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Research Article

EXPERIMENTAL EVALUATION OF ANTIDEPRESSANT ACTIVITY OF AQUEOUS AND CHLOROFORM LEAF AND SHOOT EXTRACTS OF *EICCHORNIA CRASSIPES LINN* IN MICE

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ABSTRACT

Objective: To investigate antidepressant activity of aqueous and chloroform extract of Eicchornia crassipes plant leaves and shoots in mice.

Methods: The antidepressant activity of aqueous and chloroform extract of Eicchornia crassipes plant leaves and shoots were tested by forced swim test (FST) and tail suspension test (TST) in albino mice and the results were compared for the both extracts. Imipramine was used as the standard drug for comparison.

Results: Phytochemical screening showed presence of carbohydrates, alkaloids, flavanoids, steroids, saponins, amino acids, gums and mucilage. aqueous extract of *Eicchornea crassipes* (AEEC) and chloroform extract of *E. crassipes* (CEEC) did not produce any lethal effect even upto 2000 mg/kg, p.o during acute oral toxicity study. In FST and TST, CEEC showed diminution of duration of immobility time in 200 mg/kg but not in 100 mg/kg.

Conclusion: From the above finding concluding that, shortening of immobility time in the FST and TST indicating, CEEC showed more antidepressant activity acting either by the enhancement of central 5-HT or catecholamine neurotransmission compared to AEEC in mice.

Keywords: *Eicchornia crassipes,* Aqueous extract of *Eicchornia crassipes*, Chloroform extract of *Eicchornia crassipes*, Forced swim test, Tail suspension test.

INTRODUCTION

Depression is one of the major mental disorders characterized with symptoms such as regular negative moods, decreased physical activity, feelings of helplessness, sluggish thought, and cognitive function (Galdino *et al*., 2009). According to the World Health report, approximately 450 million people suffer from a mental or behavioral disorder. This amounts to 12.3% of the global burden of disease, and will rise to 15% by 2020 [1].

Depression is caused by chemical imbalances in the brain which may be hereditary, stressful life changes, stroke, Parkinson's disease, or multiple sclerosis, stroke, social isolation, medical conditions such as hypothyroidism (underactive thyroid), medications (such as sedatives and high blood pressure medications), cancer, major illness, or prolonged pain, and sleeping problems [2].

Despite the development of new molecules for pharmacotherapy of depression, it is unfortunate that this disorder goes undiagnosed and untreated in many patients. Although the currently prescribed molecules provide some improvement in the clinical condition of patients, it is at a cost of having to bear the burden of their adverse effects.

Ayurveda, the Indian traditional system of medicine, mentions a number of single and compound drug formulations of plant origin that are used in the treatment of psychiatric disorders. On one hand, these agents have less adverse effects, and they have been shown to be comparable in efficacy to their synthetic counterparts [3,4].

Synthetic antidepressants are often associated with their anticipated side effects such as dry mouth, inability in driving skills, constipation, and sexual dysfunction and majority of patients are reluctant to take this treatment (Singh *et al*.,). Nature plants, such as *Hypericum perforatum, Cissampelos sympodialis, Terminalia bellirica* Roxb*, Bacopa monniera, Ginkgo biloba, and Pueraria lobata* may be an important source of new antidepressant drugs and the safety of nature plant extracts maybe better than that of synthetic antidepressants [5,6].

Eicchornia crassipes commonly known as water hyacinth is a free-floating perennial aquatic plant belongs to the family of Pontederiaceae. The primary chemical constituents are carbohydrates, alkaloids, flavonoids, tannins, saponin, terpenoid, alkaloids, proteins, and phenols they also contain iron, manganese, and zinc (Ryan *et al*., 2007). In the traditional medicine of *E. crassipes* used as nervine tonic, stimulant, antispasmodic, antioxidant, antidepressant (used in menopausal phase) [7].

Several studies on anti-inflammatory activity (Cheung *et al*., 2009) antioxidant activity, (Sur *et al*., 2009) of *E. crassipes* have been reported.

The antidepressant activity of *E. crassipes* is mentioned in the Indian system of traditional medicine, but there is no scientific evidence to prove its activity. Hence, the present study is designed to evaluate the antidepressant activity of *E. crassipes* using different animal models in mice.

METHODS

Plant material collection and authentication

The leaves and shoots of *E. crassipes* were collected from a pond near Parvathipuram Town, Vizianagaram District, Andhra Pradesh. It was authenticated by Dr. M. Vasubabu, Sr. Lecturer, Department of Botany, Government Degree College, Vizianagaram, the botanical nomenclature of the plants was duly identified by using standard floras and also cross-checked with Herbarium records. The plant material was shade dried for 10 days and pulverized.

Preparation of extract

The collected leaves and shoots of *E. crassipes* were shade dried at room temperature and grinded coarsely. The leaves and shoots were extracted by percolator using water as solvent and by Soxhelet apparatus using chloroform. The resulting extract was concentrated in vacuum under reduced pressure and dried in desiccators. Thus, the prepared extract was used for further pharmacological evaluation [2,8].

Materials
Imipramine was procured from Sigma Life Sciences, Bengaluru.

Preliminary phytochemical analysis
The both aqueous and chloroform extracts of leaves and shoots of *E. crassipes* were subjected for phytochemical screening and found that carbohydrates, alkaloids, flavonoids, tannins, saponin, terpenoid, alkaloids, proteins, and phenols were present.

Animals
Albino mice of either sex weighing between 20 and 30 g were used in this study. All the animals were acclimatized in the quarantine room a National Institute of Nutrition (NIN) animal house (NIN, Hubsiguda, Hyderabad), for 7 days and housed in groups of five under standard husbandry conditions such as room temperature 23°C±2°C, relative humidity 30-70%, and light/dark cycle of 12 hrs.

All the animals were fed with synthetic standard diet (NIN, Hubsiguda, and Hyderabad) and water under still supplied *ad libitum* under strict hygienic conditions. All the experimental protocols were approved by Institutional Animal Ethical Committee of Andhra University. All the animals' studies were performed as per the rules and regulations in accordance to the guidelines of CPCSEA with a registration number.

All animals were fasted 3 hrs prior to oral administration of vehicle/standard/test compounds during the experiment were carried out during the light period (9:00-17:30 hrs) to avoid circadian rhythm.

Acute oral toxicity study
OECD guidelines (425) state that, before establishing pharmacological activity of the new chemical entity is mandatory to establish maximum tolerated dose in mice (OECD, 2001). The purpose of the sighting study is to allow selection of appropriate starting dose for the main study. The starting dose for a sighting study was selected from the fixed dose levels of 5, 50, 300, and 2000 mg/kg as a dose expected to produce evident toxicity [9].

***In vivo* models for antidepressant activity**

Forced swim test (FST)
Animals were divided into four groups of five animals in each, weighing between 20 and 30 g.

The extracts of both aqueous extract of *E. crassipes* (AEEC) and chloroform EEC (CEEC).

Group I: Control (distilled water 10 ml/kg, p.o)
Group II: Standard (imipramine 10 mg/kg, p.o)
Group III: Low dose (EEC 100 mg/kg, p.o)
Group IV: High dose (EEC 200 mg/kg, p.o)

For the FST, mice of the either sex were individually forced to swim in an open cylindrical container (diameter 10 cm, height 25 cm) containing 19 cm of water at 25°C±1°C. Treatment was given 60 minutes prior to study as described by study design all animals were forced to swim for 6 minutes and the duration of immobility was observed and measured during the final 4 minutes interval of the test. Each mice was judged to the immobile when it ceased struggling and remained floating motionless in the water, making only those movements to keep its head above water. A decrease in the duration of immobility is indicative of an antidepressant-like effect (Fig. 1) [9].

Tail suspension test (TST)
Animals were divided into four groups of five animals in each, weighing between 18 and 25 g.
The extracts of both AEEC and CEEC,
Group I: Control (distilled water 10 ml/kg, p.o).
Group II: Standard (imipramine 10 mg/kg, p.o).
Group III: Low dose (EEC 100 mg/kg, p.o).
Group IV: High dose (EEC 200 mg/kg, p.o).

The tail suspension method used in this study was similar to those described by Steru *et al*., (1985). Treatment was given 6 minutes prior to study as described by study design. Mice were suspended on the edge of the table, 50 cm above the floor, with the help of adhesive tape placed approximately 1 cm from the tip of the tail. The total duration of immobility induced by tail suspension was recorded during 6 minutes of the 10 minutes period. Animal was considered to be immobile when it did not show any movement of the body, hanged passively and completely motionless (Fig. 2) [10].

Statistical analysis
Results will be presented as mean±standard error of mean. The data will be subjected for statistical analysis by one-way analysis of variance followed by Dunnet's *t*-test and $p<0.05^*$, 0.01^{**}, and 0.001^{***} were considered as significant.

RESULTS

Preliminary phytochemical screening
The extract of leaves and shoots of *E. crassipes* was subjected for phytochemical screening and found that carbohydrates, alkaloids, flavonoids, tannins, saponin, terpenoid, alkaloids, proteins, and phenols were present. The results were shown in Table 1.

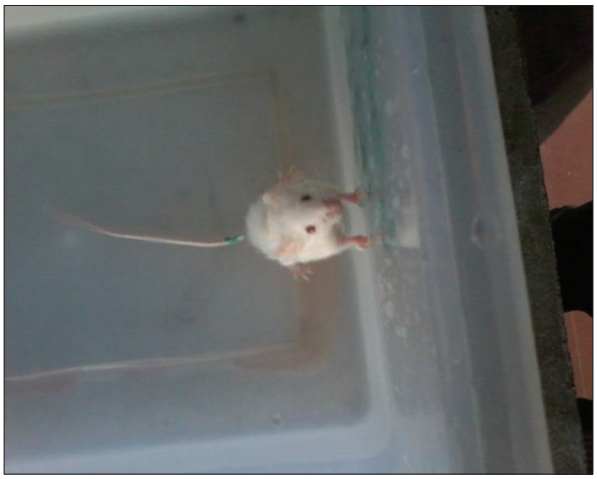

Figure 1: Representation of mice in FST

Table 1: Preliminary phytochemical screening

S. No	Phytochemical constituents	Inference	
		AEEC	CEEC
1	Test for carbohydrates		
	Molisch's test	+	−
	Fehling's test	+	−
	Barfoed's test	+	−
	Benedict's test	+	−
2	Test for alkaloids		
	Dragendorff's test	−	+
	Wagner's test	−	+
	Mayer's test	−	+
	Hager's test	−	+
3	Test for tannins	+	−
4	Test for flavonoids		
	Schinoda test	+	+
5	Test for terpenoid	+	−
6	Test for proteins		
	Biuret test	−	+
7	Test for phenols	−	+
8	Test for saponins	+	+

+: Indicates presence, −: Indicates absence, AEEC: Aqueous extract of *Eicchornia crassipes*, CEEC: Chloroform extract of *Eicchornia crassipes*

Acute oral toxicity study

The aqueous and chloroform leaf and shoot EEC were found to be safe up to the dose level of 2000 mg/kg, p.o. and did not produce any toxic symptoms. The survived animals were sacrificed, and complete absorption of the drug through gastrointestinal tract was observed. Hence, 1/20th and 1/10th of maximum therapeutic dose (2000 mg/kg) were selected for the pharmacological models.

FST

The result of the effect of aqueous and CEEC on the duration and % inhibition of immobility is shown in Table 2 the animals were treated with distilled water 10 ml/kg, p.o. as control, 100 mg/kg, p.o. of AEEC and CEEC, and 200 mg/kg, p.o. of AEEC and CEEC, imipramine 10 mg/kg, p.o. as standard (Table 3 and Figs. 3 and 4).

TST

The result of the effect of aqueous and CEEC on the duration and % inhibition of immobility is shown in Table 4. The animals were treated with distilled water 10 ml/kg p.o. as control, 100 mg/kg, p.o. of AEEC and CEEC, and 200 mg/kg, p.o. of AEEC and CEEC, imipramine 10 mg/kg, p.o. as standard (Table 5 and Figs. 5 and 6).

DISCUSSION

Depression is a heterogenous mood disorder characterized with regular negative moods, decreased physical activity, feelings of helplessness, and is caused by decreased brain levels of monoamines such as noradranline, dopamine, and serotonin. Therefore, drugs restoring the reduced levels of these monoamines in the brain either by inhibiting monoamine oxidase or by inhibiting reuptake of these neurotransmitters might be fruitful in the treatment of depression that has been classified and treated in a verity of ways. Although a number of synthetic drugs are being used as standard treatment for clinically depressed patients, they have adverse effects that can compromise the therapeutic treatment. Thus, it is worthwhile to look for antidepressants from plants with proven advantage and favorable benefits-to-risk ratio [11].

On the basis of the above information, both aqueous and non-aqueous (chloroform) leaf and shoot EEC was selected for evaluating its antidepressant activity due to its traditional use in the treatment of

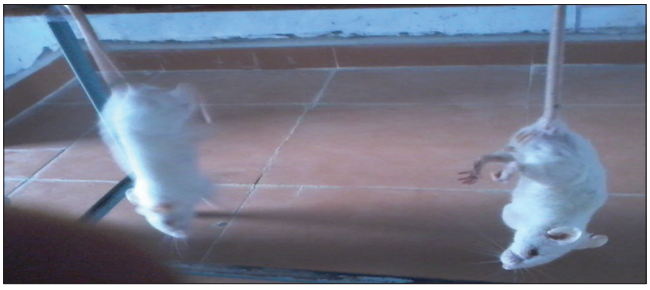

Figure 2: Representation of mice in TST

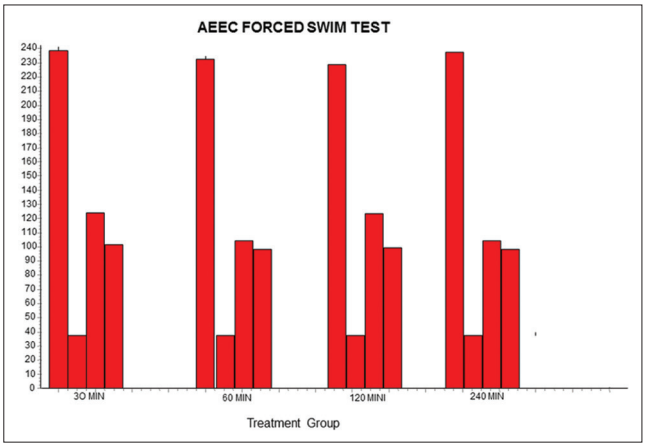

Fig. 3: Effect of aqueous extract of *Eicchornia crassipes* on immobility time in forced swim test in mice

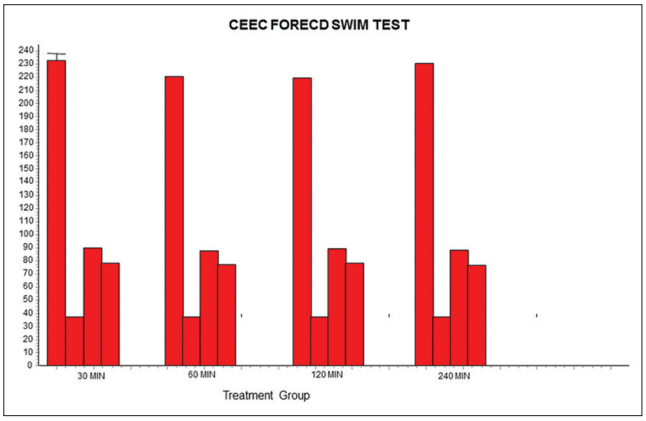

Fig. 4: Effect of chloroform extract of *Eicchornia crassipes* on immobility time in forced swim test in mice

Table 2: Percentage inhibition of immobility time in FST - aqueous extract

S. No	Treatment	Percentage of immobility			
		30 minutes	60 minutes	120 minutes	240 minutes
1.	Control (distilled water 10 ml/kg)	50.5	52.4	51.3	50.2
2.	Standard (imipramine 10 mg/kg)	25.6	28.5	26.3	27.5
3.	Low dose (100 mg/kg)	13.5	14.8	16.3	17.2
4.	High dose (200 mg/kg)	9.3	8.2	7.6	5.9

n=5 in each group. Significance at *p<0.005, **p<0.001 and NS: Not significant versus control group, FST: Forced swim test

Table 3: Percentage inhibition of immobility time FST chloroform extract

S. No	Treatment	Percentage of immobility			
		30 minutes	60 minutes	120 minutes	240 minutes
1	Control (distilled water 10 ml/kg)	50.5	51.4	52.3	50.1
2	Standard (imipramine 10 mg/kg)	26.6	27.5	28.3	28.5
3	Low dose (100 mg/kg)	14.5	15.8	15.3	16.2
4	High dose (200 mg/kg)	8.3	8.2	6.6	5.9

n=5 in each group. Significance at *p<0.005, **p<0.001 and NS: Not significant versus control group, FST: Forced swim test

Table 4: Percentage inhibition of immobility time in TST of aqueous extract

S. No	Treatment	Percentage of inhibition
1	Control (distilled water 10 ml/kg)	50.57
2	Standard (imipramine 10 mg/kg)	35.65
3	Low dose (100 mg/kg)	28.32
4	High dose (200 mg/kg)	15.85

n=5 in each group. Significance at *p<0.005, **p<0.001 and NS: Not significant versus control group, TST: Tail suspension test

Table 5: Percentage inhibition of immobility time in TST of chloroform extract

S. No	Treatment	% of inhibition
1	Control (distilled water 10 ml/kg)	51.57
2	Standard (imipramine 10 mg/kg)	33.65
3	Low dose (100 mg/kg)	27.32
4	High dose (200 mg/kg)	14.85

n=5 in each group. Significance at *p<0.005, **p<0.001 and NS: Not significant versus control group

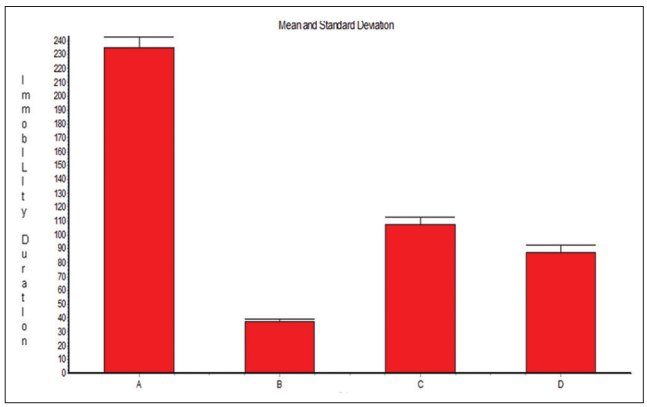

Fig. 5: Effect of aqueous extract of *Eicchornia crassipes* (AEEC) on immobility time in tail suspension test in mice, (a) Control, (b) imipramine 10 mg/kg, (c) AEEC 100 mg/kg, (d) AEEC 200 mg/kg

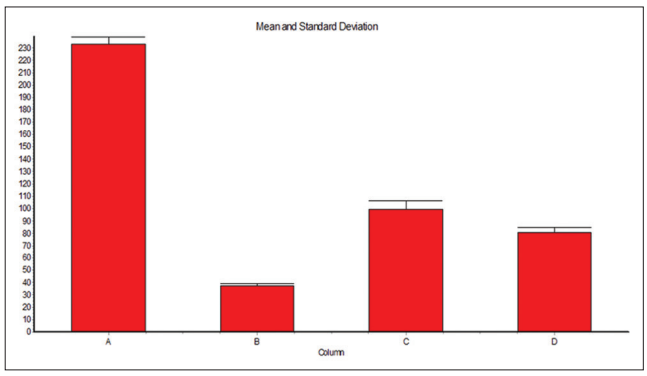

Fig. 6: Effect of chloroform extract of *Eicchornia crassipes* (CEEC) on immobility time in tail suspension test in mice, (a) Control (b) imipramine 10 mg/kg (c) CEEC 100 mg/kg (d) CEEC 200 mg/kg

depression.

In acute oral toxicity study, both AEEC and CEEC did not show any lethal effect even up to the doses of 2000 mg/kg, p.o. and test doses of 100 and 200 mg/kg, p.o. were used for the pharmacological activity.

On the basis of the clinical association of depressive episodes and stressful life events, many of the animal models for the evaluation of antidepressant drug activity assess stress-precipitated behaviors. The two most widely used animal models for antidepressant screening are the forced swimming and TSTs. These tests are quite sensitive and relatively specific to all major classes of antidepressants. In TST, immobility reflects a state of despair which can be reduced by several agents which are therapeutically effective in human depression. Similarly in the FST, mice are forced to swim in restricted space from which they cannot escape. This induces a state of behavioral despair in animals, which is claimed to reproduce a condition similar to human depression. It has been seen that the TST is less stressful and has higher pharmacological sensitivity than FST [12,13].

Results showed that the administration of the CEEC produced a diminution of the duration of immobility time of mice exposed to the both FST and TST than AEEC. In the present study, the CEEC (200 mg/kg, p.o.) administered to mice produced significant antidepressant effect in both FST and TST than CEEC (100 mg/kg, p.o.) and AEEC and their efficacies were found to be comparable to imipramine (10 mg/kg, p o).

From all the above, the antidepressant activity of chloroform extract of leaf and shoot of *E. crassipes* was found to be significant at 200 mg/kg, p o. The flavonoids components of CEEC might be interacting with 5-HT in mediating the antidepressant effect of *E. crassipes.*

CONCLUSION

The AEEC and CEEC contain carbohydrates, alkaloids, flavonoids, steroids, glycosides, saponins, amino acids, gums, and mucilage. From the above findings, the antidepressant activity of CEEC was significant at 200 mg/kg, p.o. in FST and TST. Shortening of immobility time in the forced swimming and TSTs was indicating CEEC acting either by enhancement of central 5-HT and catecholamine neurotransmission. However, more extensive pharmacological studies of this plant are required for complete understanding of the antidepressant activity of chloroform extract of leaf and shoot EEC.

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