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(RESEARCH ARTICLE)

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Diuretic activity of whole plant extract of *Leptadenia pyrotechnica*

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Abstract

In the Indian traditional medicine, *Leptadenia pyrotechnica* (Apocynaceae) whole plant is claimed to possess powerful diuretic activity. The aim of this study was to evaluate the diuretic potential of methanol extract of *Leptadenia pyrotechnica* (MELP) in male albino rats. Group I, Control (2% CMC saline solution, 10 ml/kg, b.w), Group II, serves as a standard drug furosemide (10 mg/kg b.w) and different concentrations of LP (100, 200 and 400 mg/kg b.w) were intraperitoneally administered (n = 3 per each treatment group) to hydrated rats and their urine output was monitored over a period of 5 h after drug administration. The diuretic responses with its electrolyte excretion potency of the extract were highly remarkable in comparison with control animals. The MELP at dose of 400 mg/kg shows a significant increase in volume of urine with increase in Na+ excretion accompanied by the excretion of K+ in dose dependent manner. This study suggests that the active component in L.P. had similar diuretic effect to that of furosemide. These results validate the traditional use of *Leptadenia pyrotechnica* as a diuretic agent.

Keywords: *Leptadenia pyrotechnica*; Urinary volume; Diuretic activity; Electrolyte excretion

1. Introduction

Diuretics are agents which are acts on kidney to promote the excretion of sodium and water in urine. Diuretics promote the removal of the body excess water, salts, poisons, and accumulated metabolic products for water and ionic balance [1,2]. Diuretics not only alter the excretion of Na+ but also may modify renal handling of other cations (K + , H+ , Ca2+ and Mg2+), anions (Cl- , HCO3 - and H2PO4 -), and uric acid [3,4]. In addition, diuretics may alter renal hemodynamics activation on indirectly. They serve to rid the body of excess fluid (edema) that accumulates in the tissues owing to various disease states. Diuretics are involved some pathological condition such as Lower high blood pressure, reduce fluid retention, edema, swollen ankles, provide relief from the pain, burning sensation associated with cystitis, Heart Failure, Hypertension, Liver Cirrhosis, Kidney Diseases, Pulmonary and Systemic Edema [5,6,7]. They decrease the reabsorption of Na+ and (usually) Cl from the filtrate, increased water loss being secondary to the increased excretion of NaCl (natriuresis) [8,9]. This can be achieved by: a direct action on the cells of the nephron indirectly, by modifying the content of the filtrate Classes of diuretics Carbonic Anhydrase Inhibitors e.g. Acetazolamide, Dichlorphenamide Loop Diuretics e.g. Furosemide, Ethacrynic acid Thiazides e.g. Chlorothiazide, Benz thiazide Potassium sparing diuretics e.g. Spironolactone Osmotic e.g. Mannitol, Glycerol [10,11,12]. Worldwide trend towards the utilization of natural plant remedies has created an enormous need for information about the properties and uses of the medicinal plant. The Indian Traditional Medicine like Ayurvedic, Siddha and Unani are predominantly based on the use of plant materials [13,14]. Herbal drugs have gained importance and popularity in recent years because of their safety, efficacy and cost effectiveness. The association of medical plants with other plants in their habitat also influences their medicinal values in some cases [15,16].

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The well documented uses of plants and its formulated poly herbal products are used as Diuretics for various clinical condition such as Lower high blood pressure, reduce fluid retention, edema, swollen ankles, provide relief from the pain, burning sensation associated with cystitis, Heart Failure, Hypertension, Liver Cirrhosis, Kidney Diseases, Pulmonary and Systemic Edema [17,18].

2. Material and methods

2.1. Preparation of extract

Dried plant material approximately 5 grams was coarsely crushed in small pieces of 2-5 mm using the cylindrical crusher. The successive extraction of powdered material was carried out in several batches using different solvents in increasing order of polarity in a Soxhlet apparatus by hot percolation technique. The solvents used were petroleum ether, chloroform, acetone, methanol and distilled water. The powdered material of *Leptadenia pyrotechnica* was evenly packed in a Soxhlet extractor for about 36 hours with different solvents. The temperature was maintained (25°C- 100°C) on an electric heating mantle with thermostat control. The extracts were then concentrated by evaporating the solvent under reduced pressure. Preliminary phytochemical studies were carried out on methanolic extract to assess the presence of various phytoconstituents and diuretic activity.

2.2. Experimental animals

Wistar male albino rats, weighing 150 to 200 gm, were housed in groups of 3 per cage under controlled light (12:12 light: dark cycle) and temperature (25 ± 2°C). Environmental and behavioral assessment was conducted during the light cycle. Food and water ad libitum was provided. The animals were acclimatized to laboratory conditions for seven days before commencement of experiments.

2.3. Toxicity study

Acute toxicity study was performed for Methanolic extracts *of Leptadenia pyrotechnica*. Albino rats were used for acute oral toxicity study. The animals were kept fasting for overnight providing only water, after which the various extracts were administered orally at the dose of 150 mg/kg and observed for 14 days. If mortality was observed in two animals out of three animals, then the dose administered was assigned as toxic dose. If the mortality was observed in one animal, then the same dose was repeated to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher doses such 50,100,150,200, 400,500 & 2000 mg/kg body weight. The animals were observed for toxic symptoms for 72 h.

2.4. Screening for Diuretic Activity (Lipschitz test)

Table 1 Allocation of Group

Group	Treatment	Dose	Animals required
G1	Methanolic Extract	100 mg/kg	3
G2	Methanolic Extract	200 mg/kg	3
G3	Methanolic Extract	400 mg/kg	3
G4	Standard group (furosemide)	10 mg/kg	3
G5	Control group	2% CMC in normal saline	3

2.5. Lipschitz model

- Male Albino rats were divided into 5 groups of 3 rats in each.
- Groups I, II, III were treated with low, medium, and high doses of methanolic extract of plant of *Leptadenia pyrotechnica* orally.
- Group IV received Furosemide (10 mg/kg,) orally.
- Group V serves as normal control received vehicle orally (CMC 2% in normal saline 10 ml/kg b.wt).
- Immediately after the methanolic extract (L.p.) treatment all the rats were placed in the metabolic cages (3 per cage), specially designed to separate urine and feces and kept at 21°C±0.5°C. The total volume of urine collected for 5 hrs. Was measured at the end. During this period no food and water was made available to animals.

Various parameters like total urine volume and concentration of sodium and potassium in the urine were measured and estimated respectively.

2.6. Estimation of urinary electrolyte

Urine electrolytes (sodium and potassium) were determined by Flame Photometry method at Pacific college of Pharmacy, PAHER, Udaipur.

2.7. Statistical analysis

Experimental results were expressed as mean + SEM (n=3). Statistical analysis was performed with one-way-ANOVA followed by Dunnett t-test.

3. Results and discussion

3.1. Acute oral toxicity

Oral administration of the *Leptadenia pyrotechnica* extract up to dose 2000 mg/kg in mice did not cause any mortality or any toxicity during the experimentation period However, mice after receiving *Leptadenia pyrotechnica* extract exhibited a normal action for all the group of animals. The *Leptadenia pyrotechnica* at 2000mg/kg body weight orally is safe for consumption and for medicinal uses in according with the OECD guidelines No.423. Based on the above study to fixed dose were 100, 200 and 400 mg/ kg for diuretic action.

3.2. Diuretic activity

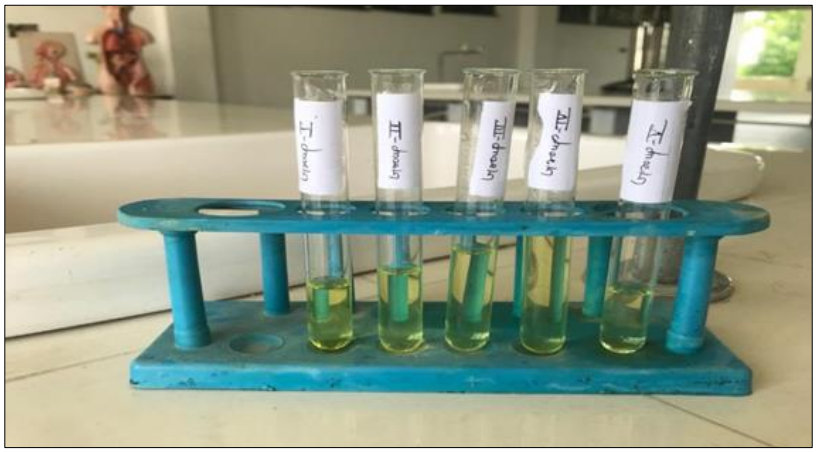

Figure 1 Collection of total urine volume after 5hrs of group I, II, III, IV and V

Table 1 Total Volume of urine and electrolyte concentration

S. No.	Groups	Total Urine Vol (ml/kg b.wt/5 h)	Na+ mmol/L	K+ mmol/L
1	methanolic extract of *Leptadenia pyrotechnica* (100 mg/kg b.wt)	7.20±0.02	60.40+2.80	32.13+1.82
2	methanolic extract of *Leptadenia pyrotechnica* (200 mg/kg b.wt)	7.41±0.02	82.99+2.00	38.93+2.67
3	methanolic extract of *Leptadenia pyrotechnica* (400 mg/kg b.wt)	10.46±0.02	92.53+2.32	42.11+1.79
4	Standard (Frusemide 10 mg/kg b.wt)	11.23±0.01	95.05+2.09	43.81+1.60
5	Control 2% CMC in normal saline (10 ml/Kg b. wt)	6.45±0.02	56.03 + 2.16	25.09 + 1.51

The results obtained after the evaluation of diuretic activity of methanolic extract of whole plant of *Leptadenia pyrotechnica.* From the result it can be observed that methanolic extract of whole plant of *Leptadenia pyrotechnica* has shown a significant diuretic activity by increasing urinary output and increased excretion of sodium and potassium when compared to standard. The effect of methanolic extract of whole plant of *Leptadenia pyrotechnica* was found to be dose dependent, i.e., among the three doses studied, higher dose produced more effect. A comparison was made with the standard diuretic drug furosemide, the diuretic effect observed after treatment with of methanolic extract of whole plant of *Leptadenia pyrotechnica* was found to be significant in terms of urinary output, sodium and potassium concentrations. Determination of urinary electrolyte concentration revealed that of methanolic extract of whole plant of *Leptadenia pyrotechnica* was effective in increasing urinary electrolyte concentrations for ions tested (Na^+, K^+).

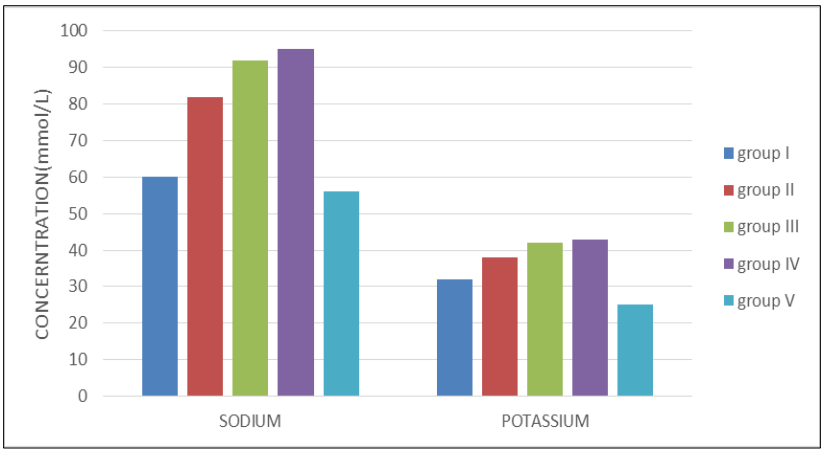

Figure 2 Effect of methanolic extract of whole plant of *Leptadenia pyrotechnica* on urinary electrolytes (sodium and potassium) in male albino rats

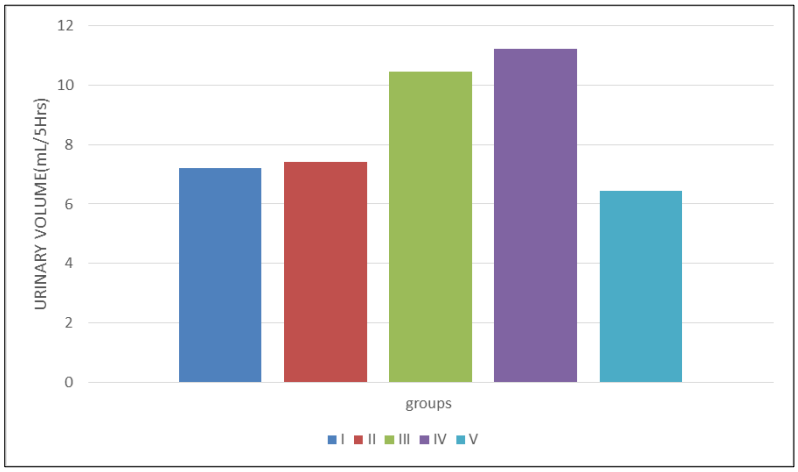

Figure 3 Effect of Frusemide and methanolic extract of whole plant of *Leptadenia pyrotechnica* on Urine Volume in male Albino Rats

4. Conclusion

Results showed that single dose administration of methanolic extract of whole plant of *Leptadenia pyrotechnica* as 100, 200 and 400 mg/Kg and standard Furosemide (10 mg/kg) have increased the urinary output along with an increase in concentration of Sodium and Potassium ions in urine. Methanolic extract of whole plant of *Leptadenia pyrotechnica* 400 mg/Kg produced a greater diuretic activity which is comparable to that of standard Furosemide (10 mg/kg). In traditional medicine the plant is used for its diuretic activity. Ours scientific study come up with identification of so many phytoconstituents reported earlier for this diuretic effect in our Methanolic extract of whole plant of *Leptadenia pyrotechnica*. Thus our study supports and justifies the rationale behind the folklore use of whole plant *Leptadenia pyrotechnica* for its diuretic activity.

Compliance with ethical standards

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Disclosure of conflict of interest

No conflict of interest.

Statement of ethical approval

The experiment complied with the guidelines for animal experimentation of our laboratory and was approved by the Institutional Animal Ethical Committee (IAEC) with IAEC Approval No. 1622/PO/Re/S/12/CPCSEA-PCP22(001).

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