

Evaluation of Diuretic activity of an Alcoholic extracts of *Boerhaavia diffusa* and *Anisochilus carnosus* in Rats

P Venkatesh^{1*}, A Dinakar², N Senthilkumar³

¹P.Rami Reddy Memorial College of Pharmacy, Department of Pharmaceutical Chemistry, Kadapa-516 003. Andhra Pradesh-India.

²Sun Institute of Pharmaceutical Education and Research Centre, Nellore-524 346. Andhra Pradesh-India.

³JKK Munirajah Medical Research Foundation-College of Pharmacy, B.Komarapalayam-638 183. Tamilnadu-India.

Abstract

The present study was undertaken to evaluate diuretic effect of alcoholic extracts of stem and leaves of *Boerhaavia diffusa* (AEBD) and leaves of *Anisochilus carnosus* (AEAC) in normal rats. The extracts were administered to experimental rats orally at doses of 150 & 300mg/kg of AEBD and 200 & 400mg/kg of AEAC. Furosemide was used as a standard drug at a dose of 20mg/kg in the present study. The diuretic effect was evaluated by measuring urine volume, sodium and potassium content in urine. Urine volume was significantly increased by the doses of AEBD and AEAC in comparison to control group. While the excretion of sodium also increased by the test drugs. The diuretic effect of the extracts was comparable to that of standard drug. Hence the present study provides a quantitative basis for explaining the folkloric use of *Boerhaavia diffusa* and *Anisochilus carnosus* as a diuretic agent.

Key words:

Boerhaavia diffusa, *Anisochilus carnosus*, Diuretic, Furosemide, Sodium, Potassium.

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*Corresponding author, Mailing address:

P. Venkatesh, M.Pharm.,
Associate Prof., Dept. of Pharmaceutical Chemistry
P. Rami Reddy Memorial College of Pharmacy,
1-35-1, Prakruthi Nagar,
Kadapa-516 003. Andhra Pradesh-India.
E.mail: pharmacy_pv@yahoo.co.in

Introduction

Diuretic is defined as a chemical that increases the rate of urine formation. The primary action of most diuretics is the direct inhibition of sodium transport

at one or more of the four major anatomic sites along the nephron where sodium reabsorption takes place. Because the sodium-transport systems at each of these locations are unique, there is a different set of relatively rigid structural features that a diuretic must possess in order to inhibit sodium reabsorption at each site. The primary and secondary effects induced by a diuretic determine its excretion pattern. A diuretic usually possesses some combination of natriuretic, chloruretic, saluretic, kaliuretic, bicarbonuretic, and calciuretic properties depending on whether it enhances renal excretion of sodium, chloride, sodium chloride, potassium, bicarbonate, or calcium respectively [1].

Boerhaavia Diffusa belongs to the family Nyctaginaceae, which is commonly known as Horse-purslane, Hogweed and Pig weed in English. Atikimamidi, Atima mamidi, Punarnava in Telugu. It is a diffuse herb with stout root-stocks. Leaves are thick-chartaceous in unequal pairs, ovate or elliptic-oblong, subfleshy. Anthocarps club-shaped, 5-ribbed, glandular hairy, top rounded; seeds erect. It is commonly distributed weed along roadsides, fields and waste places throughout the Chittoor district of Andhra Pradesh, India. The whole plant is used for the treatment of jaundice, dyspnoea, constipation, arthritis, anaemia, cardiac diseases and liver diseases.

Anisochilus Carnosus belongs to the family Lamiaceae, which is commonly known as thick-leaved lavender in English. Saugudu ganapa, ritchurodda and karpuravalli in Telugu. It is an annual erect herb, stems quadrangular, sparsely pubescent, brownish from prolonged exposure to sun. Leaves fleshy, broadly ovate, deeply crenate, obtuse or acute, base rounded, verrucose above, and pubescent beneath. It is commonly distributed in rock crevices on hills. On the way from Papanasam to Kumaradhara theertham (tirumala), dhanambanda area in Talakona. The whole plant used as diaphoretic, stimulant, expectorant, liver disorders,

cough and cold. Leaf used for cough, dropsy, indigestion and sores in the leg fingers [2].

Antibacterial activity of *Boerhaavia diffusa* L. leaves [3], Chemopreventive action of *Boerhaavia Diffusa* on DMBA-induced skin carcinogenesis in mice [4], anti-ulcer activity of *Anisochilus carnosus* leaf extract in pylorus ligated rats [5] has been reported. A detailed literature reviews indicated that, the diuretic activity of stem and leaves of *Boerhaavia diffusa* and leaves of *Anisochilus carnosus* has not been clinically evaluated so far. In the present study, the diuretic activity of alcoholic extracts of *Boerhaavia Diffusa* and *Anisochilus Carnosus* in rats is reported.

Materials and methods

Plant material

The stem and leaves of *Boerhaavia Diffusa* and leaves of *Anisochilus Carnosus* were collected from Sri Venkateswara University campus, Tirumala gardens of Chittoor district of Andhra Pradesh and the same were authenticated by Assistant Professor, Dr.K.Madhava Chetty, Department of Botany, S.V.University, Tirupati, AP. Voucher specimens were deposited at department of pharmacognosy for further reference.

Extraction and Phytochemical screening

The shade dried plant materials were reduced to moderately coarse powder and extracted successively with alcohol using Soxhlet apparatus after defatting. The prepared extracts were subjected to identify the presence of various phytoconstituents [6,7].

Experimental animals

Male wistar albino rats weighing between 200-250gm were obtained from Venkateswara Enterprises, Bangalore, Karnataka, India. The animals were housed under standard environmental conditions as per the rules and regulations of the

Institutional animal ethics committee. Experimental protocols for the pharmacological and toxicity studies were reviewed and approved by the Institutional animal ethical committee (1423/PO/a/11/CPCSEA).

Acute toxicity studies

Acute toxicity studies were performed for the extracts of AEBD and AEAC as per stair case method [8]. For the diuretic activity studies, the amount of dose administered were adjusted on the basis of observation during the toxicity studies.

Experimental Protocol [9-11]

The method of Lipschitz et al., was employed for the assessment of diuretic activity. The rats were divided into six groups of six rats each. Of these groups of animals, the first group served as a control and is feed with normal saline orally (25ml/kg). Second group of animals received the same amount of normal saline in which Furosemide at a dose of 20mg/kg is dissolved. The other groups received normal saline orally (25ml/kg) in which the test drugs are dissolved. Group three animals received 150mg/kg of AEBD, group four animals received 300mg/kg of AEBD, group five animals received 200mg/kg of AEAC and group six animals received 400mg/kg of AEAC. All the drugs were freshly prepared prior to administration. Each of these preparations is given in such manner so that the fluid intake is the same in all cases.

Immediately after administration, the animals were placed in metabolic cages (each animal per cage), specially designed to separate urine and faeces, kept at 25°C±0.5°C. The volume of urine collected was measured at the end of 24 hrs. During this period, no food and water was made available to animals. By pulling at the base of the tail, all the urine was expelled from the bladder. The urine samples are analyzed thereafter for Na⁺ and K⁺ concentration by flame photometric method.

Statistical Analysis

Experimental results were expressed as mean±SEM (n=6). Statistical analysis was performed with one way ANOVA.

Results and Discussion

The results of the evaluations carried out on the extracts are listed in table-1 and table-2. Table-1 shows the urinary volume and diuretic index while table-2 shows the electrolyte (Na⁺ and K⁺) content of the animals.

Table-1 shows, the reference diuretic Furosemide, significantly increased the urine output when compared to control (p<0.01), the diuretic index being 3.18. The test drugs AEBD 150mg/kg and 300mg/kg showed a significant increase in the urine volume with a dose dependent increase in the diuretic index to 1.49 and 2.84, whereas the AEAC 400mg/kg showed the diuretic index of 1.65.

Table 1: Effect of AEBD and AEAC on urine volume in Rats

Groups	Urine Volume (ml)	Diuretic Index
I Control	3.08±0.26	---
II Standard	9.80±0.39**	3.18
III AEBD 150mg	4.58±0.36*	1.49
IV AEBD 300mg	8.75±0.32**	2.84
V AEAC 200mg	3.25±0.29	1.05
VI AEAC 400mg	5.07±0.37**	1.65

Each value represents the mean±SEM, n=6. * p<0.05, ** p<0.01, Statistical significant test for comparison was done by ANOVA, followed by Dunnett's test. Groups II to VI are compared with group I.

Table-2 Shows the urinary electrolyte content following administration of the extracts. The dose of AEBD 300mg/kg and AEAC 400mg/kg produced a significant increase in Na⁺ excretion, compared with the control group (p<0.01). Only the standard drug and AEBD 300mg/kg dose produced significant increase in K⁺ excretion (p<0.01). The

doses of AEBD 150mg/kg and AEAC 400mg/kg showed a significant of $p < 0.05$.

Table 2: Effect of AEBD and AEAC on urinary electrolyte excretion

Groups	Na ⁺ (mEq/L)	K ⁺ (mEq/L)	Na ⁺ /K ⁺ ratio
I Control	60.17±2.23	53.83±1.74	1.1178
II Standard	97.50±2.17**	78.16±2.62**	1.2474
III AEBD 150mg	71.00±1.88*	62.00±2.13*	1.1452
IV AEBD 300mg	93.33±2.98**	74.66±1.97**	1.2501
V AEAC 200mg	65.67±2.60	55.33±2.39	1.1869
VI AEAC 400mg	79.00±2.69**	63.66±1.85*	1.2409

Each value represents the mean±SEM, n=6. * $p < 0.05$, ** $p < 0.01$, Statistical significant test for comparison was done by ANOVA, followed by Dunnett's test. Groups II to VI are compared with group I.

Conclusion

From the above results, it is concluded that *Boerhaavia Diffusa* and *Anisochilus Carnosus* used by tribal's traditionally showed significant diuretic activity. The experimental evidence obtained in the laboratory model could provide a rationale for the traditional use of this plants as diuretic.

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