

EVALUATION OF ANTIDEPRESSANT ACTIVITY OF ECLIPTA ALBA USING ANIMAL MODELSMISHRA SWATI ^{*1}, JENA MONALISA¹, PAL ABHISEK ²¹Department of pharmacology, IMS &SUM Hospital, SOA University, BBSR -India. ²School of Pharmaceutical sciences, SOA University, BBSR -India. Email: mishraswati017@gmail.com

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ABSTRACT

Objective : *Eclipta alba* (Asteraceae) is a traditional medicinal plant known as *Bhringaraj*. This plant has been used for the treatment of a variety of diseases. The leaves of *Eclipta alba* showed antihyperglycemic activity. The roots of *Eclipta alba* were found effective in wound healing.

Methods : This study was undertaken to evaluate the possible antidepressant effect of *Eclipta alba* leaf extract (EALE) using Tail suspension test (TST) & Forced swim test (FST). 36 albino rats of either sex weighing between 200-250gm were randomly selected divided into 6 equal groups. Group-I (control) received polyethyleneglycol (1ml/100gm), Group-II, III & IV received EALE in doses of 100,200,400 mg/kg orally (P.O.) respectively. Group V & VI (positive control) received Fluoxetine & Imipramine at doses of 20mg/kg & 15mg/kg p.o respectively. Drug treatment was given for seven & fourteen successive days. 60 minutes after last dose of drug or standard the immobility period was recorded.

Results : EALE produced significant antidepressant like effect at dose of 200 & 400 mg/kg administered for 7 & 14 consecutive days as indicated by reduction in immobility times of mice in TST & FST (P<0.05). The efficacy of EALE at 200mg/kg was found to be comparable to that of Fluoxetine & Imipramine at doses of 20mg/kg & 15mg/kg. **Conclusion:** The results of the present study indicate that EALE possesses significant antidepressant activity compared to that of both Fluoxetine & Imipramine.

Keywords: Eclipta alba, Forced swim test, Tail suspension test, Antidepressants, Immobility time

INTRODUCTION

Eclipta alba (L.) (*E. alba*) is commonly known as false daisy or bhringaraj. It belongs to the Family Asteraceae and is widely distributed throughout India, China, Thailand and Brazil[1]. Depression is a major clinical illness affecting 9.5% of population. Changes in the monoamine neurotransmitters have been observed in patients of depression [2]. The use of plant products for the treatment of human ailments has been a natural approach to health care since the beginning of civilization. In the search for new therapeutic products for the treatment of neurological disorders, medicinal plant research, worldwide, has progressed constantly, demonstrating the pharmacological effectiveness of different plant species in a variety of animal models [3]. It is reported to have anthelmintic, antipyretic, anti-inflammatory, antihistaminic, hepatoprotective & expectorant properties [4,5]. It has a great traditional importance because of being used as a medicinal resource in India for centuries[6]. It is used as a promoter of hair growth and blackening of hair[7]. The leaf extract of *E. alba* is a powerful liver tonic, rejuvenative and hepatoprotective[8]. The extract is also used as anti-venom against snake bites in China and Brazil[9]. Thus the present study has been undertaken to evaluate the antidepressant activity of *Eclipta alba* leaf extract (EALE) in rats employing tail suspension test (TST) & forced swim test (FST). Standard antidepressant drugs such as Fluoxetine (SSRI), and Imipramine (TCA) have been employed to standardize the animal models of depression.

MATERIALS AND METHODS**PREPARATION OF ECLIPTA ALBA LEAF EXTRACT (EALE)**

Leaves were collected and shade dried. They were crushed into coarse powder and extracted with 90% ethanol using Soxhlet's apparatus for 24 hrs. The extract was concentrated under pressure and then dried in air. The concentrated ethanolic extract was suspended in poly ethylene glycol. Freshly prepared solution was used for each experiment.

PLAN OF STUDY**ANIMALS**

About 36 albino rats of either sex weighing between 200 -250 gms. procured from disease free animal house of O.U.A.T., BBSR, Orissa were used for the present study. Animals had free access to food and water and maintained under standard laboratory conditions with a natural light and dark cycle. The animals were acclimatized for at least five days before behavioural experiments. Experiments were carried out between 9.00 and 15.00 hrs. Experimental protocol was approved by the institutional animals' ethics committee before the start of the study.

DRUGS & CHEMICALS

EALE, Fluoxetine Hydrochloride (Ranbaxy Lab.), Imipramine Hydrochloride (Sigma Aldrich).

VEHICLE

Polyethylene Glycol (PEG).

STUDY DESIGN

The animals were selected randomly for each experiment and divided into 6 equal groups. Drugs (PEG, EALE, Fluoxetine, Imipramine) administered orally (P.O.) for 7 & 14 successive days as depicted in (Table 1)

Table 1: Protocol of the study (Approved by IAEC)

GROUP	DRUG	DOSE (P.O.)
1	PEG	1 ml / 100 gm.
2	EALE	100 mg/kg
3	EALE	200 mg/kg
4	EALE	400 mg/kg
5	Fluoxetine	20 mg/kg
6	Imipramine	15 mg/kg

Sixty minutes after last dose, immobility period was recorded in two different animal models of depression like:

Forced Swim Test (FST) (Porsolt et al)[10]
 Tail suspension test (TST) (Steru et al)[11]

Laboratory Models For Testing Antidepressant Activity

Forced Swim Test (FST): FST or behaviour despair was proposed as a model to test for antidepressant activity by Porsolt et al [10]. Depression was produced by forcing the animal to swim individually in a glass jar containing fresh water of 15cm height and maintained at 25°C. This constituted pretest session. Twenty-four hour later each animal was again forced to swim. After an initial 2 min period of vigorous activity, each animal assumed a typical immobile posture. The total duration of immobility was recorded in next 4 min of a total 6 min test. The change in the immobility period was calculated after administering drugs to the groups as mentioned in the above table.

Tail Suspension Test (TST) : The total duration of immobility induced by tail suspension was measured according to the method described by Steru et al [11]. Depression was produced by suspending the animal from the edge of a table 50 cm above the floor by an adhesive tape placed approx. 1cm. from the tip of the tail. Immobility time was recorded during a 6 min. period. Changes in the immobility duration were studied after administering drugs in separate groups of animals.

The antidepressant activity was expressed as reduction in the immobility duration between the control, standard and animals treated with test drug.

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 behavioural change and death up to 72h.

STATISTICAL ANALYSIS :

All the results are expressed as Mean \pm SEM. All the groups were analysed using student's 't' test.

RESULTS

The observation of acute toxicity study indicated that there was no death in 2000mg/kg dose after 72hr. EALE at the dose of 100 mg/kg had no beneficial effect on immobility period of rats in both the models of depression i.e. FST & TST. The decrease in immobility period in both the models was observed starting from 200 mg/kg. But the increase in dose from 200 to 400 mg/kg did not produce any further reduction in immobility period, suggesting the ceiling effect at 200 mg/kg. At the dose 200 mg/kg, EALE showed antidepressant effect which is comparable to that of Imipramine and Fluoxetine at the dose of 15 & 20 mg/kg respectively (Table 2)

Table 2: Effect of EALE on Immobility Period (Secs) of rats using Forced Swim Test

GROUP	DRUG	DOSE	IMMOBILITY PERIOD (Secs)		
			PRE TREATMENT	POST TREATMENT (7 DAYS)	POST TREATMENT (14 DAYS)
1	PEG	1ml/100gm	193.13 \pm 3.01	192.6 \pm 2.36	192.54 \pm 2.36
2	EALE	100mg/kg	196.57 \pm 2.04	195.58 \pm 1.75*	193.91 \pm 2.01*
3	EALE	200mg/kg	195.05 \pm 2.02	147.67 \pm 1.63 ^{b**a} δ	142.33 \pm 1.55 ^{b**a} δ
4	EALE	400mg/kg	195.2 \pm 1.82	162.24 \pm 1.31 ^{b*a} δ	161.07 \pm 1.82 ^{b*a} δ
5	FLUOXETINE	20mg/kg	194.48 \pm 1.79	110.64 \pm 0.66 ^b	109.75 \pm 0.73 ^b
6	IMIPRAMINE	15mg/kg	192.33 \pm 1.17	137.91 \pm 1.17 ^b	134.6 \pm 1.07 ^b

Values as Mean \pm SEM, n=6, 1. a = p < 0.05, b = p < 0.001 as compared to pre treatment value, 2. * = p < 0.001, ** = p < 0.05 when compared to standard (Both Fluoxetine & Imipramine), 3. α = p < 0.001 when compared to control, 4. δ = p < 0.001 when EALE (100) is compared to EALE (200) and EALE (400)

At the dose 200 mg/kg, EALE showed antidepressant effect which is comparable to that of imipramine and Fluoxetine at the dose of 15 & 20 mg/kg respectively. The comparable anti depressant effect of

EALE with that of TCA (imipramine) and SSRI (fluoxetine) suggest possible involvement of either nor-adrenergic or serotonergic system.

Table 3: Effect of EALE on immobility period (secs) of rats using tail suspension test

GROUP	DRUG	DOSE	PRE TREATMENT	POST TREATMENT AFTER		
				4 DAYS	7 DAYS	14DAYS
1.	PEG	1ml/100gm	192.59 \pm 2.21	192.56 \pm 1.92	191.02 \pm 2.93	190.06 \pm 2.82
2	EALE	100 mg/kg	191.10 \pm 2.33	190.42 \pm 2.23	191.04 \pm 2.42	190.01 \pm 2.28
3.	EALE	200mg/kg	189.01 \pm 1.37	190.06 \pm 1.26	153.27 \pm 1.96 ^b α	152.18 \pm 1.88 ^b α
4.	EALE	400mg/kg	190.48 \pm 2.23	190.09 \pm 2.12	166.09 \pm .71 ^b α	164.26 \pm 2.42 ^b α
5.	FLUOXETINE	20 mg/kg	189.43 \pm 1.79	187.56 \pm 1.58	103.52 \pm 0.95 ^{*c}	104.68 \pm 1.01 ^{*c}
6.	IMIPRAMINE	15mg/kg	190.5 \pm 2.24	188.37 \pm 2.02	126.72 \pm 2.23 ^{*c}	124.57 \pm 2.13 ^{*c}

Values as Mean \pm SEM, Student's t test n = 6, 1.* P<0.001 when compared to pre treatment, 2.a=P<0.05, b=P<0.01, c=P< 0.001 when compared to control, 3. α =P<0.05, β =p<0.001 When compared to standard

DISCUSSIONS

In the present study, EALE (200 mg/kg) produced significant antidepressant effect in in FST & TST. These models of depression are widely used to screen new antidepressant drugs. The tests are quite sensitive and relatively specific to all major classes of antidepressant drugs including TCAs, SSRIs, MAOI, Atypical antidepressants. The forced swimming test is the most widely used tool for assessing antidepressant activity pre-clinically. The widespread use of this simple model is mainly due to its ability to detect a broad spectrum of antidepressant agents [12]. It has been

argued that TST (Tail Suspension Test) is less stressful than FST (Forced swim test) and has greater pharmacological sensitivity. The results obtained from TST are in concordance with the validated FST by Porsolt et al. Environmental factors and hereditary factors play a major role in producing deficient monoaminergic transmission in central nervous system thereby producing symptoms of depression [13]. Ecliptin alkaloid & Cumulbin, a flavonoid present in EALE may be facilitating monoaminergic transmission there by producing antidepressant effects.

CONCLUSION

Hence *Eclipta alba* leaf extract possesses antidepressant effect in animal models of depression which was comparable to that of Imipramine and Fluoxetine as demonstrated in this study. The phytochemical analysis, separation of active ingredients and further investigation in this line is essential to establish its therapeutic benefits.[14]

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