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Review

Evaluation of hydro-alcoholic extract of leaves of *Boerhaavia diffusa* for anxiolytic activity in rats

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The hydro-alcoholic extract of *Boerhaavia diffusa* of leaves was investigated for evaluation of exhibited anxiolytic activity in rats at a dose 100 and 200 mg/kg by p.o. route for elevated plus maze test, hole-board test, haloperidol induced catalepsy and ketamine induced sleep method. Results of *in vivo* activity lead to the conclusion that the hydro-alcoholic of *B. diffusa* showed predominantly significant activity which is compared to the standard drug, diazepam (0.5 mg/kg).

Key words: *Boerhaavia diffusa*, anxiolytic activity, diazepam, haloperidol, hydro-alcoholic extract, ketamine, sleep.

INTRODUCTION

Boerhaavia diffusa family: Nyctaginaceae, Sanskrit: "Punarnava is a perennial creeping weed found throughout India. The leaves of *B. diffusa* are reported for their use in the indigenous system of medicine for the treatment of dyspepsia (Table 1), jaundice, enlargement of the spleen and abdominal pain (Kirtikar and Basu, 1956). A decoction of whole plant is taken with milk in the early morning to cure jaundice and weakness However, no scientific evaluation of these claims appears to have been undertaken so far. In the present study, we were made to validate the folklore use of this plant as anxiolytic activity.

MATERIALS AND METHODS

Plant

The leaves of *B. diffusa* were collected in August, 2009 from local market of Bopal, India and were authenticated. The voucher specimen (NIPS/PC/105) was preserved in the laboratory for reference.

Preparation of extract

The leaves were dried under shade, powdered and passed through 40 meshes, and were stored in closed vessel for further use. *B. diffusa* was extracted using solvent system of 70% methanol and 30% water in soxlate apparatus at a temperature of 40 to 60°C. On the seventh day, 35 cycles of the soxlate extraction were done. The extract was filtered and concentrated in a vacuum under pressure using rotary flash evaporator.

Phytochemical analysis of the extract

The extract was screened for the presence of various constituents employing standard screening test (Trease and Evans, 1985). Conventional protocol for detecting the presence of glycosides, saponins, flavonoids, tannins, etc., was used. Several phytoconstituents like flavonoids and saponin are known to have anxiolytic activity (Ambavade et al., 2006; Nayak et al., 2004).

Toxicity studies

Toxicity studies of hydro-alcoholic extract were carried out in oral doses of 1000 to 2000 mg/kg body weight using albino rats. After test extract administration, animals were observed for 72 h period. The number of deaths was expressed as a percentile and the LD_{50} was determined by probate test using the death percentage versus the log dose (Turner RA.1965). Study protocol was approved from the Institutional Animal Ethics Committee (IAEC).

Statistical analysis

Results are expressed as mean ± standard error of the mean (SEM), and statistical difference was analyzed using Dunnet t test

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Table 1. Treatment schedule.

Group	Treatment	Dosage, route of administration
1	Distilled water (vehicle)	1 ml/kg, p.o
2	Hydro-alcoholic extract	100 mg/kg, p.o
3	Hydro-alcoholic extract	200 mg/kg, p.o.
4	Diazepam	0.5 mg/kg, i.p

n = number of animals used in each group and treatment duration (10 days).

Table 2. Effect of *B. diffusa* extract on time spent in open arm.

Treatment	Time spent in open arm
Vehicle	29.5 ± 4.21
Extract 100 mg/kg	77.0 ± 15.19*
Extract 200 mg/kg	45.5 ± 7.3
Diazepam 0.5 mg/kg	70.25 ± 1.6*

Values are mean ± SEM of observation. F_{12} = 6.38, P = 0.008; compare to respective vehicle control group. *Represents data that is significant.

and results were considered significant when $P < 0.05$.

EVALUATION OF ANXIOLYTIC ACTIVITY

Animals

Adult albino rats (150 to 200 g) were used in this study. They were housed in well ventilated rooms (temperature 22 ± 2°C, humidity 65 to 70% and 12 h light/dark cycle) and fed with standard rodent pellet diet (Lipton India Ltd., Bombay) with tap water *ad libitum*.

Elevated plus maze model

Elevated plus maze (EPM) test (Pellow et al., 1985) for studying the anxiolytic effect in rodents was used. EPM consists of two open arms (15 × 10 cm) and two closed arms (50 × 10 × 40 cm) with an open roof elevated at 50 cm. 1 h after the oral administration of drugs, the rat was placed at the centre of the maze, facing one closed arm. During a 5 min test period, the following measures were taken: the time spent in the open and closed arms; and total number of arm entries. The duration of treatment was 10 days. The result is as shown in Table 2.

Hole-board test

Placing a rat on the hole-board apparatus, elevated to 45 cm from the table, induced anxiety in it as it was exposed to a new environment. The anxiogenic agents reduce the number of head poking, whereas the anxiolytic agents increased the number of head poking.

The hole-board apparatus consist of metal plate floor (40 × 40 cm) placed at 25 cm above the ground. The metal plate consist of six hole (1.5 cm in diameter) spaced symmetrically in a diamond pattern. A rat was placed on one corner of the apparatus and was observed for the next 5 min for the number of head poking (Mohan et al., 2005). The results are as shown in Table 3.

Haloperidol induced catalepsy

Purpose and rational catalepsy in rats was defined as a failure to correct an externally imposed unusual posture over a prolonged period of time. Neuroleptics which have an inhibitory action of nigrostriatal dopamine system induced catalepsy (Costall and Naylor, 1974; Chermat and Simon, 1975) while neuroleptics with title or no nigrostriatal blockade produce relatively title or no cataleptic behavior. Furthermore, cataleptic symptoms caused Parkinson-like extrapyramidal side effects in rodents seen clinically with administration of antipsychotic drugs (Duvoisin, 1976).

Procedure

Albino rats were divided into four groups each. They were administered vehicle, that is, water by intraperitoneal (i.p) route. After 30 min, the rats were administered haloperidol 1 mg/kg i.p and both forepaws of rats were place on a wooden bar elevated 6 cm above the ground. The duration for which the rats retains the fore paws on the elevated bar was noted at 0, 30, 60, 90 and 120 min. The cut off time was 300 s.

The two doses of the extract (*B. diffusa*), 100 and 200 mg/kg for animals in each group, were administered to both groups: the 2nd group was given 100 mg/kg and the 3rd group was given 200 mg/kg p.o. After 45 min, the same group of animals was treated/ administered with haloperidol (1 mg/kg i.p) and both forepaws of rats were placed on wooden bar elevated 6 cm above the ground. The duration for which the rats retain the forepaws on the elevated bar was noted at 0, 30, 60, 90 and 120 min, though the cut off time was 300 s. The results are as shown in Table 4.

Ketamine induced sleep

Materials required are ketamine (80mg/kg), extract (dose A and B), syringe (1 ml), animals and vehicle (distilled water). The animals were divided into four groups of similar body weight of same sex, each groups were treated separately with calculated dose. Animal was treated with either vehicle or extract (p.o) and after 45 min, the animals are administered ketamine (80 mg/kg i.p), then the animals were placed in the observation table for 1 h induction of sleep and the duration of sleep was recorded (Winters et al., 1972). The results are as shown in Table 5.

RESULTS AND DISCUSSION

Elevated plus maze (EPM) model

In the animal study, we found out that *B. diffusa* plant extract significantly reduced the anxiety; the result is as shown in the Table 2. Two doses of extract were taken and observed in elevated plus maze apparatus. The

Table 3. Effect of extract on the number of head poking in hole board apparatus.

Treatment	No. of head pocking
Vehicle	5.0 ± 0.9
Extract (100 mg/kg)	7.75 ± 0.85
Extract (200 mg/kg)	6.25 ± 1.1
Diazepam (0.5 mg/kg)	7.75 ± 0.62

Values are mean ± SEM. F_{12} = 1.88, P = 0.188; compared to respective vehicle group control. Data that is not significant.

Table 4. Effect of ketamine on duration of sleep.

Treatment group	Duration of sleep
Vehicle (1 mg/kg) + ketamine (80 mg/kg i.p.)	62 ± 1
Extract (100 mg/kg) + ketamine (80 mg/kg i.p.)	54 ± 1.95*
Extract (200 mg/kg) + ketamine (80 mg/kg i.p.)	51.25 ± 2.49*

Values are mean± SEM of observations. P = 0.009; compare to respective vehicle group control. *Represents data that is significant.

Table 5. Interaction of the extract with haloperidol at 0, 30, 60 and 120 min.

Treatment	0 min (mean±SEM)	30 min (mean±SEM)	60 min (mean±SEM)	90 min (mean±SEM)	120 min (mean±SEM)
Vehicle + Haloperidol (1 mg/kg)	0 ± 0	255 ± 44.25	300 ± 0	300 ± 0	83.5 ± 0.5
Extract 100 mg/kg + Haloperidol (1 mg/kg)	0 ± 0	0 ± 0*	180 ± 9.25*	195 ± 61.53*	255 ± 28.2*
Extract 200 mg/kg + Haloperidol (1 mg/kg)	0 ± 0	0 ± 0*	0 ± 0*	0 ± 0*	16.25 ± 2.39*

Values are mean ± SEM of 5 observations. *Represents that data is significant. All results were analyzed by dunnet t test and results were considered. significant when p<0.05.

doses of extract were compared to the control group and standard diazepam (0.5 mg/kg i.p). According to the result, the low dose that is, 1st extract was found significant as compare to the high dose of 2nd extract and the value of “p” was found (P = 0.008). Elevated plus maze was used to observe the anti anxiety activity of the extract on the rats, as the dose of the extract was compared to the time spent by the vehicle (100 mg/kg) in treating rats, which shows that the extract has anxiolytic activity.

Hole-board test

Two doses of the extract were taken and observed in hole-board apparatus. The dose of the extract was compared to the control group and standard diazepam (0.5 mg/kg i.p). According to the result, both doses were found not significant and the value of “p” was found (P = 0.188).

Effect on sleep duration using ketamine

The two doses of the extract + ketamine (80 mg/kg) were taken as stated previously and the

duration and latency of sleep were observed. The two doses of the extract were compared to the control group, and the result is as shown in Table 4. According to the result, both doses of the extract significantly reduced the duration of sleep in rats. Both doses of the extract were found significant at P = 0.009.

Drug interaction with centrally acting drug

Haloperidol is a potent neuroleptics drug which induces catalepsy in rats. Two doses of the extract were taken for experiments as stated in the materials and method and the result is as shown in Table 5. The two doses of the extract were compared to the vehicle treated group, in different time interval or duration of time like 0, 30, 60, 90 and 120 min. In 0 min, both doses of the extract were not significant, but in 30 min duration, both they were found significant (P = 0.001). In 60 min, the extract dose of 100 mg/kg was found significant (P = 0.001) and the extract dose of 200 mg/kg was also found significant. In 90 min, both doses of the extract were found significant (P = 0.001) and in 120 min, it was found that both doses of the extract was found significant, that means the extract in maximum time interval decreases catalepsy with the haloperidol drug that acted similarly like the agent which is dopaminergic or which is working as a D_2 receptor agonist.

Conclusion

In the present study, we used the EPM model of anxiety to evaluate the anxiolytic effects of the hydro-alcoholic extract of *B. diffusa*. The elevated plus maze is currently one of the most widely used models of animal anxiety (Hoggs, 1996). The extracts of *B. diffusa* increased the time spent in open sided arms of the plus-maze by rat in the dose range of 100 mg/kg. Maximum activity by all the extracts were produced at 100 mg/kg and the response was reverted when the doses were increased to 200 mg/kg. Plants containing sterols, flavonoids, etc., were reported to have anxiolytic activity (Ambavade et al., 2006; Nayak et al., 2004) and preliminary phytochemical screening revealed the presence of sterols, tannins and flavonoids in the *B. diffusa*. Therefore, the anxiolytic activity of *B. diffusa* may be due to the presence of tannins, sterols, flavonoids, etc. However, further investigations are required to isolate the phytoconstituents responsible for anxiolytic activity and to find their mechanism of action.

As expected, diazepam produced significant increases in open arm time and in a number of entries into the open arms. Diazepam also increased the total number of entries. These data are in agreement with the results of other studies, where diazepam and other benzodiazepines have been shown to produce robust anxiolytic effects in a variety of anxiolytic screening procedures, including conflict model (Vogel et al., 1971), EPM procedures (Pellow et al., 1986) and other non punishment procedures (Winslow et al., 1991). The result of the present study suggests that hydro-alcoholic extract of *B. diffusa* plant may possess significant anxiolytic activity.

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