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INVESTIGATION ON THE EFFECT OF ECLIPTA ALBA ON ANIMAL MODELS OF LEARNING AND MEMORY

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Abstract : Short and long term memory loss may result from deteriorating cerebral mechanisms due to varied causes which could have a tremendous impact on the quality of life. Herbs are being constantly explored to resolve cognitive deficits. *Eclipta alba* (*Ea*) commonly called as the trailing Eclipta is being examined for its memory enhancing quality as it is traditionally used for this purpose. The shade dried leaves of *Eclipta alba* was extracted with distilled water. The suspension of *Ea* containing 100 and 200 mg/kg was administered to rats to evaluate Transfer Latency (TL) on an elevated plus maze. TL was a measure of acquisition and retrieval learning. Mice were placed at the center of open field apparatus to assess spatial habitual learning, observed for 20 minutes for rearing and time spent during rearing using varied doses for 30 minutes, 24 hours and 96 hours and 144 hrs. The results revealed significant improvement of retrieval memory.

Key words : eclipta alba, transfer latency, spatial habitual learning, explicit memory

INTRODUCTION

Lack of neural plasticity can generate pertinent cognitive deficits which indeed can affect the quality of life. In order to circumvent this problem, memory elevators are being constantly explored, of which herbs play a vital role. *Eclipta alba* (Family–Compositae) commonly called as the trailing eclipta grows widely as an annual weed in moist places. It has been bestowed with the natural gift of a tonic. *Eclipta alba* is popularly used in the Indian traditional system of medicine to prevent abortions,

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night blindness, hernias, bronchitis, leucoderma, vertigo and to promote hair growth (1). It is reported to possess hepatoprotective (2), nootropic (3), immunomodulatory (4) and free radical scavenging action (5). Phytochemically, *Eclipta alba* is rich in wadeoloctone, β-amyrin, stigmasterol and luteolin-7-glucoside (6). Traditionally, it is being used as a memory modulator and we are scientifically validating this claim by measuring transfer latency and spatial habitual learning.

METHODS

Albino rats of Wistar strain (180–200 g) and Swiss albino mice (22–25 g) were used after obtaining permission from the Institutional animal ethical committee bearing Number: SSCP/15/2004–05 dated 03–02–2005. The animals were housed under standard conditions of 55±5% relative humidity, 23±1°C temperatures and a 12 hrs light: dark cycle. They were provided with food and water *ad libitum*. The animals were housed under these conditions for 6 days prior to the experiment for acclimatization.

Preparation of the extract

The leaves of *Eclipta alba* (***Ea***) were collected locally as it abundantly grows as an annual weed in moist places. The authentication of the plant has been done by a Botanist, Prof. Siddappa, Department of Botany, Siddaganga College for Boys, Tumkur. The plant was shade dried, powdered, extracted with double distilled water in a reflux condenser to obtain the total aqueous extract of *Eclipta alba* (TAE of ***Ea***) and concentrated to obtain a semisolid mass. Suspension of this extract containing 100 and 200 mg/kg were prepared using aqueous tragacanth solution (2%) and it was administered orally.

Safety evaluation :

TAE of ***Ea*** was administered to 10 mice and 10 rats in a dose of 2 g/kg, p.o and observations were made for gross behavioral changes such as locomotion, rearing, respiration, tremors, gait, passivity, righting reflex, lacrimation and mortality for 14 days (7).

Animals :

To measure transfer latency 15 rats were used and for spatial habitual learning 15 mice were used. They were divided into three groups randomly with each group containing 5 animals.

Group 1 : served as a control and received 1 ml of 2% aqueous solution tragacanth orally 60 min. prior to the experiment.

Group 2 : received 100 mg/kg of TAE of ***Ea*** orally 60 min. prior to the experiment.

Group 3 : received 200 mg/kg of TAE of ***Ea*** orally 60 min. prior to the experiment.

Transfer latency using elevated plus maze (EPM) :

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 is 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 and the time to move from the open arm to the closed arm was recorded as transfer latency (TL). The 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 (8).

Spatial habitual learning in mice using the open field :

Mice were individually placed at the centre of open field apparatus which consists of a rectangular chamber 26 × 26 × 40 cm illuminated with a 40 W bulb. The floor of the field is divided into 16 rectangular squares with white lines. The animal was placed at the centre of the open field and observed for 20 minutes for rearing or vertical activity and time spent during rearing. Re-exposure to the open field was done after thoroughly cleaning the floor of the field at 24, 96 and 144 hrs (9).

Statistical analysis :

The statistical analysis of data was done by one way ANOVA followed by Scheffes test using SPSS package. $P<0.05$ was considered as the level of significance.

RESULTS

No untoward observations were seen in the behavior of the animals when observed for first 24 hrs. No gross behavioral changes and mortality were observed for 14 days after single drug administration.

Transfer latency using Elevated plus maze :

The transfer latency recorded for the control group using the elevated plus maze (Table I) on day-1 was 74±0.34 and on day-2 was 40±1.26 seconds. The group treated with 100 mg/kg of TAE of ***Ea*** showed a transfer latency of 68±1.24 seconds on day-1 and a transfer latency of 31±0.68 seconds on day-2. At a dose of 200 mg/kg the time elapsed to move from the open arms to the closed arms was 60±0.56 seconds on day-1 but on day-2 the time taken was further reduced to 26±0.38 seconds ($P<0.05$). TAE of ***Ea*** at a dose of 100 and 200 mg/kg produced a significant decrease in TL measured using

TABLE I: Effect of TAE of *Ea* on transfer latency (memory modulation) measured on EPM.

Sl. No	*Treatment*	*No. of Animals*	*Route of Administration*	*Transfer Latency (Seconds)±SEM*	
				Day-1	*Day-2*
1	Control	5	Oral	74±0.34	40±1.26
2	100 mg/kg TAE of *Ea*	5	Oral	68±1.24	31±0.68
3	200 mg/kg TAE of *Ea*	5	Oral	60±0.56*	26±0.38*

TAE of ***Ea*** = Total Aqueous Extract of *Eclipta alba*.
*P<0.05 compared to control.

EPM after 24 hours in comparison with the control.

Spatial habitual learning :

The number of rearing observed in the control (group-1) at 30 minutes was 15±1.21 whereas the rearing decreased to 13 ±0.98, 10±0.55 and 9.3±0.67 at 24, 96 and 144 hours respectively. The total time spent during rearing declined from 6±0.62 to 5.4±0.48, 4.9±0.34 and 4.82±0.32 minutes at 30 min, 24, 96 and 144 hours respectively. Treatment with 100 mg/kg of TAE of *Ea* (Group-2) showed rearing of 7.2±1.27 at 30 min and at 24, 96, 144 hrs the rearing recorded were 5±0.89, 4.8±0.88, 3.9±0.76 which was significantly (P<0.01) lower than the control. Time spent during rearing at 30min. was 5.2±0.76. At 24, 96, 144 hrs a significant reduction (P<0.01) in the time spent during rearing was observed at 4.35±0.66, 4.2±0.73, 3.9±0.54 min. Observations with group-3 (200 mg/kg) were in consonance with group-2 with rearing of 4.9±0.65 at 30 min, 3±0.59, 2.5±0.43, 2.1±0.11 at 24, 96, 144 hrs which was statistically significant (P<0.01) compared to the control. Time spent during rearing was significantly reduced (P<0.01) at 30 min, 24 hours, 96 hours and 144 hours to 3.9±0.32, 3.6±0.42, 2.8±0.23, 2.2±0.44 (Table II). A significant decrease in rearing as a measure of decreased exploration due to improved memory is seen with 200 mg/kg of TAE of ***Ea*** compared to the control after 96 hrs and 144 hours indicating improvement in learning and memory.

DISCUSSION

Animal models have been instrumental in shaping our understanding of the ability of the brain to process information. Simple but explicable models such as the elevated plus maze and the open field are available to evaluate learning and memory modulation. The time consumed by the animal to move from the open to the closed arm in EPM is recorded as transfer latency which exemplifies short-term memory (10). The cognitive processing of spatial information takes place when the animal navigates the maze at intervals following the first exposure. Re-exposure to the maze would enable the animal to recall places and things reflecting explicit memory.

Spatial habitual learning in the open field is a time-tested approach to assess learning and memory. The decrement in response to a novel environment after repeated exposure to the familiar environment is referred to as

TABLE II: Effect of TAE of *Ea* on rearing activity (spatial habituation learning).

S. No.	*Time of recording Treatment*	*Av. No of Rearing activity*			*Av. Time spent during each rearing (sec)*		
		Grp-1	*Grp-2*	*Grp-3*	*Grp-1*	*Grp-2*	*Grp-3*
1	30 min	15±1.21	7.2±1.27	4.9±0.65*	6±0.62	5.2±0.76	3.9±0.32*
2	24 hours	13±0.98	5±0.89*	3±0.59*	5.4±0.48	4.35±0.66	3.6±0.42*
3	96 hours	10±0.55	4.8±0.88*	2.5±0.43*	4.9±0.34	4.2±0.73*	2.8±0.23*
4	144 hours	9.3±0.67	3.9±0.76*	2.1±0.11*	4.82±0.32	3.92±0.54*	2.2±0.44*

TAE of ***Ea*** = Total Aqueous Extract of *Eclipta alba*.
Values are mean±SEM, n=5, *P<0.01 compared to control group.
Grp = group.

'spatial habitual learning'. Recurrent exposures produce a decrease in the exploratory initiatives, which is implicative of memory pertaining to a specific feature of that environment (9). Exploratory activities like rearing and locomotion may be reduced on subsequent contact with the open field. Explicit memory is ascertained when observations are recorded after 24, 96 and 144 hrs.

Cholinergic dysfunctioning and suppression of the immune system have been implicated in inducing cognitive deficits in the neuronal memory circuits (11). *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. In spatial habitual learning, the exploratory rearing is significantly reduced with time indicating improved memory. Reports on luteolins possessing credible enhancement of the central cholinergic receptors are available (12). 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 modulator.

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