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RESEARCH ARTICLE

EFFECTS OF PIPER BETLE LEAVES (PAAN) EXTRACT AS ANTI-DEPRESSANT AND ANTI-ANXIETY IN EXPERIMENTAL ANIMALS

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ABSTRACT

Objectives: The present study was undertaken to determine the anti-depressant and anti-anxiety effects of hydroethanolic extract of Piper betle leaves commonly known as "paan".

Materials and Methods: Hydroethanolic extract of Piper betle leaves in the doses of 100,200 and 400 mg were administered orally for successive 14 days to young Swiss albino mice of either sex. The antidepressant activity was evaluated by forced swim test and tail suspension test. On the other hand anti-anxiety activity was evaluated by light/dark exploration test and elevated plus maze test. Imipramine 15 mg/kg was used as standard in anti-depressant models and diazepam 2 mg/kg was used as standard in anti-anxiety models.

Results: Hydroethanolic extract of Piper betle leaves of doses 200 and 400 mg/kg showed significant activity as compared with control in reducing the immobility time in forced swim test and tail suspension test. On the other hand, gradual increasing dose of extract of Piper betle leaves also showed significant activity in improving anxiety of mice as compared with control in light/dark exploration and elevated plus maze test.

Conclusion: Hydroethanolic extract of Piper betle leaves showed anti-depressant activity probably acts through the mechanism of blocking the uptake of dopamine, noradrenaline and serotonin through their respective transporters. On the other hand, anti-anxiety activity acts probably through GABA but the role is not established.

Keywords: Anti-depressant; anti-anxiety; dopamine; Piper betle; GABA.

INTRODUCTION

In India leaves of *Piper betle* had been traditionally used for chewing purpose and have biologically active phytochemicals with great potential for medicinal use [1]. Since the time of Charaka and Sushruta many herbal medicines in different oral formulations have been recommended for the treatment of various human diseases [2]. *Piper betle* (English: Betel vine, Hindi: Paan, Sanskrit: nagavallli) belong to the genus *Piper* of the family piperaceae. Over 700 species of the plant belonging to the genus *Piper* are found distributed in both hemispheres [3]. *Piper betle* is commonly cultivated in India, Srilanka, Malaysia, Indonesia, Philippine islands and East Africa. It has been traditionally used as "breath fresheners" in India [4].

The betel plant is a slender, aromatic creeper, rooting at the nodes. The branches of the plant are swollen at the nodes. The plant has alternate, heart shaped, smooth, shining and long stalked leaves with pointed apex [5]**.** Essential oil from leaves of this plant has been used for the treatment of respiratory catarrhs and as antiseptic and the fruit is employed with honey as a remedy for cough [6]. Antioxidant, anti-bacterial and anti-fungal [7], antiinflammatory, anti-diabetic and radio protective [8] activities and contraceptive effects [9] of *P. betle* have been reported in various studies. *Nigrum* of family *piperaceae* has biological activities such as CNS stimulant, analgesic, antipyretic and antifeedent activities [10]. Futher, *Piper betle* has also shown neuroprotective effect in ethanol treated rats [11]. An example of classical ayurverdic preparation containing Piper betle is Laghu shuta shekhar rasa used as antacids [12].

From the perusal of literature it appears that the neuropsychopharmacological effect of *Piper betle* has been less investigated. Therefore, it was found of interest to evaluate these activities of betel leaves extract in experimental models.

MATERIALS AND METHODS

Plant material

The *Piper betle* leaves, commonly known as 'paan', were collected from the local market of Wardha and were authenticated from department of botany, J.B Science College, Wardha (M.S). The leaves were dried in shade and stored in air tight container for study.

Preparation of extract

The shade dried leaves were powdered using a mechanical grinder. The powder was macerated in hydro alcoholic solution (containing ethanol-70% and water 30%) in the ratio of 1:2.5 for 24h and successively extracted with 70% ethanol using a mechanical percolator. Extract was filtered and dried. Hydroethanolic extract of *Piper betle* (HEPB) was suspended in 1% gum acacia. 40 gm powder yielded 6 gm extract.

Animals

Swiss albino mice of either sex (25±5g) were used in the present study. They were raised in institutional animal house (MGIMS, Sewagram, M.S.). Animals were provided normal diet and tap water *ad libitum* and were exposed to 12-h light and 12-h dark cycle. The animals were acclimatized to the laboratory conditions prior to experimentation and were fasted overnight. The Institutional Animal Ethical Committee approved the protocol of the study.

Ethical Clearance

Ethical clearance was taken from Institutional Animal Ethics Committee of institute where research was conducted (MGIMS/IEAC/Aug/4/2012).

Drugs

Diazepam hydrochloride (2mg/kg, P.O) [13] (Calmpose injection, Ranbaxy Laboratories, Gurgaon, India) was used as a reference drug for anxiolytic activity and Imipramine hydrochloride (15

mg/kg, P.O) (Talendep, TALENT Pharma, Gujarat, India) for antidepressant activity were purchased and used in the study.

Experimental design

Animals were randomly divided in two groups A and B. Group A and B were then further divided into five subgroups containing six animals in each subgroups (n=6). Group A_I and B_I serve as a control and were treated with 1% gum acacia 10ml/kg. Subgroup II, III & IV of A and B were given test drug, HEPB in dose 100mg/kg, 200mg /kg & 400mg/kg respectively. Pilot study was done and the above doses were found effective and hence selected for study. Group A_V and B_V receive standard drug, imipramine and diazepam respectively. All the groups were treated with vehicle, test drug (HEPB) and standard drugs for period of 14 days Antidepressant and anxiolytic activity in group A and B respectively was observed on 7^{th} and 14^{th} day 60 minutes postdrug/vehicle administration .

- The antidepressant activity of the test drug was evaluated using the following experimental models of depression 1) Tail suspension test (TST) and 2) Forced swim test (FST) :

Tail Suspension test (TST)

The tail suspension test is based on total duration of immobility by a mouse induced on suspending by the tail as described by Steru et al [14] as a facile means of evaluating potential antidepressants (Immobility = Depression). Mouse was suspended by its tail approximately 50 cm above the floor by using an adhesive tape placed approximately 1cm. from the tip of the tail. The total duration of immobility in 6-min. was measured [15].

Forced swim test (FST)

This model was proposed to test for antidepressant activity by Porsolt *et al*. [16] Mouse was forced to swim individually in a glass jar (25 × 12 × 25 cm^3sub) containing fresh water of 15 cm height and maintained at 25°C (± 3°C). Each animal assumed a typical immobile posture after an initial 2 min period of vigorous activity (Immobility = Depression). A mouse was considered to be immobile when it remained floating in the water without struggling but making only minimum movements of its limbs necessary to keep its head above water. The total duration of immobility was recorded during the next 4 min of a total 6 min test. The changes in immobility duration were studied after administering drugs in separate groups of animals. Each animal was used only once.

- The anxiolytic activity of the test drug was evaluated using the following experimental models of anxiety 1) Light/dark exploration test and 2) Elevated plus maze test:

Light/dark exploration test

The Light dark apparatus consisted of two boxes measuring 25 × 25 × 25 cm and joined together. One box was made dark by covering its top with plywood, whereas other box was illuminated by a 40-W lamp placed 25 cm above the box. The mice of group B were treated with HEPB (100, 200 and 400 mg/kg, p.o.), diazepam (2 mg/kg, P.O.) or vehicle 60 min before being placed individually in the centre of the lit box and four behavioural events i.e. number of crossings to light compartment, time spent in light box, time spent in dark box were recorded for the next 5 min [17]. Anxiolytic activity was defined by increase in the time spent in the lighted box and decrease in the time spent in the dark box.

Elevated plus maze test

The elevated plus maze apparatus consisted of four arms crossed with each other. Two open arms (35 × 5 cm^2) and two closed arms (35 × 5 × 20 cm^3). All the four arms were connected together with a central square of 5 × 5 cm^2. The apparatus was elevated to the height of 25 cm in a dimly illuminated room. Mice were treated with HEPB (100, 200 and 400 mg/kg, p.o.), diazepam (2mg/kg P.O) or vehicle 60 min before being placed individually in the centre of the apparatus, facing the closed arm. The time spent and the numbers of entries in both the open and the closed arms were recorded for the period of 5 min. Entry of a mouse was considered when all the four paws were within the arm [18]. Increase in time spent in open arm and increase in number of entries in open arm defined anxiolytic activity.

Statistical Analysis

The statistical analysis of data was done by using ANOVA then student's't' test. *P*<0.001 was considered as highly significant.

RESULTS

Forced Swim Test

In the forced swin test, hydroethanolic extract of *Piper betle* of doses 200 mg/kg p.o and 400 mg/kg p.o given for 7 days and then continued for 14 days showed a gradual statistically significant decrease in the immobility time as compared with control with significant and very significant values respectively [p<0.05 and p<0.01]. The value on the 14th day is statistically more significant than the values on 7^{th} day. On the other hand, standard drug Imipramine (15mg/kg p.o) dose showed a highly significant p-value. [Table 1]

Table 1: Anti-depressant effect of ethanolic extract of *Piper betle* on immobility time in forced swim test.

S.No	Group (ml/mg/kg	Dose (mg/kg)	Immobility time (sec) Day 7	(Mean±SEM) Day 14
1	Control	10 ml/kg#	150.16±11.49	141±10.1
2	HEPBL	100 mg/kg	135±6.41	119±4.78
3	HEPBL	200 mg/kg	113±3.1*	103±3.98**
4	HEPBL	400 mg/kg	91±3.79**	86±4.67**
5	Impiramine	15 mg/kg	60±6.53***	43±2.87***

n=6, n= No. of animals. HEPBL= Hydroethanolic Extract of Piper betle Leaves. Statistical analysis was carried by one way ANOVA followed by Students paired't' test where *P<0.05 was considered significant, **P<0.01 considered very significant and ***P<0.001 was considered highly significant

Tail Suspension Test

Hydroethanolic extract of *Piper betle* leaves (100,200 and 400 mg/kg p.o) showed a gradual decrease in the immobility in the tail suspension when the doses were given for consecutive 14 days as compared with the control group which was given normal saline indicating statistically significant anti-depressant effects of the extracts. Doses of 200 mg/kg p.o and 400 mg/kg p.o showed statistically significant p-value at 14^{th} days when compared with the data at 7^{th} day. On the other hand, the standard Imipramine showed a highly statistically significant p-value as compared with control. [Table 2]

Table 2: Anti-depressant effect of ethanolic extract of *Piper betle* on immobility time in tail suspension test.

S.No	Group (ml/mg/kg	Dose (mg/kg)	Immobility time (sec) Day 7	(Mean±SEM) Day 14
1	Control	10 ml/kg#	157.16±8.84	146±5.54
2	HEPBL	100 mg/kg	140.16±5.26	132±3.62
3	HEPBL	200 mg/kg	122.5±4.25**	118.83±2.15**
4	HEPBL	400 mg/kg	112.83±3.45**	109.16±3.33***
5	Impiramine	15 mg/kg	77.83±3.19***	73.33±5.64***

n=6, n= No. of animals. HEPBL= Hydroethanolic Extract of *Piper betle* Leaves. Statistical analysis was carried by one way ANOVA followed by Students paired' test where *P<0.05 was considered significant, **P<0.01 considered very significant and ***P<0.001 was considered highly significant.

Light/Dark Exploration Test

Hydroethanolic extract of *Piper betle* leaves had showed a gradual increase in the behaviour to stay in the light zone with increasing doses of the extract especially with 200 mg/kg p.o and 400 mg/kg p.o. The significance of the results increases as the doses are given for 14 consecutive days. P-value of p<0.01 and p<0.001 are found in the group of doses 200 mg/kg p.o and 400 mg/kg p.o with gradual increase in the duration of treatment. Diazepam showed a highly statistically significant p-value of 0.001 as compared with the control. On the other hand extract of 100 mg/kg p.o showed a statistically significant p-value of <0.05 on the 14th day as compared with the 7th day. The extract showed a promising anti-anxiety effect.

Table 3: Anti-anxiety effect of ethanolic extract of *Piper betle* on time spent and number of crossing in light/dark exploration test.

S.No	Group Dose	Day 7 Time spent in light(s) (Mean±SEM)	Crossing	Day 14 Time spent in light(s) (Mean±SEM)	Crossing
1	Control	76.5±3.03	13.5±0.95	81±2.4	12.5±0.76
2	HEPBL (100mg/kg)	83.66±3.37	10.16±1.01	88.5±2.23*	10.66±0.55
3	HEPBL(200 mg/kg)	89.33±3.8	9.16±0.6**	93.33±2.06**	9.33±0.42
4	HEPBL (400mg/kg)	92.83±2**	9.16±0.4**	94±1.98***	8.16±0.3***
5	Impiramine (2 mg/kg)	108.832.46***	8.16±0.4***	115.16±1.81***	6.66±0.21***

n=6, n= No. of animals. HEPBL= Hydroethanolic Extract of *Piper bettle* Leaves. Statistical analysis was carried by one way ANOVA followed by Students paired' test where *P<0.05 was considered significant, **P<0.01 considered very significant and ***P<0.001 was considered highly significant.

Elevated Plus Maze Test

In another model of anti-anxiety, extract of doses 200 mg/kg p.o and 400 mg/kg p.o of *Piper betle* leaves showed statistically significant decrease in the time spent in the enclosed arm with gradual increase in doses as well as increase in duration of treatment with improvement in time spent in the open arm. The anti-anxiety effects are evident from the values that the animal crossed and entry into the open arm more often with increasing dose of the extract as well as with the standard, Diazepam. On the other hand, there is decrease entry into the enclosed arm with increasing dose as well as increase in the duration of the treatment. Thus hydroethanolic extract of *Piper betle* leaves showed statistically significant anti-anxiety effects on the experimental animals. [Table 4 and 5]

Table 4: Anti-anxiety effect of ethanolic extract of *Piper betle* on animal stay in the open and enclosed arm of elevated plus-maze on day 7th .

S.No	Group Dose	Time spent in the open arm (s)	Time spent in the closed arm (s)	Entries in open arm	Entries into closed arm
1	Control	43.66±1.4	240.33±1.64	5.83±0.3	11.66±0.49
2	HEPBL (100mg/kg)	45.83±1.22	233.83±3.48	6.16±0.3	10.16±0.54
3	HEPBL(200 mg/kg)	48.16±1.19*	223.33±4.31*	7.16±0.3*	9.5±0.42**
4	HEPBL (400mg/kg)	51.5±1.25**	218.83±4.02**	7.5±0.56**	9±0.36**
5	Impiramine (2 mg/kg)	58.66±0.76***	208±2.46***	9.83±0.4***	8.5±0.34***

n=6, n= No. of animals. HEPBL= Hydroethanolic Extract of *Piper betle* Leaves. Statistical analysis was carried by one way ANOVA followed by Students paired' test where *P<0.05 was considered significant, **P<0.01 considered very significant and ***P<0.001 was considered highly significant. Result expressed in Mean±SEM.

Table 5: Anti-anxiety effect of ethanolic extract of leaves of *Piper betle* on animal stay in the open and enclosed arm of elevated plus-maze on day 14th

S.No	Group Dose	Time spent in the open arm (s)	Time spent in the closed arm (s)	Entries in open arm	Entries into closed arm
1	Control	57.66±1.78	229.5±4.61	7.16±0.3	10.5±0.42
2	HEPBL (100mg/kg)	60.83±2	222.66±1.68	7.83±0.16	10±0.25
3	HEPBL(200 mg/kg)	63.83±1.42*	218.83±2.79	8.66±0.42*	8.83±2.79
4	HEPBL (400mg/kg)	67.33±1.52**	215.33±3.72*	9±0.44**	8.16±0.3**
5	Impiramine (2 mg/kg)	72.83±1.7***	207.16±2**	11.16±0.47***	7.5±0.34***

n=6, n= No. of animals. HEPBL= Hydroethanolic Extract of *Piper betle* Leaves. Statistical analysis was carried by one way ANOVA followed by Students paired' test where *P<0.05 was considered significant, **P<0.01 considered very significant and ***P<0.001 was considered highly significant. Result expressed in Mean±SEM.

DISCUSSION

Piper betle leaves are used as stimulant, antiseptic and also as breath-freshener since ancient times. Many studies have been on *Piper betle* leaves in the past and found to have anti-microbial, aromatic stimulant, antiflatulent. It is also useful in arresting bleeding or secretion and is an aphrodisiac. Piper betle leaves is also used for diuresis, obstructed or scanty urination, headaches, weakness of nerves, headaches, respiratory disorders, constipation, sore throat and inflammation [19].

Piper betle leaves found to have starch, sugars, tannins and diastases and a phenol, chavicol. Alkaloid, arakene is the most important constituents of betel leaves which have properties allied to cocaine [20]. The other constituent is essential oil known as betel oil. Betel oil contains terpene and sesqueterpene [21].

The antidepressant and anxiolytic potential of *Piper betle* leaves extract evaluated in present study showed a significant antidepressant activity in the most commonly used behaviour paradigms in animal models of depression, namely, forced swim test and tail suspension test . In this study, the *Piper betle* leaves extract in the doses of 100mg/kg, 200 mg/kg and 400 mg per kg was given once daily for fourteen days and were found have significant activity as compared to control but not quite comparable to that of standard antidepressant drug Imipramine [22] in mice for antidepressant activity. *Piper betle* leaves extract used in the present studies have allied property of cocaine [20] and it is probably due to this property, it has antidepressant activity and acts through the mechanism of blocking the uptake of dopamine, noradrenaline and serotonin through their respective transporters [23]. However further studies are required to evaluate its mechanism of action in detail.

On the other hand, *Piper betle* leaves extract used in the anxiolytic model showed significant anxiolytic activity when compared with the control but the values are not comparable with the standard anxiolytic drug, Diazepam [24]. The role of GABA (gamma amino butyric acid) as the mechanism of action attributed for its anxiolytic potential have not been established convincingly, but the results were better as compared to the control and further research with increasing doses to evaluate its anxiolytic activity.

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