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Antidepressant Activity of Ethanolic Extract of *Piper betle* Leaves in Mice

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ABSTRACT

To evaluate the antidepressant activity of ethanolic extract of *Piper betle* leaves in mice, 48 adult albino mice (Swiss strain) weighing 25-30 g were selected. Twenty four animals were allocated to Forced Swim Test (FST) and 24 to Tail Suspension Test (TST) models. In each model there were 4 groups. The control group received vehicle (10 mL kg⁻¹, p.o.), the standard, imipramine (10 mg kg⁻¹, p.o.) and the two test groups received ethanolic extract of *P. betle* leaves (PBL) 100, 200 mg kg⁻¹ p.o., respectively, 1 h prior to the acute study. In chronic study, the drugs were given orally once a day for 10 days and the last dose was given 1 h before the experiment. Duration of immobility was noted in FST and TST. Statistical analysis was performed using Mean±SEM and ANOVA followed by Dunnett's test. The p<0.05 was considered statistically significant. Ethanolic extract of PBL produced significant antidepressant effect at both the doses (100, 200 mg kg⁻¹), as indicated by reduction in the duration of immobility compared to the control. The antidepressant effect was higher at 100 mg than at 200 mg. In addition, the effect at 100 mg was greater than that of imipramine. Ethanolic extract of PBL thus has shown significant antidepressant activity greater than imipramine in mice.

Key words: Forced swim test, tail suspension test, *Piper betle* leaves, depression, imipramine

INTRODUCTION

According to WHO (2012) report "Depression is the leading cause of disability as measured by YLDs and the 4th leading contributor to the global burden of disease (DALYs) in 2000. By the year 2020, depression is projected to reach 2nd place of the ranking of DALYs calculated for all ages and both sexes. Today, depression is already the 2nd cause of DALYs in the age category 15-44 years for both sexes combined" (WHO, 2012). Suicide is the major consequence in most of the depressive illnesses. About 60% deaths are due to depression and related disorders (Shalam *et al.*, 2007).

Chronic stress is one of the main triggers of inducing depression even though the mechanism of provoking depression is not clearly established (Bolandghamat *et al.*, 2011). Most of the synthetic drugs used in the treatment of depression have various adverse effects. Insomnia and loss of libido with selective serotonin (5 HT) reuptake inhibitors and tolerance and physical dependence with

tricyclic antidepressants are very common; several drug-drug interactions may occur (Kothari *et al.*, 2010).

These limitations create a need for alternative treatment of depression such as medicinal plants and plant based antidepressant formulations.

Among the medicinal plants, *Piper betle* L. is the most important one which is widely distributed in Asian countries and has more medicinal values (Al-Adhroey *et al.*, 2010).

The leaf extracts are found to have anti-diabetic, cardiovascular, anti-inflammatory, immunomodulatory, Virulence activity, anti-ulcer and hepato-protective activities (Arambewela *et al.*, 2005; Chen *et al.*, 1995; Vaghasiya *et al.*, 2007; Kanjwani *et al.*, 2008; Nalina and Rahim, 2006; Majumdar *et al.*, 2002; Young *et al.*, 2007). The plant extract is found to have highest phenolic content (Jamal *et al.*, 2010) which can contribute to antimicrobial activity. It is reported that *Piper betle* leaves is a CNS stimulant (Guha, 2006). It is also used in the field of nanotechnology for the synthesis of silver nanoparticles (Mallikarjuna *et al.*, 2012). Though other activities have been evaluated in animals, the CNS stimulant activity has not been evaluated so far. If it has CNS stimulant activity whether it will contribute to the antidepressant action is not known. As anti depressants are more useful clinically than the CNS stimulants, it was decided to evaluate the antidepressant activity of PBL in mice.

MATERIALS AND METHODS

Animals: The experimental protocol was approved by the Institutional Animal Ethics Committee (IAEC) of Chettinad Hospitals and Research Institute in its meeting dated 18.02.11.

Adult male Swiss albino mice weighing 25-35 g from our breeding stock were used in this study. The animals were housed at 24±2°C with 12:12 h light and dark cycle. They had free access to food and water. The animals were acclimatized for a period of 7 days before the study. The animals were used according to the CPCSEA guidelines for the use and care of experimental animals.

Drugs and chemicals: The standard antidepressant drug imipramine (M/s. Alkem Ltd. Mumbai) was obtained from our institutional pharmacy. Standardized ethanolic extract of PBL was purchased from M/s. Chemill Nutraceuticals Pvt. Ltd., Bangalore.

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, normal saline (10 mL kg⁻¹) and served as the control, group 2 received the standard drug imipramine (10 mg kg⁻¹), groups 3 and 4, the test drug (PBL extract) in doses of 100 and 200 mg kg⁻¹ per orally. Single dose of Drugs/vehicle was administered to the animals 60 min prior to the evaluation in acute study. For chronic study, a new set of animals were used. They were grouped as in acute study and were administered the drug/vehicle orally once daily for a period of 10 days. Evaluation was carried out at 60 min post drug/vehicle administration on the 10th day. The antidepressant activity was evaluated by TST and FST.

Tail suspension test (TST): The method described by Steru *et al.* (1985) was used in our study. The animals were hung by the tail on a plastic string 75 cm above the surface with the help of an

adhesive tape. Mice were considered immobile only when they hung passively and were completely motionless. The duration of immobility was observed for a period of 8 min and the last 6 min value was taken for calculation.

Forced swim test (FST): The method described by Porsolt *et al.* (1977) was used in our study. Each animal was placed individually in a 5 L glass beaker, filled with water up to a height of 15 cm and was observed for a duration of 6 min, last 4 min values were taken for calculation. The mouse was considered immobile when it floated motionless or made only those moments necessary to keep its head above the water surface. The water was changed after each test.

Statistical analysis: The Mean±SEM values were calculated for each group. The data were analysed using one-way ANOVA followed by Dunnett's multiple comparison test. The p<0.05 was considered to be significant.

RESULTS

Tail suspension test (TST): A significant (p<0.01) decrease in the duration of immobility was seen with the standard drug imipramine and ethanolic extract of PBL in all the tested doses as compared to the control in acute study but in chronic study the dose of 100 mg kg⁻¹ produced a greater decrease in the duration of immobility as compared to 200 mg kg⁻¹ and the standard drug imipramine (Table 1).

Forced swim test (FST): A significant decrease in the duration of immobility was seen with the standard drug imipramine and at both the doses of ethanolic extract of PBL as compared to the control. In acute study, ethanolic extract of PBL in a dose of 100 mg kg⁻¹ is more efficacious than imipramine in reducing the duration of immobility (Table 2). However, in chronic study, ethanolic extract of PBL in both the doses (100 and 200 mg kg⁻¹) was more efficacious than imipramine. The duration of immobility was however shorter for 100 than 200 mg.

Table 1: Effect of ethanolic extract of PBL on immobility time in the tail suspension test (TST) using mice

Groups	Drug treatment	Duration of immobility (sec)	
		Acute study	Chronic study
1	Normal saline; 10 mL kg ⁻¹	233.5±11.18	211.66±16.44
2	Imipramine; 10 mg kg ⁻¹	167.16±7.05***	157.0±5.50***
3	PBL; 100 mg kg ⁻¹	159.66±6.75***	141.33±5.98***
4	PBL; 200 mg kg ⁻¹	170.66±9.06***	154.0±9.15***

Values represented Mean±SEM (n = 6), ***p<0.01 vs. control (group 1)

Table 2: Effect of ethanolic extract of PBL on immobility time in the forced swim test (FST) using mice

Groups	Drug treatment	Duration of immobility (sec)	
		Acute study	Chronic study
1	Normal saline 10 mL kg ⁻¹	118.5±4.46	120.5±5.27
2	Imipramine 10 mg kg ⁻¹	50.16±6.56***	66.33±3.59***
3	PBL 100 mg kg ⁻¹	44.33±4.03***	62.0±2.69***
4	PBL 200 mg kg ⁻¹	52.0±5.26***	65.0±4.32***

Values represented Mean±SEM (n = 6), ***p<0.01 vs. control (group 1)

DISCUSSION

Depression is a widespread psychiatric disorder affecting approximately 5% of the world population (Monteleone *et al.*, 2011). In addition to the conventional anti depressant drugs, many plant products and ritual therapies have been used for thousands of years. Among the medicinal plants *P. betle* has been used since ancient days for its CNS stimulant, anticancer, bacteriostatic and chemopreventive activity (Padma *et al.*, 1989; Bhide *et al.*, 1991a, b; Fathilah *et al.*, 2009). As it reported as a CNS stimulant, it is not known whether it will have antidepressant action. If it has antidepressant action, it will contribute to the treatment of depression as anti depressants are more useful clinically than the CNS stimulants.

Therefore, the purpose of this study was to evaluate the antidepressant effect of PBL using behavioural animal models. In this study, two animal models, the Forced Swim Test (FST) in mice developed by Porsolt *et al.* (1977) and Tail Suspension Test (TST) developed by Steru *et al.* (1985) were used. The immobility displayed by rodents when subjected to unavoidable stress such as forced swimming reflects a state of despair or lowered mood, similar to depressive disorders in humans in whom stress is an important contributory factor for depression. Moreover, a significant correlation was found between the clinical efficacy of antidepressant drugs and their efficacy in both the models.

Imipramine (10 mg kg⁻¹) and ethanolic extract of PBL (100 and 200 mg kg⁻¹) in both acute and chronic study have shown significant reduction of duration of immobility in mice, in FST and TST denoting the antidepressant activity. Among the 2 doses of ethanolic extract of PBL, 100 mg kg⁻¹ showed highly significant reduction in immobility than 200 mg kg⁻¹. This dose related action could be due to the 'window phenomenon' the extract might have. It has been reported that PBL contains an alkaloid called 'arakene' and that it has actions similar to cocaine (Nadkarni, 2007; Chopra *et al.*, 2006; Bakhru, 1992).

Cocaine is a local anaesthetic agent which has in addition sympathomimetic especially CNS stimulant activity. Sympathomimetic activity is due to the inhibition of nor-epinephrine reuptake both in the peripheral and central nervous system (Fazlul Huq, 2007). In the CNS it also inhibits reuptake of dopamine (Frank *et al.*, 1988). Increased level of dopamine in the CNS is responsible for its stimulant and euphoric action (Catterall and Mackie, 2006). But it is not known whether PBL acts by the same mechanism. Hence, the effect of PBL on spontaneous activity of the mice was studied using actophotometer. It was found that animals treated with PBL showed increased activity showing its stimulant effect.

In our study, the antidepressant effect of PBL was found to be superior to that of the standard drug imipramine. Imipramine acts by inhibiting the reuptake of nor epinephrine and 5 HT increasing their synaptic level (Baldessarini, 2006; Frank *et al.*, 1988). PBL has shown both anti depressant and stimulant effect. As discussed earlier the alkaloid arakene present in PBL is similar to cocaine. Cocaine also inhibits the reuptake of nor epinephrine. Hence, it can be stated that PBL might act similar to cocaine and imipramine. Further studies may help establish the mechanisms of action of PBL.

CONCLUSION

In conclusion *P. betle* leaves extract has shown significant antidepressant activity greater than imipramine and it has the potential to be used as an antidepressant.

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