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Research Article

EFFECT OF ETHANOLIC EXTRACT OF *MIMOSA PUDICA* ROOT L. ON ACUTE RESTRAINT STRESS-INDUCED ANXIETY AND DEPRESSION IN SWISS ALBINO MICE

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ABSTRACT

Objective: The present study is undertaken to study the ameliorative effects of ethanolic extract of *Mimosa pudica* root (EMPR) on stress-induced anxiety and depressive-like behavior in Swiss albino mice.

Methods: The animals were subjected to acute restraint stress (ARS) for 7 days, and on the 8th day after drugs administration, the antianxiety effect was evaluated using elevated plus maze (EPM) test. Antidepressant activity was evaluated by animal despair models-forced swim test and tail suspension test. Phytochemical analysis of the extract was also conducted.

Results: EMPR (1000 and 2000 mg/kg) and the combined test groups have shown significant antianxiety effects similar to the standard diazepam as indicated by an improved open arm exploratory behavior in EPM model. EMPR also significantly reduced the immobility time in the animal despair models tested.

Conclusion: Results suggest that EMPR possesses significant anxiolytic and antidepressant activity in male Swiss albino mice.

Keywords: Depression, Anxiety, Elevated plus maze, *Mimosa pudica* root.

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INTRODUCTION

Stress, which is a crucial element of existing health-care society and disease, also plays a significant role in the pathogenesis of neuropsychiatric disorder as these stressful events result in altered visceral, physiological, immunological, psychological, and neurobehavioral responses such as anxiety, depression, cognitive impairment, insomnia, anorexia, and activation of human hypothalamic-pituitary-adrenal axis in animals and human [1,2]. Pernicious consequence on cellular effects, as a result of stressful conditions, arises due to disturbance in the balance between the production of reactive oxygen species and antioxidant defense mechanisms, which has also been shown to play a role in the pathogenesis of neuropsychiatric disorders [3-5]. Furthermore, preclinical studies have demonstrated that inhibition of oxidative stress may contribute to the therapeutic effect of some antidepressants [6-9]. Central nervous system (CNS) is mainly susceptible for damage by free radicals as the brain has abundant lipid content and requires more oxygen along with the comparative paucity of antioxidant enzymes [10].

Restrain stress can induce a series of dysfunction in CNS, such as cognitive impairment, anxiety, depression, amnesia, and insomnia [11], and it can also alter the free radical scavenging enzymes in discrete regions of the brain [12]. Hence, close surveillance was paid to study the protective effects of natural products on stress-induced injury in animal models. In this regard, numerous indigenous medicinal plants, which were once used as nerve tonics due to their adaptogenic and rejuvenating properties in ancient traditional medicine, have been investigated. The drugs of the plant origin are gaining increasing popularity and are being investigated for numerous neuropsychiatric effects including antidepressant, anti-stress, and adaptogenic activities [11-13].

Epidemiological studies have indicated a positive association between the consumption of phenolic-rich food or beverages and the prevention of diseases. These effects have been attributed to antioxidant components such as various phenolic acids like flavonoids and tannins among others. Flavonoids, tannins, and phenolic components act as free radical scavengers and metal chelators, which are useful in preventing chronic disorders like cancer and neurodegeneration [14-16]. Roots of *Mimosa pudica* containing flavonoids, tannins, and steroid components may possess potential effects of anxiety and depression.

Abundant original medical plants which are safer and well tolerated are used since ages for treating neurological diseases including depression and epilepsy. *M. pudica* plant belonging to the family Fabaceae is known as "sensitive plant" in English and "lajvanti" or "chuimi" in local Hindi [17]. Previous studies on *M. pudica* have shown significant antiepileptic activity in its root part [18] and neuroprotective as well as anti-Parkinsonism activity of the whole plant extract [19]. However, so far no studies are reported about the beneficial effects of *M. pudica linn* roots in anxiety and depression induced by restraint stress. Considering substantial neuroprotective properties of *M. pudica* roots Linn., we sought to investigate the ethanolic extract of *M. pudica* root Linn. for its potential to improve cognitive impairment induced by ARS in adult Swiss albino mice.

METHODS

Plant materials and extraction

The fresh plant roots of *M. pudica* were collected during September–October (2015) from Bannari forest (Dhimbam district), Coimbatore, Tamil Nadu. It was taxonomically identified and was authenticated by Dr. Mruthunjaya, Department of Pharmacognosy, JSS Pharmacy College, Mysuru, and herbarium of the plant is preserved for future references (Specimen Voucher No. 10601/Pharma). The collected roots were washed and shade dried at room temperature for 7 days. Dried roots were coarsely powdered and fine powder was separated. The coarse powder of roots (500 g) was subjected to extraction with ethanol by Soxhlet apparatus, and the extract was concentrated to dryness by

vacuum. The extract was then weighed to calculate the percentage of yield in terms of air-dried crude material. The resultant extract of *Mimosa pudica* root (EMPR) was kept in refrigerator for further use. Before administration, the extract was freshly prepared with normal saline, and two doses (1000 mg/kg and 2000 mg/kg) were selected based on the results of previous studies.

Animals

Adult male Swiss albino mice weighing between 22 and 30 g were randomly selected from the breeding stock of Central Animal Facility of JSS Medical College, Mysuru. They were housed in polypropylene cages under standard condition (25±3)°C, humidity 45%–55%, and 12/12 h light/dark cycle. They were given free access to food and water *ad libitum*. The animals were acclimatized for 7 days before the study. The study was conducted according to the Indian National Science Academy guidelines for the use and care of experimental animals. The experimental protocols were approved by the Institutional Animal Ethical Committee of JSS Medical College, Mysuru (JSSMC/PG/13B10601), and procedures in this study were performed in accordance with guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (261/PO/ReBi/2000/CPCSEA). All efforts were made to minimize animal suffering and the number of animals used in the experiments.

Experimental design

Thirty-six mice (n=6) were randomly divided into six experimental groups for evaluating the anti-anxiety effects in elevated plus maze (EPM). Groups I (control) mice received distilled water (1.0 ml/kg, p.o.) daily for 7 days; Groups II (Stress control) mice received distilled water (1.0 ml/kg, p.o.) daily for 7 days and subjected to restraint stress on the 8th day. Groups III (Standard) mice received diazepam (1mg/kg, i.p.), Groups IV and V received EMPR (1000 mg/kg and 2000 mg/kg, p.o., respectively), while Group VI was treated with combined dose of EMPR (1000 mg/kg p.o) + standard (0.5 mg/kg, i.p.). All the drugs were administered daily for 7 days, and the mice were subjected to ARS on the 8th day.

Depressive-like behavior was assessed by subjecting the mice to behavioral paradigms such as forced swim test (FST) and tail suspension test (TST).

Another set of thirty mice (n=6) was randomly divided into five experimental groups for FST and TST tests, and these animals were devoid of ARS procedure, which may otherwise interfere with the study. Group I (control) mice received distilled water (1.0 ml/kg, p.o), Group II (standard) mice received imipramine (15 mg/kg, i.p.), Group III and IV mice were treated with EMPR (1000 mg/kg and 2000 mg/kg; p.o., respectively), and Group V mice were treated with EMPR 1000 mg/kg (p.o.) + imipramine 10 mg/kg (i.p.). The respective drugs were administered on 1st, 7th, and 14th days for animals of each group. 60 min after the last dose of the drugs, the immobility period was recorded by both the models.

Chemicals

Ethanol, normal saline, imipramine, and diazepam (Sun Pharmaceuticals) were used.

Procedure for acute restraint stress (ARS)

ARS protocols were adapted from a previously described procedure [20]. Immobilization stress was accomplished by placing individual rodent on restraint device made of wire mesh for 4 h. This restrained all physical movements for the animals, and also they were deprived of food and water during the entire period of exposure to stress. After 4 h, the animals were released from their enclosure to their respective cages. On the 8th day, about 40-min post-release, the animals were subjected to behavioral tests. In normal control group, the mice were kept in their animal cages in the experimental room.

Behavioral assessments

EPM test

This test has been proposed for selective identification of anxiolytic or anxiogenic drugs. The EPM consists of two open arms and two enclosed arms sized 50×40×40 cm^3 arranged to form a plus shape such that the two similar kinds of arms are opposite to each other. The maze is elevated to a height of 50 cm [21]. The EPM relies on rodents' proclivity toward dark/enclosed spaces and an unconditioned fear of heights/open spaces [21,22]. After treatment with each group, the rats were placed in the center of the maze facing one of the enclosed arms. During the 5 min test period, the open arm exploration (for both entries and time) was calculated from number of open arm entries divided by the total number of entries in both open and closed arms and the time spent on open arm exploration divide by total time spent in both open and close arms, respectively. The procedure was conducted in a sound-attenuated room.

FST

The test was first described by Porsolt *et al.* (1977). A vertical glass cylinder (25 cm height and 14 cm in diameter) was filled with water of temperature 27°C to a depth of 20 cm. Each mouse was gradually placed in the cylinder for 6 min and the duration of floating (i.e., the time during which mice made only the small movements necessary to keep head above water) was scored. Immobility time was analyzed during the past 4 min period of the test. Readings were taken on the 1st, 7th, and 14th days of treatment.

TST

The TST was conducted as previously described by Steru *et al*. (1985) with slight modification. Mice were suspended from the edge of a table 50 cm above the floor by an adhesive tape placed approximately 1 cm from the tip of the tail. The total duration of immobility was recorded during the past 4 min of the 6 min test period. Mice were considered to be immobile only when they hung passively and were completely motionless. Recording of the duration of immobility in animals was done by an observer blind to the treatment given to the animal under study. Readings were taken on 1st, 7th, and 14th days of treatment.

Statistical analysis

The results were computed using GRAPH PRISM PAD version 7 software, and one-way ANOVA test followed by *Post hoc* Dunnett's multiple comparison tests was applied for analysis. Observations were expressed as means±standard error of mean. The differences between means were considered to be statistically significant at $p<0.05$ (90% confidence interval).

RESULTS

Phytochemical screening test

The freshly prepared EMPR was subjected to phytochemical screening tests for the detection of various active constituents. The extract showed the presence of flavonoids, tannins, and steroids in the crude extract of EMPR as depicted in Table 1.

Acute oral toxicity study

The acute toxicity was carried out in adult Swiss albino mice by fixed dose method of the Organization for Economic Cooperation and Development guideline No-423 [23]. Ethanolic extract of roots of *M. pudica* was given orally up to the dose level of 4000 mg/kg [24].

Table 1: Phytochemical screening of ethanolic EMPR

Sl. no	Chemical constituents	Test	EMPR
1	Test for alkaloids	Mayer's test	-
		Wagner's test	-
		Hager's test	-
		Dragendroff's test	-
2	Test for carbohydrates	Molisch's test	-
3	Test for saponins	Foam test	-
4	Test for triterpenoids/steroids	Salkowski's test	+
		Liebermann–Burchard test	-
5	Test for flavonoids	Shinoda test	+
6	Test for tannins	Ferric chloride test	++
		Gelatin test	+

Phytochemical screening of the crude EMPR extract, EMPR - Ethanolic extract of *Mimosa pudica* roots, +: Present, −: Absent, ++: Reaction intensity is high, +: Reaction intensity is normal

Behavioral studies

EPM

Analysis of EPM data revealed that ARS (4 h/6 consecutive days) in control stress group induced a significant reduction in the percentage of open arm entries and time spent in open arms as compared to the normal control group ($p<0.05$), while post-treatment with ethanolic EMPR (1000 and 2000 mg/kg) for 6 days reversed the restraint stress-induced changes in both number of open arm entry and time spent in open arms as compared to the control stress group ($p<0.01$). Post-EMPR treatment with 1000 mg/kg showed a significant increase in the time spent in open arm as compared to the control group., while there was a significant increase in both number of open arm entries and time spent in open arms indicating improvement in exploratory attitude and anti-anxiety activity among the animals treated with EMPR 2000 mg/kg and in combined group when compared to the control group (Table 2).

Table 2 shows the effect of pretreatment on ARS-induced changes in the EPM test.

FST

The mice treated with EMPR were also evaluated for depressive-like behavior elicited by forced or despair swim test. The immobility period during FST test was measured (Table 3). This immobility is known to reflect a state of depression in mice. In the FST, our results showed that the immobility time decreased significantly on the 1st, 7th, and 14th days in the group treated with the combined drugs ($p<0.001$). In test groups treated with EMPR (1000 mg/kg and 2000 mg/kg), a significant decrease in immobility period was observed on 7th and 14th days as compared to the control group ($p<0.01$) (Table 3).

In Table 3, the FST results show significant anti-depressive effects by EMPR.

TST

Mice treated with the standard drug imipramine (15 mg/kg) exhibited a highly significant decrease in the immobility period ($p<0.0001$) as compared to control group, whereas in EMPR test group, a significant decrease in immobility time was observed at the dose of 2000 mg/kg on days 7 and 14. However, in the combination test group (EMPR 1000 mg/kg+imipramine 10 mg/kg), the significant antidepressive effect improved from day 1 onward to day 14 as evidenced by the decrease in immobility period ($p<0.01$, $p<0.001$, and $p<0.001$ on 1st, 7th, and 14th days, respectively) (Table 4).

Table 4 presents that the TST results show significant antidepressive effect in EMPR 2000mg/kg and combination test groups.

DISCUSSION

Stressful episode influences the non-specific response of the body to any demand imposed on it [25]. Stress is known to alter the physiological homeostasis of the organism and complex mechanisms contributing to the breakdown in adaptation processes, resulting in various visceral, endocrinal, and behavioral changes [1]. Stress also plays the major role in the pathogenesis of various mental disorders. Anxiety and depressive disorders in particular affect about 1/8th of the total population worldwide and have become one of the important research fields in psychopharmacology during this decade [26]. ARS type of stressful events has been reported to induce anxiety and

Table 2: Effect of EMPR on EPM

Groups (n=6)	Treatment	Percentage of protection	
		Number of Open arm entries	Open arm time (s)
I	Control (distilled water)	20.4±0.8ns	10±1.15*
II	ARS control (distilled water)	8.6±0.8	4.6±0.50
III	Diazepam (1.5mg/kg)	36.8±1.3****	23.6±1.2****
IV	EMPR (1000mg/kg)	21.8±0.8ns	13.8±0.8*
V	EMPR (2000mg/kg)	25.2±0.03**	14.6±0.5**
VI	EMPR (1000mg/kg)+ Diazepam (1mg/kg)	26.6±0.5***	16.4±0.8***

EMPR: Ethanolic extract of *Mimosa pudica* roots. Results are expressed as mean±SEM (n=6). Statistical test was performed by one-way ANOVA followed by *post hoc* Dunnett's multiple comparison tests. ****p<0.0001, ***p<0.001, **p<0.01, *p<0.05. ns: Non-significant. EPM: Elevated plus maze

Table 3: Effect of EMPR on FST in mice

Groups (n=6)	Drug treatment	Immobility time (s)		
		Day 1	Day 7	Day 14
I	Control (distilled water 1 ml)	130.3±2.3	137±1.5	141.2±0.6
II	Imipramine (15 mg/kg)	97.1±2.3****	96.5±3.2****	83.3±1.8****
III	EMPR (1000 mg/kg)	129.3±1.3ns	129.8±1.4**	134.3±0.17***
IV	EMPR (2000 mg/kg)	129.8±0.3 ns	125.3±0.4****	126.8±0.7****
V	EMPR (1000 mg/kg)+ Imipramine (10 mg/kg)	118.8±2.5****	116.5±0.7****	114.5±1.5****

EMPR: Ethanolic extract of *Mimosa pudica* roots. Comparison was performed between control and all the other groups. Values expressed as mean±SEM. Statistical analysis is performed by one-way ANOVA followed by *post hoc* Dunnett's multiple comparison test. *p<0.05, **p<0.01; ***p<0.001; ****p<0.0001; ns: Non significant

Table 4: Effect of EMPR on TST in mice

Groups (n=6)	Drug treatment	Dose (ml/kg or mg/kg)	Immobility time (s)		
			Day 1	Day 7	Day 14
I	Control (distilled water)	1.0	146.2±2.1	148.8±2.8	149.3±2.4
II	Imipramine (15 mg/kg)	15	100.8±0.07****	97.3±0.6****	94.5±1.4****
III	EMPR (1000 mg/kg)	1000	126.3±17.09ns	137.3±1.3ns	137.5±0.7ns
IV	EMPR (2000 mg/kg)	2000	130.2±0.4ns	135.2±4.8*	124.2±2.3*
V	EMPR (1000 mg/kg)+Imipramine (10 mg/kg)	1000+10	114.8±0.9**	115.3±1.1***	113.3±2.3***

EMPR: Ethanolic extract of *Mimosa pudica* root. Test values compared to control group are expressed as mean±SEM. Statistical test is done by one-way ANOVA followed by *post hoc* Dunnett's multiple comparison test, ns: Non significant, *p<0.05, **p<0.01; *** p<0.001

depression-like behavioral models effectively. ARS is a reliable model for anxiety and depression induced by a mental stress produced due to a restraint or an inescapable state, in addition to impairment in the *in vivo* antioxidant defense mechanism [27].

Stress-induced behavioral alteration can be monitored in the rodent. EPM is the most commonly employed test to study the effect of anxiolytics on behavioral parameters of animals. In the present study, ARS (4 h) causes anxiety-like behavior and reduction in exploratory attitude as evidenced by a significant reduction in the EPM parameters such as the number of open arm entries and time spent in open arms. The results are in agreement with previous studies [28,29]. Increase in the percentage of open arm entries and the time spent in open arms of EPM following the treatment with EMPR at the two different dosage levels might be due to the chemical constituents present in it such as tannins, flavonoids, and others. The combination group with lower doses of EMPR and standard diazepam significantly increased the number of open arm entries and time spent in open arms almost comparable to a standard dose of drug diazepam.

EMPR extract reduced the duration of the immobility on the 1st, 7th, and 14th days of readings in both the models of depression, namely, FST and TST in comparison with control and exhibited a dose-dependent effect. The characteristic behavior evaluated in these tests, termed as immobility, has been considered to reflect behavioral despair similar to that seen in the human depression, and hence, any reduction in this parameter reflects antidepressant activity. There is a significant correlation between the clinical efficacy of antidepressant drugs and their potency in FST which is not found in any other model. Furthermore, our data indicated that higher doses of plant extracts were more effective than smaller doses both in FST and TST.

Our study is in accordance with the previous study which demonstrated that ethyl acetate extract of leaves of *M. pudica L* in the dose up to 400 mg/kg p.o. significantly alleviated the symptoms of anxiety, depression, and dementia in Swiss albino mice [27]. Another study also showed similar results of antidepressant and anxiolytic properties of the 100–500 mg/kg of lyophilized aqueous leaf extract of *M. pudica* in mice [28].

As a mentioned in the results, EMPR possesses various phytochemicals such as flavonoids, tannins, and steroids. CNS depressant and anxiolytic activity of EMPR reflected in results of the study may be attributed to these phytochemicals found in the extract. Several plants have been reported to have CNS depressant and anxiolytic activity due to the presence of flavonoids [29,30], tannins, saponins [30], and triterpenoids/steroids [29], from their phytochemical analysis. Phytochemical analysis of EMPR also revealed the presence of flavonoids, tannins, and steroids. Flavonoids, tannins, and triterpenoids are reported to have agonist/facilitatory activities at $GABA_A$ receptor complex, which led to hypothesis that they can act as benzodiazepine-like molecules. This is supported by their behavioral effects in animal models of CNS depression and anxiety [31,32].

CONCLUSION

From the results obtained, we conclude that EMPR possesses significant CNS depressant and anxiolytic activity. Flavonoids, tannins, and steroids may be the phytochemicals responsible for this activity. Central antidepressant and anxiolytic activity along with significant antiepileptic activity as reported in our previous studies may complement each other and thus may be useful in the treatment of different types of epilepsy, depression, and anxiety-like conditions.

AUTHOR'S CONTRIBUTION

Authors Shashikumara and Amrutheshwari B are involved in the conduct of experiments. Author Prathima C and Shashikumara are involved in preparing of graphs, manuscript writing, and editing.

CONFLICTS OF INTEREST

Authors declare no conflicts of interest.

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