Academic Sciences International Journal of Pharmacy and Pharmaceutical Sciences

ISSN- 0975-1491 Vol 5, Suppl 3, 2013

Research Article

DIURETIC ACTIVITY OF RHYNCHOSIA BEDDOMEI LEAVES EXTRACT IN RATS

M.CHINNA ESWARAIAH*, A. ELUMALAI, NARASIMHA ROA, PRASANTHI P AND CHINNAM PRAVEEN KUMAR

Department of Pharmacognosy, Anurag Pharmacy College, Ananthagiri (v), Kodad (M), Nalgonda (Dt), Andhra Pradesh, India, 508206.
Email: eswarphd@gmail.com, malairx@gmail.com

Received: 22 May 2013, Revised and Accepted: 14 Jun 2013

ABSTRACT

Objective: The present study was undertaken to explore the diuretic effect of ethanol extract of leaves of *R. beddomei*.

Method: Ethanol extract of *R. beddomei* leaves were administered to experimental rats orally at doses of 150 and 300 mg/kg p.o. Furosemide (10 mg/kg) was used as positive control in study. The diuretic effect of the extract was evaluated by measuring urine volume, sodium and potassium content and pH.

Result: Urine volume was significantly increased by ethanol extract in comparison to control group. While the excretion of sodium and potassium was also increased at a dose of 300 mg/kg. There was no significant change in the pH of urine after administration of the *R. beddomei*. The diuretic effect of the ethanolic extract was comparable to that of the reference standard (Furosemide) and possesses an advantage of a potassium-conserving effect.

Conclusion: We can conclude that aqueous and methanol extracts of *R. beddomei* produced notable diuretic effect which appeared to be comparable to that produced by the reference diuretic Furosemide. The present study provides a quantitative basis for explaining the folkloric use of *R. beddomei* as a diuretic agent.

Keywords: *R. beddomei,* Furosemide, Diuretic activity.

INTRODUCTION

Diuretic agents have very wide application in the treatment of various chronical diseases associated with edema. They are generally prescribed for the treatment of hypertension, congestive heart failure, glaucoma, diabetes insipidus and liver aliments. The modern era of diuretic therapy began in 1949 when sulphanilamide was discovered to possess diuretic and natriuretic properties [1]. The genus *Rhynchosia* belongs to the family Fabaceae (Leguminosae). It is known as Adavi-kandi, Adavivuluva, Vendiaku, Vendaku and Vendichettu.It is mainly founds in Eastern Ghats of Andhra Pradesh, India. The chief constituent of leaves are flavonoid compounds viz., Flavones, flavonols and flavanones. It also contains Alkaloids, Glycosides, Fatty acids, Lignans, Triterpenoids and Essential oil. It possesses as abortifacient, antibacterial, antifungal, diabetic and hepatoprotective properties. The leaves are also used for wounds, cuts, boils and rheumatic pains by Adivasi tribes [2] [3] [4]. However, no systematic pharmacological studies have been carried out in order to confirm its diuretic activity. Hence, in the present study diuretic activity of *R. beddomei* was investigated to justify the rationale behind using this plant as diuretic in hypertension. The present investigation was undertaken to confirm traditional medicinal use of the plant.

MATERIAL AND METHODS

Plant material

The *R. beddomei* leaves were collected from medicinal garden of Anurag Pharmacy College. The plant and plant material were identified and authenticated by Department of Pharmacognosy, Anurag Pharmacy College and Voucher herbarium specimens was deposited in the Department of Pharmacognosy of our College. The plant material was dried in sun shade, pulverized, passed through sieve no.40 and stored in air tight container and used for further extraction.

Processing of Plant Material

Dried coarse powder (40-mesh) leaves (500g) of *R.beddomei*, was placed in a glass stoppered conical flask and macerated with 200ml ethanol shaking frequently, and then allowing it to stand for 24 hours. Filter it rapidly through what man No. 1 filter paper. The extract was concentrated to ¾ of its original volume by rotary evaporator. The concentrated extracts were taken in a china dish and evaporated on a thermostat controlled water bath till it forms a thick paste and dried over a desiccator to obtain greenish brown colored residue (12.3% w/w) was subjected to preliminary phytochemical analysis.

Drugs and Chemicals

All the drugs, chemicals, and reagents were procured from S.D. Fine Chemicals, (Mumbai, India). All the chemicals were of analytical grade.

Acute Toxicity Studies

Healthy albino mice of either sex weighing 25-30g, maintained under controlled conditions of temperature (20 –25^{0}C) and humidity (55%) were used for toxicity study as per Up & Down or Staircase method. The maximum no-lethal and the minimum lethal dose are thus determined using only about 10 mice, once the approximate LD50 or the range between the maximum non-lethal and minimum lethal dose is found, a final and more reliable LD50 assay is planed using at least 3 or 4 dose levels within this range with longer number of animals in each group. LD50 is expressed in term of mg/kg. The maximum no-lethal dose was found to be 5000mg/kg body weight; hence 1/10th of the dose was taken as effective dose (500mg/kg body weight) for the ethanol extract of *R. beddomei* leaves for diuretic activity.

Evaluation of diuretic activity

Treatment

Albino Wistar male rats (200-250g) procured from CPCSEA approved breeder were used for diuretic studies. Animals were kept at room temperature (26 ± 2°C) for one week to acclimatize to laboratory conditions before starting the experiment; they were given free access to water and standard rat feed but 18h prior to the experiment, the rats were deprived of food but water ad libitum.

Diuretic Activity

Male healthy Wistar albino rats (200-250g) were divided into different groups of six animals each. All the animals received priming dose of 0.9% sodium chloride solution (20 ml/kg body weight p.o.). The first group received vehicle saline (20 ml/kg body weight p.o.), served as control; the second group received the standard drug Furosemide (10 mg/kg body weight p.o.), served as standard. The third group received *R. beddomei* in a single dose (150 mg/kg body weight p.o.) and fourth group received the *R. beddomei*

in a single dose (300 mg/kg body weight p.o.) suspended in normal saline. After oral administration, each animal was placed in an individual metabolic cage specially designed to separate faeces and urine at room temperature. The volume of urine collected was measured at the end of 5 hr and the total urine volume and concentrations of Na+, K+ and Cl− in the urine were determined [5] [6]. The concentration of the electrolytes in urine were expressed in terms of mmol/L and the urine volume was expressed in ml/5 h. Na+ and K+ concentrations were measured by Flame photometer and Cl- concentration was estimated by titration with silver nitrate solution (N/50) using 3-5 drops of 5% potassium chromate as an indicator. The ratio of the concentration of Na+/K+ at the end of 5 h, were calculated to assess the diuretic potential of *R. beddomei*.

Statistical Analysis

The values were expressed as mean ± SEM. The results were analyzed by using ANOVA followed by Dunnett's t-test. Statistical significance on comparison with standard drug and control group are indicated by *mark *P<0.01, was considered significant.

RESULTS

The results of different diuretic parameters are shown in Table 1. Furosemide treated animals significantly ($p < 0.01$) increased the urinary output (by 87%) and electrolyte excretion of Na+ (by 52%), K+ (by 85%) and Cl− (by 36%) as compared to control. Ethanol extract treated animals significantly ($p < 0.01$) increased the urinary output (by 73%) and electrolytic excretion of Na+ (by 46%) and K+ (by 72%), without significant renal excretion of Cl− as compared to control. The observed Na+/K+ ratio for Furosemide and ethanol extract were 1.61 and 1.45 respectively, as compared to 1.70 for control. The present result shows significant diuretic potency and their effect on electrolyte excretion of *R. beddomei* comparable to the standard drug Furosemide.

Table 1: Effect of oral administration of ethanol extract of *R. beddomei* leaves on urinary volume and electrolytic excretion

Groups	Dose	Total Urine Volume (ml)	Na+ (mmol/L)	K+ (mmol/L)	Cl- mmol/L)	Na+/K+
Control	20 ml/kg	5.2±0.48	80.32±3.71	47±2.92	107.78±6.62	1.70
Standard	10mg/kg	14.0±0.87*	122.83±4.63*	87±4.75*	147±6.13*	1.61
Ethanolic extract	250mg/kg	7.2±0.23 *	90.12±2.61*	62±1.21*	111.14±4.52	0.95
	500mg/kg	11.6±0.73*	109.89±4.57*	81±5.67*	127.34±4.52	1.45

Values are expressed as mean ± SEM (n = 6); *p < 0.01 compared with control (ANOVA followed by Dunnett's t-test).

DISCUSSION

In this study, the diuretic action of *R. beddomei* was evaluated using Furosemide which is a high-ceiling loop diuretic, under controlled laboratory conditions. As diuretic therapy may lead to number of life-threatening electrolytic disorders and toxicities, so safety profile studies was carried out following a sub chronic administration of extracts. A complex set of interrelationships exists among the cardiovascular system, the kidneys, the central nervous system (Na+, appetite, thirst regulation) and the tissue capillary beds (distribution of extracellular fluid volume), so that perturbation at one of these sites can affect all the remaining sites. A primary law of the kidneys is that Na+ excretion is a steep function of mean arterial blood pressure (MABP) such that small increase in MABP cause marked increase in Na+ excretion. Results showed that there was absence of mortality and overt signs of toxicity. This would amplify the heterogeneous array of diuretic curatives available for safe and effective treatment of edema and cardiovascular diseases. The results of the present study revealed that *R. beddomei* induced diuresis was strong and accompanied with high natriuresis, chloruresis, and kaliuresis ($p < 0.01$). Further there was low Na+/ K+ ratio, so the *R. beddomei* seem to be acting like loop diuretics which inhibit Na+, K+ and Cl-co-transport at thick ascending loop of Henle. K+ excretion was increased perhaps due to high

Na+ load reaching the distal tube. The preliminary phytochemical analysis revealed that alkaloids, carbohydrates, tannins, steroids, carotenoids, sesquiterpenes and amino acids are present in *R. beddomei*. These natural products might be acting individually or synergistically to produce diuresis. It is also possible that the alcohol extract might manifest cumulative effect of several active principles in the extract. Since hypertension can be treated with diuretics, this study will provide basis for the traditional use of this plant in hypertension.

CONCLUSION

In conclusion, the extract of *R. beddomei* has diuretic effect supporting the ethno pharmacological use as diuretics and our results have shown that the *R. beddomei* administered at the dose of 500 mg/kg body weight (p.o.) has significant effects on urinary excretion of electrolytes and support the claims of diuretic efficacy of the title plant. The present study also provides basis for the traditional use of *R. beddomei* in hypertension.

REFERENCE

1. Schwartz WB. Effect of sulphanilamide on salt and water excretion in congestive heart failure. N Engl J Med. 1949;240 : 173.
2. Adinarayana D, Gunasekar D, Seligmann O and Wagner H. Rhyncosin- A new 5- deoxyflavonol from *R. beddomei* . *Phytochemisry*; 1980;19 :483-484.
3. Murthy KRK, Yoganarasimhan SN. Flora of Coorg (kodagn)– Karnataka Bangalore India with data on Med. Vishmat publishers; 1990: 157-158.
4. Bakshu L, Venkataraju RR. Antimicrobial activity of *R. beddomei*. Fitoterpia. 2001;72 :579-582.
5. Haloui M, Louedec L, Michel JB and Lyoussi B. Study of phytochemical and diuretic potential of methanol and aqueous extracts of aerial parts of Phyla nodiflora Linn. International Journal of Pharmacy and Pharmaceutical Sciences.2009;1(1) : 85-91.
6. K. Amala, A. Saraswathy, S. Amerjothy. Hepatoprotective and diuretic activities of alcoholic and aqueous extracts of *Epaltes pygmaea* DC. (Asteraceae). International Journal of Pharmacy and Pharmaceutical Sciences.2013;5(2, Suppl2) : 502-506.