

ANTIDEPRESSANT AND ANXIOLYTIC EFFECTS OF ALCOHOLIC EXTRACT FROM *TEPHROSIA PUMILA* (L.) PERS

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Article Received on
24 July 2017,

Revised on 14 August 2017,
Accepted on 03 Sept. 2017

DOI: 10.20959/wjpr201710-9517

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ABSTRACT

Tephrosia pumila (L.) Pers (Leguminosae (Fabaceae)), is an herbaceous climber that has been generally utilized in Indian traditional medicine for the treatment of different central nervous system (CNS) disorders. Nevertheless, the available scientific information about this species is rare and there are no reports identified with its conceivable impact on the CNS. In this work, the effects of ethanolic extract of *Tephrosia pumila* (L.) Pers (TPP) were assessed in rats utilizing behavioral tests sensitive to clinically effective antidepressant and anxiolytic effects compounds. The extract (200 and 400mg/kg), administered intraperitoneally, was able to decrease the

immobility time of rats dose-dependently when subjected to both tail suspension and forced swim tests for antidepressant activity and elevated Plus maze test, actophotometer test for anxiolytic effect and the effects are comparable to that of standard drugs i.e., Diazepam (20mg/kg). Neither the extracts of TPP and Diazepam, at the doses tested, produced significant effects on locomotor activity when subjected to open field behavioral test. These results demonstrated that TPP had specifically antidepressant effects *in vivo*. In conclusion, the present study recommended that TPP extracts possessed potential antidepressant and anxiolytic effects which could be of therapeutic interest for using in the treatment of patients with depressive disorders.

KEYWORDS: *Tephrosia pumila* (L.) Pers, Antidepressant activity, Anxiolytic effect, tail suspension test, forced swim test, elevated Plus maze test, actophotometer test.

INTRODUCTION

As indicated by 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.^[2,3] Depression is the most prevailing mental disorder and depression is recognized to be symptomatically, psychologically and biologically heterogeneous.^[4] 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.^[5] In spite of the accessibility 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 maintain to be a major medical problem.^[6] Fundamental 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 utilized in complementary and alternative medicines for management of mood disorders.^[7]

The plants of *Tephrosia pumila* (L.) Pers (TPP) was chosen 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. The literature reports that antimicrobial activities,^[8] antiprotozoal activity,^[9] anticancer activity.^[10] The experiential documents reports that chemical constituents in TPP include flavonoids and alkaloids.^[11] A few reports have pointed out the flavonoids and alkaloids as the bioactive constituents of TPP, one of the species of *Tephrosia* that have been extensively studied chemically and biologically.^[12] So far there has been no scientific report in literature about the antidepressant activity (in experimental animal models) of this plant. Therefore, the present study has been undertaken to investigate the effect of ethanolic extract of *Tephrosia pumila* (L.) Pers depression in rats.

MATERIALS AND METHODS

Plant material

The plants of *Tephrosia pumila* (L.) Pers (TPP) (Family: Leguminosae (Fabaceae))^[13,14] was gathered from Ranga Reddy dist, Telangana State in the month of August and was identified and authenticated from Department of Botany Osmania University, Hyderabad, Telangana State. The plant material was cleaned, reduced to small fragments, air dried under shade at room temperature and coarsely powdered in a blender. The powdered material was stored or taken up for extraction process.

Preparation of extract

Fresh plants of TPP were collected and dried under shade. The extracts used were prepared by using soxhlet apparatus by taking containing 500 ml of EtOH equivalent to two portions. Boiled upto 50-60°C for 4-5 hrs, the filtrate was boiled until the concentrated residue is formed. Powdered Drug is extracted with EtOH yielding a crude extract. Extract obtained was passed through the Whatman filter paper No.1 and the ethanol was evaporated (at 40°C) with the help of heating mantle and dried in a desiccator.

Experimental animals

Healthy adult albino wistar rats weighing 200-250 grams of either sex were chosen for the study. Animals were housed in appropriate cages in uniform hygienic conditions and fed with standard pellet diet (Amrul Laboratory Animal Diet) and water ad libitum. They were fasted overnight before the day of test. Animals were housed within the departmental animal house and the room temperature was maintained at 27° C. Animal studies had approval of IAEC. An authority regulating animal experiments and was approved by the Institutional Animal Ethics Committee Reg. No. 1567/PO/A/11/CPCSEA formed as per CPCSEA guidelines.

Drugs and chemicals

Diazepam (Nicholas Piramal Ltd, India) and Ethanol (ChangshuYangyuan Chemicals, China.) were used reference standards for antidepressant activity.

Preliminary phytochemical analysis of the extract

The ethanolic extract of TPP (EATPP) was subjected to preliminary phytochemical screening. Phytochemical studies were performed to identify the presence of various phytochemical constituents.

Experimental protocols

Overnight fasted animals were selected haphazardly 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 four groups of six animals each as follows: Group I (n=6) – Control, received distilled water, i. p; Group II (n=6) – (Standard) Diazepam (20 mg/kg, i. p); Group III (n=6) – EATPP 200 mg/kg, i. p; Group IV (n=6) – EATPP 400 mg/kg, i. p. The antidepressant activity and Antianxiety Activity was carried out using different models. Further the effect of drugs was assessed in open field test.

Screening for Antidepressant Activity

Forced Swim Test^[15-17]

For the determination of antidepressant activity, forced swim test (FST) protocol was employed. During the test, animals were separately placed in a glass cylinder (20 cm in height, 14 cm in diameter) filled with water up to a height of 10 cm, at $25 \pm 2^\circ\text{C}$. All animals were forced to swim for 5 min and the duration of immobility was observed and measured during the 5 min interval of the test. Immobility period was regarded as the time spent by the rats to float in water with no struggle and making only those movements necessary to keep its head above the water. In order to check the fitness level of each test animal, a pre-test was carried out 24 h before the FST by subjecting each test animal to a session of 15 min swimming.

Tail suspension test^[18-21]

Tail suspension test was performed in view of the technique. The rats were suspended 58 cm above the floor by means of an adhesive tape, placed approximately 1cm from the tip of the tail. The total duration of immobility was quantified during a test period of 5 min. Rats were considered immobile when they were completely remain motionless.

Screening for Antianxiety Activity

Elevated Plus Maze (EPM) model^[22-26]

The apparatus comprises of two open arms (35x5cm) and two closed arms (30x5x15cm) that extend from a common central platform (5x5cm). The floor and dividers of the closed arms are made of wood and painted black. The entire maze is elevated to a height of 50 cm above the ground level. Rats weighing (200-250 gms) were housed in a pair of 10 days prior to the

test in the apparatus. During this time the rats were handled by the investigator on alternate days to reduce stress. 30 min and 60min after oral administration of the drug treatment, each rat was placed in the center of the maze facing one of the enclosed arms. During five minutes session, number of entries into open arm and time spent in the open arm were noted. The procedure was conducted preferably in a sound attenuated environment.

Actophotometer test^[27-31]

The locomotor activity can be easily studied with the help of actophotometer, the rats were grouped and treated with drugs. Turn on the equipment (check & make sure that all the photocells are working for accurate recording) and placed individually each rat in the activity cage for 10 minutes. Note the basal activity score of all the animals. Inject the drug diazepam (Dose: 20 mg/kg, ip; make a stock solution containing 2 mg/ml of the drug & inject 1 ml/100 g body wt of rats), and after 30 mins re-test each rat for activity scores for 10 mins. Note the difference in the activity, before & after diazepam. Calculate percent decrease in motor activity.

Statistical Analysis

Statistical analysis was done by one way analysis of variance ANOVA followed by Dunnet's t-test and value of $p < 0.05$ was used as statistical significance. Data are expressed as mean \pm SEM. A probability value of < 0.05 was accepted as statistically significant difference between the vehicle-treated control and drug- treated groups. The Chi-square test was utilized for the quantal data in the rotarod test.

RESULTS

Evaluation of Antidepressant Activity

Forced Swim Test

Antidepressant activities of ethanolic extract of TPP were studied at a dose of 200 and 400 mg/Kg by using Forced Swim Test. The anti-depressant activities of various extracts were surveyed utilizing Forced Swimming Test in albino wistar rats were illustrated in Table No 1. It was observed that all the test extracts has shown significant reduction in immobility time when compared to control. Similarly the animals treated with diazepam (20 mg/kg) were shown significant decrease in immobility time.

Table 1: Effects of the Ethanolic extract of TPP and Diazepam on the duration of immobility in the rats Forced Swim Test (mean \pm S.E.M.)

S.No	Groups	Dose (mg/kg)	Immobility period in min		% change in activity
			Before	After	
1.	Normal control	-	77.16 \pm 1.94	----	-----
2.	Diazepam	20	58.00 \pm 2.83***	16.33 \pm 1.86	71.84 %
3.	EATPP	200	71.83 \pm 2.23***	24.50 \pm 2.17	65.89%
4.	EATPP	400	60.16 \pm 1.72***	17.00 \pm 1.41	71.74 %

n=6, ^{ns}p- non-significant, *p<0.05, **p<0.01- significant, ***p<0.001- more significant v/s control, SEM= standard error mean, SD = standard deviation, n= number of animal

Tail Suspension Test

In tail suspension test, the ethanolic extract of TPP at a dose of 200 and 400 mg/kg significantly increased the immobility time. The magnitude of the antidepressant effects of TPP shows same significant effect as that of standard drug Diazepam (20 mg/kg i.p.) whereas TPP is highly significant. (Table 2).

Table 2: Effects of the Ethanolic extract of TPP and Diazepam on the duration of immobility in the rat's tail suspension test (mean \pm S.E.M.)

S.No	Groups	Dose (mg/kg)	Immobility period		% change in activity
			Before	After	
1.	Normal control	-	92.83 \pm 2.99	----	-----
2.	Diazepam	20	62.66 \pm 3.50***	17.83 \pm 1.33***	71.54 %
3.	EATPP	200	70.16 \pm 2.71***	25.83 \pm 2.93***	63.18 %
4.	EATPP	400	82.66 \pm 3.20***	18.16 \pm 2.93***	78.03 %

n=6, ^{ns}p- non-significant, *p<0.05, **p<0.01- significant, ***p<0.001- more significant v/s control, SEM= standard error mean, SD = standard deviation, n= number of animal

Evaluation of Antianxiety Activity

Elevated Plus Maze Test

Anxiolytic property of ethanolic extracts of plant of TPP and their Combination were studied at a dose of 200 and 400 mg/Kg by using Elevated plus maze experiment.

The magnitude of the antianxiety impacts of ethanolic extracts of TPP and their Combination was compared with the standard drug diazepam 20 mg/kg i. p. (Table 3). In elevated plus-maze test (EPM), the extracts of TPP significantly increased the number of entries and time spent into the open arm.

Table 3: Effects of the Ethanolic extract of TPP and Diazepam on Elevated Plus Maze test in rats.

S.No	Groups	Dose (mg/kg)	Average time spent (sec)		No.of rearings
			open	Closed	
1.	Normal control	-	12.83 ± 1.47	19.16 ± 2.14	12
2.	Diazepam	20	33.33 ± 3.01***	22.83 ± 1.47 ^{ns}	9
3.	EATPP	200	26.83 ± 2.14***	36.33 ± 3.27***	7
4.	EATPP	400	20.00 ± 1.41***	50.33 ± 3.98***	8

n=6, ^{ns}p- non-significant, *p<0.05, **p<0.01- significant, ***p<0.001- more significant v/s control, SEM= standard error mean, SD = standard deviation, n= number of animal

Actophotometer Test

Anxiolytic property of ethanolic extracts of plants of TPP and their Combination were studied at a dose of 200 and 400 mg/Kg by using Actophotometer test.

The percentage of reduction in locomotor activity with diazepam (20 mg/kg i.p) is 68.32 % i.e. there is highly significant decrease in locomotor activity compare to control, where as doses of (200 and 400mg/kg, i.p) shown dose dependent decrease in locomotor activity in TPP that is 68.77 % and 74.46% respectively when compared to standard.

Table 4: Effect of Ethanolic extracts of TPP on Locomotor activity.

S.No	Groups	Dose (mg/kg)	Locomotor activity (scores) in 10 min		
			Before	After	%change in activity
1.	Control	-	210.17 ± 8.50	--	---
2.	Diazepam	20	285.67 ± 4.50***	90.00 ± 2.53***	68.32 %
3.	EATPP	200	249.83 ± 4.83***	78.00 ± 1.41***	68.77 %
4.	EATPP	400	271.50 ± 4.93***	69.33 ± 2.34***	74.46 %

n=6, ^{ns}p- non-significant, *p<0.05, **p<0.01- significant, ***p<0.001- more significant v/s control, SEM= standard error mean, SD = standard deviation, n= number of animal.

DISCUSSION

The phytoconstituents are known to play an important role in bioactivity of medicinal plants. In qualitative phytochemical investigation reveals the presence of alkaloids, flavonoids, tannins, terpenoids and saponins presence of chemical constituents in ethanolic extracts of TPP.

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*^[32] 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 ethanolic extract of TPP in rats 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.^[32] 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, rats 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.^[33] It has been seen that the TST is less stressful and has higher pharmacological sensitivity than FST.^[34]

Results showed that the administration of the AETPP 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 rats exposed to the both forced swimming and tail suspension tests. In the present study, ethanolic extract (200 and 400 mg/kg, i.p) administered to rats, produced significant antidepressant-like effect in both TST and FST and their efficacies were found to be comparable to Diazepam (20 mg/kg, i.p).

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 AETPP and Diazepam (20 mg/kg, i.p) upon the open field test demonstrated that these products do not modify the spontaneous locomotor activity of rats, 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.^[35]

The fear due to height induces anxiety in the animals when placed on the EPM. The ultimate manifestation of anxiety and fear in the animals is exhibited by decrease in the motor activity and preference to remain at safer places. Anti-anxiety agents are expected to increase the motor activity, which is measured by the time spent by the animal in the open arms. The

conventional EPM is highly sensitive to the influence of both anxiolytic and anxiogenic drugs acting at the GABAA-benzodiazepine complex. This animal model is considered one of the most widely validated tests for assaying sedative and anxiolytic substances like the benzodiazepines. In EPM, rats will normally prefer to spend much of their allotted time in the closed arms. This preference appears to reflect an aversion towards open arms that is generated by the fears of the open spaces. Drugs that increase open arm exploration are considered as anxiolytics. The data of Anti-anxiety activity of plant extracts has evaluated by elevated plus-maze have been presented. The AETPP exhibit significant anti-anxiety effect which is comparable with the effect of standard drug Diazepam and individual extracts.

Results showed that basal activity score (mean \pm standard error) in all groups recorded for 10 minutes. Locomotor activity is considered as an index of alertness, and the spontaneous decrease in basal activity score implicates the reduction of anxiety. Such types of effect can be found in the case of sedatives. There is a significant decrease in the locomotor score in case of animal treated with the EATPP and standard drug Diazepam. The Results shows the comparison of the locomotor score among TPP and standard drug diazepam.

CONCLUSION

The findings in this study suggest that the *Tephrosia pumila* (L.) Pers possess Antidepressant Activity and Anti- anxiety activity. The results have been obtained carefully from the controlled experiments model with laboratory animals. The statistical validity of the findings has been proven and they provide a scientific foundation for the use of the biologically active ingredients of *Tephrosia pumila* (L.) Pers in Antidepressant and anxiety for explain the clinical importance of the *Tephrosia pumila* (L.) Pers. 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. K.S. murali Krishna, Marri Laxman Reddy Institute of Pharmacy, Dundigal, Hyderabad, Telangana, India for providing facilities to carry out research work.

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