



Research Paper

Study of Muscle relaxant and Locomotor activity of an ethanol extract of *Boerhavia Diffusa* Mice

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ABSTRACT

Boerhavia diffusa (BD) is a plant of rasayana category as per ayurvedic claims. It is reported to possess antiaging, disease prevention, and life strengthening activities which hold enormous influence in disease burden and affordability/availability of healthcare in the world.

Objective. This paper has been compiled to comment on the studies reported for BD to highlight its chemical and therapeutic potential along with its muscle relaxant activity by rotarod and locomotor activity by actophotometer.

Methods. In the present paper, a detailed account of chemical constituents and pharmacological activities has been presented. All the findings were correlated with modern pharmacological activities to appraise the value of BD.

Results. Chemical analysis of BD gives a wide variety of chemical constituents, namely, rotenoids, flavonoids, xanthenes, purine nucleoside, lignans, and steroids. Various pharmacological reports emphasize its role in disorders of reproductive system, gastrointestinal system, respiratory system, urinary system, hepatic system/jaundice, cardiovascular system, and cancer.

Conclusions. The studies on the therapeutic activities of BD range from studies on crude extracts to isolated compounds. Muscle relaxant activity is present in our extract.

Keywords: -*Boerhavia diffusa* (BD) Linn, locomotor, Rota-rod, Anticonvulsant, anti-anxiety activity.

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I. INTRODUCTION

The traditional use of herbal medicines implies substantial historical use, and this is certainly true for many products that are available as 'traditional herbal medicines'. In many developing countries, a large proportion of the population relies on traditional practitioners and their medicinal plants in order to meet health care needs. Although modern medicine may exist side-by-side with such traditional practice, herbal medicines have often maintained their popularity for historical and cultural reasons. Such products have become more widely available commercially, especially in developed countries. In this modern setting, ingredients are sometimes marketed for uses that were never contemplated in the traditional healing systems from which they emerged. In Germany, for example, where herbal products are sold as 'phytomedicines', they are subject to the same criteria for efficacy, safety and quality as are other drug products. In the USA, by contrast, most herbal products in the market place are marketed and regulated as dietary supplements, a product category that does not require pre-approval of products on the basis of any of these criteria. These matters are covered extensively in Section 3 below. The pharmacological treatment of disease began long ago with the use of herbs (Schulz et al., 1001). Methods of folk healing throughout the world commonly used herbs as part of their tradition. Some of these traditions are briefly described below, providing some examples of the array of important healing practices around the world that used herbs for this purpose. Ayurveda is a medical system primarily practised in India that has been known for nearly 5000 years. It includes diet and herbal remedies, while emphasizing the body, mind and spirit in disease prevention and treatment (Morgan, 1002).

The WHO regional office for the western pacific invited a group of experts to develop criteria and general principles to guide research work on evaluating herbal medicines (WHO, 1993). This group recognized the importance of herbal medicines to the health of many people throughout the world, stating: 'A few herbal medicines have withstood scientific testing, but others are used simply for traditional reasons to protect, restore, or improve health. Most herbal medicines still need to be studied scientifically, although the experience obtained from their traditional use over the years should not be ignored. As there is not enough evidence produced by common scientific approaches to answer questions of safety and efficacy about most of the herbal medicines now in use,

Boerhaviadiffusa (BD) Linn. (Nyctaginaceae) is a well-known medicinal plant in traditional Indian medicine as well as other parts of world, for example, Southern American and African continent. Its various parts and especially roots have been used for gastrointestinal, hepatoprotective, and gynecological indications in above mentioned parts of the world and also throughout India. In ayurvedic texts, more than 35 formulations of different types contain it as major ingredient. In Ayurveda, BD has been classified as "rasayana" herb which is said to possess properties like antiaging, reestablishing youth, strengthening life and brain power, and disease prevention, all of which imply that they increase the resistance of the body against any onslaught, in other words, providing hepatoprotection and immunomodulation. BD has been widely studied for its chemical constituents and therapeutic activities. The roots are the source of a novel class of isoflavonoids known as rotenoids, flavonoids, flavonoid glycosides, xanthenes, purine nucleoside, lignans, ecdysteroids, and steroids. Various animal studies and trials have confirmed the presence of activities, for example, immunomodulation, hepatoprotection, antifibrinolysis, anticancer activity, antidiabetic activity, anti-inflammation, and diuresis. In this paper, traditional uses, chemical constituents, and reported pharmacological activities have been summarized to present the chemical and therapeutic potential of this plant. Present review also provides an ethnopharmacological appraisal of an important medicinal herb

II. MATERIAL AND METHODS

Selection of plant:- The plant selection on their availability and folk usage of the plant. The plant were chosen.

Collection of Plant Material: The Plant material of *Boerhaviadiffusa* (BD) Linn was collected from Sehore (M.P.), during the month of October 2022.

Authentication of plant:- The plant was identified and authenticated by Dr. Zia ul Hasan H.O.D. Department of Botany, Saifia Sciences College Bhopal (M.P.) and stored in the herbarium of the Institute and a specimen voucher no. 512/Bot./Saf./22 was assigned.

Defatting of plant material:- The shade-dried plant material are coarsely powdered and fats and oil removed by Soxhlation process with petroleum ether. The extraction proceeded until the substance was defatted.

Extraction by Soxhlation process:- Accurately weight 150 gram of dried powdered of aerial portion (leaf) of *Boerhaviadiffusa* (BD) Linn were extracted with Hydroalcoholic solvent using a 48-hour Soxhlation procedure, filtered and dried with vacuum evaporator at 200°C, and prepared extract was also subjected to colour, odour and consistency.

Determination of percentage yield of the extract:- The crude extract after the Soxhlation extraction process, extract was further on vacuum evaporator dried extract of aerial part of *Boerhaviadiffusa* (BD) Linn was done by using solvent Hydroalcoholic (ethanol:water, 70:30 v/v). The percentage yield of extract were calculated 20 gm.



Fig. Soxhlation of extract

Quantitative phytochemical analysis

Estimation of Total polyphenol content (TPC)

The total polyphenol content of the extract was estimated using the FolinCiocalteau reagent based assay. 5-50 µg/ml methanolic gallic acid solutions were used as standards and methanol was used as a blank. The absorbance of the developed colour was recorded at 706 nm using a UV-Vis spectrophotometer. All determinations, for gallic acid as well as the plant extract, were carried out in triplicate. Data are represented as an average of the three determinations. Using these readings, a calibrated gallic acid standard curve was made. Based on the measured absorbance of the plant extract, the concentration of phenolics was estimated (µg/ml) from the calibration line. The content of polyphenols in the extract was calculated and expressed in terms of gallic acid equivalent (mg of GAE/g of dry weight material).

Estimation of Total flavonoids content (TFC)

Total flavonoid content was based on aluminium chloride method. The 10 mg quercetin was dissolved in 10 ml methanol and various aliquots of 5,10,15,20 and 25 µg/ml were prepared in methanol. And the 10 mg of dried extract of were dissolved in 10 ml methanol and filter. 3 ml (1 mg/ml) of this solution was used for the evaluation of flavonoid. In addition, 1 ml of 2% AlCl₃ methanolic solution was added to 3 ml of extract or normal and allowed to stand at room temp. for 15 min. absorption was measured at 420 nm.

PHARMACOLOGICAL ACTIVITY

Literature reveals that Boerhaviadiffusa (BD) Linnhas been explored for its pharmacological activity

Animals

Swiss albino mice weighing between 25-35 gm are used in the experiments. The animals were placed randomly and allocated treatment group. All the experiments were performed between 9:30 to 16:30 hours to overcome diurnal and circadian variations. All the animals were housed at a temperature of 25±2⁰C and in a relative humidity of 65±5%. A 12:12 light: day cycle was followed. All the animals were housed in polypropylene cages with paddy husk as bedding with free access to water and fed with standard commercial pelleted chow (Hindustan Lever). All the experimental procedures and protocols used in this study were reviewed by institutional animal ethics committee of Vivekanand college of pharmacy , Bhopal (M.P.) proposal number IAEC/Rips/2022/03 and were in accordance with the guidelines of the IAEC.



Fig. during dosing to mice

Acute oral toxicity study.

The acute oral toxicity study was conducted according to the OECD-423 (Acute toxic class method) guidelines. Six group of mice n=6 were administered orally for 7 days with HETP (50, 300, and 1000 mg/kg) and the animals were kept under observation for mortality and any behavioural changes.



Fig. Drug dosing to mice

Effect of HETP on locomotor activity of mice on Actophotometer.

Swiss albino mice weight 25-30 gm were taken and divided in groups each consisting of 6 animals. The first group was marked as control and second as standard group. Rest two groups were marked for different doses (100 and 200 mg/kg.p.o.) HETP .The was turned on checked to make sure that all the photocell are working for accurate recording and each mice was placed individually in the activity cage for 5 minute. Basal activity score of all the animals were noted. Diazepam (2 mg/kg) was injected. and after 30 minute placed each mouse in activity case for 5 minute. Note the score, the difference in theactivity before and after diazepam treatment. Repeat the above procedure for different doses of hydroalcoholic extracts (100,200mg/kgp.o.). Percentage change in motor activity was calculated. (Kulkarni 1005)

Effect of HETP on muscle grip performance of mice on Rota-rod apparatus.

Swiss albino mice of about 30-35gm weight were taken and divided into 4 groups each consisting 6 animals. The first group was marked as controland second as standard group. Rest 2 group weremarked for different doses (100 and 200 mg/kg. p.o.) of theHETP.Rota-rod was turned on setting the speed of rotation at 22-25 rpm. The animals were placed singly one by one on rotating rod .the fall off time ,when the mouse falls from the rotating rod was noted down .

The drug (diazepam,2 mg/kg i.p.)was injected to animal of second group and after 30 minute,the above mentioned parameter was observed.after that the same procedure was followed for the test group. Comparison was made between the fall-off time of all the animal. (kulkarni,1005)



Effect of HETP on parameter of anxiety on elevated plus- maze in mice.

Swiss albino mice of about 25-35gm were taken and divided into 4 groups each consisting of 6 animals. The first group was marked as control and second as standard. Rest of 2 groups were marked for different doses of (100 and 200 mg/kg. p.o.) HETP. Animals were placed individually at the centre of the plus maze with their head facing towards the open arm and their following behaviours were noted for five minutes.

First preference of mice to open or enclosed arm.

Number of entries in open and enclosed arms (An arm entry defined as the entry of four paws into the arm).

Average time of each animal spends in each arm (Average time = total duration in the arm/number of entries).

Standard drug (Diazepam 2 mg/kg.i.p.) and different doses of HETP(100 and 200 mg.kg. p.o.) was injected to the animals of 3rd and 4th group and after 30 minutes. The above mentioned parameters were observed. Comparison were made among the preferences of the animal to open/enclosed arm. average time spent in open arm and number of entries in open arm for each group (Kulkarni. 1005).

Effect of HETP on MES induced convulsion in rat.

Swiss albino Rats of about 80-140 gm were taken for experiments. Animals were marked and divided in 5 groups. each group consisting of 6 animals. First group was marked control and second and third group were designated for standard drug treatment (Phenytoin 120 mg/kg and Phenobarbitone 45 mg/kg i.p.). Rest 2 group were marked for 2 different dose of HETP(100 and 100 mg.kg. p.o.) respectively. Care was taken to hold the animal properly. Corneal electrodes was placed on the cornea and a current of 150 mA was applied for a duration of 0.2 sec. Different stages of convulsions i.e. (a) tonic flexion. (b) tonic extensor phase. (c) clonic convulsions. (d) stupor, and (e) recovery or death was noted after electric current application. The time (sec) spent by the animal in each phase of the convulsions was noted. The same procedure is repeated with all animals of the group.

The standard drug and HETP injected to the animals of all respective groups. After 30 minutes the same current was applied for similar duration and time spent in different stages was noted. The reduction in time or abolition of tonic extensor phase of MES-convulsions for ever groups was noted (Kulkarni. 1005).



Fig. MES induced convulsion in mice

III. RESULT

Table 1: Qualitative analysis of Boerhaviadiffusa (BD) Linnhydroalcoholic extract of presence of different phytoconstituents

S.NO.	TEST	OBSERVATION	INFERENCE
1	Alkaloid		
	Wagner's reagent	Reddish brown ppt	+ ve
	Dragendorff's reagent	Reddish brown ppt	+ ve
	Mayer's reagent	Cream colourppt	+ ve
2	Glycoside		
	Keller Killiani test.	Appearance of reddish brown colored ring at the junction of two layers	+ ve
	Conc. sulphuric acid test	reddish color precipitate	+ ve
	Molish's test	Formation of reddish-purple colored ring at the junction of two layers.	+ ve
3	Steroid		
	Solkowski Test	brown or red colored ring on the sulphuric acid layer given the confirmatory test.	+ ve
	LibermannBurchard's Test	translucent green colour given the confirmatory test.	+ ve
4	Carbohydrates		
	Molisch Test	Formation of the red violet ring at the junction of the solution and its disappearance on addition of excess alkali solution indicates the presence of carbohydrates.	+ ve
	Benedict's Test	Depending on the concentration of the reducing sugar, the amount and colour of the precipitate produced varied. A positive Benedict's test appears green, yellow, orange, or	+ ve

		red.	
5	Phenolic compounds		
	Ferric chloride test	Formation of blue, green or violet colour indicates the presence of phenolic compounds.	+ ve
	Lead acetate test	Formation of white precipitate indicates presence of phenolic	+ ve
	Dilute iodine solution test	Formation of transient red colour indicates the presence of phenolic compounds	+ ve



Fig. phytochemical analysis of extract

Table:-2 Estimation of total phenolic content (TPC)

S.No.	Concentration $\mu\text{g/ml}$	Absorbance
0	0	0
1	5	0.142 \pm 0.001
2	10	0.301 \pm 0.003
3	15	0.419 \pm 0.002
4	20	0.598 \pm 0.002
5	25	0.675 \pm 0.003

Table:-2 Estimation of total flavonoid content (TFC)

S.No.	Concentration $\mu\text{g/ml}$	Absorbance
0	0	0
1	5	0.315 \pm 0.002
2	10	0.445 \pm 0.003
3	15	0.619 \pm 0.002
4	20	0.898 \pm 0.003
5	25	1.075 \pm 0.001

Table:-3 Effect of HETP on locomotor activity of mice on Actophotometer.

Groups	Dose (mg/kg)	Locomotion Score (M \pm SEM) (Min.)		% Change in locomotor activity
		Basal	After 30 min. drug administration	
Vehicle control	5 ml/kg/p.o.	195.5 \pm 10.500	-	-
Diazepam	2 mg/kg/i.p.	133.5 \pm 1.500	5.5 \pm 1.500***	95.88
HETP	100 mg/kg/p.o.	455.5 \pm 262.50	161 \pm 101.00**	64.65
HETP	200 mg/kg/p.o.	535 \pm 55.000	153.5 \pm 28.500*	71.30

Values are expressed as mean \pm S.E.M. (n = 6). Values are statistically significant at ***P<0.001, ** P<0.01, * P<0.05 vs. control group respectively (One-way ANNOVA followed by Tukey' s post hoc test).

Table:- 4 Effect of HETP on muscle grip performance of mice on Rota-rod apparatus.

Groups	Dose (mg/kg.)	Fall off time in Sec. (M±SEM)		% Change in fall off time
		Basal reaction time (M±SEM)	After 30 min. drug administration (M±SEM)	
Vehicle control	5 ml/kg/p.o.	1125 ±135.00	-	-
Diazepam	2 mg/kg/i.p.	717.5±155.50	75±25.000***	89.19
HETP	100 mg/kg/p.o.	212.5±57.500	145±55.000**	31.76
HETP	200 mg/kg/p.o.	144±4.000	72±3.000*	50

Values are expressed as mean±S.E.M. (n = 6). Values are statistically significant at***P<0.001, ** P<0.01, * P<0.05 vs. control group respectively (One-way ANNOVA followed by Tukey' s post hoc test).

Table:-5Effect of HETP on MES induced convulsion on rat.

Group	Dose mg/kg	Flexon phase in sec. (M±SEM)	Extensor phase in sec. (M±SEM)	Clonus phase in sec. (M±SEM)	Stuper phase in sec. (M±SEM)	Recovery/Death
Vehicle control	5 ml/kg/p.o.	10.5±1.500 ^{ns}	12.5±1.500 ^{ns}	22.5±1.500 ^{ns}	345±135.00 ^{ns}	Recovery
Phenytoin	120mg/kg/i.p.	Absent	Absent	12.5±0.5000**	170±10.000**	Recovery
HETP	100 mg/kg/p.o.	12.5±0.5000 ^{ns}	7.5±0.5000 ^{ns}	27±2.000 ^{ns}	187.5±17.500 ^{ns}	Recovery
HETP	200 mg/kg/p.o.	14.5±0.5000 ^{ns}	4.5±0.5000.**	17±3.000*	215±5.000**	Recovery

Values are expressed as mean±S.E.M. (n = 6). Values are statistically significant at***P<0.001, ** P<0.01, * P<0.05 vs. control group respectively (One-way ANNOVA followed by Tukey' s post hoc

IV. DISCUSSION

The hydroalcoholic extract of Boerhaviadiffusa (BD) Linnshow the presence of steroid, tannins and phenolic compounds.,alkaloids,glycoside,carbohydrate. The result are shown in table -1The Percentage yied of hydroalcoholic extract was (20 gm).This study was conducted on several central nervous system related experimental models e.g., locomotor activity, rotarod. elevated plus maze,and MES induced convulsion to investigate the possible central effect of *thespesiapopulnea* . theclassical models for screening CNS action providing information on depressant property of psychomotor performance anxiolytic and myorelaxant activity. There has been a considerable popular interest in the use of the natural remedies or herbal products to treat anxiety and depression. Recently several plants have been reported to possess anxiolytic effects in different animal models of anxiety. Various traditional herbal medicines have also been suggested to possess anxiolytic activity. The plant was found to be rich in steroidal and flavonoid content.The phytoconstituents which are responsible for many pharmacological activities.

Locomotor activity is considered as an index of alertness and a decrease in it is indicative Sedative activity. thespesiapopulnea significantly decreased locomotor activity in all the tested doses that act as a centrally acting muscle relaxant interacting with specific receptors enhancing chemical and mission (Tripathi, 1002). Decrease in locomotion reveals depressant effect on GABAergic transmission due to increase in the concentration of GABA in brain (Kumar et al., 1007).

The HETPCNS depressant the reduce grip strength and mice may fall from the rota-rod due loss of muscle or muscle coordination.. HETP decreases the fall off time ofmice from the rotating rod .Based on the exposure of animal to an elevated plus maze The fear due to height induces anxiety in the animals when placed on the elevated plus maze (EPM). The animal being exposed to the new environment tends to avoid And tries prefer to stay in closed arm due to fear (Vishwanatha et al.. 1009; Sharma et al. 1009: ilambujanaki et al., 2010). The ultimate manifestation of anxiety and fear in the animals is inhibited by decrease in the motor activity and preference to remain at safer places. Anxiolytic spent by the animal in the open arms (Sharma et al., 2011). an anxiolytic effect expressed by an increased number of open arm entries and time spent in the EPM. Diazepam produced significant increase in open arm duration and also number of entries into the open arms. Plus maze model is considered one of the most widely validated tests for assaying sedative and anxiolytic substances acting at the GABA benzodiazepine complex (Chakraborty et al., 2010). Current study data are consistent with the results of numerous previous studies, which have shown that diazepam and other benzodiazepines produce significant anxiolytic effects in a variety of anxiolytic screening procedures, including elevated plus-maze test procedures. In our finding the HETP treated.

Epilepsy is one of the most common serious neurological conditions. drugsthatinhibit voltage-dependent Na⁺ channels, such as phenytoin. The effect of HETP on MES-induced convulsion in rats are tabulated in table -6. The tonus and and extension phase was decreased in dose dependent manner. Treated group change in duration of clonus phas in all theHETP treated group was non-significant compared to the control. The animals were recovered in vehicle treated. phenytoin, and all doses of HETP.

V. CONCLUSION

pharmacological investigation of the plant Boerhaviadiffusa (BD) Linnproduce depressant action on the CNS. hydroalcoholicextract of *Thespesiapopulnea* induce, act as hypnotic, also decrease anxiety, means act as anxiolytic agent due to hypnosis. Boerhaviadiffusa (BD) Linn also exert muscle relaxant and locomotor, anti-anxiety effect of mice. it is pharmacological safe with good bioavailability with least toxicity Boerhaviadiffusa (BD) Tectona. This review gives some phytochemicals as well as the detailed pharmacological information of Tectonagrandis. The main focus on the pharmacological potentials of Boerhaviadiffusa (BD) Linn which is very helpful to researcher to add more about this valuable plant. Apart from this still there are few options to investigate the unexplored potential of plant based on its uses. The active constituent needs to be isolate and should be considered for further in-vivo or in-vitro studies to confirm the traditional claims and to explore the potential of development of drug.

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