Research Article

Evaluation of the Antidepressant Effects of *Phyllanthus amarus* in Mice

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ABSTRACT

Depression is one of the leading causes for many disorders in both developed and developing countries. The objective of the present study is to evaluate the antidepressant activity of *Phyllanthus amarus* and to compare with the standard drug imipramine and control group of Swiss albino mice by tail Suspension test (TST) and force swimming test (FST). Present study showed that *P. amarus* has significant antidepressant activity at doses of 50, 100 mg / kg in acute models of depression. The results showed that the alcoholic extract of *P. amarus* decreased immobility time with the increase swimming time. Treatment drug doses at (50, 100 mg / kg, p.o.) significantly (P<0.01) reduced the immobility time as compared to the immobility time of control in both the screening models. Imipramine at doses of (20 mg/kg, p.o.,) was selected as reference standard. These results suggested the anti-depression activity of the plant extract. The effect of the alcoholic extract was less potent than standard imipramine group which was used as a standard. As an indicator of the antidepressant effect, it was shown that the immobility time of animals in the forced-swimming and tail-suspension experiments was shorter, i.e. the activity of the animals was higher. Alcoholic extract of *P. amarus* displayed dose-dependent antidepressant effect at doses of 50 and 100 mg / kg. Both models have proved to be equally valuable for demonstration of substances with a potential antidepressant effect. The pharmacological approach to the treatment of depression includes the action of *Phyllanthus amarus*schum and thonnis the natural antidepressant.

Keywords: Antidepressant activity, Force swimming test, *Phyllanthus amarus*, Tail suspension test.

INTRODUCTION

Depression is a psychiatric disorder characterized by state of low mood and aversion to activity that can affect a person's thoughts, behaviour, feelings and sense of well-being. Depressed people feel sad, anxious, empty, hopeless, worried, helpless, worthless, guilty, irritable, and sometimes restless.[1] Depression can be associated with number of infectious diseases, neurological conditions including hypo androgenism, Addison' s disease, stroke, cancer, Lyme disease, multiple sclerosis, diabetes, chronic pain, and sleep apnea. To date, the efficacy of the drugs for these conditions are very limited so the need for newer, better-tolerated and more efficacious treatments is remaining high. It is difficult to predict which patient will respond to any given treatment.[2] In the traditional systems of medicine, many plants and formulations have been used to treat depression for thousands of years. Therefore, herbal therapies should be considered as alternative / complementary medicines. Recently, the search for novel pharmacotherapy from medicinal plants for psychiatric illnesses has progressed significantly.[3] This has been reflected in the large number of herbal medicines whose psychotherapeutic potential has been assessed in a variety of animal models. A great number of preclinical and clinical studies have not only confirmed but have also extended the medicinal uses of species of the genus *Phyllanthus* mentioned in traditional medicine. *Phyllanthus amarus* (family: Euphorbiaceae) is a reputed drug of Ayurveda. It is a small erect annual herb that grows up to 15 to 50 cm high. It is indigenous to the rainforest of the Amazon and other tropical areas of the world including the Bahamas, southern India, and Africa.[4]

It is used in traditional medicine to treat various nervous disorders. It is also used as a stomachic, a digestive; rejuvenate, for promoting memory and intellect, for skin disorders, and as an antiepileptic, antipyretic, and analgesic. All parts of the plant are employed therapeutically. *Phyllanthus* species can also be found in other countries including China, The Phillipines, Cuba, Nigeria and Guam.[5] *Phyllanthus* genera contain many important phytoconstituents which are responsible for various type of pharmacological activity like very effective hepatoprotective agents in the Indian indigenous systems of medicine and are considered bitter, astringent, stomachic, diuretic, febrifuge, deobstruant and antiseptic.[6] In western parts of India it was used as a diuretic in gonorrhoea and acidity of the urine. The root with rice water was a remedy for menorrhagia. In chronic dysentery, the plant along with fenugreek for menorrhagia. In chronic dysentery the plant along with fenugreek was given. It was found that mainly as weed in waste lands, agricultural lands and riverbanks.[7] It was found that mainly as weed in waste lands, agricultural lands and riverbanks.

MATERIALS AND METHODS

Collection and authentication of plant material

Plant material *Phyllanthus amarus* were procured from local market of Mumbai and it was authenticated at Nicholus Piramal Pvt Ltd. Goreagoan, Mumbai India.

Preparation of extracts

The leaves material of *P. amarus* was extracted successively with petroleum ether (60-80°C), chloroform, and ethanol in a Soxhlet extractor. The different extracts were evaporated under reduced pressure to obtained dry masses. The extracts were then stored in desiccator till further pharmacological studies.

Animals for the depression study

Adult male Swiss albino mice (25-35g) each were procured from Central Animal House, Jamia Humdard, New Delhi, India. They were housed in an environmentally regulated room on a 12 h light: 12 h dark cycle with 25±2°C and had free access to food and water. The experimental protocol was approved by the institutional animal ethical committee and experiment conducted according to the Committee for the purpose and Supervision of Experimental animals (Ref. no: 837 CPCSEA 29th Dec 2009). Experiments were carried out between 09:00 and 18:00 h. Pharmacological evaluation of alcoholic extracts of *P. amarus* were conducted on depression studies in mice.

Experimental design

On the day of the experiment, the animals were divided randomly into control and experimental groups (n=6). Group 1 received the vehicle, 1% Na CMC (10 ml / kg) and served as the control group, groups 2 received the standard drug imipramine (20 mg / kg) per orally, groups 3 and 4 received the test drug (PAE) in doses of (50, 100 mg /kg). Drugs/vehicle was administered to the animals 60 minutes prior to the behavioural evaluation in study. Behavioural evaluation was carried out 60 minutes post drug/vehicle administration on 8th day. The antidepressant activity of the test drug was evaluated using the following experimental models of depression TST and FST.

Tail suspension test (TST)

Tail suspension test (TST) was used to evaluate the effects of various extracts on anti depressive study in mice as described by Steru et al., (1985).[8] In tail suspension test, mice were suspended 50 cm above the floor by means of an adhesive tape, placed approximately 1 cm from the tip of the tail in a sound – isolated room. The time during which mice remained immobile was quantified during a period of 6 min.[9] The duration of immobility was recorded during the last 4 minutes of the observation period. Mice were considered to be immobile only when they hung passively and were completely motionless. All results were summarized in (Table 1).

Forced Swim Test (FST)

The method described by Porsolt, et al., was used in our study (Porsolt RD, et.al., 1977). In this model, mice are forced to swim in a restricted space from which they cannot escape. The apparatus consisted of clear plexi-glass cylinder (25 cm height x 20 cm diameter) filled to 20 cm depth with water (24 ± 1°C) and were observed for duration of 6 minutes.[10] The duration of immobility was recorded during the last 4 minutes of the observation period. The mouse was considered immobile when it floated motionlessly or made only those moments necessary to keep its head above the water surface[11]. The water was changed after each test [(10), (11)]. All the results summarized in (Table 2).

Statistical analysis

The mean ± S.E.M. values were calculated for each group. The data were analysed using one-way ANOVA followed by Dunnet's multiple comparison test. P< 0.05 was considered to be statistically significant.

RESULTS

Tail suspension test

The tail suspension test (TST) has become one of the most widely used animal models for assessing antidepressant–like activity in mice. The results are summarized in (Table 1 and figure 1). In tail suspension test, control group of animal showed immobility for duration of 83.166 ± 1.706 seconds, whereas immobility duration of standard drug imipramine group animal 15.166 ± 3.421 sec. test drug PAE at dose of 50 mg/kg showed immobility time 48.66 ± 3.127 and higher dose of PAE showed immobility time 43.67 ± 3.405.In this model, test drugs and standard drug compared to control group of animals, there was a significant difference in immobility duration of both the groups compared to control. Alcoholic extract of PAE at doses of (50,100 mg / kg) and standard drug imipramine significantly (P<0.01) decrease in the duration of immobility was seen as compared to the control (group 1). In both doses of PAE (50, 100mg/kg) produced a greater decrease in the duration of immobility as compared to the control group.

Table 1: Tail suspension test of PAE

Treatment groups	Duration of immobility time (seconds) ± SEM
Control group	83.166 ± 1.706***
Imipramine	15.166 ± 3.421***
50 mg/kg PAE	48.66 ± 3.127***
100 mg/kg PAE	43.67 ± 3.405***

PAE means *Phyllanthus amarus*

Force swimming test (FST)

The force swimming test (FST) has become one of the most widely used animal models for assessing antidepressant–like activity in mice. The results are

summarized in (Table 2 and figure 2).The force swimming test (FST), control group of animal showed immobility for duration of 93.33 ± 2.79 seconds, whereas immobility duration of standard drug imipramine group of animal showed6.83 ± 1.25. test drug PAE at dose of 50 mg/kg showed immobility time 64.66 ± 6.97 and higher dose of PAE showed immobility time 57.62 ± 6.47 when test drugs and standard drug when compared to control group of animals, there was a significant difference in immobility duration of both the groups compared to control.

Alcoholic extract of PAE at doses of (50,100 mg/kg) and standard drug imipramine significantly (P<0.01) decrease in the duration of immobility was seen as compared to the control (group 1). In both doses of PAE (50,100mg/kg) produced a greater decrease in the duration of immobility as compared to the control group. The results are summarized in (Table 2 and figure 2). Effects of PAE on immobility time in the force swimming test (FST) using mice.

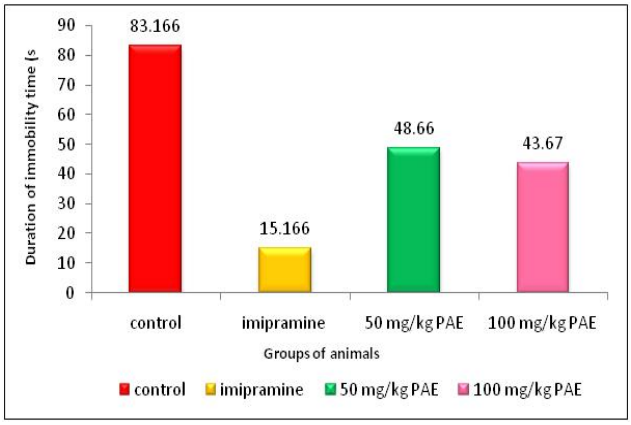

Figure 1: Effects of PAE on tail suspension test

Values are expressed as mean ± SEM of six observations. The P value is <0.01, considered significant. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's test; ** p < 0.01.

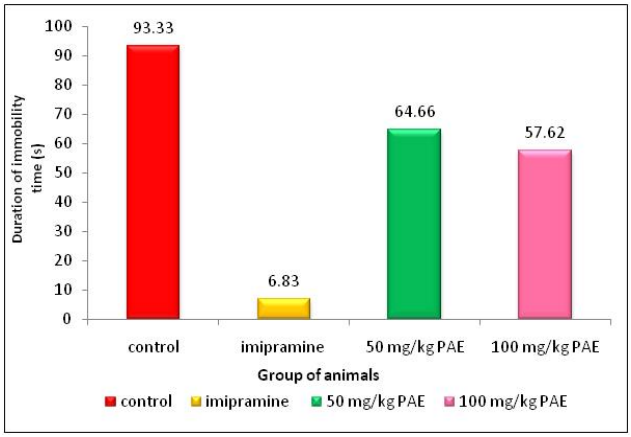

Figure 2: Effects of PAE on force swimming test (FST)

Values are expressed as mean ± SEM of six observations. The P value is <0.01, considered significant. Statistical significant test for comparison was done by ANOVA, followed by Dunnet's test; ** p < 0.01.

Table 2: Force swimming test of PAE

Groups	Duration of Immobility time (S) ± SEM
Control	93.33 ± 2.79 **
Imipramine	6.83 ± 1.25 **
50 mg /kg PAE	64.66 ± 6.97 **
100 mg /kg PAE	57.62 ± 6.47 **

DISCUSSION

Phyllanthusamarus, a traditional Ayurvedic medicinal plant used for centuries as a memory enhancing, antidepressant, analgesic antipyretic sedative and antiepileptic agent. In present study, behavioural models namely forced swim test and tail suspension test were employed. Both represent the behavioural despair models, claimed to reproduce a condition similar to human depression. The tests were based on the observation that animals, following initial escape oriented movements, develop an immobility posture when placed in an inescapable chamber. The test model of depression (forced swim test and tail suspension test) are based on the observation that mice when forced to swim or suspended in a restricted space from which there is no possibility of an escape, eventually cease to struggle, surrendering themselves (despair or helplessness) to the experimental conditions. This suggested that this helplessness or despair behaviour reflected a state of lowered mood in laboratory animals and could serve as a valuable test for screening antidepressant drugs. In this study mice were treated with alcoholic extract of *P. amarus* at dose of (50,100 mg/kg) for 7 days once daily which was compared to control group which didn't receive any treatment except the vehicle 1 % Na CMC at the dose of 10 ml / kg. Standard drug imipramine (20 mg/kg) which was given daily once for 7 days to evaluate the antidepressant activity using two well established animal models i.e. force swim test (FST) and tail suspension test (TST). Lower dose of PAE was found to be less effective in altering the immobility duration of mice. However, other doses significantly reduced the immobility time of mice in FST and TST. This reduction in immobility time was found to be dose dependent in both the models. The minimal effective dose of extract was 50 mg/kg in FST and TST model. The results indicate that alcoholic extract of *P. amarus* may have an antidepressant-like effect and the immobility time observed in the test reflected a state of lowered mood or hopelessness in animals, thus, this animal model is the most widely used tool for preclinical screening of putative antidepressant agents. The FST shows a strong sensitivity to monoamine alterations and is a very specific cluster of stress-induced behaviours that are not related to depression symptoms in humans, but which are nonetheless exquisitely sensitive to monoaminergic manipulations. It also provides useful model to study neurobiological and genetic mechanisms underlying stress and antidepressant responses. This suggests that *P.*

amarus has reproducible antidepressant like activity irrespective of the model.

CONCLUSION

To conclude, alcoholic extract of *P. amarus* has antidepressant property at doses of 50 and 100 mg/kg where it has shown significantly better results than control group and also antidepressant activity is comparable with standard antidepressant drug imipramine. The exact mechanism(s) involved in these effects will have to be elucidating in further studies which may be because of antioxidant property or some other mechanisms.

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