International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 3, Issue 1, 2011

Research Article

ANTIDEPRESSANT ACTIVITY OF METHANOLIC EXTRACT OF PASSIFLORA FOETIDA LEAVES IN MICE

SANTOSH P, VENUGOPL R, NILAKASH A S, KUNJBIHARI S, DR. MANGALA L

National Institute of Pharmaceutical Education and Research (NIPER), Guwahati, Assam, India Email: dr_mlahkar@rediffmail.com

Received: 03 Oct 2010, Revised and Accepted: 05 Nov 2010

ABSTRACT

Passiflora foetida (Passifloraceae), popularly known as stinking passion flower, is an herbaceous climber that has been widely used in Mexican traditional medicine for the treatment of different central nervous system (CNS) disorders. Nevertheless, the available scientific information about this species is scarce and there are no reports related to its possible effect on the CNS. In this work, the effects of methanolic extract of leaves of P. foetida (PF) were evaluated in mice using behavioral tests sensitive to clinically effective antidepressant compounds. The extract (100,200 and 300mg/kg), administered intraperitoneally, was able to decrease the immobility time of mice dose-dependently when subjected to both tail suspension and forced swim tests and the effects are comparable to that of standard drugs i.e., fluoxetine (20mg/kg) and imipramine (15mg/kg). Neither the extracts of PF nor fluoxetine, at the doses tested, produced significant effects on locomotor activity when subjected to open field behavioral test. These results demonstrated that PF had specifically antidepressant effects in vivo. In conclusion, the present study suggested that PF extracts possessed potential antidepressant effects which could be of therapeutic interest for using in the treatment of patients with depressive disorders.

Keywords: Passiflora foetida, antidepressant activity, tail suspension test, forced swim test, open field test.

INTRODUCTION

According to the World Health report[1], 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[2]. Psychiatric illness is also often associated with suicide and there are between 10 and 20 million suicide attempts every year. Depression is the most prevalent mental disorder and depression is recognized to be symptomatically, psychologically and biologically heterogeneous[3].The disorder was characterized by apathy, loss of energy, retardation of thinking and activity, as well as profound feelings of gloominess, despair and suicidal ideation. In spite of the availability of antidepressant drugs like tricyclic antidepressants, selective reversible inhibitors of monoamine oxidase-A (MAO-A), selective serotonin reuptake inhibitors (SSRIs) and selective noradrenaline reuptake inhibitors (SNRIs), depression continue to be a major medical problem[4]. Basic neuroscience offers the promise of improving our understanding of disease pathophysiology, identifying novel mechanisms that can be targeted by more effective pharmacotherapies and screening of herbal sources of drugs. These considerations implicate the search for new antidepressant agents that have a fast onset of action, with less side effects and a wider safety margin. Various plants are being used in complementary and alternative medicines for management of mood disorders.

On the basis of the above information, the leaves of PF was selected for evaluating its anxiolytic and antidepressant activity due to its traditional use in the management of anxiety, stress, insomnia, hysteria, skin inflammation, cough and fever. Chemical constituents in PF include hydrocyanic acid, groups of flavonoids and harman alkaloids[5]. Some reports have pointed out the harman alkaloids as the bioactive constituents of Passiflora incarnata Linn, one of the species of Passiflora that have been extensively studied chemically and biologically[6,7]. Harman alkaloids were also found to be present in PF. Unlikely P. incarnata; so far there has been no scientific report in literature about the antidepressant activity (in experimental animal models) of this species. Therefore, the present study has been undertaken to investigate the effect of methanolic extract of passiflora foetida Linn. on depression in mice.

MATERIALS & METHODS

Plant material

The leaves of P.foetida L. were collected from botanical garden in madhapur, Hyderabad, Andhrapradesh. The plant material was identified and authenticated by the botanist, Botanical garden, Hyderabad. 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 dried plant material was crushed into fine particles (powder) using a mixer. The powdered plant material (500 g) was packed in a soxhlet apparatus and subjected to continuous hot percolation for 8h using methanol (1:4) as solvent. Extract obtained was passed through the Whatman filter paper No.1 and the methanol was evaporated (at 40^0C) with the help of heating mantle and dried in a desiccator.

Animals

Male Swiss albino mice weighing between 20 – 25 g were used for the present study. The animals were maintained under standard environmental conditions (25 + 2° C and relative humidity of 45 to 55%) and were fed with standard pellet diet and water ad libitum. The study was approved by Institutional Animal Ethics Committee (01/NIPER/CPCSEA/351). CPCSEA guidelines were adhered during the maintenance and experiment. All experiments were carried out between 10:00- 17:00 hours.

Drugs & chemicals

Fluoxetine hydrochloride (FLUDAC®, Cadila Pharmaceuticals, Ahmedabad, India) and imipramine hydrochloride (Sigma-Aldrich, St. Louis, USA) were used reference standards for antidepressant activity.

Experimental protocols

Overnight fasted animals were selected randomly on the day of experiment for administration of vehicle, standard drug and study drug. The animals were acclimatized one hour before for behavioral tests. Thirty minutes and 1 hour time interval between drug administration and behavioral tests were maintained in case of intraperitoneal and oral administrations respectively.

The animals were divided into five groups of six animals each as follows:

Group I (n=6) – Control, received distilled water, i.p
Group II (n=6) – (Standard) Imipramine (forced swim test) 15mg/kg & fluoxetine (tail suspension test) 20 mg/kg, p.o; Diazepam (1mg/kg, i.p) in open field test.

Group III (n=6) – MEPF 100 mg/kg, i.p
Group IV (n=6) – MEPF 200 mg/kg, i.p
Group V (n=6) – MEPF 300 mg/kg, i.p

The antidepressant activity was carried out using two different models. Further the effect of drugs was evaluated in open field test.

Behavioral tests

Tail suspension test (TST)

Tail suspension test commonly employed behavioral model for screening antidepressant-like activity in mice, was first given by Steru .,et.al. Animals were moved from their housing colony to laboratory in their own cages and allowed to adapt to the laboratory conditions for 1-2 hr. Each mouse was individually suspended to the edge of a table, 50 cm above the floor, by adhesive tape placed approximately 1 cm from the tip of the tail. Each animal under test was both acoustically and visually isolated from other animals during the test. The total period of immobility was recorded manually for 6 min. Animal was considered to be immobile when it didn't show any body movement, hung passively and completely motionless. The test was conducted in a dim lighted room and each mouse was used only once in the test. The observer, recording the immobility of animals, was blind to the drug treatments given to the animals under study[8,9].

Forced swim test (FST)

Forced swim test, the most frequently used behavioral model for screening antidepressant-like activity in rodents, was first proposed by Porsolt., et. al. The procedure was same as followed previously. Mice were individually forced to swim in open glass chamber (25 × 15 × 25cm) containing fresh water to a height of 15 cm and maintained at 26°±1°C. At this height of water, animals were not able to support themselves by touching the bottom or the side walls of the chamber with their hind-paws or tail. Water in the chamber was changed after subjecting each animal to FST because "used water" has been shown to alter the behavior. Each animal showed vigorous movement during initial 2 min period of the test. The duration of immobility was manually recorded during the next 4 min of the total 6 min testing period.

Mice were considered to be immobile when they ceased struggling and remained floating motionless in water, making only those movements necessary to keep their head above water. Following swimming session, mice were towel dried and returned to their housing conditions[8,9].

Open Field

This test utilizes behavioral changes in rodents exposed to novel environments and is used to confirm that the observed antidepressant effect is not due to stimulation of general motor activity. Various types of Open field apparatus have been used to test the mice. The open field test was carried out on the dark grey floor subdivided into 16 equal parts in a wooden box (100 cm x 100 cm x 30 cm). Respective treatment was given to the animals and 30 min later, the animals were individually placed in the corner square of the open field. The following parameters were observed for 5 min[10]:

- Activity in the centre (number of central squares crossed)
- Spontaneous ambulation (number of squares crossed at periphery)
- Rearing (No. of times the animal stands on the rear paws).

STATISTICAL ANALYSIS

All the data represent mean±S.E.M. values. The data were analyzed by means of analysis of variance (ANOVA). Whenever ANOVA was significant, further multiple comparisons were made using Tukey's test as the post hoc test. All analyses were performed using the SPSS statistical software. The levels of statistical significance ranged from p<0.05 to p<0.001.

RESULTS

Tail suspension test (TST)

In this test (Fig. 1), animals treated with three doses of MEPF (100, 200 and 300 mg/kg, i.p) showed decreases in their immobility times, which was significant (135.33±3.19, 113.17±2.81 and 96.17±2.45 respectively; p<0.001) when compared with control (160.17±3.62). Similarly, animals treated with fluoxetine (20 mg/kg), as expected, showed a significant decrease in the immobility time (73.33±2.11; p<0.001).

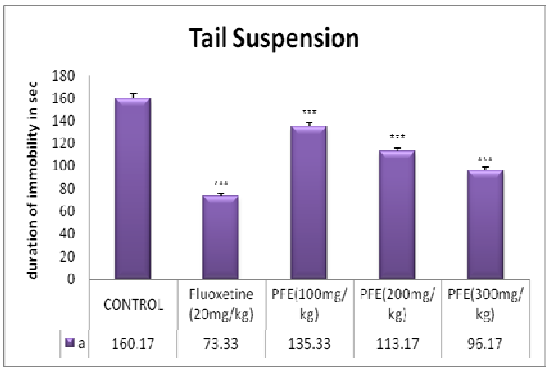

Fig. 1: Effects of MEPF and diazepam on duration of immobility in the TST. Results are expressed as mean±S.E.M (n=6). ***P < 0.001 as compared to respective control group.

Forced swim test (FST)

The possible antidepressant effect of MEPF after intraperitoneal administration was studied in the forced swimming test. In this test (Fig. 2), animals treated with three doses of MEPF (100, 200 and 300 mg/kg, i.p) showed decreases in their immobility times, which was significant (82.83±2.87, 60.67±1.98 and 47.67±2.46 respectively; p<0.001) when compared with control (150.17±4.9). Similarly, animals treated with imipramine (15 mg/kg), as expected, showed a significant decrease in the immobility time (38.5±2.92; p<0.001).

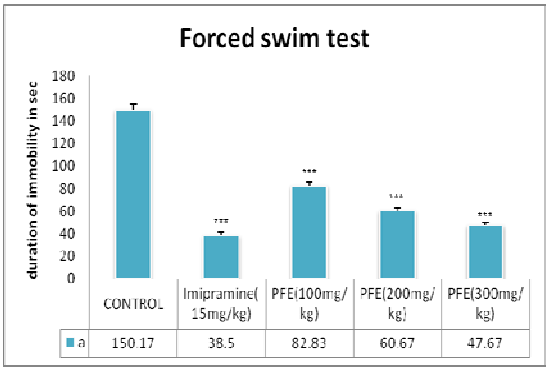

Fig. 2: Effects of MEPF and diazepam on duration of immobility in the FST. Results are expressed as mean±S.E.M (n=6). ***P < 0.001 as compared to respective control group.

Open field test

Though there was slight increase in the number of squares crossed (peripheral) by mice in MEPF treated groups (100,200 and 300mg/kg, i.p.) as compared to control, it was not statistically significant (Fig.3 & 4). The number of central Squares crossed in the control and standard (Diazepam) group were 10.17 ± 1.33 and 31.33±3.67 sec respectively. There was a significant increase in no. of crossings in diazepam group as compared to control group. But when different doses of MEPF were used alone the increase in no. of central square crossings was not statistically significant. There was significant increase in the rearing of animals with diazepam in comparison to the control group (p<0.01). There was also increased number of rearing in test drug treated groups which was not statistically significant.

Table 1: Effects of diazepam and MEPF on behaviour of mice in Open field paradigm

Treatments	N	No. of squares crossed (Mean±SEM)		No. of Rearings (Mean±SEM)	Total	
		Centre		periphery		
Control	6	10.17±1.33		81.50±10.15	91.67±10.03	3.67±0.49
Diazepam(1mg/kg)	6	31.33±3.67***		109.83±6.79ns	141.17±9.19**	7.17±0.65**
MEPF(100mg/kg)	6	15.00±2.08ns		88.17±7.16ns	103.17±8.55ns	4.00±0.58ns
MEPF(200mg/kg)	6	19.83±2.3*		90.17±4.13ns	110±4.77ns	5.33±0.49ns
MEPF(300mg/kg)	6	20.50±1.31*		98.33±4.58ns	118.83±4.38ns	6.00±0.73ns

Values are expressed as mean±SEM. ns= not significant, *P<0.05, **P<0.01, ***P<0.001, compared to respective vehicle treated control group.

DISCUSSION & CONCLUSION

The incidence of anxiety and depression in the community is very high and is associated with lot of morbidity. Hence, it is very important to address these problems and find effective remedies. Though several drugs are available, all are associated with some limitations and there is an urgent need for alternative medications for these disorders. Despite the widely popular use of Passiflora foetida for treating nervous disorders, there is an absence of scientific reports about the evaluation of its pharmacological effects. In this work, it was demonstrated that the administration of different doses of the methanolic extract of P.foetida in mice was able to induce antidepressant effects.

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 tail suspension tests. These tests are quite sensitive and relatively specific to all major classes of antidepressants[11]. 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[12]. It has been seen that the TST is less stressful and has higher pharmacological sensitivity than FST[13].

Results showed that the administration of the MEPF produced a diminution of immobility time (a posture thought to reflect a state of ''behavior despair'' in which animals have given up the hope to escape) of mice exposed to the both forced swimming and tail suspension tests. In the present study, methanolic extract (100, 200 and 300 mg/kg, po) administered to mice, produced significant antidepressant-like effect in both TST and FST and their efficacies were found to be comparable to imipramine(15 mg/kg, po) and fluoxetine (20 mg/kg, po).

Data in the literature demonstrated that drugs that alter general motor activity may give false-positive/negative results in the forced swimming test. The effects produced by MEPF and DZP (1.0mg/kg) upon the open field test demonstrated that these products do not modify the spontaneous locomotor activity of mice, which indicates that the plant extract exerts antidepressant effects without modifying significantly this parameter. Therefore, it is probable that these effects are not related to the stimulation of general motor activity[14].

It has been established that the shortening of immobility time in the forced swimming and the tail suspension tests depends mainly on the enhancement of central 5-HT and catecholamine neurotransmission[15]. Early evidence of a role for noradrenaline in depression came from the discovery that drugs, either causing or alleviating depression, acted to alter the noradrenaline metabolism. Furthermore, depletion studies carried out in treated and untreated patients indicated a role for serotonin and noradrenaline in depression[16].

Harmaline alkaloids present in P.foetida act as reversible monoamine oxidase inhibitors[17] and in common with other beta carboline binds to 5-hydroxy tryptamine (HT) receptors[18]. MAO regulates the metabolic degradation of catecholamines, serotonin and other endogenous amines in central nervous system. Inhibition of this enzyme causes a reduction in metabolism and subsequent increase in the concentration of biogenic amines. Also the flavonoid components of MEPF might be interacting with adrenergic and serotonergic systems in mediating the antidepressant effects of MEPF. However, the precise mechanisms by which the extract produced antidepressant-like effect are not completely understood. Further studies would be necessary to evaluate the contribution of active chemical constituents for the observed antidepressant activity as it still remains to be determined which components were responsible for these effects.

ACKNOWLEDGEMENT

The authors are grateful to Dr. (Mrs.) C. C. Barua, Veterinary College, AAU, Guwahati, Assam, India, for her valuable suggestions during the project work.

REFERENCES

1. The World Health Report. Mental health: new understanding new hope. WHO, Geneva, 2001.
2. Reynolds EH. Brain and mind: a challenge for WHO. Lancet 2003; 361:1924-1925.
3. Thase ME, Howland RH. Biological processes in depression: an update and integration. In: Beckham EE, Leber WR, editors. Handbook of Depression, 2nd ed., New York, Guilford, 1995; 213–279.
4. Yu ZF, Kong LD and Chen Y. Antidepressant activity of aqueous extracts of Curcuma longa in mice: Ethnopharmocol 2002; 83: 161.
5. Narongchai P, Omboon L and Leena S. Rapid reversed-phase high performance liquid chromatography for vitexin analysis and fingerprint of Passiflora foetida. Current science 2007; 93 (3): 378-382.
6. Dhawan K, Kumar S and Sharma A. Antianxiety studies on extracts of Passiflora incarnate Linneaus. J. Ethnopharmacol 2001; 78: 165-170.
7. Soulimani R, Younos C, Jarmouni S, Bousta D, Misslin R and Mortier F. Behavioral effects of Passiflora incarnata L. and its indole alkaloid and flavonoid derivatives and maltol in the mouse. J. Ethnopharmacol 1997; 57: 11-20.
8. Dunham NM, Miya TS. A note on simple apparatus for detecting Neurological deficit in rats and mice. J.Am. pharm. 1957; 46: 208-9.
9. Dhingra D and Sharma A. Antidepressant-like activity of Glycyrrhiza glabra. Neuropsychopharmacol Biol Psychiatry 2006; 30: 449.
10. Rogoz Z, Skuza G, Khodzinska A. Anxiolytic like effects of the preferential dopamine D3 receptor agonists in an animal model. Pol J Pharmacol 2003; 55 (3):449-454.
11. Porsolt RD, Bertin A and Jalfre M. Behavioral despair in mice: a primary screening test for antidepressants. Archives Internationales de Pharmacodynamie et de Therapie 1977; 229: 327.
12. Willner P. The validity of animal models of depression. Psychopharmacology 1984; 83: 1.
13. Thierry B, Steru L, Simon P and Porsolt RD. The tail suspension test: ethical considerations. Psychopharmacology 1986; 90: 284.

14. Novas ML, Wolfman C, Medina JH, De Robertis E. Proconvulsant and anxiogenic effects of n-butyl-h-carboline-3-carboxylate, on endogenous benzodiazepine binding inhibitor from brain. Pharmacol Biochem Behav 1988; 30: 331– 6.
15. Borsini F, Meli A. Is the forced swimming test a suitable model for revealing antidepressant activity? Psychopharmacology (Berl.) 1988; 94: 147–60.
16. Brunello N, Mendlewicz J, Kasper S, Leonard B, Montgomery S, et al. The role of noradrenaline and selective noradrenaline reuptake inhibition in depression. European Neuropsychopharmacology 2002; 12: 461–75.
17. Abdel Fattah AFM, Matsumoto K and Murakami Y. General Pharmacology 1997; 28: 405.
18. Abdel Fattah AFM, Matsumoto K, Gammaz and Watanabe H. Pharmacol Biochem Behaviour 1995; 52: 421.