

EVALUATION OF ANTIAGGRESSIVE ACTIVITY OF ECLIPTA ALBA IN EXPERIMENTAL ANIMALS

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ABSTRACT

The occurrence of aggression for prolonged periods of time could have a hazardous impact on the health and social wellbeing of the individual. Aggression can ensue due to exposure to an intimidating situation. Aggression is prominently seen when a disturbance occurs in the fine balance of neurotransmitters such as 5-hydroxytryptamine, gamma-aminobutyric acid, dopamine and their receptor subtypes. The present study investigated the ability of 100 and 200mg/kg of aqueous extract of *Eclipta alba* to circumvent aggression. Foot shock induced aggression and water competition test were utilized as models for screening of antiaggressive activity. *Eclipta alba* significantly minimized dominance ($p < 0.05$) which is correlated to the level of aggression particularly with 200mg/kg in the water competition test. A tangible behavioral submission was observed with 100 and 200mg/kg and of *Eclipta alba* in the foot shock induced test.

Keywords: *Eclipta alba*, water competition, foot shock induced aggression.

INTRODUCTION

Anxiety is categorized under neuropsychiatric disorders, which encompass aberrant expressions of defensive and offensive behavior. An exaggerated or fearful response to an appropriate or inappropriate condition may be observed during anxiety (Blanchard *et al.*, 2001). Increased anxiety provokes elevated aggression. Such exaggerated responses may induce subtle alterations of different integrated systems resulting in undesirable symptoms of emotional reactivity reflected as aggression. Therefore aggression is an "overt behavior with the intension of inflicting physical damage on the opponent" (Moyer, 1968). Aggression generally ensues due to conflicting interests associated with restricted territory, electrical, sensory, chemical stimulation or with the removal of positive re-enforcements (Katherine, 2001). Although aggression is an adaptive response, if it is prolonged it can have serious repercussions on the health and social behavior of the individual.

Numerous natural remedies have found acceptance as anxiolytic agents as they diffuse the unwarranted effects produced by synthetic agents. *Eclipta alba* (Family-Compositae) commonly called as the trailing *Eclipta* is phytochemically rich in wedelolactone, β -amyryn, stigmaterol and luteolin-7-glucoside (Asolkar *et al.*, 1992). The hepatoprotective (Singh *et al.*, 2001), analgesic (Sawant *et al.*, 2004), immunomodulatory (Jayathirtha and Mishra, 2004) and free radical

scavenging action (Bhattacharya *et al.*, 1997) have already been demonstrated with *E. alba* so far. Traditionally, it has been used to produce beneficial neuropsychiatric alterations. Validation of its traditional claim would be instrumental in amalgamating *E. alba* to the legion of natural remedies used to treat neuropsychiatric disorders. Keeping this in mind, this study was undertaken.

MATERIALS AND METHODS

Wistar albino rats (150-200g) and Swiss albino mice (20-25g) of either sex were housed in cages at an ambient temperature of $25 \pm 2^{\circ}\text{C}$ in a 12 hr. light/dark cycle. Animals were allowed to acclimatize for 6 days prior to the experiment. The institutional animal ethical committee approved the protocol bearing number SSCP/2004-05 dated 03-02-2005. Animals were divided randomly into 4 groups with each group containing 10 animals. Group-1 served as a control and received 2% aqueous solution of tragacanth orally, Group-II received Diazepam (2.5mg/Kg body weight dose) which served as standard, Group-III received 100mg/Kg TAE of *Ea* (Total aqueous extract of *Eclipta alba*) and Group-IV received 200mg/Kg of TAE of *Ea*. Doses of TAE of *Ea* have been selected based on the safety study conducted in our laboratory which corresponds to 1/10th and 1/20th of the maximum safe dose.

Extract preparation

The entire plant of *Eclipta alba* (*Ea*) was collected locally as it is abundantly grown in moist places, authenticated by a Botanist and a voucher specimen is maintained in our

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herbarium bearing No. E/SCP/12. The plant was wiped, shade dried and powdered. 250g of the powder were refluxed with water for 18 hours. The total aqueous extract of *Eclipta alba* was then evaporated to obtain a semisolid mass (8.6g). The desired quantity of the extract was suspended in 2% aqueous solution of tragacanth as a suspending agent and administered in a dose of 100 and 200 mg/kg body weight orally, 60 minutes prior to the experiment. Diazepam was also suspended in 2% aqueous solution of tragacanth and was given in a dose of 2.5mg/Kg body weight by oral route.

EXPERIMENTAL

Safety evaluation

TAE of *Ea* was administered to 10 mice and 10 rats in a dose of 2g/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 (Ghosh, 1984).

Foot shock induced aggression

Male Swiss albino mice treated with the vehicle control (1ml of 2% tragacanth) were placed in pairs on the aggressometer (Inco Ltd., Punjab, India) which consists of steel rods at the bottom with a diameter of 6mm and enclosed within a perspex chamber for clear visibility of the animals. A 60 Hz current is delivered to the foot of the animal for a total of 3 minutes. A period of relaxation of 5 seconds is provided between two successive shocks of 5 sec during the 3 minutes recording. The animals were observed for 3 minutes for i) vocalization ii) leaping iii) running iv) rearing v) facing each other to attack. Administration of Diazepam (2.5mg/kg), TAE of *Ea* in a dose range of 100 and 200mg/kg was done 60 minutes prior to the recording of the behavioral response to group-2, group-3 and group-4 respectively. The observations were observed for 3 minutes (Ambavade *et al.*, 2006).

Water competition test

Albino rats of Wistar strain (250-400g) were paired and housed in different cages. After 6 days of acclimatization, animals were deprived of water for 23 hrs. At the end of this, pairs of water deprived animals were administered with the vehicle and 60 minutes later, a water bottle having a spout was introduced such that only one animal of a pair can drink at a time. Frequency and time in seconds of spout possession were recorded for 5 minutes and the aggressive animal of the pairs was marked for identification. Animals were then provided with water for 55 minutes after which the water bottle was withdrawn for 23 hours. The test was repeated on the second day. On day-2 instead of the control drug, the aggressive animals received Diazepam (2.5mg/kg), 100mg/kg and 200mg/kg TAE of *Ea* which was administered 60 minutes prior to the recording and aggressive bursts were observed for 5 minutes (Gerhard, 2002).

STATISTICAL ANALYSIS

Results are expressed as mean \pm SEM. Data for foot shock induced test was analyzed using unpaired student 't' test. Data from the water competition test was analyzed using paired student't' test. $p < 0.05$ was considered as the level of significance.

RESULTS

No untoward observations such as gross behavioral changes and mortality were seen for 14 days of observations.

Foot shock induced aggression

The delivery of 60 Hz foot shock current induced 9.85 ± 1.03 number of vocalization and 6.7 ± 0.56 number of rearing following the administration of 2% aqueous solution of tragacanth to the control group. Leaping, running and facing each other with an attempt to fight were also observed in the control group. Diazepam treated group (Group-2) showed a significantly steady decline in vocalization to 1.5 ± 0.51 compared with the control ($p < 0.05$). Leaping was significantly curtailed while running, rearing and facing each other were totally absent. A dose of 100mg/kg body weight of TAE of *Ea* produced a reduction in vocalizations to 5.2 ± 0.73 along with lowering of leaping, running, rearing and facing each other. The vocalization was significantly reduced to 2.5 ± 0.56 and leaping was reduced to 2.3 ± 0.36 in the group treated with 200mg/kg TAE of *Ea* with the abolition of running, rearing and facing each other compared with the control ($p < 0.05$) indicating its efficacy in aggression (table 1).

Water competition test

The average time of spout possession and frequency of spout possession in the water competition test are depicted in fig. 1 and fig. 2. The frequency and time of spout possession following administration of the vehicle in the aggressive animal of the pairs was 9.3 ± 1.12 and 202 ± 2.16 seconds respectively. Group-2 exhibited a significant reduction ($p < 0.05$) in the frequency of spout possession to 3 ± 0.51 in the aggressive animal of the pairs. The time of spout possession was also consistently reduced to 124 ± 1.41 seconds. TAE of *Ea* at a dose of 100mg/kg produced a frequency of spout possession of 8.3 ± 0.67 which was not significant compared to the control. However at a dose of 200mg/kg, it produced a significant reduction in spout gaining frequency of 5.4 ± 0.27 along with a reduction in time spent to 132 ± 1.78 which was found to be statistically significant ($p < 0.05$) compared to the control.

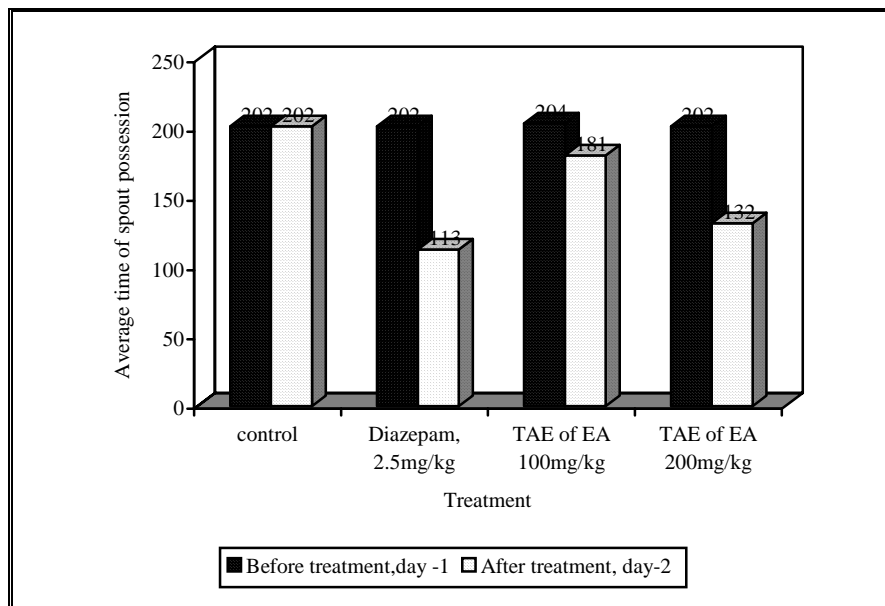


Fig. 1: Effect of TAE of Ea on time of spout possession.

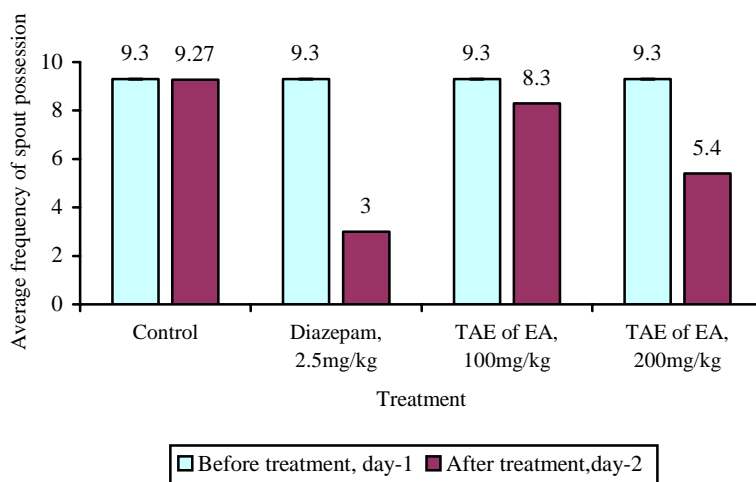


Fig. 2: Effect of TAE of Ea on frequency of spout possession.

DISCUSSION

One of the difficult medical and social problems associated with neuropsychiatric disturbances is aggression. Analysis of aggression in different animal species could provide a firm understanding of human violence and the therapeutic measures to be taken to combat it (Klaus *et al.*, 2002). Exposure of an animal to a threatening situation elicits a behavioral repertoire referred to as aggression. The behavioral profile may be manifested in the form of offensive and defensive aggression (Adams, 1979). Offensive behavior is characterized by initiative of the aggressor and devastation to the opponent (Blanchard *et al.*, 1977;

Oliver *et al.*, 1990). On the other hand defensive behavior lacks initiative and the animal does not impose intentional damage (Oliver *et al.*, 1990). The paradigm such as foot shock induced aggression and water competition test provide an assessment of the rodents level of defensive behavior, which in turn would reflect the extent of anxiousness or fear the animal experiences (Gerhard, 2002). In rats, defensive behavior is composed of a host of responses like vocalization, leaping, rearing, running and facing each other which depend on the nature of threat the animal envisages. In the water competition test, water deprivation induces high rank motivation resulting in competition for a common goal which is "water". Therefore it serves to demonstrate that the animal with

Table 1: Effect of TAE of Ea on foot shock induced aggression.

| No. of animals | Behavioral response | Group-I (Control) ± SEM | Group-II (Diazepam) ± SEM | Group-III 100mg/kg TAE of Ea ± SEM | Group-IV 100mg/kg TAE of Ea ± SEM |
|----------------------|---------------------|-------------------------------|---------------------------------|--|---|
| 10 animals per group | Vocalization | 9.85±1.03 | 1.5±0.51 * | 5.2±0.73 | 2.5±0.56 * |
| | Leaping | 8.55±0.82 | 1.37±0.29* | 4.8±0.48* | 2.3±0.36 * |
| | Rearing | 6.7±0.56 | --- | 3.0±0.41 * | 2.0±0.14 * |
| | Running | 3.0±0.75 | --- | 1.0±0.17 * | -- |
| | Facing each other | 4.0±0.28 | --- | 1.0±0.22 * | -- |

*p<0.05 compared to control. Diazepam: 2.5mg/kg body weight.

greater dominance or aggression will take hold of the spout faster and for a longer duration. If the drug administered minimizes this urge it would indicate the anxiolytic property of the drug.

In animal aggression, the involvement of different neuronal circuits has been demonstrated. The initiation and execution of aggression is related to the disturbance in the fine balance of serotonin, dopamine, gamma-aminobutyric acid (GABA) and their particular receptor subtypes. Aggressive laboratory animals generally display reduced brain 5-HT turnover (Nelson *et al.*, 2001).

Our investigation has revealed that the aggressive bouts as tested on the foot shock aggression paradigm was substantially reduced with both 100 and 200mg/kg of TAE of Ea. Running, rearing and facing each other which are significant features of defensive aggression have been attenuated with 200mg/kg of TAE of Ea indicating it has potential anti-aggressive qualities. The dominating trait which reflects aggression was also subdued in the water competition test when tested with 100 and 200mg/kg of TAE of Ea. The ability to suppress dominance was predominately observed with 200mg/kg of the extract. The placidity in behavior induced in animals by TAE of Ea may be due to its ability to alter the level of neurotransmitters and Na⁺/K⁺-ATPase. The enzyme Na⁺/K⁺-adenosine triphosphatase (Na⁺/K⁺-ATPase) has an accomplished role in regulating brain function, such as repolarization of the neuronal membranes and neurotransmitters uptake/release. Individual variations in the activity of brain Na⁺/K⁺-ATPase may result in differences in the functioning of the brain, which, in turn, could produce behavioral divergences (Rosana *et al.*, 2005). *Eclipta alba* has been found to activate Na⁺/K⁺-ATPase which could produce an elevation in the intracellular concentration of Ca²⁺. Stimulation of the Ca²⁺ receptor could induce the release of 5-hydroxytryptamine (Kuo-peing *et al.*, 2003). The enhanced turnover of 5-HT can cause blunting of

aggression and could be a plausible reason for the anti-aggressive property of *Eclipta alba*.

In conclusion, the total aqueous extract of *Eclipta alba* was found to be efficacious in producing serenity and masking the constellation of behavioral changes encountered during aggressive bouts making it a promising naturally derived product.

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