

Phytochemical Investigation and Evaluation of Diuretic Activity of Aqueous and Alcohol Extracts of *Baliospermum montanum* (Willd) roots in Male Wistar Rats.

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Summary

The objective of the present investigation was to study diuretic effect of alcohol and aqueous extracts of roots of *Baliospermum montanum* in male wistar rats. The dried roots of plant *Baliospermum montanum* were subjected to successive extraction using the solvents (alcohol and water). The prepared extracts were then subjected to preliminary phytochemical analysis. It was found that the plant possesses triterpenoids, glycosides, flavonoids and tannins. The alcohol and aqueous extracts were selected for further pharmacological studies. The diuretic activity of alcohol and aqueous extracts of *Baliospermum montanum* were given to experimental rats orally at doses of 200 mg/kg p.o and frusemide (100 mg/kg i.p) was used as standard positive control in this study. The total urine volume, urinary concentration of sodium, potassium and chloride ions were the parameter of the study. The results indicate that alcohol and aqueous extract at 200 mg/kg body weight shows significantly increased ($p < 0.01$) in the urine volume and electrolyte excretion ($p < 0.01$ and $p < 0.001$), when compared to the control group. Both the extracts showed significant diuretic activity. The diuretic effect of the extract was comparable to that of the standard drug (frusemide). From the present study we can conclude that the alcohol and the aqueous extracts of *Baliospermum montanum* gives significant diuretic activity which appeared to be comparable to that of standard drug frusemide.

Keywords: *Baliospermum montanum*, Phytochemical investigation, Diuretic activity, Alcohol and aqueous extracts, frusemide.

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Introduction

Baliospermum montanum Willd (Euphorbiaceae) is commonly known as Danti in Hindi, Nikumbha in Sanskrit and Kaduharala in Kannada¹. It is distributed throughout India from Kashmir to Arunachal Pradesh, up to an altitude of 1000 m and southwards into peninsular India, ascending to an altitude of 1800 m in hills². It is a stout under shrub, 0.9-1.8 m in height with numerous erect from the perennial roots^{1,2,3}. The plant is common in shady places and cultivated by using root suckers. It is externally brownish or grayish brown and internally buff colour without mottling⁴. The plant is known to possess varied medicinal properties. The roots are acrid, thermogenic, purgative, anodyne, diuretic and febrifuge. They are useful in jaundice, leprosy, lithiasis.^{1,3,5,8} They are also useful in lymphocytic leukemia and free radical scavenging activity^{9,10}. The alcohol and aqueous extracts of *B.montanum* roots has been reported to exhibit hepatoprotective activity against paracetamol induced liver damage in male albino rats as well as *invitro* anthelmintic activity against *Pheritima posthuma* and *Ascardia galli*.^{11,12} However, there are no reports on the diuretic activity of the plant. Hence the present study was designed to verify the claims of traditional use, in ayurveda system of medicine. frusemide was selected as the standard drug.

Methods

Plant material and extraction procedure

Fresh roots of *B.montanum* were collected during the month of July 2009, from Dharwad, Karnataka and authenticated at regional medical research centre (ICMR), Nehru Nagar Karnataka, Belgaum and herbarium deposited at KLE University's college of pharmacy, Belgaum. The fresh roots of the plant were air dried and powdered to 40 mesh and kept in air tight container. Powder (400 g) was subjected to successive solvent extraction with the solvent in the order of increasing polarity i.e. Petroleum ether (60-80°C), followed by chloroform, alcohol using Soxhlet extractor. After extraction the solvent was distilled off using a rotary flash evaporator to a syrupy mass. The marc was finally extracted with water by cold maceration method to get the aqueous extract. The extracts were subjected to preliminary phytochemical screening and diuretic activity.¹³

Experimental protocol

Male albino Wistar rats weighing 180-200 gm were selected for the experiment. The experimental protocols have been approved by the institutional animal ethical committee of KLE University's college of pharmacy Belgaum, Karnataka (Reg.No.221/CPCSEA). The method of Lipschitz et al was used for the assessment of diuretic activity.^{14,15} The rats were randomly divided into four groups of six animals each and were fasted and deprived of water for eighteen hours prior to the experiment.

The first group of animals serving as control received normal saline (25ml/kg, p.o); the second group received frusemide (100mg/kg i.p) in saline; the third and fourth groups received the alcohol and aqueous extracts at the doses of 200 mg/kg, respectively. Immediately after administration the animals were placed in metabolic cages (1 per cage) specially designed to separate urine and faeces and allow collection of urine into volumetric flask, through funnels at the lower portion of the cages. After a period of 5 hours the urine collected in each of collecting flasks was measured using a graduated measuring cylinder and the volume of urine

corresponding to each of the groups was noted. During this period no food or water was made available to animals. The parameters taken for each individual was total urine volume, urine concentration of Na⁺ and K⁺ and Cl⁻ were measured by flame photometry (TCM-35). The urine concentration of the electrolytes expressed in terms of MEq/L and the urine volume is expressed in ml/kg body weight.

Statistical analysis

All the data obtained by various parameters was statistically evaluated by one way analysis of Variance test (ANOVA) followed by Dunnett's test. Significance level was $p < 0.01$ and $p < 0.001$.

Results

The preliminary phytochemical analysis showed the presence of Flavanoids, Glycosides, Triterpenoids and Tannins in all the extracts [Table 1]. Present study shows that the alcoholic and aqueous extracts of *Baliospermum montanum* roots possess good diuretic activity. Total urine volume, cation and anion excretion were increased. Significant increase in Na⁺, K⁺ and Cl⁻ excretion was observed in aqueous and alcoholic extract treated animals but less than frusemide control drug.

The volume of urine were significantly increased ($p < 0.01$) in alcohol and aqueous extracts treated group as compared with that of control group; but the value of standard drug was significant ($p < 0.01$) as compare to other groups.

The excretion of Na⁺ was high in alcohol extract treated group ($p < 0.01$) when compared with the control and standard group ($p < 0.001$). But in aqueous extract treated group the values are slightly significant ($p < 0.05$) when compared with the control.

The excretion of K⁺ was high in aqueous extract treated group ($p < 0.01$) when compared with the control and standard group ($p < 0.001$).

The excretion of Cl⁻ was significantly increased in the alcohol and aqueous extract treated group ($p < 0.01$) when compared with the control and standard group ($p < 0.001$).

Table 1: Preliminary Phytochemical test of *Baliospermum montanum* Alcohol, Aqueous extracts.

S.No	Phytochemical tests	Alcohol extracts	Aqueous extracts
1	Triterpenoids	+	+
2	Glycosides	+	+
3	Flavonoids	+	+
4	Proteins	-	-
5	Alkaloids	+	+
6	Tannins	+	+

+ : represent presence of phyto-constituents respectively. - : represent absence of phyto-constituents respectively

Table-2: Parameters of diuretic activity of different extracts of *Baliospermum montanum* (willd)

Treatment	Dose (mg/kg)	No. of rats used	Urine volume (Mean± SE)	Na ⁺ (mEq/L) (Mean± SE)	K ⁺ (mEq/L) (Mean± SE)	Cl ⁻ (mEq/L) (Mean± SE)
Normal saline	25	6	1.60±0.6	11.2±0.45	63.03±0.9917	9.09±0.222
Alcohol extract	200	6	4.733±0.14**	19.24±0.5662**	70.45±0.71	14.373±0.3728**
Aqueous extract	200	6	3.517±0.13**	13.6±0.9503*	78.27±2.882**	12.45±0.9328**
frusemide	100	6	8.400±0.11**	22.5±0.5654***	80.53±0.7875** *	21.28±0.399***

Statistical analysis by ANOVA and Dunnett's test. Results are expressed as mean± standard error, N=6, P<0.05*, P <0.01**, P <0.001***, Urine volume expressed in ml, Concentration of urine expressed in M Eq/L

Discussion

According to previous literature survey the roots of *Baliospermum montanum* are used for the treatment of renal diseases, but to the best of our knowledge, no previous pharmacological or clinical study has been carried out to test the diuretic activity of this plant.

Both the extracts of *Baliospermum montanum* showed increase in urinary excretion at 200 mg/kg. Thus the diuretic effects of both extracts are indicated by increase in water followed by excretion of sodium, potassium and chlorine. The active principles responsible for the diuretic effect of the extract of this plant have not been elucidated but preliminary phytochemical analysis showed the presence of compounds such as glycosides, flavonoids, tannins and triterpenoids in aqueous and alcohol extract only. Active principles such as flavonoids, tannins and triterpenoids are known to be responsible for diuretic activity.^{16,17,18}

The diuretic effect may be produced by the inhibition of tubular reabsorption of water and ions or by producing stimulation of regional blood flow.^{19,20}

Primary law of kidney is that sodium excretion is steep function of mean arterial blood pressure (MABP), such as small increase in sodium excretion.²¹ One of the earliest strategies for the management of hypertension was to alter sodium balance by restriction of salt in the diet. Diuretic agents having antihypertensive effects were used alone and had greater efficacy and all other antihypertensive drugs. In this study pharmacological evaluation of diuretic action of alcohol and aqueous extracts *Baliospermum montanum* roots were evaluated using frusemide under control lab condition. As diuretic therapy may lead to number of life threatening electrolytic disorder and toxicities, so safety profile studies have been carried out, following a sub chronic administration of extracts. This amplifies the heterogeneous array of diuretic curatives available for safe and effective treatment of edema and cardiovascular disorders.²²

Conclusion

The alcohol and aqueous extracts of *Baliospermum montanum* roots has diuretic effect supporting the ethanopharmacological use as diuretics at dose of 200mg/kg body weight. Urine concentration of Na⁺ and K⁺ and Cl⁻ were measured. The excretion of Na⁺ was high in alcohol extract treated group, K⁺ was high in aqueous extract treated group and Cl⁻ was significantly increased in the alcohol and aqueous extract treated group when compared with the control and standard group. Thus the extracts of *Baliospermum montanum* roots have showed significant diuretic activity.

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