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Research Article

Memory Enhancing Activity of Eclipta Alba in Albino Rats: A Correlation with Anticholinesterase Activity

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ABSTRACT

Memory is the ability of an individual to record sensory stimuli & retain them over short or long periods of time & recall the same at a later date when needed. Short and long term memory loss may result from deteriorating cerebral mechanisms due to different causes having impact on the quality of life. Memory enhancer can improve thinking, memory, and alertness in people with Alzheimer's disease that affect the mind. Indigenous drugs are being constantly explored for this purpose. *Eclipta alba* is being examined for its memory enhancing quality as it is traditionally used for this purpose. Ethanolic extract of Eclipta alba (EEEA) was evaluated for memory enhancing activity using rodent models. Piracetam was the standard drug used. EEEA was administered to albino rats to evaluate transfer Latency (TL) on an elevated plus maze (EPM). TL was a measure of acquisition and retrieval learning. Radial arm maze (RAM) was used to evaluate the latency to find the food. Also time taken to reach the reward chamber was calculated by using Hebb – Willium's maze.Biochemical analysis was done for acetyl cholinesterase enzyme level. EEEA at a dose of 100 and 200 mg/kg produced a significant decrease in TL measured using EPM in comparison with the control. In RAM the activities of EEEA (100,200mg/kg) showed significant memory enhancement. Time taken to reach the reward chamber is significantly decreased in test groups in comparison to control using Hebb – Willium's maze. The increase in AChE activity by scopolamine was significantly reduced by EEEA (100 &200mg/kg p.o).

Keywords: EEEA, Piracetam, EPM, RAM, Hebb – Willium's Maze

INTRODUCTION

Memory is one of the important functions of brain which ultimately involves multiple neuronal pathways & neurotransmitters. [1] Prevalence of impairments of learning & memory in cognitive disorders like Alzheimer's disease, amnesia, delirium, depression & Schrizophrenia is still increasing.[2] For the management of these disorders, new memory enhancers are being constantly explored, of which herbs play a vital role. *Eclipta alba* (Family–Compositae) commonly called as BHRINGARAJ grows widely as an annual weed in moist places. It is reported to possess hepatoprotective [3], nootropic[4], immunomodulatory [5] and free radical scavenging action [6]. Phytochemically, *Eclipta alba* is rich in wadeoloctone, β- amyrin, stigmasterol and luteolin-7-glucoside [7]. Traditionally, it is being used as a memory modulator. So the present study was undertaken to investigate the effect of *Eclipta alba* on learning and memory in rats. Both working memory and long term memory were evaluated.

MATERIALS AND METHODS

Wiatar albino rats weighing between (180–200 g) were used after obtaining permission from the Institutional animal ethical committee. The animals were housed under standard conditions, room temperatures and a 12 hrs light: dark cycle. They were provided with food and water *ad libitum*. The animals were acclimatized under these conditions for 6 days prior to the experiment.

Drugs & chemicals used:

Piracetam →UCB India Ltd, Gujurat,
Diazepam → Ranbaxy laboratory limited
Scopolamine →Sigma – Aldrich, Bangalore
Ethanolic extract of Eclipta alba → from Shaharanpur

Acute toxicity study: Acute toxicity study was done according to OECD (Organization for Economic Co-operation and Development) Guideline, fixed dose method; with starting dose of 2000mg/kg body weight was adopted. Starting dose of 2000mg/kg (per oral) of each was given to 5 animals (albino rats), animals were kept for observation of behavioral change and death up to 72h.

Various behavioral assessments were done using various standard methods.

Transfer latency using elevated plus maze (EPM): Rats were divided into six groups consisting of 5 animals per group. Group I animals were treated with control vehicle (NS) taken as control group, group II animals were administered with scopolamine (1mg/kg ip) served as inducing group. Group III, IV, V were treated with EEEA at a dose of 50, 100, 200 mg/kg PO respectively along with Scopolamine served as test groups and group VI was given Piracetam 200mg/kg PO with scopolamine as positive control or standard group. All the extract & standard drug

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Table 1: Study protocol design: (n=5)

Groups	Treatment (mg/kg)	Remark
I	NS(10ml/kg)PO	Vehicle control
II	Scopolamine [S](1mg/kg) IP	Inducing group
III	S +EEEA(50)PO	Test
IV	S+EEEA(100)PO	Test
V	S +EEEA (200)PO	Test
VI	S + Piracetam (200)PO	Standard

Elevated Plus Maze

treated animals were subjected to scopolamine (1 mg/kg i.p)[8] 60 minutes after administration of extract & Piracetam, except the first group which served as vehicle control.

The animals were placed individually on the maze which consists of two open arms, 50 cm (length) × 10 cm and two enclosed arms, 50 cm (length) × 10 cm (width) × 40 cm (height) which lies opposite to each other. The maze was elevated to a height of 50 cm. 60 min after drug administration the animal was placed at the end of the open arms facing away from the centre of the maze. Transfer latency (TL) was recorded which the time was taken by the rats to move in to the covered arm with all its four paws. If the animals did not enter in to one of the covered arms with in 90 s, it was gently pushed in to one of the two covered arms and transfer latency was assigned as 90s. The rat was allowed to explore the maze for 10s and returned to the home cage. Twenty four hours later TL was recorded again. The measurement of transfer latency on the day recording was done on the first day and after 24 hours for 90seconds. TL on the first day served as a measure of acquisition learning and TL after 24 hrs for retrieval or explicit learning [9].

Radial Arm Maze (RAM): Animals were divided into 5 groups of 5 animals each. Group I received NS served as negative control, Group II,III, IV animals were given EEEA in a dose of 50, 100 & 200mg/kg respectively taken as test groups. Group V animals were treated with Piracetam 200mg/kg PO taken as positive control.Drugs were given 30mins prior to test. Time taken by the animals to reach reward arm was recorded after 24 hr for the animals of each groups.

Locally fabricated wooden RAM elevated above the 50cm floor consisted of an octagonal central hub 36cm in diameter with eight radial arms. Each arm was 46cm long, 15cm width with 12cm sides & had small plastic cups mounted at 30cm from the central hub. The rats were trained for RAM performance by conducting daily training trial for 7 days which consisted of two sessions wherein one food pellet was placed in fixed arm and then in the variable arm to record the effect of extract on spatial reference and spatial working memory respectively. Rats maintained at 85% of their total diet were placed individually in the central hub and were allowed to choose the arm freely to get the food with upper cut off limit of 300 sec.

The time taken by each rat to find the food along with number of re-entries was considered to assess RAM performance. Rat was considered to be learned when found the food with maximum one re-entry for three consecutive days. The number of days required for making the rat learned and the latency to find the food along with number of initial correct entries (i.e. before first re-entry) of learned rat were recorded as the effects of the drug on learning and memory process. 1 hour interval was kept between the spatial reference and spatial working memory evaluation. The apparatus was cleaned with damp cloth after each trial to avoid place preference and the influence of olfactory stimuli [10].

Hebb – Willium's Maze (HWM): Hebb – Willium's maze is an incentive based exteroceptive behavioral model useful for measuring working memory of rodents.The 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.

In the present study the animals were divided into5 groups of 5 animals in each group.Group-I served as the control.TRC (time taken to reach the reward chamber) was tested by using Hebb-William maze. Group-II treated with Diazepam (1mg/kg, i.p) alternatively for 10 days. TRC was tested by using Hebb-William maze. Group-III treated with Piracetam (200mg/kg, po) for 15 days. The amnesia

Table 2: Study protocol design: (n=5)

Groups	Treatment (mg/kg)	Remark
I	NS(10ml/kg)PO	Negative control
II	EEEA(50)PO	Test
III	EEEA(100)PO	Test
IV	EEEA (200)PO	Test
V	Piracetam (200)PO	Positive control

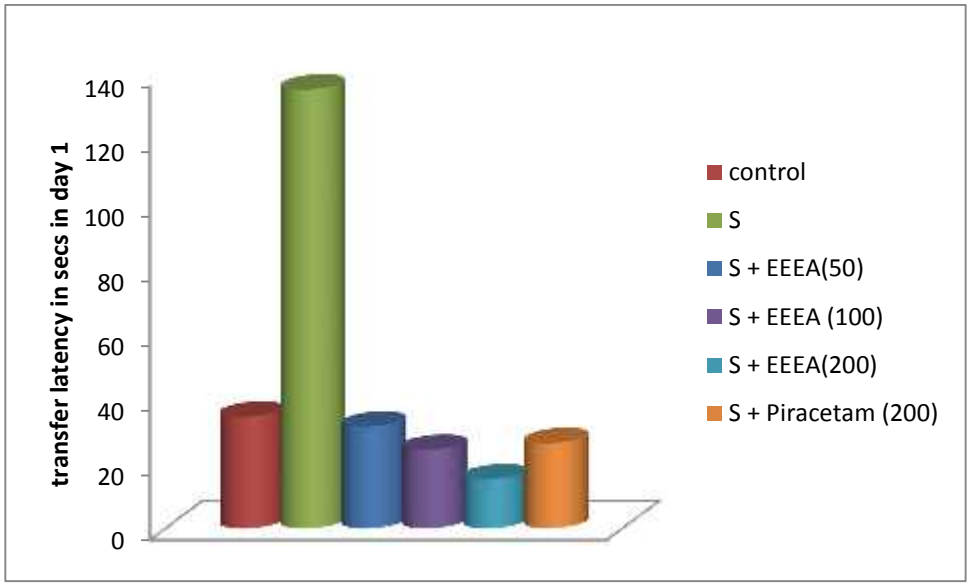
Table 3: Study protocol design: (n=5)

Groups	Treatment (mg/kg)	Remark
I	NS(10ml/kg)PO	Negative control
II	Diazepam[D] (1mg/kg)IP	Inducing group
III	D +Piracetam (200)PO	Standard
IV	D+EEEA(100)PO	Test
V	D + EEEA(200)PO	Test

Table 4: Effect of EEEA on transfer latency in rats using an elevated plus-maze.

Treatment groups	Transfer latency in secs	
(mg/kg)PO	Acquisition day 1	Retention after 24hr
Control (NS 10ml/kg) p.o)	34. 66± 2. 53	27. 33±1. 40
Scopolamine(1) ip	135. 33±3. 58$^{\#}$	133. 16±4. 85$^{\#}$
EEEA(50)+S	31. 53±2. 63*	16. 83±1. 51*
EEEA(100)+S	24. 50±0. 88*	9. 00±1. 46*
EEEA(200)+S	15. 33±1. 054*	6. 16±1. 13*
Piracetam (200)+S	26. 50±2. 43*	7. 20±1. 18*

$^{\#}$ p< 0.05, * p<0.001



Graph 1: Effect of EEEA on transfer latency (day 1) in rats using an elevated plus-maze.

inducing agent Diazepam (1mg/kg, i.p) was given alternatively for 10 days. TRC was tested by using Hebb-William maze. Group-IV treated with EEEA (100mg/kg) orally for 15 days. The amnesia inducing agent Diazepam (1mg/kg, i.p) was given alternatively for 10 days. TRC was tested by using Hebb-William maze. Group-V treated with EEEA (200mg/kg) orally for 15 days. The amnesia inducing agent Diazepam(1mg/kg, i.p) alternatively for 10 days. TRC was tested by using Hebb-William maze.

Acetylcholinesterase enzyme activity: Exactly 60 minutes after scopolamine treatment the rats were sacrificed by decapitation and the whole brain were taken out quickly. The brain was dissected out then suspended in phosphate buffer and weighed accurately and was homogenized in a tissue homogenizer. To this, 100µl of Ellman's reagent was added and then taken into the photocell. The absorbance was set to zero at 412nm. 20µl of the substrate (Acetyl thicholine iodide) was added. A change in the absorbance per minute was noted. The rate of moles of substrate hydrolyzed per minute per gram of tissue was later calculated as per the following equation.

$R = \Delta A/1.36\ (10^4)\ X\ 1/(400/3120)C_0 = 5.74X10^{-4}\ X\ \Delta A/C_0$

Where,ΔA= Change in absorbance per minute

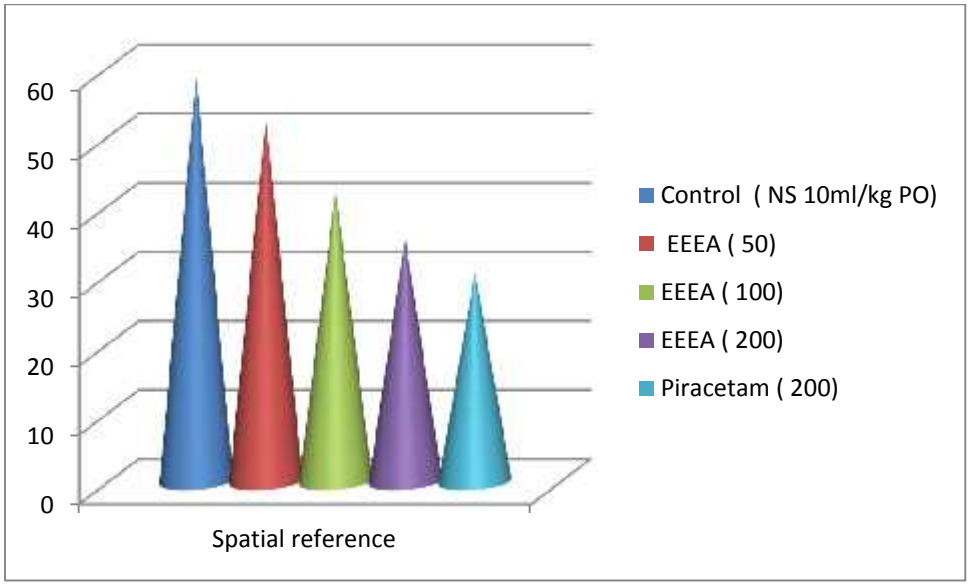
C_0 = Original concentration of the tissue.

R = Rate in moles substrate hydrolyzed per minute per gram of tissue.

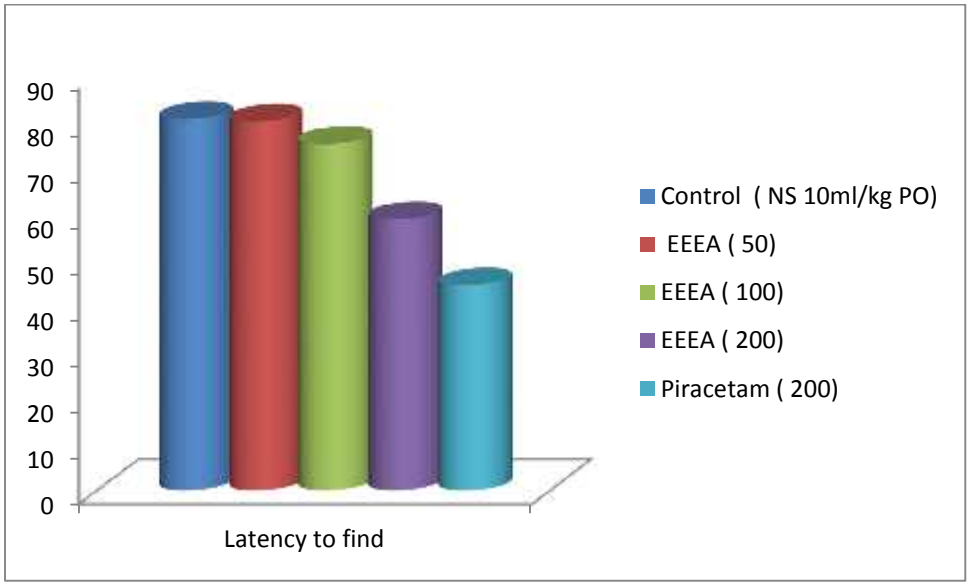
Table 5: Time taken to reach reward arm for EEEA on Radial Arm Maze

Drug treatment (mg/kg)PO	Spatial reference			Spatial working		
	Days to make rat learned	Latency to find food (sec)	Number of initial correct entries	Days to make rat learned	Latency to find food (sec)	Number of initial correct entries
Control (NS 10ml/kg PO)	16.0±0.2	58.6±0.50	6.9±0.5	18.2±2.1	80.6±2.5	7.3±0.8
EEEA (50)	14.56±0.89	52.6± 2.6	6.9±0.1	17.8±1.1	80.1±3.3	7.2±0.4
EEEA (100)	13.83±0.1*	42.2±0.9*	6.7±0.3	14.6±0.2**	75.3±1.6*	7.1±0.9
EEEA (200)	10.19±0.8**	35.3±1.58*	6.5±0.6	13.4±0.9**	58.9±1.3**	6.8±1.1
Piracetam (200)	9.03±0.3**	30.2±0.41*	6.3±0.4	11.8±0.6**	44.5±0.7**	6.7±1.0

*$p < 0.05$, **$p < 0.01$ vs. control*



Graph 2: Spatial reference in RAM (latency to find food)



Graph 3: spatial working in RAM (latency to find food)

Table 6: Effect of EEEA on learning scores of albino rats on day 1- 4 in HWM :

Groups with Dose in mg/kg PO	Learning scores in secs			
	Day1	Day2	Day3	Day4
Control	58.9±2.15	33.2±4.16	46.6±4.61	45.4±8.04
Diazepam (1 mg/kg)IP	80.3±14.4$^{\#\#}$	117.8±10.1$^{\#\#\#}$	96.2±10.9$^{\#\#}$	83.4±8.49$^{\#\#}$
Piracetam (200)+Diazepam	74.7±12.4*	67.5±4.51*	54.8±6.04*	60.6±6.01*
EEEA (100)+Diazepam	69.9±9.84*	44.5±3.09**	39.2±4.22*	44.9±2.23*
EEEA (200)+Diazepam	50.4±2.46**	40.2±4.67**	39.4±4.87*	51.8±4.92*

** p<0.05, ** p<0.01,$^{\#\#}$p<0.01 $^{\#\#\#}$p<0.001*

Table 7: Effect EEEA on acetyl cholinesterase enzyme activity(n=5)

Groups	Treatment with mg/kg	Acetylcholinesterase Enzyme activity (In moles/g of tissue)
I	Control	10.38±0.30
II	Scopolamine	23.11±0.08$^{\#}$
III	S + EEEA (100)	18.927±0.23*
IV	S+ EEEA (200)	9.56±0.39**
V	S+ piracetam(200)	8.71±0.30**

p<0.01$^{\#}$,p<0.0*, p<0.001**

STATISTICAL ANALYSIS

The data were analyzed by using one way ANOVA followed by Dunnet's multiple comparison tests.

OBSERVATION & RESULTS

Acute toxicity test: The observation indicated that there was no death in 2000mg/kg dose after 72hr.

The effect of vehicle, scopolamine control, EEEA (50 mg/ kg, 100 mg/kg, and 200 mg/kg) and piracetam were evaluated at 1st day & after 24hrs of administration of drugs. Transfer latency on 1st day of drug treatment reflected learning behavior of animals, where as transfer latency of next day reflected retention of information or memory. Scopolamine hydro bromide (1 mg/kg i. p) group showed a significant increase in transfer latency values on acquisition as wells as on the retention days as compared with vehicle control rats, indicating impairment in learning and memory. EEEA at dose level of 50, 100, 200 mg/kg orally demonstrated significant decrease in transfer latency on transfer latency on 1st day and 24hr after in elevated plus maze test as compared to scopolamine control and successfully reversed memory deficit induced by scopolamine (p<0.05). Piracetam used as positive control at a dose of 200 mg/kg PO also improved learning and memory in rats and reversed the amnesia induced by scopolamine. (table 5). The results obtained was statistically significant (p<0.001).

In RAM,the activities of EEEA showed significant memory enhancement when compared to the normal control. *EEEA* (100 and 200 mg/kg) showed significant reduction in number of days required to make the rat learned in both spatial reference as well as spatial working memory. On the contrary, similar doses showed reduction in latency to find the food by the learned rat only in spatial working memory when compared to control rat. *EEEA* pretreatment did not show any significant (p> 0.05) change in the number of initial correct entries in either model at any dose level. The effect of leaf extract was comparable to standard control piracetam.

The time taken by the animal (Learning score) to reach the reward chamber (B) from the entry chamber (A) in EEEA 100mg/kg treated animals was reduced significantly in comparison to Diazepam control on day1,2,3 and 4 respectively.

The time taken by the animal (Learning score) to reach the reward chamber (B) from the entry chamber (A) in EEEA200mg/kg treated animals was reduced significantly in comparison to Diazepam control on day 1,2,3 and 4 respectively. All the learning scores were comparable to that of piracetam treated animals

Acetylcholinesterase enzyme activity**:** The acetylcholine esterase activity was significantly increased by scopolamine as compared to control (Table no 7). The increase in AChE activity by scopolamine was significantly reduced by both Piracetam (200mg/kg p.o) and EEEA (100 &200mg/kg p.o).

DISCUSSIONS

Alzheimer's disease is a neurodegenerative disorder without an effective treatment. Alzheimer's disease is a/w decline in cognitive ability, patients also have non cognitive symptoms, such as depression, apathy& psychosis that impairs learning [11] Progressive memory loss, dementia, cognitive deficits are currently seen as medical & social problems[12]. The administration of antimuscarinic agent Scopolamine produces transient memory deficit. Scopolamine amnesia test is widely used as primary screening test for antialzheimer drug[13]. The present study suggests that EEEA possess memory enhancing activity in scopolamine induced amnesia model. EEEA treated rats showed decrease in TL in EPM model which is an indicator of cognitive enhancement. RAM performance is an appetitive motivated task and is also useful to assess the spatial reference as well as spatial working memory performance and agents that affect these processes [14]. The time taken by the animal (Learning score) to reach the reward chamber (B) from the entry chamber (A) in EEEA200mg/kg treated animals was reduced in HWM test showed memory enhancing activity of the extract.

A deficient cholinergic system has been implicated for the progressive decline of learning and memory in various

neuropsychiatric disorders[15]. Our study suggested a good correlation of loss of memory and loss of cholinergic activity which include acetylcholinesterase enzyme activity. We also observed that retention of memory was associated with decreased acetyl cholinesterase enzyme activity. Madepalli *et al.*, (1994)[16] reported that acetylcholine content and acetylcholinersterase enzyme activity have an inverse relationship on memory retention. In the present study, we measured the acetylcholinesterase enzyme activity of rat brain and that supports the findings of Madepalli *et al*., (1994)[16] because acetyl cholinesterase enzyme activity increased after scopolamine treatment, which decreased by treatment with EEEA. The results from the present study suggest that EEEA *possess* antiamnesic activity as it reversed the memory impairment produced by scopolamine & diazepam. The results suggest that these extracts improve cognition by decreasing the level of acetylcholinesterase enzyme activity.

CONCLUSION

These results indicated possible use of the extract as a part of therapy to treat poor learners and patients with impaired spatial memory functions. *Eclipta alba* produces a significant reduction in the transfer latency when tested after an interval of 24 hours in the EPM indicating that it improves the ability to retrieve information and therefore strengthens explicit memory. Many factors like experimental conditions, employed experimental protocol, modulation of specific neurotransmitters and involved neurochemicals can affect the extract activity on reference and working memory[17]. Reports on luteolins possessing credible enhancement of the central cholinergic receptors are available [18]. Luteolins being an active constituent in the extract of *Eclipta alba* may be responsible for minimizing cognitive deficits due to cholinergic dysfunctioning. Their profound free radical scavenging action could insulate neuronal tissues from degeneration probably by preserving these areas from stress perturbations. Protection of neuronal tissues may be possibly due to the immunomodulatory action of *Eclipta alba.* Therefore, *Eclipta alba* can serve as a potential memory modulatorThus, the exact mechanism of action and responsible phytochemicals will be revealed after detailed biochemical and phytochemical investigations.

In the present study scientific evaluation was carried out by using ethanol extract of leaf of *Eclipta alba* to prove nootropic potential. In conclusion data produced from the study shows significant memory enhancement by extracts of *Eclipta alba*, which might also be useful as supportive adjuvant in treatment of elderly memory loss, hence *Eclipta alba* can be used for the management of Alzheimer's disease and other neurodegenerative disorders.

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