



**EVALUATION OF DIURETIC AND NATRIURETIC ACTIVITY OF ETHANOLIC
EXTRACT OF *BOERHAVIA DIFFUSA* LINN ROOT IN ALBINO WISTAR RATS**

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ABSTRACT

Boerhavia diffusa commonly known as punarnava is a perennial herbaceous plant belonging to family Nyctaginaceae. The medicinal value of this plant in the treatment of a large number of human ailments is mentioned in Ayurveda, Charaka Samhita, and Sushruta Samhita. The aim of present study was to evaluate diuretic and natriuretic activity of ethanolic extracts of *Boerhavia diffusa* root in albino wistar rats. The results of preliminary phytochemical evaluation revealed the presence of Alkaloids, Glycosides, Flavonoids, Proteins, amino acids, Carbohydrates, Steroids, Fats and oils, Tannins and phenols. In acute oral toxicity, animals didn't show any visible signs of morbidity and mortality. Four groups of Albino wistar rats were used to evaluate diuretic activity of ethanolic extract of roots of *Boerhavia diffusa* by using Lipschitz test model. Oral administration of *Boerhavia diffusa* at doses 100 mg/kg and 200 mg/kg produced a significant ($P < 0.01$) increase in the urine volume when compared to control and standard. Determination of urinary electrolyte concentration in urine was done by using a flame photometer. It has revealed that ethanolic extract of *Boerhavia diffusa* roots was effective in increasing urinary sodium concentration. The results were analyzed by One Way Analysis of Variance (ANOVA). Thus, in conclusion the *Boerhavia diffusa* has a significant diuretic and natriuretic action in rats.

KEYWORDS: Diuretic, Natriuretic activity, Ethanolic extract, Lipschitz test, *Boerhaavia diffusa*, Furosemide.

INTRODUCTION

Diuretics are substances or drugs that produce a deficit of water and sodium from the body by net activity on renal system. Diuretics increase the quantity of sodium ions in urine. Their predominant effect is to reduce the reabsorption of chloride and sodium from glomerular filtration, become greater loss of water being secondary to the enhanced elimination of salt.

This phenomenon plays important role to regulate the formation of body fluids in a several classes of clinical conditions, which includes edematous states such as treatment of heart failure with thiazide diuretics, treatment of kidney disease and renal failure with loop diuretics, treatment of hepatic cirrhosis with potassium sparing diuretics. Secondly nonedematous states such as treatment of diabetes insipidus, hypertension and nephrolithiasis with thiazide diuretics, treatment of hypercalcaemia with loop diuretics, and treatment of glaucoma with carbonic anhydrase inhibitors. There are many classes of diuretics are available but they adversely affect quality of life including impotence, fatigue and weakness.^[1,2]

Boerhavia diffusa (Nyctaginaceae) plant is a perennial species growing prostrate or ascending upward in

habitats. Genus *Boerhavia* is found in Ceylon, Australia, and Sudan, extending to China, Africa, America and Islands of the Pacific. Among 40 species of *Boerhavia*, 6 species are found in India, namely *B. diffusa*, *B. erecta*, *B. rependa*, *B. chinensis*, *B. hirsute* and *B. rubicunda*. The plant is mentioned in the Atharvaveda with the name 'Punarnava'. The plant has gained lot of importance in the field of phytochemistry because of its various pharmacological and biological activities such as immunomodulatory effects, immunosuppressive activity, antioxidant activity, antidiabetic activity, analgesic and anti-inflammatory activity, antibacterial activity, antistress activity, hepatoprotective activity, anti-viral activity, bronchial asthma, anti fibrinolytic activity, chemopreventive action, and anticonvulsant activity. The roots are reputed to be laxative and are given for the treatment of anasarca, ascites and jaundice.^[3,4]

MATERIALS AND METHODS

Plant Collection and Authentication: The fresh roots of *Boerhavia diffusa* were purchased from local traders in the month of February, 2019. The roots of *Boerhavia diffusa* Linn were botanically authenticated as *Boerhavia diffusa* Linn. Roots (Family: Nyctaginaceae). The properly cleaned and dried sample of *Boerhavia diffusa* root was grinded by mechanical grinder. The coarse

powdered material was stored in an air tight container at room temperature for further investigation.^[5]

Extraction of Phytoconstituents of Plant: The ethanolic extract of *Boerhavia diffusa* roots was prepared by using soxhletation. About 100gm powder material of *Boerhavia diffusa* roots was taken in soxhlet apparatus along with about 300ml of 90% v/v ethanol. The extraction process was continued for 12 - 14 h at 60°-70°C until the solvent in the thimble become clear or the appearance of colorless solvent in the side tube. After complete extraction the *Boerhavia diffusa* root extract was evaporate at temperature of 70°C on a water bath and maintained physicochemical properties.^[6] 14 gm solvent free semisolid dark brownish extract was obtained. The resulting extract was weighed and stored in well closed air tight containers for further investigation.

Phytochemical Investigation: Chemical test for *Boerhavia diffusa* root extract have been performed for the detection of Alkaloids, Glycosides, Flavonoids, Proteins, amino acids, Carbohydrates, Saponins, lipids, Steroids, Tannins, phenols Fats and oils using standard procedures.^[7]

Animals: Healthy Albino Wistar rats of either sex and of approximately the same age, weighing about 180-200 g were kept under standard conditions in the animal house. All the experimental protocols were approved by the Institutional Animal Ethical Committee. (Approval No. 1888/PO/Re/S/16/CPCSEA/2019/02) and were carried out strictly in accordance with the norms of CPCSEA, New Delhi.

Drugs and Chemicals: furosemide tablets (frusenex, geno pharmaceuticals Ltd), Ethanol, Deionised water and Carboxymethyl cellulose (CMC) etc.

Acute Oral Toxicity Study: Healthy Female Albino Wistar rats weighing between 180 to 200 g were used in the study. Acute oral toxicity was performed as per OECD-423 guidelines. The starting dose of 5 mg/kg of ethanolic extract was administered orally to three animals in each group. Mortality was not observed; the procedure was repeated for further higher doses such as 50, 100, 200, 400, 1000 and 2000 mg/kg body weight. Animals were observed for the symptoms related to toxicity such as any visible signs of toxicity, aggressive behavior, stress, and ataxia and other postural changes, diarrhea, dermal irritation etc.^[8]

Evaluation of Diuretic Activity

Purpose and Rationale: A method for testing diuretic activity was performed in rats using Lipschitz test. This method was based on water and sodium excretion in test animals and compared to rats treated with a high dose of furosemide. The "Lipschitz- value" was the quotient between excretion by test animals and excretion by the furosemide control.

Drug Administration: The animals were divided into 4 groups of 5 animals each and treated with single dose of the following drug orally with the help of oral feeding tube in a volume of 1 ml. All the test & standard drug were administered orally 30 minutes prior to test.

Group 1 (Control): 10 ml/kg body weight, normal saline in 2% CMC suspension.^[9]

Group 2 (Standard): 10 mg/kg body weight, standard drug Furosemide in 2% CMC suspension.^[9]

Group 3 (Test 1): 100 mg/kg body weight, plant extracts in 2% CMC suspension.

Group 4 (Test 2): 200 mg/kg body weight, plant extracts in 2% CMC suspension.

Procedure: The diuretic activity of ethanolic extract of roots of *Boerhavia diffusa* in Wistar albino rats were studied by the Lipschitz Test using metabolic cage.^[9-11] Before the standard and test treatment all the animals were hydrated with physiological saline (0.9% NaCl) at a dose of 15 ml/kg body weight by gavage. Five animals per group were placed in metabolic cages. Metabolic cages were especially designed to separate urine and faeces and keep at 21°C±0.5°C. After that the total volume of urine was collected from control, standard and extracts treated groups after 5 hours and urine volume was measured by standard measuring cylinder. Urinary pH was estimated using a calibrated pH meter. The urine volume was again collected after 24 hours in beakers from all four groups and subjected for urinary analysis especially natriuretic activity.

Evaluation of Natriuretic Activity: Before estimating the Sodium and Potassium content, the fresh urine samples from control, standard and extract treated groups of animals were diluted with deionized water. Before dilution, the fresh urine samples were filtered to remove debris and shedding. Estimation of Sodium and Potassium content of the fresh urine samples from all four groups of animals were done by using a flame photometer.^[10-11] Natriuretic activity was expressed in value of the ratio Na⁺/K⁺.

Statistical Analysis: The data was expressed as mean ±SEM and statistically analysed using one way ANOVA followed by Dunnett's test. The results were considered statistically significant when p< 0.05.

RESULTS

Phytochemical Studies:- The results of preliminary phytochemical evaluation performed on crude extracts of *Boerhavia diffusa* Linn roots revealed the presence of Alkaloids, Glycosides, Flavonoids, Proteins and amino acids, Carbohydrates, Steroids, Fats and oils, Tannins and phenols. Saponins and lipids were absent in the extracts of *Boerhavia diffusa* Linn roots.

Acute Toxicity Study:- In oral acute toxicity study with ethanolic extracts of *Boerhavia diffusa* Linn roots no mortality was observed during the period of 14 days even

with the higher dose tested i.e. 2000 mg/kg and none of the animals showed any symptom related to toxicity such as behavioral changes, convulsions, diarrhea, autonomic or physical changes and neurological changes. Oral administrations of *Boerhavia diffusa* Linn roots were found to be safe for the present study.

Evaluation of Diuretic and Natriuretic Activity: The present investigation focused on pharmacological evaluation of ethanolic extract of roots of *Boerhavia diffusa* for diuretic and natriuretic activity at doses of 100 mg/kg and 200 mg/kg body weight, orally. The diuretic activity was carried out as per the method of Lipschitz et. al.

Effect on Urine Volume: The animals were observed normal with no signs of dehydration at 12 h intervals. The effect of the ethanolic extract of *Boerhavia diffusa* linn roots on the urination of rats was observed for 5 h which showed that the ethanolic extract has a significant dose dependent diuretic effect in test animals compared with the control group. At the end of 24 hr urine output was a significant ($p < 0.01$) with standard and test drug treated groups compared to control groups. The pH of urine samples of all the groups were as shown in the Table-1.

Table 1: Effect of ethanolic extract of *Boerhavia diffusa* linn roots on urine volume in wistar rats.

S. No.	Treatment group	Dose (mg/kg)	Urine volume (ml)		pH of the urine
			5hr	24hr	
1	Group- I (normal)	10ml/kg, orally	0.9	3.30±0.069	6.94
2	Group-II (Standard)	10mg/kg, orally	1.52	8.27±0.094**	6.10
3	Group- III (Test I)	100mg/kg, orally	1.12	4.95±0.168**	6.89
4	Group - IV (test - II)	200mg/kg, orally	1.50	6.90±0.233**	6.34

Values expressed as \pm S.E.M. * $p < 0.05$, ** $p < 0.01$; when compared with the control group.

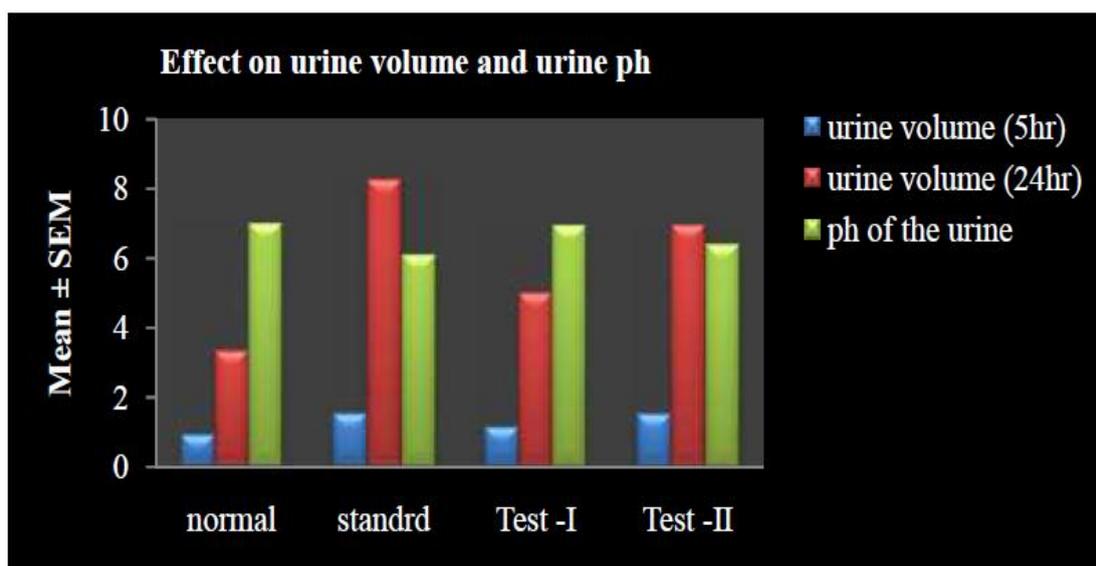


Fig no. 1: Showing Effect of *Boerhavia diffusa* linn roots extract on urine volume and urine pH on experimental Rats.

Diuretic index of control, standard and extract treated groups are 1.11, 2.50, 1.5 and 2.09 respectively. Whereas diuretic activity of standard and extract treated groups are 1, 0.60 and 0.83 respectively. The diuretic index value > 1.50 , shows a good diuretic activity, whereas the diuretic index values ranging from 1.00–1.50 and 0.72–0.99 demonstrate moderate and mild diuretic activity, respectively.

In the present study, the diuretic activity values of the two maximum doses of test extract (0.63 and 0.83) were as closer to standard drug (1.00). The Lipschitz value of standard and extract treated groups are 1.00, 0.59 and

0.83 respectively. The percent urine excretion of control, standard and extract treated groups are 4.40, 9.18, 5.50 and 7.66 percent respectively. The results show the good diuretic action of *Boerhavia diffusa* linn roots extract as compared to standard group.

Table 2: Effect of ethanolic extract of *Boerhavia diffusa* linn roots on urinary excretion and related parameters in wistar rats.

S.No.	Treatment group	% urinary excretion	Diuretic action	Diuretic index	Diuretic activity	Lipschitz value
1	Group- I (normal)	4.40	1.00	1.11	--	--
2	Group-II (Standard)	9.18	2.08	2.50	1.00	1.00
3	Group- III (Test - I)	5.50	1.25	1.5	0.60	0.59
4	Group - IV (test - II)	7.66	1.74	2.09	0.83	0.83

- % urinary volume excretion = volume excreted/ volume administered \times 100
- Diuretic action = urinary excretion of treatment group/ urinary excretion of control group.
- Diuretic activity = diuretic action of test drug/ diuretic action of standard drug
- Diuretic Index=Mean urine volume of test/Mean urine volume of control.
- Lipschitz value = Mean urine volume of test/Mean urine volume of standard.

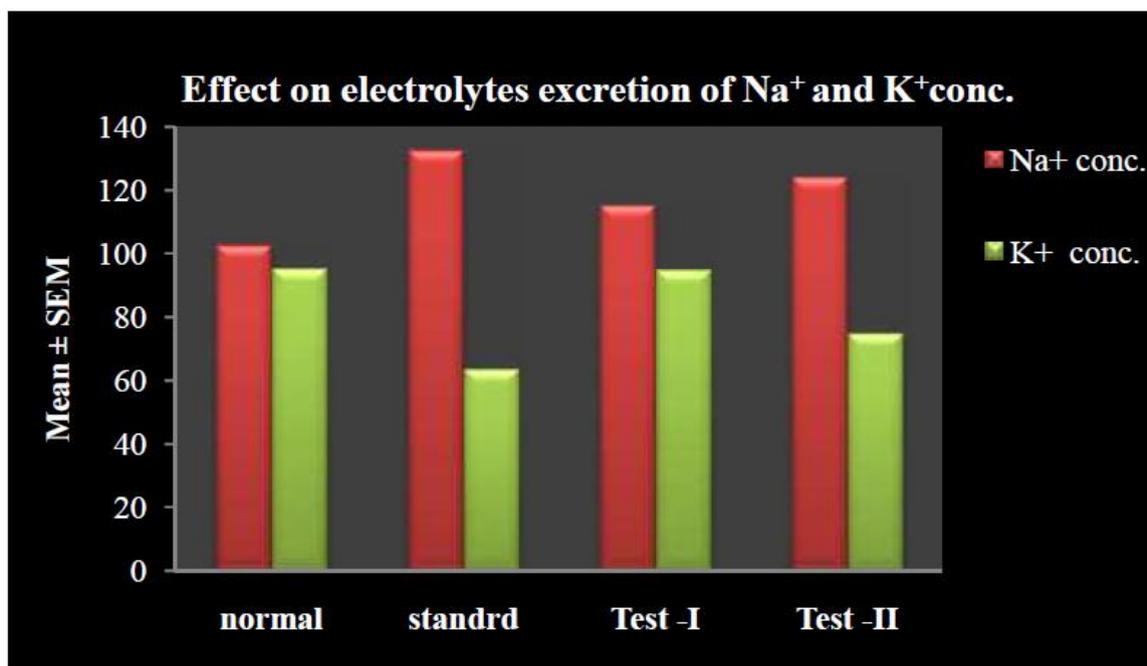
Effect on Urinary Electrolyte Excretion: - The urinary electrolytes excretion was calculated at the end of 24 hr urine collection. The amount of Urinary Na⁺ excretion in all test groups was significantly increased when compared with control. The amount of Urinary K⁺ excretion was significantly decreased in Ethanolic extracts treated groups when compared with control.

Determination of urinary electrolyte Concentration revealed that ethanolic extract of *Boerhavia diffusa* roots was effective in increasing urinary Na⁺ excretion which was comparable to that of furosemide. The ratio Na⁺/K⁺ was calculated for Natriuretic activity. It was revealed that the extract treated groups shown favourable Natriuretic effect.

Table 3: Effect of ethanolic extract of *Boerhavia diffusa* linn roots on 24h urinary electrolytes concentration excretion in wistar rats.

S.No.	Treatment group	Urinary electrolytes conc. (mmol/L) on 24 hr. urine		Natriuretic activity (Na ⁺ /K ⁺)
		Na ⁺ conc.	K ⁺ conc.	
1	Group- I (normal)	102.52 \pm 1.142	95.25 \pm 0.853	1.07
2	Group-II (Standard)	132.52 \pm 1.191**	63.56 \pm 1.066**	2.08
3	Group- III (Test - I)	114.52 \pm 2.555**	94.36 \pm 0.823**	1.21
4	Group - IV (test - II)	123.51 \pm 0.983**	74.45 \pm 1.615**	1.65

Values expressed as \pm S.E.M. *p<0.05, **p<0.01; when compared with the control group.

**Fig. 2: Showing effect of *Boerhaavia diffusa* roots extract on electrolytes excretion of Na⁺ and K⁺ conc. on experimental Rats.**

DISCUSSION

The diuretics are generally defined as substances which increase the excretion of urine by acting in the kidney and support the elimination of electrolytes. Substances which increase the urinary output are called diuretics and the substances which increase the sodium excretion are called natriuretics. These diuretics and natriuretic substances act generally on the different parts on the cells of the nephron and increase the formation of urine.^[1,2] Clinically, Diuretics are used in the treatment of many life threatening diseases such as, hypertension, congestive heart failure, liver cirrhosis, edema, influenza, nephritic syndrome, poisoning, pregnancy toxemia, renal failure and certain kidney diseases.^[1,2]

In this present study Furosemide was taken as standard diuretic for comparing the pharmacological responses. Furosemide is substances that enter the tubular lumen firstly through secretion into the proximal tubule. They reduce Na⁺ reabsorption by blocking the Na⁺, K⁺ importer located in the apical membrane of these cells. An herbal plant contains different types of chemical constituents which are responsible for many types of therapeutic activity and a particular chemical constituent are responsible for a particular therapeutic effect. Recent studies have shown that *Boerhavia diffusa* linn roots contain Alkaloids, amino acids that are responsible for diuretic and natriuretic activity.

Acute oral toxicity studies did not show any physiological, behavioral and neurological changes at 2000mg/kg maximum dose as well. In the present study, the diuretic index values of the two maximum test doses (1.5, 2.09) were as closer to standard drug (2.50) and > 1.50 which suggest that the chemical constituents of the *Boerhavia diffusa* Linn roots responsible for good Diuresis effect. Moreover analysis of 24 hr urinary excretion showed a significant increase in the Na⁺ and decrease K⁺ excretion in both the test groups. The natriuretic activity of *boerhaavia diffusa* at a dose of 200 mg/kg (1.65) extract was relatively close to furosemide (2.08). As a result, it is suggest that the *boerhaavia diffusa* roots extract at maximum doses might have a furosemide-like action.

CONCLUSION

From the study, it is concluded that the *Boerhavia diffusa* Linn roots used by tribal's traditionally showed significant diuretic activity. In this present study the ethanolic extract of *Boerhavia diffusa* Linn roots contain certain active phytoconstituents that may be responsible for the diuretic and natriuretic activity. From the above observations and results proved that single dose of ethanolic extract of roots of *Boerhavia diffusa* Linn 100 and 200 mg/kg and standard Furosemide (10 mg/kg) increased the urinary volume along with an increase in Sodium concentration in urine. Hence from overall investigation it can be concluded that ethanolic extract of *Boerhavia diffusa* Linn roots has got significant potential to be utilized as a diuretic and natriuretic substances and

it may be due to presence of chemical constituents such as amino acids and flavonoids etc. but further studies are needed to isolate these pharmacologically active principles and to find out their exact mechanism of action.

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