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Research Article

DIURETIC ACTIVITY OF *CALOTROPISPROCERA* (AIT.) R. BR. LEAVES IN NORMAL RATS

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ABSTRACT

Calotropisprocera (Ait.) R. Br. (Asclepiadaceae), a xerophytic shrub widely distributed in the tropics of Asia and Africa, is used in various forms as panacea for diverse health problems in different parts of the world. In view of its medicinal importance, an ethanolic extract, water and *n*-butanol fraction of ethanolic extract of *Calotropisprocera* (Ait.) R. Br. leaves were investigated for *in-vivo* diuretic activity by using Lipchitz method. Results of diuretic activity showed that ethanolic extract and water fraction produced notable diuretic effect which appeared to be comparable to that produced by the furosemide. The increase in urine output was statistically significant at all the treatment levels.

Keywords: *Calotropisprocera,* diuretic activity, Lipchitz method.

INTRODUCTION

Diuretics, either alone or in combination with other drugs, are valuable in the treatment of hypertension, congestive heart failure, ascites, and pulmonary edema.[1-5]Plant medicine is commonly used in the traditional treatment of some renal diseases, and many plants are reported to possess significant diuretic activity.[6-8].*Calotropisprocera*(Ait) R.Br. belongs to the family Asclepiadaceae and is a soft wooded, evergreen perennial shrub having few stems, few branches and relatively few leaves concentrated near the growing tip.The morphological studies revealed the leaves are sub-sessile, 6-15 cm by 4.5-8 cm, broadly ovate, ovate-oblong, elliptic or obovate acute, pubescent, when young and glabrous on both sides.[9]

Different extracts of *Calotropisprocera* (Ait) R.Br. leaves have been found to possess following diverse biological activities like antidiarrhoeal[10,11], antioxidant[12-14], antipyretic[15], analgesic[16], antimicrobial[17,18], spasmolytic[19], schizonticidal[20], cytotoxic[21-23], anthelmintic[23], hepatoprotective[24], hypoglycemic[25]and abortifacient[26] but diuretic activity of *Calotropisprocera* (Ait.) R. Br. leaves is not reported in literature. Since, the diuretic activity of leaves of this plant has not been investigated in scientifically controlled studies, the aim of the present study was to evaluate the diuretic potential of ethanolic extract, water and *n*-butanol fraction of ethanolic extract of *Calotropisprocera* (Ait.) R. Br. leaves in normal rats.

MATERIALS AND METHODS

Plant material and preparation of the extracts

The leaves of*Calotropisprocera*(Ait) R. Br. were collected in the month of February from the local field of Mathura (Uttar Pradesh), India. The leaves were cleaned by washing with running water and shade dried, then powdered to pass through 100 mesh size. Powdered leaves were extracted with ethanol by maceration for seven days at room temperature. The solvent was recovered under reduced pressure and ethanolic extract was obtained as brownish green viscous residue. The dried residue was suspended in water and further extracted with *n*-butanol to obtained water and *n*-butanol fraction. The *n*-butanol fraction and water fraction was concentrated under reduced pressure and vacuum dried. The ethanolic extract, *n*-butanol fraction, and water fraction were investigated for their *in vivo* diuretic activity.

Phytochemical screening

Qualitative assay, for the presence of plant phytoconstituents such as carbohydrates, alkaloids, glycosides, flavonoids, tannins and saponins were carried out on the ethanolic extract, *n*-butanol fraction and water fraction of *Calotropisprocera* (Ait) R.Br leaves following standard procedure.[27,28]

Experimental animals

Wistar rats weighing between 140-150 gm were used for the present investigation.All experiments were carried out in a human manner after receiving approval from Institutional Animal Ethical Committee (GLAIPR/IAEC/03/12/PharmChem/R7), and in accordance with the regulations for animal experiments and fundamental guidelines.

Reference drug

Furosemide (Lasilix), a high-ceiling loop diuretic, was used as the reference drug (positive control).

Diuretic activity

The *in-vivo* diuretic activity of ethanolic extract and different fractions were carried out on the rats. The method of Lipschitzet. al., (1943) with some modification was employed for the assessment of diuretic activity.[29]Thewistar rats (150-200g) were divided in to eight groups of six rats in each group. On the day of experiment the group I animals serving as control received normal saline 25 mL/kg. The group II and group III animals received ethanolic extract 100 mg/kg and 200 mg/kg respectively. The IV and V group received *n*-butanol fraction of ethanolic extract 100 mg/kg and 200 mg/kg respectively. The VI and VII group received water fraction of ethanolic extract 100 mg/kg and 200 mg/kg respectively. The VIII group received 20 mg/kg furosemide in normal saline and served as standard. Immediately after administration of drug animalswere kept in metabolic cage specially designed to separate urine and fecal matter and kept at room temperature (25±0.5°C). Throughout the experiment the total volume of urine was collected at the end of 24 hr after dosing. The urine volume was measured in the measuring cylinder and sodium and potassium content was measured by flame photometry.

Statistical analysis

Results are expressed as mean±S.E.M. Statistical analysis of the data was performed with one-way analysis of variance (ANOVA) followed by Dunnet test. Significant differences were indicated by P values lower than 0.01 & 0.001.

RESULT AND DISCUSSION

Preliminary phytochemical screening of ethanolic extract revealed the presence of carbohydrates, glycosides, alkaloids saponins, tannins, flavanoids and steroids. Water fraction showed the presence of carbohydrates, glycosides, alkaloids and tannins while *n*-butanol fraction showed presence of glycosides, alkaloids, saponins and flavanoids.

Invivo diuretic activity results of ethanolic extract, water and *n*-butanol fraction of ethanolicextract are given in the table 1 showed variation of urine volume (mL/100mg/hr), electrolyte (Na^+ and K^+) content (meq/kg/mL) and diuretic index of urine.

Effect on urine volume

Results showed that the reference drug furosemide increased the urine output when compared to control ($P<0.01$), the diuretic index being 1.88. The ethanolic extract (100 mg/kg and 200 mg/kg) and water fraction (100mg/kg and 200 mg/kg) significantly increased the urine output when compared to control ($P<0.01$), the diuretic index being 1.637 and 1.917 for ethanolic extract and 1.398 and 1.621 for water fraction at 100 mg/kg and 200 mg/kg dose respectively. On the other hand diuretic index for *n*-butanol fraction was 0.896 and 1.207 at 100 mg/kg and 200 mg/kg dose respectively as shown in figure: 1, which revealed that *n*-butanol fraction showed insignificant result.

Effect on urinary electrolyte excretion

As shown in table 1 the ethanolic extract (100 mg/kg and 200 mg/kg) and water fraction (100 mg/kg and 200 mg/kg) produced significantly increase in Na^+ excretion ($P<0.001$), andethanolic extract (100 mg/kg and 200 mg/kg) and water fraction (200 mg/kg) produce significantly increase in K^+ excretion ($P<0.001$). Ethanolic extract and water fraction showed increase in water, Na^+ and K^+ excretion (figure 2) which indicate them as a diuretic drug and *n*-butanol fraction did not showed any increment in Na^+ and K^+ excretion.

In vivo diuretic activity results of ethanolic extract, water and *n*-butanol fraction of ethanolic extract results showed that ethanolic extract and its water fraction have significant diuretic activity.The pharmacological response was compared with that produced by furosemide, a widely used diuretic drug.The notable diuretic effect observed from the ethanolic extract and its water fraction as well as having an interesting effect on the conservation of potassium is an interesting property in a phyto-diuretic. In conclusion, the

present studies support the ethnomedicaluse of *Calotropisprocera* (Ait) R.Br. plant for their diuretic effect.[30]

Table 1: *In-vivo* diuretic activity result of ethanolic extract and different fractions

Name of Drug/Extract	Dose mg/kg	No. of Rats Used	Urine Volume (mL)	Electrolyte Excretion (Meq./kg/24hr)		Diuretic index
				Na^+	K^+	
Ethanolic Extract	100	6	3.16±0.0176**	78.90±0.512**	19.91±0.408**	1.637
	200	6	3.7±0.115**	81.42±0.345**	21.37±0.674**	1.917
n-Butanol Fraction	100	6	1.73±0.079*	51.59±0.775*	15.07±0.579*	0.896
	200	6	2.33±0.115*	51.83±1.176*	14.96±0.407*	1.207
Water Fraction	100	6	2.7±0.120**	77.45±1.209**	21.38±0.652**	1.398
	200	6	3.13±0.083**	83.18±1.121**	17.73±0.393*	1.621
Control	-	6	1.93±0.086	53.75±0.870	16.65±0.313	---
Furosemide	20	6	3.63±0.071**	92.29±0.512**	30.13±0.425**	1.880

All the values are given as Mean ± SEM; N=6; *P<0.01 & **P<0.001 as compared to control by Dunnet test

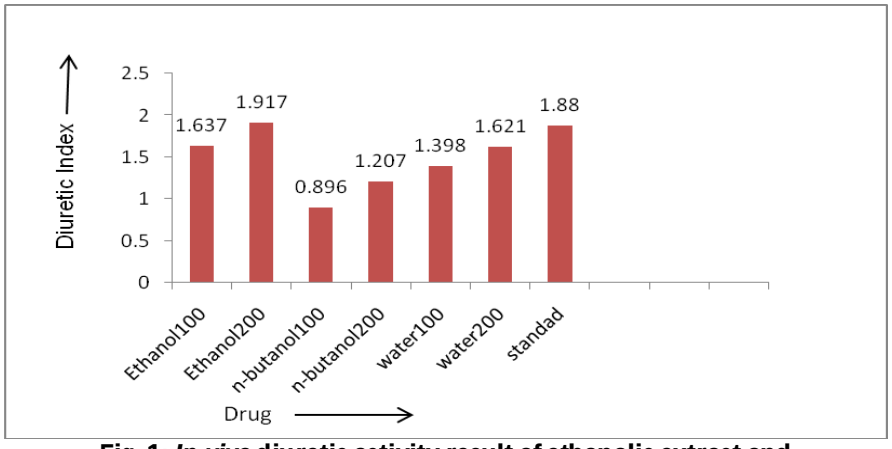

Fig. 1: *In-vivo* diuretic activity result of ethanolic extract and different fractions of *Calotropisprocera*(Ait) R.Br.leaves

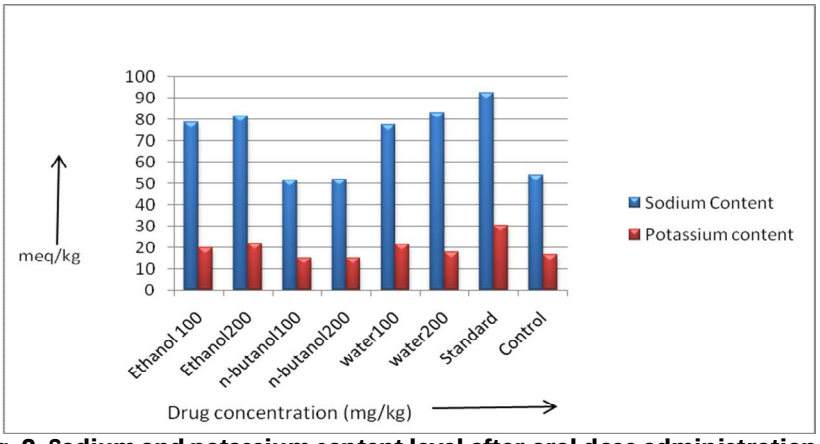

Fig. 2: Sodium and potassium content level after oral dose administration of *Calotropisprocera*(Ait) R.Br.leaves extract and fractions

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