and clinical trials have shown that traditional herbal medicine are safe and effective.

The roots of *Smilax zeylanica* is a belong to the Smilacaceae family. It is commonly distributed in the forest and hills of south India and found in tropical and subtropical hills from Himalayan region in the north to Kerala in south. It is widely in hilly region of Karnataka, Kerala and Tamil Nadu between altitudes of 500-1800 meter. *S. zeylanica* is used ethnomedicinally for the treatment of different conditions such as abscesses, boils, dysentery, psoriasis, rheumatism, skin diseases, swellings, toothache and venereal diseases[6-10]. This plant is shown to exhibit several bioactivities such as antimicrobial and analgesic[11,12], antioxidant[12,13], cytotoxic[13], immunomodulatory and antiarthritic[14], anthelmintic[15], antipyretic[16], anticonvulsant[16], antidiabetic[17], cytoprotective[18], hepatoprotective[19], anti-inflammatory[20], antiepileptic[21], pesticidal[22], thrombolytic[23], and antidepressant[24] activities. Therefore, the present investigation focused to evaluate the Neuroprotective Effect of Ethanolic extract of root of *Smilax zeylanica on* Aluminium Chloride Induced Alzheimer's Disease in Wistar rats

MATERIALS AND METHODS

1. Collection and Identification of Plant materials

The roots of *Smilax zeylanica*were collected form Kulithalai,Kanyakumari District, Tamil Nadu, India. Taxonomic identification was made from Botanical Survey of Medical Plants Unit Siddha, Government of India. Palayamkottai. The roots of *Smilax zeylanica* were dried under shade, segregated, pulverized by a mechanical grinder and passed through a 40-mesh sieve.

2. Preparation of Extracts

The above powdered plant materials were consecutively extracted with petroleum ether (40-60^{0}C) by hot continuous percolation method in Soxhlet apparatus[25] for one day. The marc was dried out and extracted with chloroform and then marc was extracted with ethyl acetate (76-78^{0}C) for one day, then this marc was dried out after that it was extracted with ethanol for one day and then marc was extracted with water. All the three extracts were concentrated by utilizing a rotary evaporator and undergone to freeze drying using a lyophilizer until dry powder was acquired. The ethanolic extract gave more yield and more phytoconstituents were present. So, the ethanolic extract of *Smilax zeylanica* was selected for the further investigation.

EVALUATION OF NEUROPROTECTIVE ACTIVITY

Experimental

Both the genders of Wister rats with 8 weeks weighed between 150 and 200 g of body weight was used for the present study. Rats were acclimatized for experimental conditions for about two weeks. The rats housed in plastic cages at 25°C with relative humidity of 70% under 12/12 hours day/ night cycle. Rats were fed with food and water *ad libitum*. The experiments were approved as per the strategy of CPCSEA, New Delhi, India and approved by the Annamalai University IAEC **(Approved number: AU/IAEC/1199/1/18).** Animals were grouped randomly into five different groups with six rats in each group:

Group 1: Control – received saline (5 ml/ kg p.o.).

Group 2: Negative control group i.e received aluminium chloride ($AlCl_3$) at dose of 100mg/ kg of body weight p.o.

Group 3: $AlCl_3$+ Ethanolic extract of *Smilax zeylanica* (150mg/ kg of body weight p.o.)

Group 4: $AlCl_3$+ Ethanolic extract of *Smilax zeylanica* (300mg/ kg of body weight p.o.)

Groups 5: $AlCl_3$ + piracetam (0.5mg/ kg body weight p.o.)

Alzheimer was induced to the all the rats, expect in the group I, by Aluminium Chloride at a dose of 100mg/ kg/ body weight for 60 days through oral gavage. From 61st day onwards the group 3 and 4 animals were treated with the ethanolic extract of *Smilax zeylanica* for 60 days. At the end of 24 h of the last administration of dose, the rats were exposed to multiple behavioral studies.[26]

Behavioural experiments

Five behavioural studies with various degrees of stress were used as an integrative testing sequence to allow measuring the most behavioural changes in rat's models.

Locomotor Activity

Locomotor activity was evaluated among animals by using digital photoactometer (INCO, Ambala, India). The physical movements were noted over a period of 15 min and expressed in terms of total photo beam counts for 15 min per animal. Locomotor activity was evaluated on 7th and 14th day before probe trial in Morris water maze.[27]

Elevated plus maze test (EPM)

It is used to measure anxiety and examine spatial learning and memory. The EPM test apparatus had two components (i) two open arms (50 cm x 10 cm) and at right angle to it and (ii) two closed arms (50 cm x 10 cm x 40 cm) and connected with a central square (10 cm x 10 cm) of dimensions. The roof is opened, and total length of the apparatus is 50 cm. Acquisition memory test for rats were tested on 7th day. Each rat was kept independently at one end of the open arm. Initial transfer latency (ITL) was measured as each rat travelled from open arm to closed arm in the EPM apparatus. Later animal was allowed to explore the maze for 25 seconds and then replaced in the same cages. Retention latency was measured on 14th day, by keeping rats in open arm.[28]

Y – Maze Test[29, 30]

It is well known that spontaneous alternation is a measure of spatial working memory. The Y-maze can be used as a measure of short-term memory, general locomotor activity and stereotypic behaviour. The maze is constructed in wood and painted with black colour. Each arm is 40 cm length, 15 cm height, 5 cm wide in bottom and at top 10 cm wide and with an equal angle converges. Individually animals are kept at the end of one arm and allowed to move until its tail fully enters into next arm in the maze for 10 minutes. For each animal the Y-maze testing was carried out for 5 minutes. The series of arm entries is recorded manually, the arms being marked 1, 2 or 3. An alternation is defined as entry into all three arms successively, for example; 123, 21, 1, 2, 3, 2, 3 here the animal(s) made 10 arm entries 7 of which are correct alternations.

Maximum number of spontaneous alternations = Total number of arms entered – 2

% alternation = [actual alternations/ maximum spontaneous alternations] x 100.

Passive Avoidance Test

In 1970's King and Glasser developed this test to evaluate retention of memory in long term.[31] The passive avoidance (PA) behavior test was assessed by using light - dark chambers separated by a grid door and metal grid floor.[32] On 1st day, each rat was kept in the light chamber. Once rats enter into the dark chamber electric shock (40 V; 0.5 mA; 1 s) was applied through the grid floor. Animals were withdrawn as soon as from the apparatus and replaced in the same cage. On 2nd day, the rats were once again kept in the light chamber and time spent to re-enter into the dark chamber was noted as step-through latency.[33]

Morris water maze test

The apparatus contains a circular tank was 150 cm diameter, 45 cm in height and in it up to 30 cm water was filled. Tank was divided into four equal compartments. In the acquisition phase, each animal was kept in the different compartments of tank for each group, towards the tank wall and allowed 60 seconds to place the tiny visible platform, which was kept around 1 cm above the water level. Rats were allowed to stay in the platform for 10 seconds. The animals not reached the platform were helped to reach the goal and allowed to remain there for next 10 seconds. The rats were trained two successive training sessions on 1st and 12th day. Acquisition latency was the average time taken by rat's travel from the wall to reach platform. On 7th and 14th day, after administration of aluminium chloride, average time required to find the hidden platform was noted as 1st and 2nd retention latencies respectively.[34]

Acetylcholinesterase Activity

The acetylcholinesterase activity was estimated quantitatively by Ellman's method. The activity of enzyme was calculated by increasing yellow colour generated during reaction between thiocholine and 5,5'-dithiobis-(2-nitrobenzoic acid) [DTNB].[35]

Statistical analysis

Data were presented as mean ± SEM. One-way ANOVA using Tukey's employed for post hoc test for multiple comparisons. The value of $P < 0.05$ was considered statistically significant.

RESULTS AND DISCUSSION

Effect of EESZ on First Transfer Latency and Retention Transfer Latency of Rats by EPM Test:

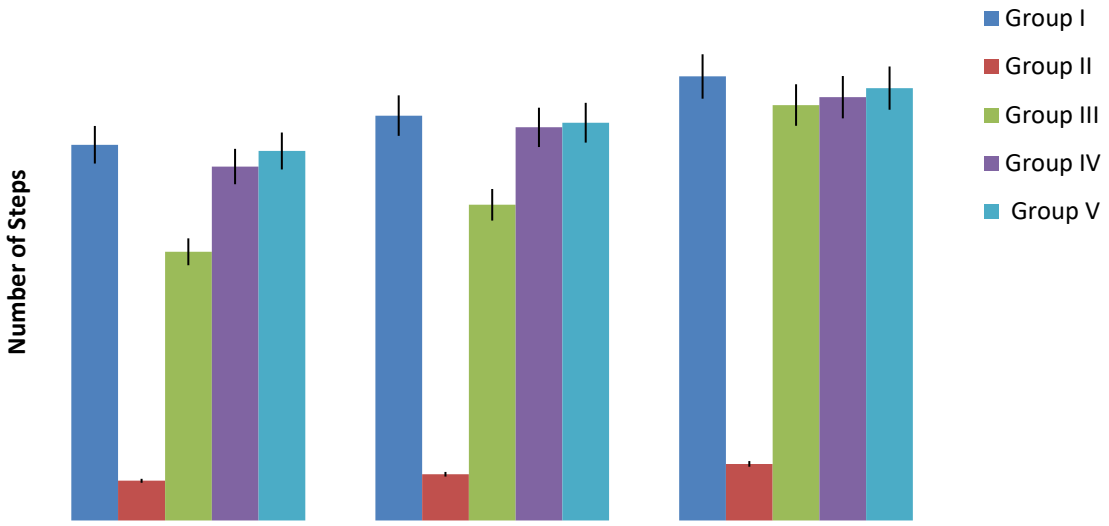
The first transfer latency (FTL) was measured on 1st day and retention transfer latency (RTL) was measured on 7th and 14th day. The RTL was compared between group IV with group I and III it was found to be significantly decreased ($P < 0.001$). The RTL was compared between group IV with group V it was found to be significantly decreased ($P < 0.01$). The RTL was compared between group III with group I, IV and V it was found to be significantly decreased ($P < 0.05$). The inhibition of cognitive impairment by EESZ in aluminium chloride induced rat as dose increase, dose dependent manner. There was less significant RTL in cognitive impairment observed between group III and IV. The data are

presented in Table No. 1, Fig. No.1.Behavioral and brain tonic activity can be assessed by EPM test to study spatial long-term memory for EESZ with 150 and 300 mg/ kg of body weight. FTL and RTL parameters were examined using this test. FTL was recorded for each rat whenever starts to move from one end to other as time (seconds) spent. The retention of learning memory task was studied after one day and denoted as RTL. In the present work there is a decrease in RTL on 7th and 14th day when compared with ITL on 1st and 14th day (after one day of FTL) showed enhancement of spatial long-term memory among the rats as when compared to control rats. Md. Sahab Uddin *et al.* Studies that on *Persicaria flaccida* in rats reported similar significant increase in learning and memory in EPM test[36].

Table 1: Effect of EESZ on First Transfer Latency and retention transfer latency of Rats by EPM Test

Groups	1st day	7th day	14th day
I	87.12 ±0.16	93.88 ±0.40	102.95 ±0.15
II	9.20 ±0.23	10.72 ±0.31	13.12 ±0.20
III[a]	62.30 ±0.24	73.20 ±0.47	96.33 ±0.17
IV [b,1]	82.07 ±0.18	91.15 ±0.22	98.15 ±0.22
V [c, 2]	85.67 ±0.32	92.23 ±0.59	100.25 ±0.63

Values are expressed as mean± SEM of 6 animals. Statistical significance tested by one-way ANOVA, followed by Tukey's "t" test. a – P < 0.05; b – P < 0.01; c – P < 0.005; 1 – group I compared with group II,III,IV &V; 2 – group II compared with group III,IV &V.



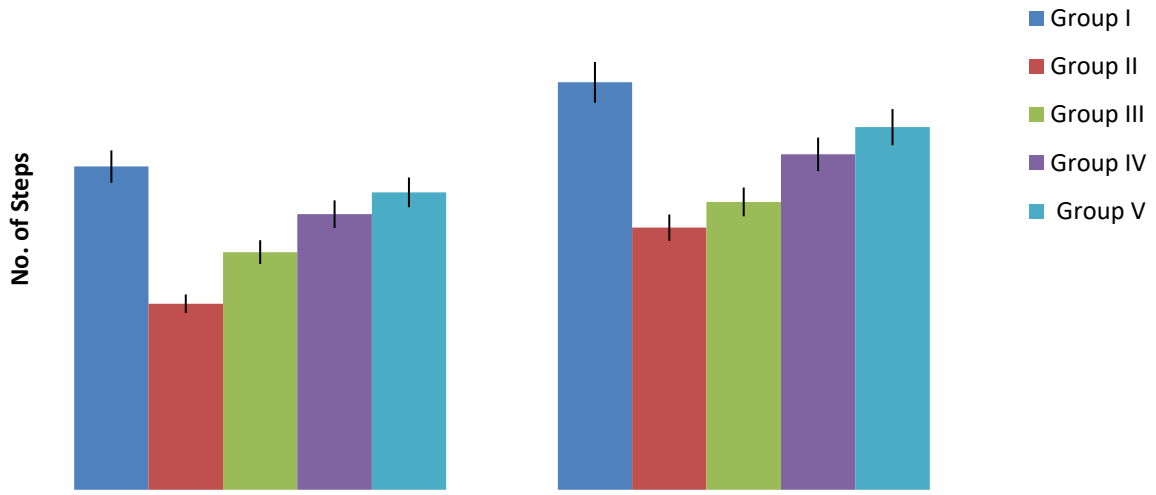
Effect of EESZ on Spatial Memory Ability in Aluminium Chloride Induced Rats by Y Maze Test

The spontaneous change was compared between group IV with group I and III it was found to be significantly decreased (P < 0.001). The spontaneous change was compared between group IV with group V it was found to be significantly decreased (P < 0.01). The spontaneous change was compared between group III with group I, IV and V it was found to be significantly decreased (P < 0.05). The spontaneous change was measured in terms of number of entries in every arm. When linear regression was determined less significant positive correlation between spontaneous alterations vs. number of entries in the maze (P < 0.1) was obtained. The data are represented in the Table No.2 and Fig. No.2. There was less significant (P < 0.05) between group III and IV in spontaneous change.

Table 2: Effect of EESZ on Spatial Memory Ability in Aluminium Chloride Induced Rats by Y Maze Test

Groups	BT	AT
I	39.67±1.12	50.00±1.69
II	22.83±0.87	32.17±0.48
III[a]	29.17±0.48	35.33±0.33
IV [b]	33.83±0.60	41.17±0.54
V [c]	36.50±0.76	44.50±0.43

Values are expressed as mean± SEM of 6 animals. Statistical significance tested by one-way ANOVA, followed by Tukey's "t" test. a – P < 0.05; b – P < 0.01; c – P < 0.005; 1 – group I compared with group II,III,IV &V; 2 – group II compared with group III,IV &V.



In the present research work, Y-maze test was employed to evaluate the effect of the EESZ the $AlCl_3$ induced in rat for AD. Y – maze test was also utilized to study the spontaneous alternation behavior, which is an indicator for spatial short-term memory[37]**.** In the present research study, spontaneous alternation behavior in $AlCl_3$ induced rats was significantly less than in rats treated with CMC vehicle only. In dissimilarity, EESZ significantly reversed the cognitive deficit induced by $AlCl_3$ in the Y-maze task. These finding suggests that EESZ improve short term memory. In the same behavioral task, in contrast with piracetam, EESZ,particularly at the dose of 300 mg/kg of body weight, significantly decreased the number of zones crossed by the rats. In EPM test treated with both the extracts at same dose i.e. EESZ higher doses, the motor activity at open area was decreased and time spent was increased at the middle of the maze when compared to control group i.e. group I and there was a decrease of retention time latency in closed area when compared to compared to control group i.e. group I. There is direct relationship between anxiety and memory, among the rats treated with EESZ at two doses (150 and 300 mg/ kg of body weight), whenever there is increase in spatial memory ability the anxiety is lowered, which is evident from this EPM and Y – maze tests. In Y-maze the time spent is increased may be due to memory facilitation by the EESZ that resulted in increased searching and improved spatial memory that observed in the present study.

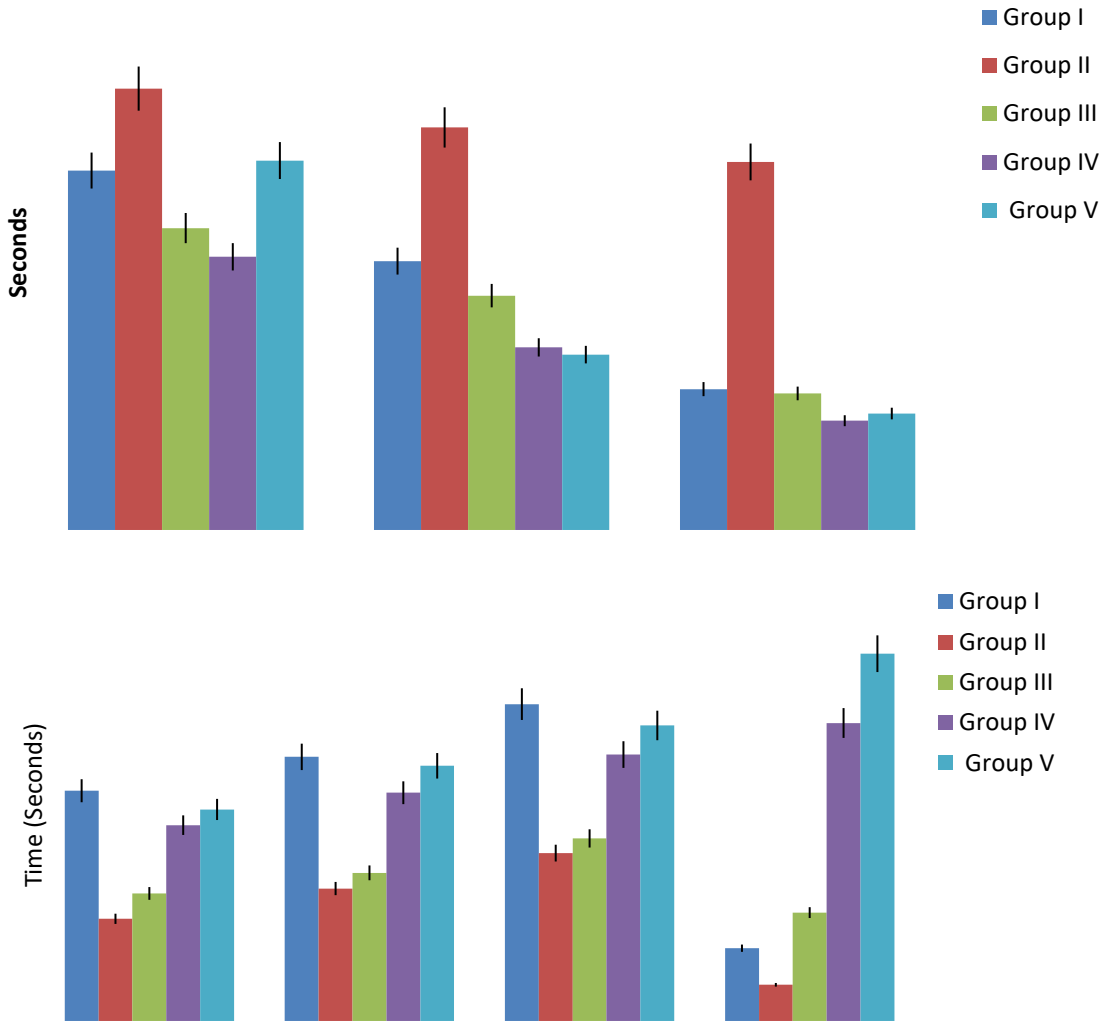
Effect of EESZ on Learning and Memory in Aluminium Chloride Induced Rats by Morris Water Maze Test

The EL and Spent time in the target zone (STTZ) for gaining trial was assessed on 1st, 7th and 14th day. The EL and STTZ was compared between group IV with group I and III it was found to be significantly decreased (P < 0.001). The EL and STTZ was compared between group IV with group V it was found to be significantly decreased (P < 0.01). The EL and STTZ was compared between group III with group I, IV and V it was found to be significantly decreased (P < 0.05). The EL and STTZ was measured in terms of seconds. The data are represented in the Table No. 3 and Fig. No.3. There is less significant difference in learning and memory activity was noticed between group III and IV.

Table 3: Effect of EESZ on Learning and Memory in Aluminium Chloride Induced Rats by Morris Water Maze Test

Groups	Escape Latency (sec)			Spent time in the target zone			STP
	1st day	7th day	14th day	1st day	7th day	14th day	14th day
I	41.67±1.05	31.17±0.17	16.33±0.14	15.98±0.91	18.33±0.97	21.99±1.72	5.05±0.27
II	51.17±1.19	46.67±0.67	42.67±1.14	7.09±0.89	9.19±0.74	11.65±1.21	2.51±0.19
III[a]	35.00±0.73	27.17±0.60	15.83±0.95	8.85±0.62	10.28±0.81	12.67±1.56	7.52±0.34
IV[b]	31.67±0.42	21.17±1.25	12.67±0.61	13.59±0.86	15.84±0.95	18.49±1.64	20.68±1.52
V[c]	42.83±1.19	20.33±0.71	13.50±0.72	14.68±0.98	17.71±0.83	20.51±1.88	25.49±1.68

Values are expressed as mean± SEM of 6 animals. Statistical significance tested by one-way ANOVA, followed by Tukey's "t" test. a – P < 0.05; b – P < 0.01; c – P < 0.005; 1 – group I compared with group II,III,IV &V; 2 – group II compared with group III,IV &V.



Hippocampus based spatial memory and leaning behavioral activities was studied by MWM test for EESZ with 150 and 300 mg/ kg of body weight and on cognitive functioning of rats using parameters like EL, STTZ and STP and these parameters is indicating learning and memory improving effect. In MWM test, in standard training period a reduction in EL and improvement in STTZ on 7th and 14th day and during test period an improvement in STTZ and STP on 14th day was showed an increase of spatial learning and memory of rats when compared with control rats i.e. group I. A similar study conducted by Taati *et al*., reported an improvement of spatial learning and memory[38] .

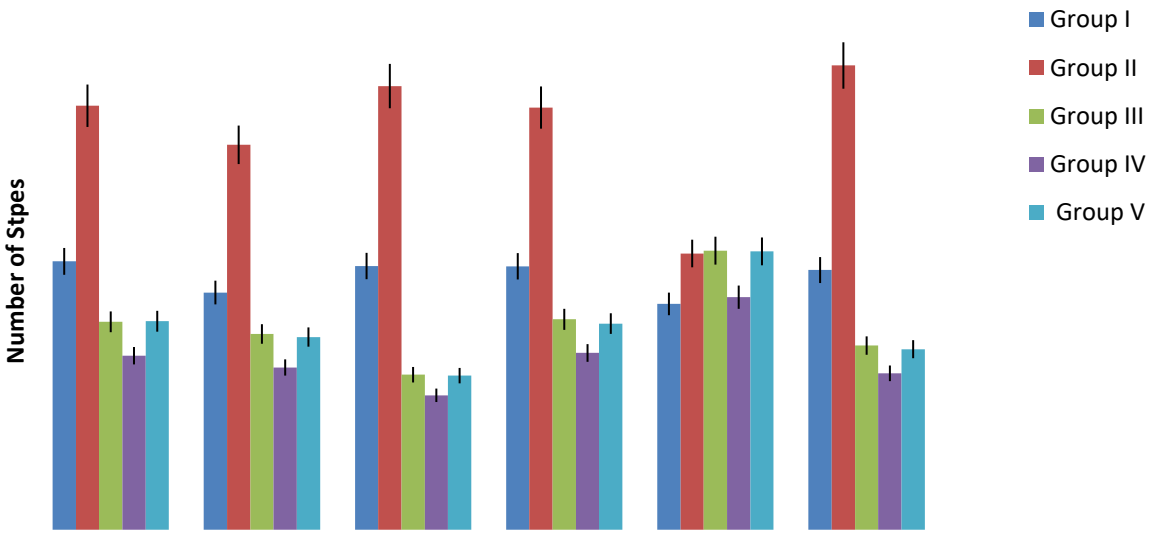
Effect of EESZ on Escape Latency and Step through Latency in Aluminium Chloride Induced Rats by Passive Avoidance Test

The EL and STL was significant (P < 0.001) decrease and increase when compared between group IV with group I and III. The EL and STL was significant (P < 0.001) decrease and increase when compared between group IV and group V. The EL and STL was significant (P < 0.001) decrease and increase when compared between group III with group I, IV and V. The data are represented in the Table No.4 and Fig. No.4. There was a significant increase (P < 0.01) in STL on 12th, 13th and 14th day respectively when compared with 7th day in the EESZ treated animals between the group I, III, IV and V, which showed enhanced retention of learning and learning. In both EL and RTL there was less significant (P < 0.05) difference in EL and RTL noticed between group III and group IV.

Table 4: Effect of EESZ on Escape Latency and Step through Latency in Aluminium Chloride Induced Rats by Passive Avoidance Test

Groups	EL		STL			
	6th day	11th day	7th day	12th day	13th day	14th day
I	51.85±0.70	45.83±0.43	50.95±0.61	50.90±0.78	43.63±0.47	50.17±0.64
II	81.92±0.43	74.37±0.62	85.72±0.55	81.57±0.55	53.37±0.54	89.70±0.58
III[a]	40.17±0.62	37.82±0.59	29.95±0.33	40.65±0.62	53.92±0.45	35.58±0.42
IV[b]	33.62±0.37	31.35±0.46	25.97±0.43	34.15±0.26	44.93±0.39	30.22±0.52
V[c]	40.28±0.53	37.23±0.67	29.77±0.44	39.83±0.43	53.78±0.50	34.88±0.92

Values are expressed as mean± SEM of 6 animals. Statistical significance tested by one-way ANOVA, followed by Tukey's "t" test. a – P < 0.05; b – P < 0.01; c – P < 0.005; 1 – group I compared with group II,III,IV &V; 2 – group II compared with group III,IV &V.



Learning and memory ability was investigated for EESZ and EESC with 150 and 300 mg/ kg of body weight by PA test for their natural leaning of animals formed after an aversive stimulus. The latency times to re-enter the dark chamber were measured as STL in this test[39]. In PA test, STL was increased on 7th (after one day EL) and 12th, 13th, 14th day (after 1, 2 and 3 days of EL) after the training period showed enhancement in learning and memory ability of rats when compared with control group i.e. group, I rat. In the present research work, EESZ and EESC on aluminium chloride treated memory impairment in mice by Weon *et al* reported for a similar improved latency time in PA test[40].

Effect of EESZ on Acetylcholine Esterase in Aluminium Chloride Induced Rats

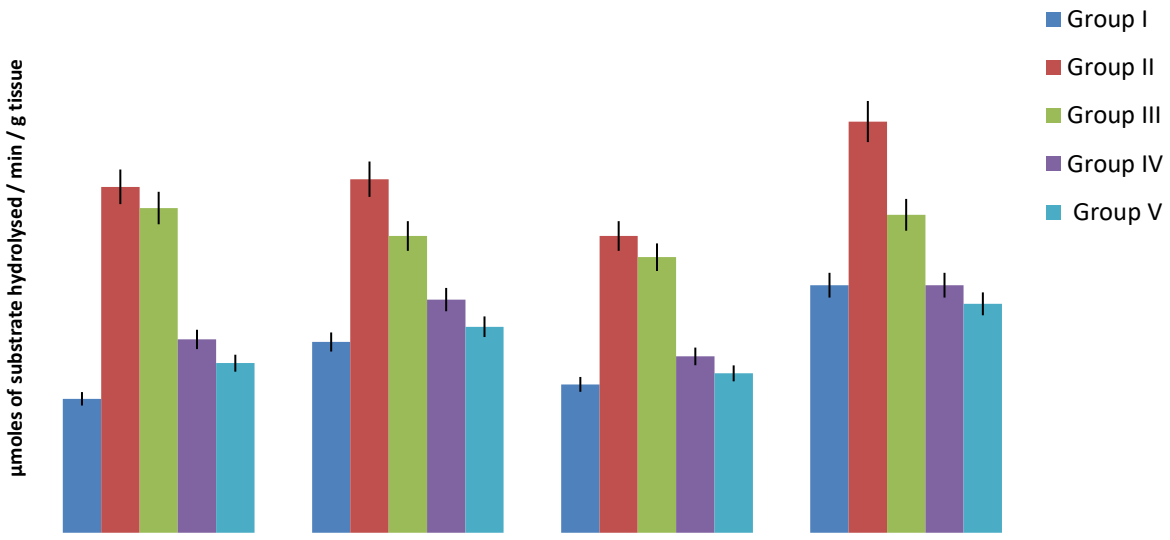
The AChE activities by EESZ in rat brain was shown in Table No. 5 and Fig. No.5.The activities of AChE in the tissue like hippocampus and cortex, significantly (P<0.001) increased in rats with AlCl3-treated animals (group II) than control group animals. Administration of EESZ (doses of 150mg/kg b.wt and150mg/kg b.wt) on brain tissues like, hippocampus and cortex AChE levels were significantly reduced when compared to $AlCl_3$-

treated animals (group 11). In both brain regions there was less significant alteration in AChE activity noticed between 124 zones among group III and IV. The AChE was measured in terms of µmoles of substrate hydrolysed / min / g tissue.

Table 5: Effect of EESZ on Acetylcholine Esterase in Aluminium Chloride Induced Rats

Groups	AChE activity (µmoles of substrate hydrolysed / min / g tissue)			
	Hippocampus		Cortex	
	7th day	14th day	7th day	14th day
I	1.58±0.15	2.25±0.21	1.75±0.21	2.92±0.15
II	4.08±0.19	4.17±0.26	3.50±0.13	4.85±0.11
III[a]	3.83±0.11	3.50±0.18	3.25±0.21	3.75±0.28
IV[b]	2.28±0.08	2.75±0.13	2.08±0.15	2.92±0.11
V[c]	2.00±0.20	2.43±0.25	1.88±0.24	2.70±0.29

Values are expressed as mean± SEM of 6 animals. Statistical significance tested by one-way ANOVA, followed by Tukey's "t" test. a – P < 0.05; b – P < 0.01; c – P < 0.005; 1 – group I compared with group II,III,IV &V; 2 – group II compared with group III,IV &V.



Acetylcholine esterase is one of the enzymatic markers for cholinergic neurons and is responsible for the breakdown of acetylcholine, a neurotransmitter related to short-term memory. Our results show that the aluminum treatment for 60 days significantly elevated the activity of AChE in both cortex and hippocampus a result that is consistent with previous findings[41]. The inhibition of the activities of AchE and the reduction of the neuroinflammatory markers by the peel aqueous extract and flavonoids fraction increased the acetylcholine with positive impact on the cognitive function. Our study clearly showed that PE possesses anticholinergic, antineuroinflammatory, anti-amyloidgenic, and antioxidant properties thus could reverse aluminum-induced cognitive dysfunction. In this study, we also investigated the histological and behavioral changes caused by AlCl3 exposure and the possible effect of PE treatment. The stress induction was done by aluminum chloride which is a neurotoxin that exerts its toxic effect by interfering with pathways involved in metabolism and normal iron homeostasis[42]

CONCLUSION

AD is a progressive neurodegenerative disorder and difficult to treatment. In AD, initially there will be memory loss and decreased cognitive behaviour, which may be significant sign and symptom. Learning and memory is controlled by the central cholinergic system. In the present study, EESC (150 mg/ kg and 300 mg/ kg of body weight) administered orally increased learning and memory in rats evaluated by the behavioral models like Elevated plus Maze, Morris water maze, Y-maze and acetylcholine esterase.

Disclaimer statements

Contributors:

All authors contributed equally.

Conflict of interest:

The authors declare that they have no conflict of interest that might have influenced the views expressed in this manuscript.

Ethics approval:

The experiments were approved as per the strategy of CPCSEA, New Delhi, India and approved by the Annamalai University IAEC **(Approved number: AU/IAEC/1199/1/18)**

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***Corresponding Author:**
B. Sabari Senthil*
Email: sabarisenthilcology@gmail.com