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SEDATIVE & ANTIANXIETY ACTIVITY OF ETHANOLIC EXTRACT OF *ECLIPTA ALBA* IN ALBINO RATS

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ABSTRACT

Anxiety is a most common emotional disorder affecting 20% of adult population worldwide. *Eclipta alba* is having memory enhancing quality & traditionally used for this purpose. Since paucity of data available for this clinical use, the present work was undertaken to study the anti anxiety & sedative effects of Ethanolic extract of leaves of Eclipta alba(EEEA) in albino rats using thiopental sodium induced sleeping time (TIS), locomotor activity by actophotometer, elevated plus maze test(EPM).EEEA was administered in 50,100,200&400mg/kg doses PO. In the TIS time, the extract in a dose of 200 & 400 mg/kg induced the sleep at an earlier stage & prolonged the duration of sleep. The extract at the dose of 400mg increased % of entries & time spent in the open arms significantly. The EEEA also decreased the locomotor activity in the same dose. The EEEA was found to possess both sedative & anxiolytic activity.

KEY WORD: **EEEA, sedative, anxiolytic activity, actophotometer**

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INTRODUCTION

Human anxiety is defined as a feeling of apprehension, uncertainty or tension stemming from the anticipation of imagined or unreal threat. [1]Anxiety disorders are the most common emotional disorders affecting people worldwide among which around 20% of adult population suffer from these conditions at some stage during their life [(1,2)].Benzodiazepines **(** Diazepam, nitrazepam, lorazepam & alprazolam etc) are the most frequently prescribed synthetic drugs for variety of conditions particularly anxiety, depression, epilepsy & insomnia commonly known as anxiolytics. Although medications cannot fully cure anxiety disorders they can, to a great degree, relieve the symptoms & reduce their occurrences. But these psychoneural drugs have very serious side effects like chronic use of BZDs cause deterioration of cognitive function, physical dependence & tolerance.[3] In this context, resurgence of interest in medicine from natural sources (mainly plant products) is seen & there is tremendous hope that the drugs of plant origin will have significantly less side effects than that observed with synthetic drugs while having comparable efficacy. Eclipta alba (commonly known as Bringaraj) is a perennial shrub grown widely in the moist tropical countries. It is reported to have anthelmintic, antipyretic, anti-inflammatory, antihistaminic, hepatoprotective, expectorant properties [4,5,6] and useful in disease of skin, spleen, stomatitis, toothache, hemicranias as well as vertigo [4]. It is also having memory enhancing quality & traditionally used for this purpose.[7]

AIMS & OBJECTIVES

Since the report has shown new dimension to one important clinical use & paucity of data available in this regard the present work was undertaken to study the sedative & antianxiety effects of *Eclipta alba* in albino rats.

MATERIALS & METHODS

The drugs & chemicals for the entire work were obtained in pure powdered form from the following pharmaceuticals

1. Diazepam→ Ranbaxy Lab Ltd, Mumbai
2. Thiopental sodium → Abbott laboratories, Mumbai
3. Ethanolic extract of leaves of *Eclipta alba* (EEEA)→ Envin Bioceuticals, Saharanpur, Uttarpradesh

Experimental animals

Wistar albino rats of either sex weighing between 100-200 gms were purchased from OUAT, Bhubaneswar. Animals were housed maintaining 12:12 hr dark:light cycle. They had free access to standard chow diet & water ad libitum. The animals were quarantined for 7 days before starting the experiment. Food but not water was withdrawn from rats 12 hours prior to the experiment. The experiments were carried out during 10am to 4pm. All the experiments were conducted under isolated & noiseless conditions. The animals were acclimatized to laboratory conditions one week prior to the experiments.

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.

(i) Thiopental sodium induced sleeping time

The experiment was conducted following the method described by Ferrini et al (1974). 36 male adult albino rats weighing between 150-200 gms were used. Animals were assigned to groups, each consisting of 6 animals each & the study protocol was shown below in table no 1.

Table no 1
Study protocol design for thiopental sodium induced sleeping time in rats (approved by IAEC)

Groups	Drugs in mg/kg	Mode of administration	Nature of group
1	Distilled water(20ml/kg)	Through pediatric nasogastric tube	Control
2	Diazepam - 5	Dissolved in 1ml of DW followed by 20ml/kg of DW	Standard
3	EEEA-50	-do-	Test
4	EEEA-100	-do-	Test
5	EEEA-200	-do-	Test
6	EEEA-400	-do-	Test

One hour after administration of test substances & distilled water, the rats received 25mg/kg thiopental sodium by intraperitonial injection [8] to induce sleep.

(ii) Spontaneous motor activity

This test was determined using actophotometer. Test involves placing the rats in a cage which has photoelectric cells connected to circuit with a counter. The animals were divided into 6 groups having 6 animals each shown in table 2.When the beam of light is cut off due to movement of rats, locomotion count was recorded by the instruments.[9] & analyzed statistically using one way ANOVA followed by Dunnet's multiple comparison test.

Table 2
Study protocol design of spontaneous motor activity of rats (approved by IAEC)

Groups	Drugs in mg/kg	Mode of administration	Nature of group
1	Distilled water (0.5ml)	Through pediatric nasogastric tube	Control
2	Diazepam - 1	Dissolved in 1ml of DW followed by 20ml/kg of DW	Standard
3	EEEA-50	-do-	Test
4	EEEA-100	-do-	Test
5	EEEA-200	-do-	Test
6	EEEA-400	-do-	Test

The rats were singly placed in the cage & spontaneous motility was recorded by actophotometer for 10mins. All the animals were treated as the protocol above. After 30mins they were again placed individually in actophotometer to score locomotor activity & analyzed statistically using one way ANOVA followed by Dunnet's multiple comparison tests.

(iii) Elevated plus maze (EPM) test

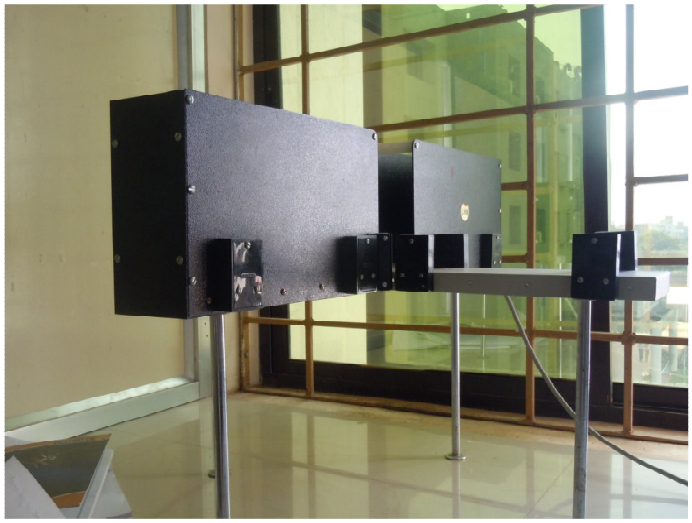
EPM test [10] for studying the anxiolytic effect in rodents was used. The method initially suggested by Handley & Mithani [11].EPM consists of two open arms (15cm X 10cm) & two closed arms (

50cm X 10cm X 40cm) with an open roof & elevated at 50cm. Adult albino rats (150- 200gms) were used in this study with the study protocol depicted in table no 3.

Table no 3
Study protocol design of EPM test in rats (approved by IAEC)

Groups	Drugs in mg/kg	Mode of administration	Nature of group
1	[12]Distilled water(0.1ml)	Through pediatric nasogastric tube	Control
2	[12]Diazepam - 2	Dissolved in 1ml of DW followed by 20ml/kg of DW	Standard
3	EEEA-50	-do-	Test
4	EEEA-100	-do-	Test
5	EEEA-200	-do-	Test
6	EEEA-400	-do-	Test

Sixty minutes after administration of the test drugs, each animal was individually placed in the centre of the EPM &were allowed 5 min for free exploration. Next, the number of open arm entries & time spent on open arms were recorded.[13]Data were analyzed statistically using one way ANOVA followed by Dunnet's multiple comparison tests.


Elevated Plus Maze

OBSERVATIONS & RESULTS

The observation indicated that there was no death in 2000mg/kg dose after 72hr

Table 4
Thiopental sodium induced sleeping time

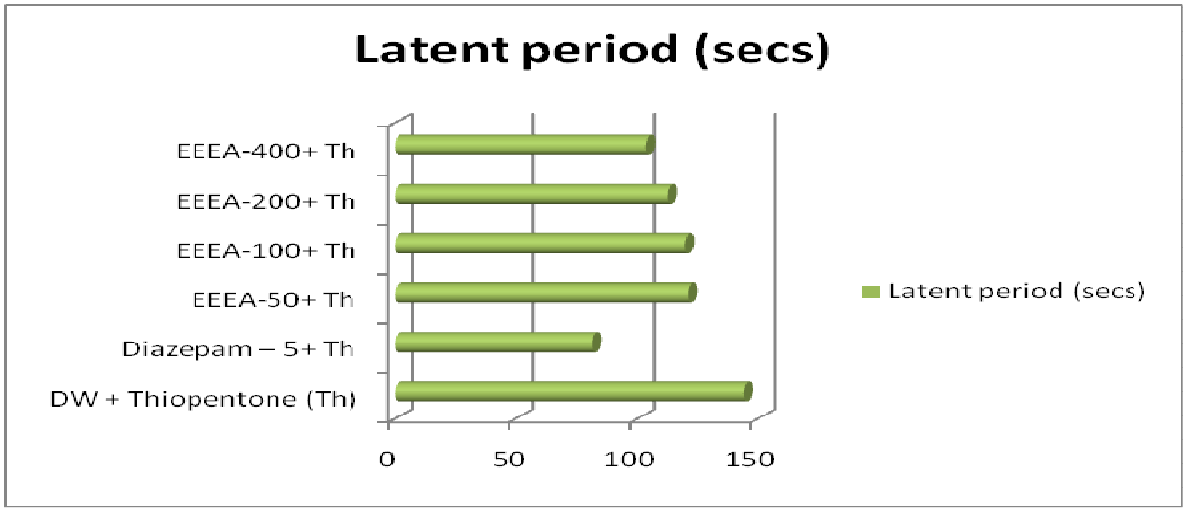
Groups with treatment	Latent period (secs)	Duration of sleep in mins
DW + Thiopentone (Th)	144.16±2.16	36.67±4.79
Diazepam – 5+ Th	81.5±0.92***	481.67±24.09***
EEEA-50+ Th	121±1.97**	74.67±6.39*
EEEA-100+ Th	119.83±3.31***	139.67±7.77***
EEEA-200+ Th	112.83±2.07***	243.17±6.77***
EEEA-400+ Th	103.33±5.02***	283.17±9.60***

n=6,* p<0.05, **p<0.01, ***p<0.001

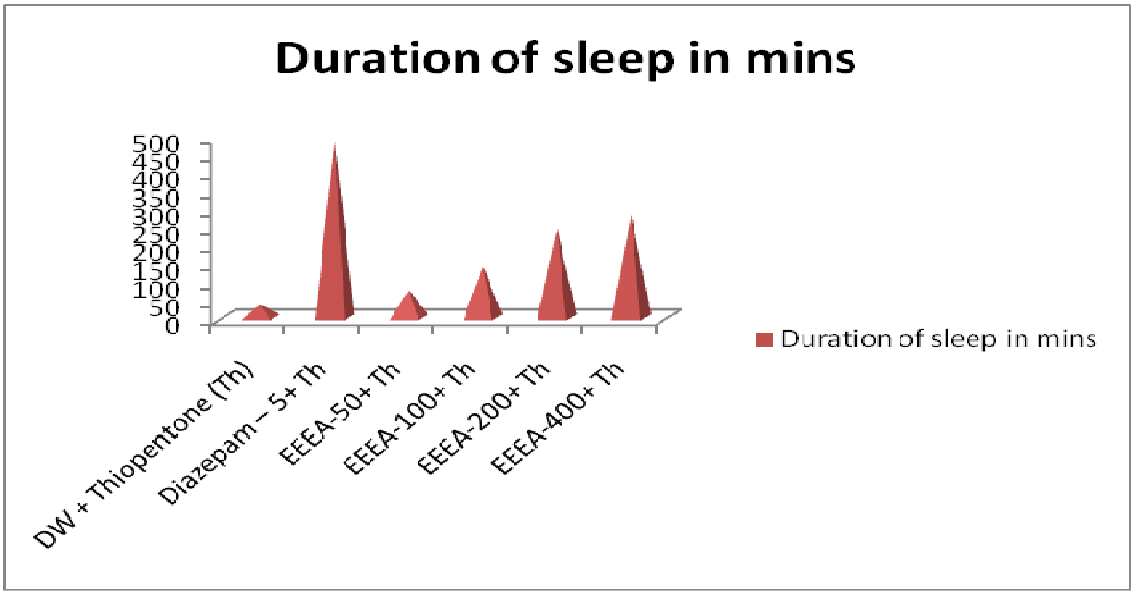
After administration of thiopental sodium, the beginning of sleeping time was taken to be when the animal assumed to be in a supine position. When the animals turned into a quadruped prone position(righting reflex) this was used as the end point of sleeping time. Sleeping time was measured with a stop watch. The effect of EEEA on sleeping time(Duration of sleep→ time between the loss & recovery of righting reflex) & latent period (time between thiopental sodium administration to loss of righting reflex)[14] were compared to

that of the control & diazepam group & analyzed statistically using one way ANOVA followed by Dunnet's multiple comparison test. In this test, EEEA at a dose of 100,200& 400mg/kg induced the sleep at an earlier stage. There was significant potentiation of thiopental induced sleeping time. In addition, EEEA prolonged the duration of sleeping time in comparison to control.

Graph 1
Effect of EEEA on latent period of sleep in Thiopental sodium induced sleeping time in albino rats



Graph 2
Effect of EEEA on duration of sleep in Thiopental Sodium induced sleeping time in albino rats



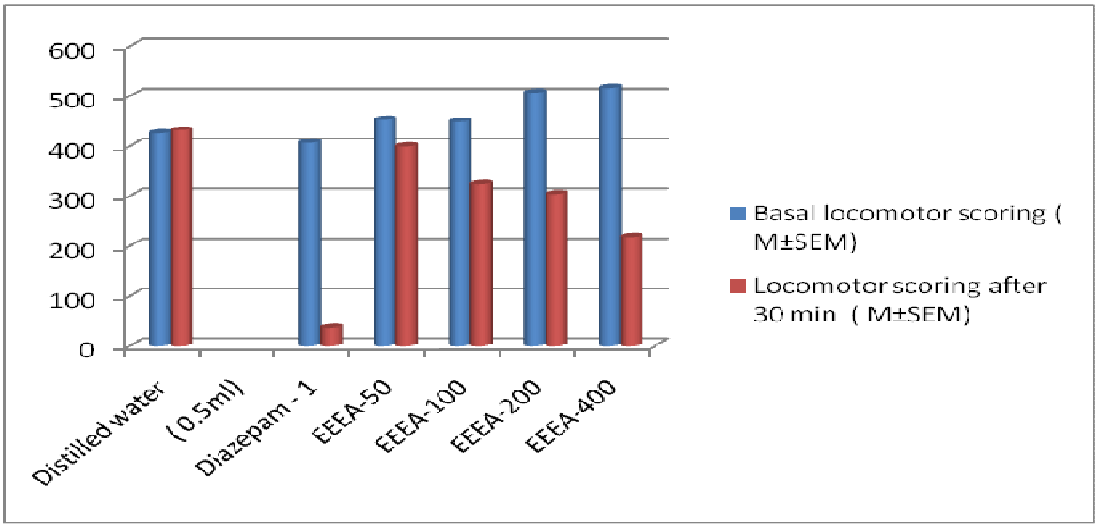
(i) Spontaneous motor activity

Table 5
Effect of EEEA on spontaneous motor activity in albino rats

Drugs in mg/kg	Basal locomotor scoring (M±SEM)	Locomotor scoring after 30 min (M±SEM)	% decrease in locomotor activity
Distilled water(0.5ml)	426±3.92	432±10.72	-
Diazepam - 1	407±4.93	37±8.18***	90.90
EEEA-50	453±9.84	400±10.88	5.50
EEEA-100	450±9.98	325±14.79**	28.25
EEEA-200	507±5.50	304±12.89***	40.04
EEEA-400	516±7.57	218±16.89***	57.43

n=6, **p<0.01,p<0.001***

Graph 3
Effect of EEEA on spontaneous motor activity in albino rats



The EEEA at dose 100,200 &400 mg/kg produced significant % reduction in locomotor activity as compared with control animals receiving only vehicle. However, diazepam treated group revealed 90.90 % decrease in locomotor activity compared with the control group.

(ii) Elevated plus maze (EPM) test

Table 6
Effect of EEEA on EPM test in albino rats

Groups with treatment	% of open arm entry	% of time (sec) spent in open arm
Distilled water(0.1ml)	22.31	25.56
Diazepam - 2	59.52	60.39
EEEA-50	32.10	38.40
EEEA-100	30.94	34.77
EEEA-200	52.88	43.50
EEEA-400	53.07	46.83

% of open arm entries= 100XOpen / Total entries (both open & closed arm)
% of time spent in open arms = 100X open / Open +enclosed

In elevated plus maze diazepam treated groups significant increase in open arm entries. The EEEA at the dose 200 & 400mg/kg significantly increase the % of entries into open arms % of time spent in the open arms.

DISCUSSION

It is well known that the drugs which increase the GABA, adenosine or other inhibitory neuromediator produced the sedative effect. The higher doses of EEEA (100,200,400) potentiated sleep induced by thiopental suggesting that the leaves of the plant possess a sleep inducing property. "Thiopental" basically a hypnotic agent, given at appropriate dose, induced hypnosis by potentiating GABA mediated postsynaptic inhibition through allosteric modification of $GABA_A$ receptors. Substances which possess CNS depressant activity either decrease the time for onset of sleep or prolong the duration of sleep or both. [15,16] Locomotor activity is considered as an index of alertness & a decrease in it is indicative of sedative activity.[17] Since locomotor activity is a measure of the level of excitability of the CNS [18], this decrease in spontaneous motor activity could be attributed to the sedative effect of the plant extracts.[19] However, the anxiolytic effect was evidenced by the EPM test that has been recognized as a valuable model able to predict anxiolytic effects of drugs in rodents [20]. The anxiolytic effect is observed when the experimental drug increases open arms entries without altering the total number of arm entries.[21] . These results could indicate an anxiolytic effect of the extract of *Eclipta alba* in albino rats. The compound identified from the leaves of *Eclipta alba*[22] contain ursolic acid, oleanolic acid act as $GABA_A$ agonists & this property could be attributed to CNS depressant effect. However, further studies are necessary to evaluate the contribution of other substances that are isolated for the activity observed, because it still remains to be determined which components exactly were responsible for these effects.

CONCLUSION

The results from the experiments confirmed that the ethanolic extract from *Eclipta alba* leaves possesses a strong sedative and anxiolytic potential. Therefore, we advance the suggestion that this extract may fulfill the therapeutic need for the treatment of anxiety and related neuropsychiatric disorders. However, further studies are required to evaluate the contribution of any other substances for the sedative & antianxiety activity seen in our study as it is not clear which components were exactly responsible for these effects.

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