IMPACT OF THE AQUEOUS EXTRACT OF ECLIPTA ALBA ON MATERNAL AGGRESSION IN RATS

DAVID BANJI*[a], OTILIA JF BANJI[a], AR ANNAMALAI[b] AND SHANTHMURTHY M[c]

[a]**Department of Pharmacology, Nalanda College of Pharmacy, Hyderabad Road, Nalgonda-508001, Andhra Pradesh, India*

[b]*Department of Pharmacology, Rajah Muthiah Medical College, Annamalai University, Annamalainagar, Tamilnadu, India*

[c]*Sree Siddaganga College of Pharmacy, Tumkur, Karnataka-572102, India*

ABSTRACT

Parturient females display impulsive behavior represented in the form of aggressive bouts when exposed to conspecifics. Prolonged aggression during the postpartum period could affect maternal care. *Eclipta alba* is traditionally known to induce neuropsychiatric alterations, however its ability to circumvent maternal aggression has not been elucidated. The present study was aimed to investigate the ability of the aqueous extract of *Eclipta alba* to suppress maternal aggression. In the single dose study, 100, 200 and 500mg/kg body weight of the aqueous extract of *Eclipta alba* was administered to parturient females 30 minutes prior to maternal aggression testing against intruder males. In the multiple dose study, 100, 200 and 500mg/kg of the extract were administered for 15 and 30 days and maternal aggression was quantified. Administration of the extract for 15 and 30 days in dose schedules of 200 and 500mg/kg body weight significantly suppressed agonistic encounters by the dams and therefore had beneficial anti-aggressive activity.

Keywords: Maternal aggression, *Eclipta alba.*

INTRODUCTION

One of the most important frameworks for a mammal or human being is to establish interaction between members of its family and to develop a social organization. The alterations in the social organization can be triggered by fear, anger or pleasure which can culminate in aggressive bouts or bursts. Although it can be foreseen as a behavioral aberration, an aggressive behavior is a trait which is manifested as an adaptive response. Agonistic behavior generally does not have a pathological origin but is displayed when an animal perceives provocation or aims at maintaining a social hierarchy or is subject to frustration, it in turn exhibits impulsive or thoughtless actions referred to as aggression (Katherine, 2001). Another deliberate yet fruitful form of aggression is protection of the young ones by the parturient females from predators and conspecifics referred to as maternal aggression. Disturbance in nest building and grooming also affects maternal care. Under such circumstances the dam displays fierce protection of her young ones (Stephen *et al*., 2004). Aggression can therefore be categorically defined as a behavior directed against another individual or animal carried out with the proximate intent to harm (Moyer, 1968). Aggression could be correlated to anxiety as it represents an exaggerated or fearful response to an appropriate or inappropriate situation (Oliver *et al*., 2005).

Prolonged anxiety could have serious repercussions on maternal care during the post partum period. Anxiety could be contained with an array of drugs, however their propensity to produce side effects are high. Natural products therefore have a sublime advantage over the other drugs largely due to their safety during this delicate phase of motherhood. *Eclipta alba* (Family:Compositae) called as the trailing eclipta has hepatoprotective (Singh *et al*., 2001), analgesic (Sawant *et al*., 2004), immunomodulatory (Jayathirtha *et al*., 2004) and free radical scavenging action (Bhattacharya *et al*., 1997). Phytochemically it is rich in wedelolactone which is a coumestan, a type of phytoestrogen, β amyrin and luteolin-7-glucoside (Asolkar *et al*., 1992). The anti-aggressive profile of the aqueous extract of *Eclipta alba* has been evaluated in male mice by the foot shock induced paradigm (Otilia *et al*., 2008), however, its impact on maternal aggression has not been explored. This delicate and precise model can provide us with greater insights into the complex and interesting subject of postpartum anxiety and aggression.

EXPERIMENTAL

The institutional animal committee approved the protocol bearing number SSCP/ IAEC Clear/ 15/ 2004-05 dated 03-02-2005.

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 herbarium bearing No. E/ SCP/ 12. The plant was wiped, shade dried and powdered. 200g of the powder were refluxed with water for 18 hours. The aqueous extract of *Eclipta alba (AeEA)* was then evaporated to obtain a

**Corresponding author:* davidbanji@gmail.com

semisolid mass (8.0g). 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, 200 and 500mg/Kg body weight orally, 30 minutes prior to the experiment. Diazepam was also suspended in 2% aqueous solution of tragacanth and was given in a dose of 2 mg/Kg body weight by oral route.

Safety evaluation

AeEA was administered to 10 rats up to 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).

Conditions

Virgin female Wistar albino rats (180-200g) were housed in cages at an ambient temperature of 25±2^{o}C in a 12 hr. light/dark cycle. They were mated and at the bottom of each cage, a gauze was placed to collect the ejaculation plugs. The male is left for another one week with the female. Evidence of copulation was confirmed by observing the lumps of spermatozoa in the vaginal smear. This was considered as day one of pregnancy. The pregnant rats were housed in groups of 5 in standard rat cages and from day 18 of pregnancy rats were housed individually.

Grouping of animals

Following parturition, animals were allocated randomly into 5 groups with each group containing 6 animals for the single dose studies. Group-1 received 1 ml of 2% aqueous solution of tragacanth, group-2 received diazepam (1mg/Kg), group-3 received 100mg/kg, group-4 received 200mg/kg and group-5 received 500mg/kg of the aqueous extract of *Eclipta alba* orally 30 minutes prior to the experiment. For the multiple dose studies, the animals were divided into 8 groups with each group containing 6 animals. They were administered with 100, 200 and 500mg/kg of *AeEA* for 15 and 30 days from day-1 of pregnancy.

Maternal aggression test

The window of peak maternal aggression occurs between postpartum days 4-10, hence this period was selected. On postpartum day (ppd) 4 and 8, each female rat was exposed to a previously group housed intruder male rat for 15 minutes in her home cage between 0900 and 1300 hrs. The pups were removed from the cage 2 minutes prior to the behavioral test, in order to prevent injury to the pups. Removal of pups from the dam just before an aggressive test does not diminish the expression of maternal aggression. Each session was quantified to assess maternal aggression. For quantification of maternal aggression, offensive features such as latency to first attack, number of attacks, total duration of attacks, amount of time spent in attacking different regions of the male including head/neck, flank/back and amount of time spent lunging was observed. Females were considered aggressive if they exhibited at least one attack. In case of time of first attack, a maximum cut off time of 300 seconds was assigned if the animal did not exhibit aggressiveness. After 5 minutes the pups were randomly scattered in their home cage (Zachary *et al*., 2006).

STATISTICAL ANALYSIS

As behavioral data was not normally distributed, nonparametric Kruskall Wallis test followed by Dunn's method of multiple comparisons was adopted for analysis of data. The mean latency to attack was analyzed using student't' test. Mean comparisons were considered to be significant if $p<0.05$.

RESULTS

Safety evaluation

No untoward observations such as gross behavioral changes and mortailiy were seen for 14 days implying that *AeEA* was safe up to 2g/kg body weight.

Maternal aggression test

High level of maternal aggression was displayed by dams treated with aqueous tragacanth solution. This was reflected in terms of the reduced attack latency of 60±2.78 and 58±2.16 seconds on ppd 4 and 8. Treatment with diazepam prolonged the latency to attack to 198±2.85 and 199.4±2.66 seconds on ppd 4 and 8 which was significant compared with the control ($p<0.01$). Treatment with 500mg/kg body weight of *AeEA* in a single dose also prolonged the time taken to attack implying significance compared with the control (fig-1). The numbers of attacks were significantly reduced ($p<0.01$) following treatment with 500mg/Kg AeEA compared with the control (Table-1). The elevated latency to attack of 210±2.42 seconds was observed in the multiple dose study following the administration of diazepam which exhibited significance compared with the control ($p<0.01$) (Fig-2). 200 and 500mg/kg of *AeEA* administered for 30 days reduced number of attacks both on ppd 4 and 8 (table 2).

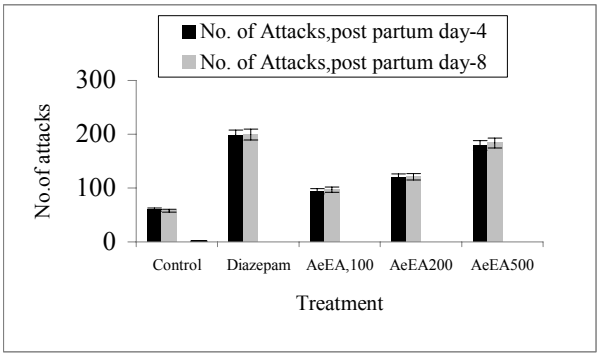

Fig. 1: Effect of aqueous extract of *Eclipta alba* (AeEA) on attack latency in the maternal aggression test: single dose study.

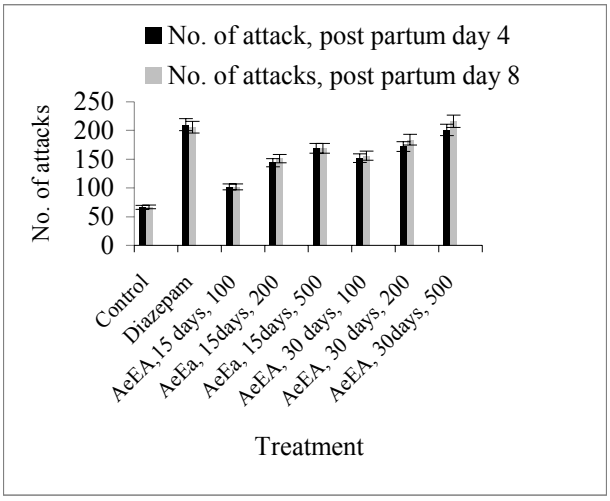

Fig. 2: Effect of aqueous extract of Eclipta alba (AeEA) on attack latency in the maternal aggression test: multiple dose study.

A decrease in the time spent and number of aggressive bouts was particularly observed with 500mg/kg body weight of *AeEA* as a single dose compared with the control ($p<0.01$) (table 3).

The total time spent and number of agonistic encounters on ppd-4 was significantly minimized following the administration of the aqueous extract of *Eclipta alba* for 30 days particularly with high doses of the extract (200 and 500mg/kg) (table 4). A significant diminution in the number of attacks was observed on ppd-8 in animals treated with 500mg/kg of the extract (table 5). The number of attacks was also altered with the prior administration of aqueous extract of *Eclipta alba* for 15 and 30 days on ppd 4 and 8 (tables 4 & 6). Administration of 100mg/kg in the single dose and in the multiple dose study produced only a slight decline in maternal aggression.

DISCUSSION

Animal models have played a pivotal role in behavioral neuroscience to gain insights into behavioral patterns. This could provide us with a comprehensive understanding of aggressive behavior during the postpartum period in human subjects.

Table 3: Effect of aqueous extract of *Eclipta alba* (*AeEA*) on postpartum day-4 in maternal aggression test: single dose study.

S. No.	Treatment	Dose mg/kg body weight	No. of attacks	Time in sec. of each attack	Bite on head	Bite on body	Upright posture	Nipping	Lunge	On top
1	Control	--	18.0±1.02	22.0±0.48	6.0±0.25	6.0±0.36	3.0±0.20	--	2.0±0.04	1.0±0.12
2	Std (Diazepam)	1.0	2.0±0.12*	10.0±0.50**	--	1.0±0.08**	1.0±0.12*	--	--	--
3	*AeEA*	100	9.8±0.56*	16.0±1.04	3.6±0.10	2.0±0.48**	2.0±0.16*	1.0±0.02	--	1.2±0.14
4	*AeEA*	200	8.8±0.74*	14.0±0.92*	2.0±0.28*	3.0±0.24*	1.0±0.14*	1.8±0.20	--	--
5	*AeEA*	500	5.2±0.64**	8.8±0.56**	1.0±0.10*	2.0±0.02**	1.2±0.04	--	--	--

Route of administration: Oral, Values are mean ± SEM, *p<0.05, **p<0.01 compared to control, n=6

Table 4: Effect of aqueous extract of *Eclipta alba* (*AeEA*) on postpartum day-4 in maternal aggression test: Multiple dose study

S. No.	Treatment	No. of days	Dose mg/kg	No. of attacks	Time in sec of each attack	Bite on head	Bite on body	Upright posture	Nipping	Lunge	On top
1	Control	15	--	15.0±1.20	22.0±1.04	4.0±0.32	4.0±0.28	3.0±0.18	--	2.0±0.30	2.0±0.10
2	Std	15	1.0	2.0±0.12**	5.0±0.72**	--	1.0±0.10*	1.0±0.02*	--	--	--
3	*AeEA*	15	100	9.5±0.84	10.0±1.25*	2.5±0.40	2.0±0.15*	2.0±0.14	--	1.0±0.28*	1.0±0.05*
4	*AeEA*	15	200	8.4±0.70*	8.0±0.80*	2.0±0.12	3.0±0.14	2.0±0.26	--	0.5±0.01*	--
5	*AeEA*	15	500	7.5±0.28*	7.2±0.58*	2.0±0.30	3.0±0.28*	2.0±0.50	--	0.5±0.53**	--
6	*AeEA*	30	100	3.8±0.16**	5.5±0.42**	--	2.0±0.16*	1.0±0.30*	--	--	--
7	*AeEA*	30	200	3.5±0.14**	5.0±0.36**	1.5±0.08*	2.0±0.12*	--	--	--	--
8	*AeEA*	30	500	3.0±0.22**	2.8±0.42**	1.0±0.12*	2.0±0.06*	--	--	--	--

Route of administration: Oral, Values are mean±SEM, *p<0.05, **p<0.01 compared to control, n=6

Female animals exhibit less aggressiveness than males in most of the mammalian species. However, the behavior of female animals is clearly controlled by their reproductive state (Jay *et al*., 1988). Parturient females portray an innate and conspicuous quality that surfaces after the birth of her pups. The dam displays flexible protective strategies so as to avoid risky social interaction. The violence pursued by the mother is significant even at the cost of her own life. This may be because the litters are solely dependent on the mother not only for nutrition but also for thermoregulation (Andrea *et al*., 2002). Threat towards the reproductive success is dealt with firmly by the female. Maternal aggression or nest defense is staunch against males who utilize the tactic of infanticide, so that the females can be induced into estrous to facilitate mating (Bruce, 1960). Although the dam salvages her pups from intruders, the impact of aggression on the biological system could be immense.

In several behavioral paradigms for anxiety testing, it has been observed that, lactation reduces the threshold of fear due to attenuation of the hypothalamic pituitary axis to stressors (Mos *et al*., 1987). However the introduction of the intruder into the nest area could reverse the reduced reactivity of the HPA axis. This is why the pups are removed from the dam's home cage only 2 minutes prior to behavioral testing.

Hormonal alterations during pregnancy and lactation are largely responsible for maternal behavior. A positive correlation exists between the elevated levels of estrogen during pregnancy and maternal aggression. Programmed neuronal circuits are activated by hormones like estrogen and progesterone. Binding of estrogens to specific brain areas such as the median preoptic nucleus is largely responsible for maternal behavior. Priming of the maternal brain with estrogen is responsible for enhancing the density of dendritic spines which provides a greater surface area for reception of nerve signals. This could escalate aggressive behavior following reproductive challenge (Russel *et al*., 2001). Estrogens like substances produced by plants bear a nonsteroid structure and are designated as phytoestrogens. They bear a functional similarity to endogenous estrogens. They can function as natural selective estrogen receptor modulators throughout the physiological systems. They have a distinct ability of behaving as estrogen agonists at specific tissue sites and exert an antiestrogenic action at the same time (Edwin *et al*., 2005). These phytoestrogens may have affinity for the ERα and ERβ isoforms (Dolores *et al*., 2001).

When female rats were administered with the aqueous extract of *Eclipta alba* for 15 and 30 days, a relative decrease in maternal aggression was observed in terms of number of attacks and time spent during each attack. This could be co-related to the presence of phytoestrogens. The submissiveness endured by the females following the administration of the extract for longer periods and in higher doses may be associated with antagonism of brain ER β receptors by the phytoestrogens. Administration of the extract for 30 days accentuated the antiestrogenic action possibly by endearing the ER β receptors and obliterating the action of endogenous estrogens. Therefore the anti-estrogenic action of the extract could be related to their affinity for the ER β receptors.

It could also be speculated that the level of circulating hormones could be diminished by these phytoestrogens both systemically and locally and this may be due to the ability of phytoestrogens to alter the total amount of hormone binding globulin synthesis in the liver cells (Patisaul, 2005). This could produce a valid decline in the levels of circulating estrogen.

Therefore *AeEA* serves as a competent agent in suppressing maternal aggression and could be used to produce serenity in postpartum anxiety.

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