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DIURETIC ACTIVITY OF METHANOL EXTRACT OF *JUSTICIA SIMPLEX. D. DON*

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ABSTRACT

Justicia simplex D. Don (Family: Acanthaceae) is traditionally used as diuretic, stomachic, expectorant, anthelmintic, diaphoretic. The present study was aimed to carry out the diuretic activity of methanolic extracts of *Justicia simplex* D. Don in rats. The Methanolic extracts was administered orally at a dose of 200mg/kg and 400mg/kg using furosemide as a standard drug. The results showed that both the dose (200mg/Kg & 400mg/Kg) of methanolic extract showed significant diuresis relatively the dose of methanolic extract 400mg/Kg has shown more activity than methanolic extract of 200mg/Kg. The findings concluded that *Justicia simplex* D. Don exhibit diuresis.

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INTRODUCTION

Plant medicine is commonly used in the traditional treatment of some renal diseases, and many plants are reported to possess significant diuretic activity (Bellakhdar, J et al., 1997). The diuretic activity of a number of plants used in ethnomedicine as diuretic agents has been confirmed in experimental animals (Benjumea, D et al., 2005).

Volume and composition of body fluids in a various clinical Disorders. But drug-induced diuresis is very much beneficial in such type of life-threatening disorders like CHF, hypertension, renal failure, Liver Cirrhosis and often pregnancy toxemia (Agunu A et al., 2005). Naturally occurring diuretics include caffeine, alcohol and wine, which inhibit Na⁺ reabsorption and inhibit secretion of ADH but have the adverse effect including impotence, fatigue, weakness etc (Agus ZS et al., 1971; Stookey JD et al., 1999). Hence search for a new diuretic agent that retains therapeutic efficacy and devoid of above adverse effects.

The Acanthaceae family, order Scrophulariales, superorder Lamiiflorae, comprises almost 250 genera with 2500 species. Its species are widespread in tropical regions of the world (Wasshausen DC et al., 2004) and are poorly represented in temperate regions

(Mabberley DJ 1997). *Justicia* is the largest genus of Acanthaceae, with approximately 600 species that are found in pantropical and tropical regions (Durkee LH 1986).

Justicia simplex is a diminutive annual occurring rather abundantly on a hilly ridge having its several regions. The plant is diuretic, stomachic, expectorant, anthelmintic, diaphoretic and aperient; removes indigestion, biliousness, fever and burning of the body; strengthens the lungs, the teeth, stops vomiting; good in diseases of the spleen. Along with black pepper given in ague. Leaf juice is dropped into eyes in case of ophthalmia (Yusuf M et al., 2009).

MATERIALS AND METHODS

Plant material:

The whole plant of *Justicia simplex* D.Don were collected from Western Ghats. It was authenticated by DR.V.Chelladurai, (Retired) Research Officer – Botany C.C.R.A.S Govt. of India, Tirunelveli. The collected material was dried at room temperature under shade for one week, then it was blended into coarse powder by electrical grinder. The powdered drug was passed through sieve No.22. To get uniform particle size.

Extraction:

Cold maceration method:

The whole part of the plant were dried under shade and coarsely powdered. About 1kg of dried powder was macerated in methanol for 5 days with occasional shaking, after completion of extraction, the methanolic extracts was filtered and concentrated till it acquires maximum concentration. A dark residue was obtained.

Preparation of sample:

Control : saline 25ml/kg p.o.

Standard : Furosemide 20mg/kg p.o.(Furosemide a high-ceiling loop diuretic,It was dissolved in water prior to administration.)

Test:

Methanol extract: 200 mg/kg p.o,(It was dissolved in water prior to administration.)

Methanol extract: 400 mg/kg p.o,(It was dissolved in water prior to administration.)

Experimental animals:

Adult male Wistar rats weighing between 150 and 200 g procured from our animal house were housed under standard environmental conditions (25±1 °C, 55±5% humidity and 12 h/12 h light/dark cycle). The animals were allowed free access to tap water and standard laboratory rat food.

Diuretic activity:

Each animal was placed in an individual metabolic cage 24h prior to commencement of the study for adaptation. The method of Lipschitz et al., (1943) and Murugesan et al., (2000) was employed for the assessment of diuretic activity. According to this method, the animals, deprived of food and water for 18 hours prior to the experiment, were divided into 4 groups (n=6). The Group I animals received saline 25ml/kg p.o, Group II animals received Frusemide at the dose of 20 mg/kg, Group III animals received methanol extract of *Justicia simplex* at the dose of 200 mg/kg and Group III animals received methanol extract of *Justicia simplex* at the dose of 400 mg/kg. Before treatment, all animals received physiological saline (0.9% NaCl) at an oral dose of 5ml/100g body weight. All the drugs were freshly prepared prior to administration. Immediately after administration, the animals were placed in metabolic cages (each animal per cage), specially designed to separate urine and faeces, kept at 20°C±0.5°C. The volume of urine collected was measured at the end of 5hrs. During this period, no food and water was made available to animals. The parameters noted were total urine volume, and concentration of Na⁺, K⁺ and Cl⁻ in the urine. Na⁺, K⁺ were determined by Flame photometer (Jeffery et al., 1989) while Cl⁻ concentration was estimated titrimetrically using 0.02N AgNO₃ with 5% potassium chromate as an indicator. Appearance of brick red precipitate was taken as the end point (Beckette et al., 1997).

Statistical analysis:The results were expressed as a mean ± S.E.M. Statistical differences between control and treated groups were tested by one way ANOVA followed by Student's *t*-test. The differences were considered significant at *P* < 0.05 (Woodson et al., 1987).

RESULTS AND DISCUSSION**Effect of methanol extract of *Justicia simplex* on urine output in rats:**

The effects of methanol extract of *Justicia simplex* on urinary excretion are shown in Table 1. The total urine output of the rats administered methanol extract of *justicia simplex* were elevated, and particularly the rats which received methanol extract of *justicia simplex* at the dose of 400mg/kg body wt. excreted nearly same volume of urine as compared to the standard group (Furosemide 20mg/kg). The results showed that both the dose (200mg/Kg & 400mg/Kg) of methanol extract showed significant diuretic activity relatively the dose of methanol extract 400mg/Kg has shown more activity than methanol extract of 200mg/Kg (Fig 1).

Table 1

Cumulative urine output in rats over a 5-hour period following oral administration of methanol extract of *Justicia simplex* D.Don.

S.NO	Treatment	Total Urine output (ml)
1	Control	05.20± 0.12
2	Standard	10.20 ± 0.13*
3	Methanol extract 200mg/kg	06.41 ± 2.13*
4	Methanol extract 400mg/kg	09.74 ± 0.08*

n=6, Mean ± SEM, P < 0.05 indicate the significant difference compared with control

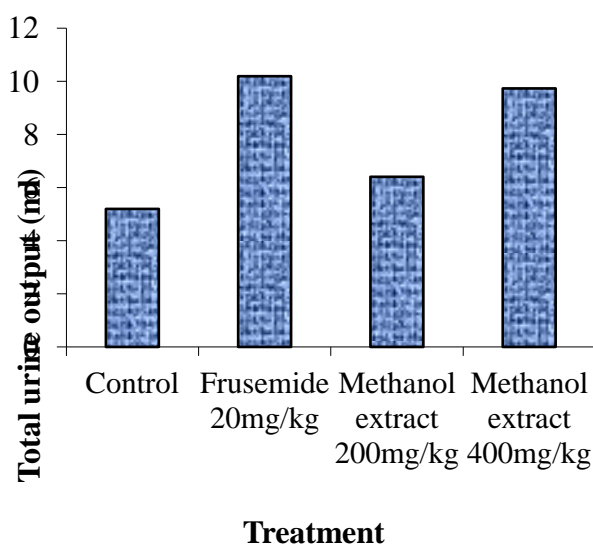


Fig. 1. Effect of methanol extract of *Justicia simplex* D.Don (200mg/kg and 400mg/kg body weight) and Furosemide (20mg/kg body weight) on urine output at 5h.

Effect of methanol extracts of *Justicia simplex* on electrolyte excretion in rats:

Table 2 shows the content (mEq/L/5h) of Na⁺, K⁺ and Cl⁻ in the urines of rats treated with methanol extracts of *Justicia simplex* D.Don, Frusemide and control. Other urinary excretion parameters as pH and conductivity of the sample urine at 5 h. The concentrations of Na⁺, K⁺ and Cl⁻ in rats treated with methanol extracts of *Justicia simplex* D.Don at low dose and high dose were 41.25mEq/L, 62.35mEq/L, 60.14mEq/L and 49.20mEq/L, 76.50mEq/L and 71.40mEq/L respectively. The excretion of sodium, potassium and chloride ions were increased. All the results were comparable with those for Furosemide (standard) and observed significant diuretic activity (Fig 2).

Table 2

Effect of orally administrated methanol extract of *Justicia simplex* D.Don on some urine parameters (up to 5 h) of rats

S.NO	Treatment	Electrolytes mEq/L			pH of urine	Conductivity (mS/cm)
		Na ⁺	K ⁺	Cl ⁻		
1	Control	35.50 ± 3.70	56.19 ± 5.88	50.30 ± 2.73	7.00±0.85	11.07±0.94
2	Standard	51.02 ± 3.09*	79.10 ± 4.97*	79.00 ± 2.87*	6.71±0.06*	13.73±0.14
3	Methanol extract 200mg/kg	41.25 ± 0.01*	62.35 ± 3.81	60.14 ± 6.4*	6.73±0.06	13.08±0.42
4	Methanol extract 400mg/kg	49.20 ± 4.54*	76.50 ± 10.74*	71.40 ± 9.31*	6.66±0.09*	13.41±0.23

n=6, Mean ± SEM, P < 0.05 indicate the significant difference compared with control

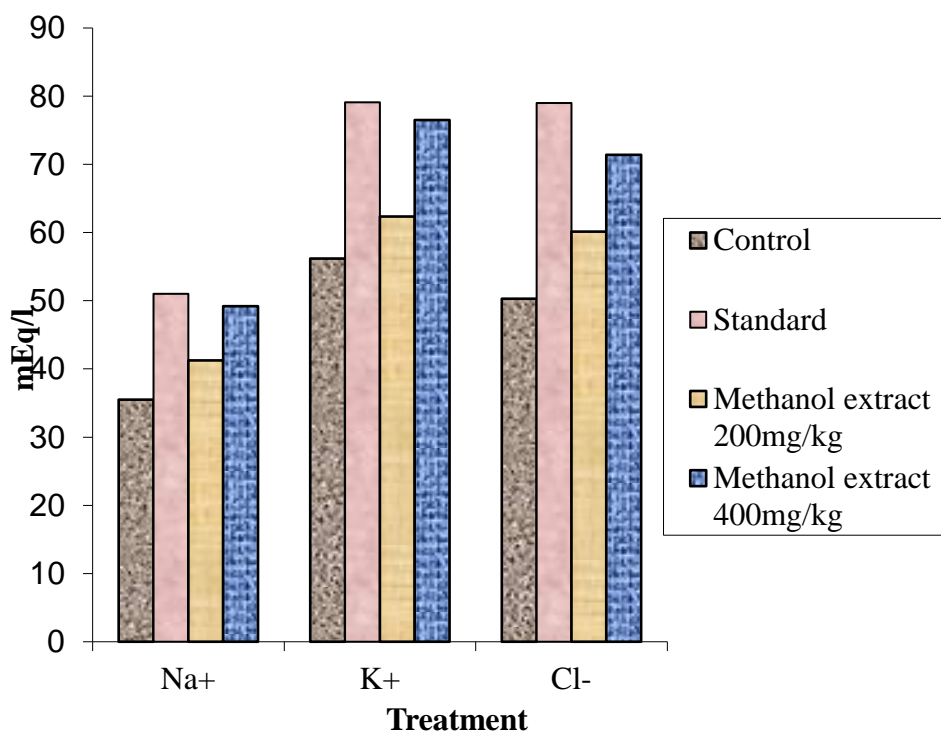


Fig. 2. Effect of methanol extract of *Justicia simplex* D.Don (200mg/kg and 400mg/kg body weight) and Furosemide (20mg/kg body weight) on electrolyte secretion in urine at 5 h.

Effect of methanol extracts of *Justicia simplex* on some other physicochemical parameters:

Table 2 shows also the results obtained for the pH and specific conductivity of the urine of rats treated with methanol extracts of *Justicia simplex*, Frusemide and control. It can be seen that the pH reduced significantly as compared to control, perhaps this reduction was due to an active substance present in this fraction which was capable of causing metabolic changes, and thus reduce the pH. The specific conductivity, which is an indirect measure of the ionic content of the urine, was increased in methanol extracts and standard treated group as compared to control group.

The experimental results demonstrated that *Justicia simplex* acts as a diuretic in rats, with increased excretion of total volume of urine as well as of cations and anions. The results showed that both the dose (200mg/Kg & 400mg/Kg) of methanol extracts showed significant diuretic activity relatively the dose of methanol extract 400mg/Kg has shown more activity than methanol extract of 200mg/Kg. The maximum diuretic activity was noted in the animals administered methanol extract of *Justicia simplex* at 400 mg/kg body wt. The diuretic effect was quite comparable with that of the standard drug Furosemide. In ion excretion, the highest dose of methanol extract notable excretion sodium and potassium, with values nearly similar to those produced by the Furosemide. Here, the methanol extracts of *Justicia simplex* D.Don increases the Na⁺ and K⁺ excretion, which may be acting like a loop diuretic. The extracts caused a significant and dose-dependent diuretic activity. The present study provides the pharmacological evidence to support traditional use of *Justicia simplex* D.Don for its diuretic effect.

CONCLUSION

It can be concluded that methanol extract of *Justicia simplex* D.Don whole plant have showed dose dependent increase in urine and electrolyte excretion. Relatively the dose of methanol extract 400mg/Kg has shown more activity than methanol extract of 200mg/Kg. The present studies support the folk claim for the *Justicia simplex* D.Don as an effective diuretic agent.

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