

EVALUATION OF ANXIOLYTIC ACTIVITY OF *BOERHAAVIA DIFFUSA* HYDRO-ALCOHOLIC EXTRACT OF LEAVES IN RATS

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ABSTRACT

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, Ketamine induced Sleep and Haloperidol Induced Catalepsy method. Results of in-vivo activity lead to the conclusion that the hydro-alcoholic of *Boerhaavia diffusa* showed predominantly significant activity which is compared to the standard drug Diazepam (0.5mg/kg).

Key words: *Boerhaavia diffusa*, anxiolytic activity, Diazepam.

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, jaundice, enlargement of the spleen and abdominal pain¹. 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 made to validate the folklore use of this plant as hepatoprotective against experimentally produced liver injury.

MATERIALS AND METHODS

Plant material

The leaves of *Boerhaavia diffusa* were collected in August 2009 from local market of Bhopal & authenticated. The voucher specimen (NIPS/PC/105) is preserved in laboratory for reference.

Preparation of extract

The leaves were dried under shade, powdered and passed through 40 meshes and stored in closed vessel for further use. *Boerhaavia diffusa* extracted with using solvent system 70% methanol and 30% water in Soxhlet apparatus at the temperature 40°C to 60°C. On the seventh day it was filtered and the alcohol extract was concentrated in 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 test². 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^{3,4}.

Toxicity Studies

Toxicity studies of Hydro-alcoholic extract were carried out in oral doses of 100 to 200 mg/kg- body weight using albino rats. After test extract administration, animals were observed 72 hr. period. The number of deaths was expressed as a percentile and the LD₅₀ was determined by probate a test using the death percentage versus the log dose⁵. Study protocol was approved from the Institutional Animal Ethics Committee (IAEC).

Extraction *Boerhaavia diffusa* extracted with using solvent system 70% methanol and 30% water in Soxhlet apparatus at the temperature 40°C to 60°C.

Treatment Schedule

Groups (n= 4)	Treatment	Dosage, Route of administration
1	Distilled water (vehicle)	1 ml/kg p.o
2	Hydroalcoholic Extract	100 mg/kg p.o
3	Hydroalcoholic Extract	200 mg/kg p.o.
4	Diazepam	0.5 mg/kg i.p

n = number of animals used in each group. Treatment duration 10 days

EVALUATION OF ANXIOLYTIC ACTIVITY

Animals

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

Methods

Elevated Plus Maze Model

Elevated Plus Maze (EPM) test⁶ for studying the anxiolytic effect in rodents was used. EPM consists of two open arms (15cm x 10 cm) and two closed arms (50 cm x 10 cm x 40 cm) with an open roof and elevated at 50 cm. 1 hour after the oral administration of drugs, the rat was placed in 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 are shown in table No.01

Hole Board Test

Placing a rat is on the hole board apparatus, elevated to 45 cm from table, induced anxiety as it exposed in new environment. The anxiogenic agents reduce the no. of head poking, whereas the anxiolytic agents increased the no. of head poking.

The hole board apparatus consist of metal plate floor (40 X 40 cm) placed 25 cm above the ground. The metal plate consist six hole (1.5 cm in diameter), spaced symmetrically in a diamond pattern. A rat was placed on one corner of the apparatus and observed for the next five minutes for number of head poking⁷. The Result are shown in table No.02

Ketamine induced Sleep

Material Required- Ketamine (80mg/kg), Extract (dose A.B.), Syringe (1ml), Animals, Vehicle (distilled water). The animals were divided into four groups of similar body weight of same sex, the each groups were treated with separately with calculated dose. The animal was treated with either vehicle or extract (p.o) and after 45 minutes the animals are administered ketamine (80 mg/kg i.p) then the animals were placed in the observation table for 1hr induction of

sleep and duration of sleep was recorded⁸. The Result are shown in table No.03

Haloperidol Induced Catalepsy

Purpose and Rational Catalepsy in rats is defined as a failure to correct an externally imposed, unusual posture over a prolonged period of time. Neuroleptics which have an inhibitory action nigrostriatal dopamine system induced catalepsy^{9, 10} while neuroleptics with title or no nigrostriatal blockade produce relatively title or no cataleptic behavior. Furthermore, cataleptic symptoms in rodents have been to Parkinson-like extrapyramidal side effects seen clinically with administration of antipsychotic drugs¹¹.

Procedure

Albino rats were divided into four groups each. They were administered vehicle i.e Water by intraperitoneal route. After 30 minutes the rats were administered haloperidol 1mg/kg i.p and both the forepaws of rats were place on a wooden bar elevated 6cm 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 minutes. The cut off time was 300 seconds.

Extract treated we had taken two doses of extract (*B. diffusa*) 100 mg/kg and 200 mg/kg four animals in each group. They were administered extract 2nd group 100 mg/kg and 3rd group 200 mg/kg p.o After 45 minutes the same group of animals is treated /administered haloperidol 1mg/kg i.p and both forepaws of rats placed on wooden bar elevated 6cm above the ground. The duration for which the rats retains the forepaws on the elevated bar was noted at 0,30,60,90 and 120 minutes the cut off time was 300 seconds. The Result are shown in table No.04

RESULT AND DISCUSSION

Elevated Plus Maze Test

In the animal study we found that *Boerhaavia diffusa* plant extract significantly reduced the anxiety, the result was shown in the table (1). Two dose of extract were taken i.e. (100 mg/kg & 200 mg/kg) and observed in Elevated plus maze apparatus. The dose 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 means (100 mg/kg) was found significant as compare to the high dose of extract (200 mg/kg) shown in the Fig (1) & the value of "p" was found (P=0.008). Elevated plus maze is used to observe the Anti anxiety activity of the extract on the rats, as the dose of extract was compare from vehicle treated to 100 mg/kg time spent in open arm of a rats was increasing which shown that extract is having anxiolytic activity.

Hole Board test

Two dose of extract were taken i.e. (100 mg/kg & 200 mg/kg) and observed in Hole board apparatus. The results are shown in the table & Fig No (2). The dose of extract were compared to the control group and standard diazepam (0.5 mg/kg i.p), according to the result both of dose was found not significant & the value of "p" was found (P=0.188).

Effect on sleep duration using Ketamine

The two dose of extract + (Ketamine 80 mg/kg) were taken as stated previously i.e. (100 mg/kg & 200 mg/kg) and observed duration and latency of sleep. The two dose of extract were compared to the control group, the result was shown in the table & fig No. (3), according to the result both dose of extract significantly reduced the duration of sleep in rats. Both the doses of extract were found significant at p = 0.009.

Drug interaction with centrally acting drug

Haloperidol is a potent Neuroleptics drugs which induces catalepsy in rats. There were two doses of extract was taken for experiments as stated in material method and the result was shown in table No.(4).The two dose of extract was compare to the vehicle treated group, in different time interval or duration of time like 0, 30, 60, 90

and 120 minutes. In 0 minutes both the extract dose was not significant, but in 30 minutes duration both the dose of extract i.e. (100 mg/kg & 200 mg/kg) was found significant (p = 0.001). In 60 minutes the extract dose of (100 mg/kg) was found significant (p = 0.001) and the (200 mg/kg) was found significant. In 90 minutes both dose of extract was found significant (p= 0.001) and in 120 minutes same cases was seen that both doses of extract was found significant that means the extract in maximum time interval decreases the catalepsy with the drug haloperidol and was acting similarly like agent which is dopaminergic agonist or working as D₂ receptor agonist.

Statistical analysis

Results are expressed as mean ± S.E.M. and statistical difference were analyzed using duntt's test and results were considered significant when p<0.05.

DISCUSSION

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 anxiety¹². Extracts of *B. diffusa* increased the time spent in open sided arms of the plus-maze by the rat in the dose range 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. are reported to have anxiolytic activity^{3,4} and preliminary phytochemical screening revealed the presence of sterols, tannins and Flavonoids in the aerial parts of *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 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 model¹³, EPM procedures¹⁴ and other non punishment procedures¹⁵.

CONCLUSION

The result of the present study suggests that hydro-alcoholic extract of *Boerhaavia diffusa* plant may possess Significant Anxiolytic activity.

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Table no.1 Effect of *B. diffusa* extract on time spent in open arm

S. No	Treatment	Time spent in open arm
1	Vehicle	29.5 ± 4.21
2	Extract 100 mg/kg	77.0 ± 15.19*
3	Extract 200 mg/kg	45.5 ± 7.3
4	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 is significant.

Table No 2 Effect of extract on number of head poking in Hole board apparatus

S. No.	Treatment	No. of head Pocking
1	Vehicle	5.0 ± 0.9
2	Extract (100mg/kg)	7.75 ± 0.85
3	Extract (200mg/kg)	6.25 ± 1.1
4	Diazepam (0.5mg/kg)	7.75 ± 0.62

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

Table 3 Effect of Ketamine on duration of sleep

S. No	Treatment groups	Duration of sleep
1	Vehicle (1 ml/kg) + ketamine (80 mg/kg i.p.)	62 ± 1
2	Extract (100 mg/kg) + ketamine (80 mg/kg i.p.)	54 ± 1.95*
3	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 is significant.

Table: 4 Interaction of extract with Haloperidol at Zero min, 30 min, 60 min, 120 min.

S. No	Treatment	0min (mean ±SEM)	30 min (mean ±SEM)	60 min (mean ±SEM)	90 min (mean± SEM)	120 min (mean ±SEM)
1	Vehicle + Haloperidol (1 mg/kg)	0 ± 0	255±44.25	300±0	300 ± 0	83.5 ± 0.5
2	Extract 100 mg/kg + Haloperidol (1 mg/kg)	0 ± 0	0 ± 0*	180± 9.25*	195 ± 61.53*	255±28.2*
3	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

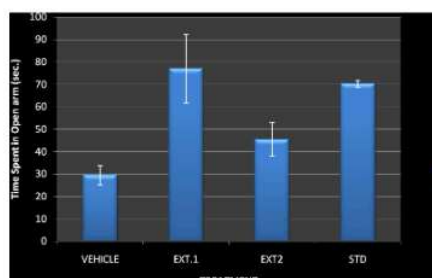


Fig. 1 Effect of *B. diffusa* extract on time spent in open arm

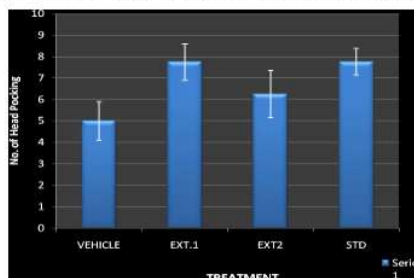


Fig. 2 Effect of extract on number of head poking in Hole board apparatus

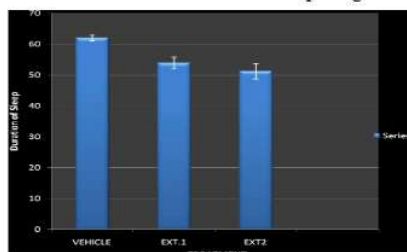


Fig. 3 Effect of Ketamine on duration of sleep