PRELIMINARY PHYTOCHEMICAL SCREENING AND DIURETIC ACTIVITY OF *BOMBAX CEIBA* L. FRUIT EXTRACTS

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Summary

The aim of the present study was to evaluate diuretic activity of aqueous and ethanolic extract of *Bombax ceiba* L. fruit in rats. Urea (1000 mg/kg, p.o) and Frusemide (25 mg/kg, p.o.) were used as standard diuretic drugs. Total volume and urinary excretion of sodium, potassium and chloride were estimated in urine collected after 5 and 24 h of oral administration of extracts. The ethanolic extract (200 and 400 mg/kg, p.o.) significantly increased total urine volume and excretion of electrolytes compared to control group. The results of preliminary phytochemical study revealed the presence of carbohydrates, glycosides, flavonoids, tannins and phenolic compounds in both extracts of *B. ceiba* fruit.

Keywords: *Bombax ceiba*, Diuretic activity, Frusemide, Lipschitz value

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Introduction

Diuretics are the drugs that increase the rate of urine flow, sodium excretion and are used to adjust the volume and composition of body fluids in a variety of clinical situations. Drug-induced diuresis are beneficial in many life-threatening disease conditions such as congestive heart failure (CHF), nephritis, hypertension and pregnancy toxemia[1]. However, most diuretic drugs have been associated with numerous adverse effects, such as electrolyte imbalance, metabolic alterations, development of new-onset diabetes, activation of the renin-angiotensin and neuroendocrine systems, and impairment of sexual function[2]. Shervin Eshaghian *et al.* (2006) reported that higher loop diuretic dosages identify patients with CHF at particularly high risk for mortality. Hence the need for novel diuretics such as plant-based substances, which are considered to be relatively safe and possessing lower potential for adverse effects, is advocated.

Bombax ceiba L. Syn. *Bombax malabaricum* Schott. (Family - Bombacaceae), is a tall deciduous tree, found throughout the hotter forest regions of India and Ceylon. Fruit is considered as demulcent, tonic, diuretic and astringent.and used traditionally as an ethnomedicine for various ailments including calculous affections, chronic inflammation and ulceration of the bladder and kidneys[3]. Phytochemical studies on various parts of *B. ceiba* revealed that it is rich in phenolic compounds. Few compounds such as shamimicin, lupeol and mangiferin, isolated from *B. ceiba* were found to possess significant analgesic, antioxidant and hypotensive properties[4,5].

Review of literature indicates that *B. ceiba* fruit have not yet been screened for its diuretic activity. Therefore, the present study was carried out to provide pharmacological evidence for the folklore consideration of the plant fruit as diuretic.

Methods

Plant materials

Fresh young fruits of *B. ceiba* were collected during May 2008, from Jamboti forest in Belgaum district, Karnataka, India and authenticated at Botanical Survey of India (BSI), Govt. of India, Ministry of Environment and Forests, Pune, India by Mr. P. G. Diwakar. A voucher specimen of the plant (NBGBCP-1) was deposited in the BSI herbarium.

Extraction

Aqueous extract (AqE) of dried and powdered fruit of *B. ceiba* was prepared by maceration for 24 h (Yield: 22.07%). Additionally, ethanolic extract (EtE) was prepared using soxhlet extractor (Yield: 13.76%). A suspension of each extract in 1% tween 80 was used for oral dosage administration in experimental rats.

Preliminary phytochemical screening

The extracts were screened for various groups of phytoconstituents viz. alkaloids, anthraquinone glycosides, cardiac glycosides, steroids, tannins, proteins, saponins, flavonoids, mucilage, fats and oils, carbohydrates and organic acids[6,7].

Animals used

Male Wistar albino rats, weighing 150-200 g, were selected for diuretic activity. Rats were acclimatized to standard laboratory conditions and provided with rat chow (Lipton India Ltd., Mumbai) and water *ad libitum*. The animal care and experimental protocol was in accordance with Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines and was approved by the Institutional Animal Ethics Committee (IAEC) wide resolution no. JNMC/IAEC/Res-2/7/2008 dated 19th December 2008.

***Acute oral toxicity*[8]**

The acute oral toxicity study was carried out as per the guidelines (Number 423) set by Organization for Economic Co-operation and Development (OECD) using three female rats for each extract per step. Aqueous and ethanolic extracts (starting dose of 2000 mg/kg, p.o.) were administered by oral gavage. Immediately after dosing, the animals were observed continuously for the first 4 h for behavior. They were then kept under observation up to 14 days after extract administration to find out the mortality. In another sets of three female rats each, the same dose of each extract was administered. Based on the mortality in either step, the next lower dose (300 mg/kg, p.o.) was administered and the LD_{50} cut-off dose was determined in accordance with the Global Harmonization System (GHS) classification.

***Assessment of diuretic activity*[9]**

The test was performed according to Lipschitz *et al.* on male rats. The rats fasted and deprived of water for 18 h prior to the experiment, were divided in seven groups of eight rats each. The first group of animals, serving as control received normal saline (25 ml/kg, p.o.) the second and third groups received urea (1000 mg/kg, p.o.) and frusemide (25 mg/kg, p.o.) in saline as standard, respectively; the fourth and fifth groups received the aqueous extract of *B. ceiba* fruits (200 and 400 mg/kg, p.o.) in saline, respectively and the sixth and seventh groups received the ethanolic extract of *B. ceiba* fruits (200 and 400 mg/kg, p.o.) in saline, respectively. Immediately after dosing, the animals were placed in metabolic cages suitable for collection of urine in graduated measuring cylinders, in group of two rats per cage. Urine was collected after 5 h and 24 h while animals were deprived of food and water. Urine volume (ml/100 g) and Na^+, K^+ and Cl^- concentrations (mEq/l/100 g) in the urine were determined[10,11]. To determine the extent of diuretic action, various indices were calculated using following formulae.

Diuretic index = Urine excretion of test group / Urine excretion of control group;
Lipschitz Value = Urine excretion of test group / Urine excretion of urea treated group
Saluretic index = Urine excretion of electrolyte in test group / Urine excretion of electrolyte in control group;
Natriuretic index = Urine excretion of Na^+ / Urine excretion of K^+

Statistical analysis

Results were expressed as mean ± SEM. Significance of difference between control and treated group was determined using Student's t-test (unpaired). Level of significance was kept at $P<0.05$.

Results

The results of preliminary phytochemical tests showed the presence of carbohydrates, glycosides, flavonoids, tannins and phenolic compounds in both the extracts. Alkaloids, triterpenoids and steroids were not detected in either extract (Table 1).

Table 1: Preliminary phytochemical screening of *B. ceiba* fruit extracts

Phytoconstituents	Aqueous extract	Ethanolic extract
Alkaloids	-	-
Carbohydrates	+	+
Glycosides	+	+
Steroids	-	-
Saponins	-	-
Triterpenoids	-	-
Flavonoids	+	+
Tannins and Phenolic compounds	+	+
Proteins and amino acids	-	-
Fats and oils	-	-

'+' indicates present; '-' indicates absent

Orally administered to rats with starting dose of 2000 mg/kg, all extracts of *B. ceiba* were found to be safe. No mortality was observed with any extract. Since, all extracts were categorized as class-IV (> 300 to ≤ 2000 mg/kg, p.o.) under GHS classification, the oral dose of 2000 mg/kg was taken as LD_{50} cut-off dose and $1/10^{th}$ dose was taken as effective dose for all extracts of *B. ceiba*. On treatment with aqueous and ethanolic extract of *B. ceiba* fruit (200 and 400 mg/kg, p.o.) in rats, significant increase in total urine volume (Table 2 and 3) and urinary excretion of Na^+ and Cl^- was observed over a period of 24 h (Table 4). Moreover, both extracts increased the volume of urine in dose dependent levels. The obtained effect was comparable to that of urea (1000 mg/kg, p.o.) and frusemide (25 mg/kg, p.o.). In comparison with aqueous extract, the ethanolic extract showed higher diuretic index and Lipschitz value. Also, it was found to be more saluretic and natriuretic.

Table 2: Effect of *B. ceiba* fruit extracts on urinary excretion volume in rats

Group	Dose (mg/kg, p.o.)	5 h		
		Urine Excretion (ml/100g)	Diuretic index	Lipschitz Value
Control	-	0.26 ± 0.02	1.00	-
Urea	1000	0.35 ± 0.02*	1.35	1.00
Frusemide	25	0.55 ± 0.06**	2.12	1.57
AqE	200	0.392 ± 0.09	1.51	1.12
AqE	400	0.430 ± 0.07*	1.66	1.23
EtE	200	0.459 ± 0.13	1.77	1.31
EtE	400	0.521 ± 0.08*	2.01	1.49

n (number of pairs in each group) = 4; Values are mean ± SEM; *$P<0.05$, **$P<0.01$, ***$P<0.001$, vs Control (Saline 25mg/kg, p.o.); Student's *t*-test (unpaired)

Table 3: Effect of *B. ceiba* fruit extracts on urinary excretion volume in rats

Group	Dose (mg/kg, p.o.)	24 h		
		Urine Excretion (ml/100g)	Diuretic index	Lipschitz Value
Control	-	1.20 ± 0.11	1.00	-
Urea	1000	1.51 ± 0.05*	1.26	1.00
Frusemide	25	3.04 ± 0.31**	2.53	2.01
AqE	200	2.181 ± 0.57	1.82	1.47
AqE	400	2.499 ± 0.35*	2.08	1.68
EtE	200	2.523 ± 0.28**	2.10	1.70
EtE	400	2.860 ± 0.20***	2.38	1.92

n (number of pairs in each group) = 4; Values are mean ± SEM; *$P<0.05$, **$P<0.01$, ***$P<0.001$, vs Control (Saline 25mg/kg, p.o.); Student's *t*-test (unpaired)

Table 4: Effect of *B. ceiba* fruit extracts on urinary excretion volume in rats

Group	Dose (mg/kg, p.o.)	Concentration of ions (mEq/l/100 g)†			Saluretic index			Natri-uretic index
		Na^+	K^+	Cl^-	Na^+	K^+	Cl^-	
Control	-	2.88 ± 0.23	1.08 ± 0.13	3.72 ± 0.13	1.00	1.00	1.00	2.67
Urea	1000	4.74 ± 0.22*	1.41 ± 0.08	5.02 ± 0.22**	1.65	1.31	1.35	3.36
Frusemide	25	5.43 ± 0.27***	1.47 ± 0.09*	5.52 ± 0.24***	1.89	1.36	1.48	3.69
AqE	200	3.18 ± 0.23	1.18 ± 0.04*	3.71 ± 0.22	1.10	1.10	1.00	2.69
AqE	400	3.40 ± 0.21	1.28 ± 0.04**	3.97 ± 0.08*	1.18	1.19	1.07	2.65
EtE	200	3.95 ± 0.23**	1.09 ± 0.09	4.13 ± 0.10**	1.37	1.01	1.11	3.63
EtE	400	4.98 ± 0.33***	1.21 ± 0.07	4.93 ± 0.28**	1.73	1.12	1.32	4.13

n (number of pairs in each group) = 4; Values are mean ± SEM; *$P<0.05$, **$P<0.01$, ***$P<0.001$, vs Control (Saline 25mg/kg, p.o.); Student's *t*-test (unpaired); † Urine collected for 24 h after treatment;

Discussion

The result shows that the cumulative urine excretion over 24 hr was significantly increased in EtE treated groups in dose-dependent manner (Figure 1). The diuretic effect of EtE appears to be superior to that of AqE. Not only the diuretic index is found to be increased by more than 2.0; but also, the effect on electrolyte excretion is more pronounced in EtE treated group at 400 mg/kg, p.o.

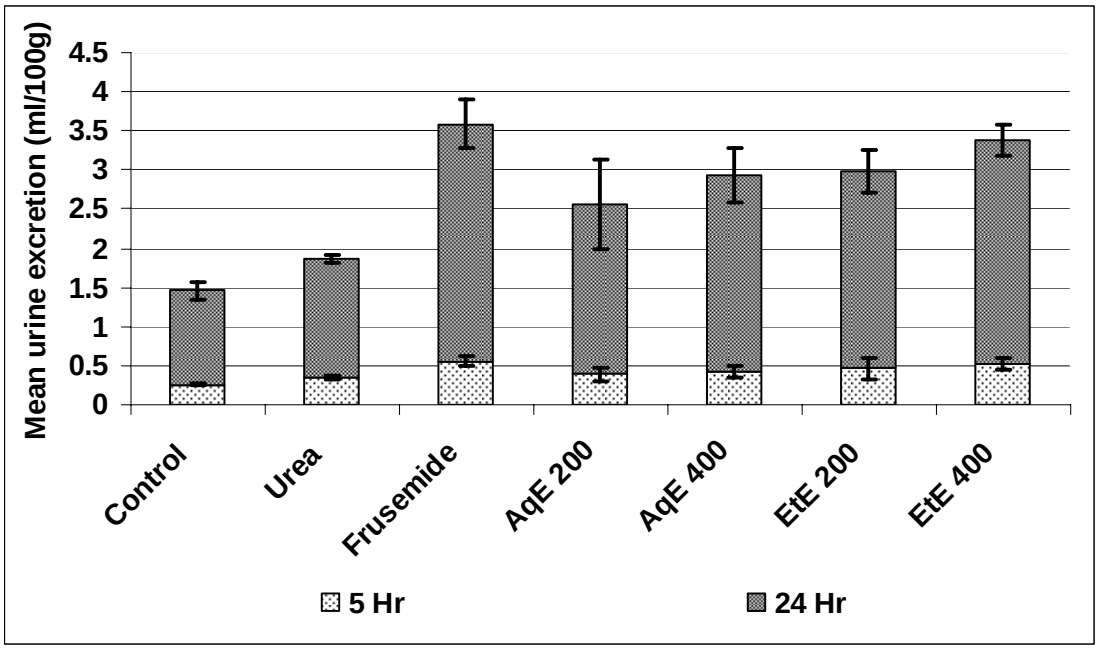

Figure 1: Cumulative urine excretion (ml/100g) in control, urea, frusemide, aqueous (AqE 200mg/kg, AqE 400mg/kg) and ethanolic (EtE 200mg/kg, EtE 400 mg/kg) extract of fruit of *B. ceiba* treated rats

Treatment with potent dose (400 mg/kg, p.o.) of AqE shows kaliuretic action; while, EtE shows mild potassium sparing effect (Table 4). The result of present study indicate that *B. ceiba* root extracts contained compounds that mediate diuretic effect by increasing the rate of urine output as well as electrolyte excretion.

In conclusion, the diuretic potentials exhibited by *B. ceiba* fruit support for its folklore use. However, further studies are necessary to better evaluate its safety and modes of action.

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