

(RESEARCH ARTICLE)



Evaluation of diuretic activity of hydroalcoholic extract of *Leptadenia pyrotechnica* in rats

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World Journal of Biology Pharmacy and Health Sciences, 2024, 18(02), 091–101

Publication history: Received on 26 February 2024; revised on 09 April 2024; accepted on 12 April 2024

Article DOI: <https://doi.org/10.30574/wjbphs.2024.18.2.0183>

Abstract

In traditional Indian medicine, the whole plant of *Leptadenia pyrotechnica* (Asclepiadaceae) is said to have a strong diuretic effect. The aim of this study was to evaluate the diuretic potential of the hydroalcoholic extract of *Leptadenia pyrotechnica* (HALPE) in rats. Group I, control (0.5% CMC saline, 10 ml/kg, b.w.), group II, furosemide (10 mg/kg b.w.) as a standard drug, and different concentrations of L.P. (100, 200, and 400 mg/kg b.w.) were administered intraperitoneally (n = 5 per treatment group) to hydrated rats, and their urine output was monitored at 6 h, 12 h, and 18 h after drug administration. The diuretic effect of the extract was highly significant compared to control animals. Halpe at a dose of 400 mg/kg shows a significant increase in urine volume with increased urine output. This study suggests that the active component of L.P. had a diuretic effect similar to that of furosemide. These results confirm the traditional use of *Leptadenia pyrotechnica* as a diuretic agent. Since ancient times, medicinal plants, sometimes called herbs, have been used in various traditional medical procedures. *Leptadenia pyrotechnica* is a plant commonly known as the chimp. It is traditionally used in rural areas for the treatment of many diseases, such as antifungal, antibacterial, anticancer, antioxidant, wound healing, anthelmintic, atherosclerosis, hypolipidemia, diuretic, diabetes, and hepatoprotective. In rural areas, this plant is also used as medicine and as a vegetable. First, the plants are dried and turned into powder, which is then used as a paste for ringworm, antifungal, wound treatment, and new plants for many other diseases.

Keywords: *Leptadenia pyrotechnica*; Hydro-alcoholic extract; Phytochemical study; Diuretic activity

1. Introduction

Diuretics, sometimes called water pills, help rid your body of salt (sodium) and water. Most of these medicines help your kidneys release more sodium into your urine. The sodium helps remove water from your blood, decreasing the amount of fluid flowing through your veins and arteries. This reduces blood pressure. Diuretics are drugs that cause a net loss of sodium and water in urine. However, sodium balance is soon restored, even with continuing diuretic action, by the compensatory homeostatic mechanism of the body, albeit with a certain degree of persisting sodium deficit and a reduction in extracellular fluid volume. Diuretics are among the most widely prescribed drugs. The application of diuretics in the management of hypertension has outstripped their use in edema. The availability of diuretics has also had a major impact on the understanding of renal physiology. Diuretics are involved in some pathological conditions such as low blood pressure, reduced fluid retention, oedema, swollen ankles, relief from pain, burning sensations associated with cystitis, heart failure, hypertension, liver cirrhosis, kidney seases, an and pulmonary and systemic oedema.

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1.1. Plant profile

1.1.1. Taxonomical Classification

- Gentianales Family: Asclepiadaceae
- Genus: *Leptadenia*
- Specific: *Pyrotechnica*
- Phylum: Magnoliophyta
- Subphylum: Spermatophytina
- Class: Magnoliopsida
- Subclass: Lamiidae
- Kingdom: Plantae
- Subkingdom: Viridaeplantae.

Since ancient times, medicinal plants, sometimes called herbs, have been used in various traditional medical procedures. *Leptadenia pyrotechnica* is a plant commonly known as the chimp. It is traditionally used in rural areas for the treatment of many diseases, such as antifungal, antibacterial, anticancer, antioxidant, wound healing, anthelmintic, atherosclerosis, hypolipidemia, diuretic, diabetes, and hepatoprotective. In rural areas, this plant is also used as medicine and as a vegetable. First, the plants are dried, turned into powders, and used as a paste for ringworm, antifungal, wound treatment, and many other . diseases.

1.2. Distribution geographic

Leptadenia pyrotechnica may be found in arid environments, particularly in desert areas, throughout the whole state of Rajasthan. In India, it is frequently found in Punjab, Banswara, Palod, Dungarpur, Kota, and western Uttar Pradesh.

1.2.1. Synonyms

Khhip, Kheep, and Khimp in India; in Pakistan, Kip, Khip, and Kheep; in Sudan, Kalumbo, Saabe, and Kalimbo.

1.2.2. Morphology

The erect, climbing shrub *Leptadenia pyrotechnica* can reach a height of 0.5 to 2.6 metres. It features a green stem and alternating, bushy branches with fluid sap that are a light green colour. When present, the leaves are sessile, narrowly linear to linear lanceolate, and range in size from 2.6 to 6.5 cm by 0.2 to 0.3 cm. Flowers are grouped in lateral umbellate cymes and have a greenish-yellow colour. The stamina corona is composed of valuate corolla lobes, a five-scaled outer corona, and a raised, undulating fleshy ring. Each flower is actinomorphic, bisexual, and pentamerous, with sympetalous corollas and sepals that are only joined at the base. Glabrous, terete, lanceolate, and measuring 7.0–14.0 x 0.5–0.8 cm, folicles are. The ovatelanceolate, 5-7 mm long, glabrous, hairy seeds have tufts of hair that range in length from 2.6 to 3.7 cm. Flowers blossom and fruit bear between August and January.



Figure 1 Dry and Crushed plant

1.2.3. Traditional uses of *Leptadenia pyrotechnica*

- A plant maceration is used as an eye ointment and eye wash in rural areas.

- For the treatment of dermatitis and smallpox, plant sap (fluid) is applied to the skin.
- For the treatment of renal diseases, kidney stones, and cough, aerial portions are infused.
- A root infusion is used in rural areas to cure constipation.
- Crushed stems are used to cure wound healing and cure ringworm.
- The fibre of *Leptadenia pyrotechnica* is used as an antihistaminic and expectorant. The whole plant is used for the treatment of wounds in Yemen, and it has proven to have antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis*.
- Fresh juice from the plant is used for abortion. Plant sap is applied to eczema and other skin diseases and is also used to treat diabetes.
- The latex or leaf paste is applied over the thorn injury for thorn removal.
- A whole plant infusion is mixed with buttermilk and given for uterine prolapse and stomach disorders in the Sariska region of Rajasthan.
- It is used to cure constipation and is considered good for health in the Bikaner region of Rajasthan.
- In the Sudanodecanian region of central Sahara, it is traditionally used for fever, cough, kidney disorders, stones, and urinary disease.

2. Material and methods

2.1. Preparation of Whole Plant Extract by the Maceration Method

I collected this plant, *Leptadenia pyrotechnica*, commonly known as kheep, from a nearby farm. Then it was dried in sunlight, and the dried plant material was coarsely ground and stored for further use.

Maceration Principle: In this process, solid ingredients are placed in a stoppered container with the whole of the solvent (hydroalcoholic) and allowed to stand for a period of at least 7 days (7–14 days) with frequent agitation until soluble matter is dissolved. The mixture is then strained (through sieves or nets), the marc is pressed, and the combined liquids are clarified (cleaned by filtration or decantation) after standing. Stoppered containers are generally used to reduce the loss of solvents by evaporation. If the volume of solvent is reduced by evaporation, then the extract may become concentrated, which may not be desired. The drug is allowed to stand for a few days.

- To help the solvent penetrate the cells of the drugs,
- To provide the time for partitioning the active ingredient into the solvent and
- To transfer the drug out of the cells into the bulk of the solvent.

Frequent agitation is required to reduce the localised concentration around the cells and tissues. As indicated in the pharmacopoeia, the process consists of the following:

- Placing the solid materials with the whole menstruum in the closed vessel and allowing it to stand for 7 days, shaking occasionally.
- Strained, press the marc, and the liquid is obtained.
- Liquid (i.e., the extract) is clarified by subsidence or filtration.

The process is normally used for the preparation of tinctures or extracts, and menstruum is usually alcoholic, hydroalcoholic (in the case of tinctures), or may be aqueous.



Figure 2 Extracts of *Leptadenia pyrotechnica*

Apparatus: A glass bottle or any other container that can be well-stoppered can be used for the maceration process. A closed container is essential to prevent the evaporation of menstruum, which is mostly concentrated alcohol. Otherwise, this may lead to variations in strength as no adjustment in volume is made.

2.1.1. Method

- Water or alcohol is used as menstruum, and the drug-to-menstruum ratio is 70:30.
- The drug is placed with the whole of the menstruum in a closed vessel for seven days. During this period, shaking is done occasionally.
- After 14 days, the liquid is strained and the marc is pressed.
- The expressed liquid is mixed with the strained liquid.
- It is then filtered to make a clear liquid.
- The final volume of drug is contained in an airtight glass container.

The extraction process yielded a total weight of 20 grams of dried *Leptadenia pyrotechnica* plant. This weight represents the combined mass of the extracted compounds obtained from the plant using the maceration method.

2.2. Evaluation of the drug

Ash value: Ash values are helpful in determining the quality and purity of a crude drug, especially in the powdered form. The object of ash vegetable drugs is to remove all traces of organic matter that may otherwise interfere in an analytical determination of incineration. Crude drugs' normally leave an ash usually consisting of carbonates, phosphates, and silicates of sodium, potassium, calcium, and magnesium. The total ash of a crude drug reflects the care taken in its preparation. A higher limit of acid-insoluble ash is imposed, especially in cases where silica may be present or when the calcium oxalate content of the drug is very high. Some analysts favour the mixing of sulfuric acid with the powdered crude drug before ash, and this sulfurated ash is normally less fusible than ordinary ash.

2.2.1. Procedure

Determination of total ash

- Weigh accurately about 2 g of the powdered drug in a tared silica crucible.
- Incinerate the powdered drug by gradually increasing the heat until it is free from carbon and cools. Keep it in the desiccator.
- Weight the ash and calculate the percentage of total ash with reference to the air-dried sample.

Determination of acid-insoluble ash

- Boil the total ash obtained as above for 5 minutes with 25 ml of dilute hydrochloric acid.

- Filter and collect the insoluble matter on ash-less filter paper; wash the filter paper with hot water; ignite in a tared crucible; cool and keep in a desiccator. Weigh the residue and calculate the acid-insoluble ash of the drug with reference to the air-dried drug.

Determination of water-soluble ash

- The total ash was boiled for 5 min. with 25 ml of water, collected on tared filter paper, dried, and weighed.
- The weight of the insoluble matter was subtracted from the weight of the ash to get water-soluble ash.

Ash value: The total ash value of the *Leptadenia pyrotechnica* compound was determined to be 10%. This value indicates the amount of inorganic material present in the compound. The acid-insoluble ash value was measured at 7.5%, representing the portion of ash that is insoluble in dilute hydrochloric acid.

2.2.2. Observation

- Weight of the empty silica crucible: 67.95 g
- Weight of powdered crude drug: 2 g
- Weight of the crucible with ash: 68.10 g

Calculation: -

$$\begin{aligned}\text{Weight of ash} &= \text{weight of silica crucible with ash} - \text{weight of empty silica} \\ &= 68.10 - 67.90 \\ &= 0.20 \text{ gm}\end{aligned}$$

The ash value of 2 grammes of powdered drug is 0.20 grammes.

Therefore, the ash value of 100 grammes of drug is $0.20/2 \times 100$.

$$\text{Percentage (\%)} \text{ ash value} = 10\%$$

2.2.3. Calculation for acid-insoluble ash

- Weight of the empty crucible: 67.95 g
- Weight of crucible + acid insoluble ash = 68.10 g
- Weight of acid-insoluble ash = $68.10 - 67.95 = 0.15 \text{ g}$
- Percentage (%) acid insoluble ash 100 gm of drug = $0.15/2 \times 100$

(%) acid insoluble ash is 7.5%.



Figure 3 Determination of Ash value

2.2.4. Calculations for water-soluble ash

- Weight of empty crucible = 67.95 g
 - Weight of crucible + total ash = 69.95 g
 - Weight of crucible + water insoluble ash = 69 g
 - Weight of water insoluble Ash = 69 - 67.95 = 0.05 g
 - Weight of water-soluble ash = Total Ash (2 g) - water-insoluble ash (1.05 g)
 - Weight of water-soluble ash = 0.95 g
- Percentage (%) of water-soluble ash in 100 g of drug = $0.95/2 \times 100$

(%) water-soluble ash = 47.50%

2.2.5. Loss on drying

Five grammes of powder were dried in an oven at 105°C and weighed.

Our result is that 5 g of powder was dried in an oven at 105°C and weighed at 4.8 g.

2.2.6. Phyto-chemical screening

Table 1 List of phytoconstituent present in plant

S.no	Chemical tests	Alcoholic extract
1.	Alkaloids	(+)
2.	Carbohydrates	(+)
3.	Protein	(-)
4.	Steroids	(-)
5.	Sterols	(+)
6.	Phenols	(-)
7.	Tannin	(-)
8.	Flavonoids	(+)
9.	Glycoside	(+)
10.	Saponin	(+)

2.3. Experimental animals

Albino Wistar rats of either sex, approximately in the in the same age group, were used after being acclimatised for a week at laboratory conditions. They were provided with a with a standard rodent pellet diet (Lipton India) and water ad libitum. The animals had free access to food and water and were maintained under a 12:12-hour light and dark cycle. All experiments were carried out during the day.. The protocol was approved by the institutional animal ethical committee, and care of the animals was taken as per the guidelines of the committee for the purpose of control and supervision in experiments on animals (CPCSEA), representative of animal welfare, Govt. of India. The experimental protocol was approved by the Institutional Animal Ethics Committee, IAEC Ref. No. 870/PO/Re/S/05/CPCSEA.

2.4. Toxicity study

Herbal toxicity according to OECD Guideline 423: The acute oral toxicity test is intended to provide information on the likely health hazards that appear upon high-dose exposure to a test item. The test item is administered once at one of the pre-defined doses (5/50, 300, or 2000 mg/kg) in the animals (preferably female rats or mice) in step I.

About 48 or 72 hours after the step-I dosing, the presence or absence of toxic clinical signs, including the number of deaths, helps to decide whether to proceed with additional testing with the same dose or with an increase or decrease in the dose for step II.

Thus, the procedure helps to classify the test item by fixed LD50 cut-off values.

2.4.1. Acute Toxicity Procedure:

- At the end of the acclimatisation period, the animals are fasted overnight (~16–18 h in the case of rats), but water is provided.
- The experiment is performed in two steps, viz., steps I and II, with a minimum 48/72-hour interval between the steps. Three animals are used in each step.
- The bodyweight of the overnight fasted animal is measured prior to dosing.
- The animal is administered with one of the pre-defined doses and water is withdrawn for the next 4 h thereafter.
- Following the dosing, the animal is intensively monitored for toxic clinical signs for the next 4 h (preferably at 30 min, 1, 2 and 4 h). After 4 hours of intensive monitoring, food and water are provided to the animals.
- Animals are observed for the below-mentioned clinical signs.
- Once a week, a complete functional observation battery is performed to record the signs of toxicity, if any.
- The animals are observed at least once daily for mortality or morbidity for a total of 14 days. The bodyweight of the animal is measured once in a week and before the necropsy.
- Any animal(s) found to be severely mutilated during the experiment period is euthanized humanely for animal welfare reasons and recorded accordingly (OECD 2000). At the end of 14 days of monitoring, all the test animals are euthanized and subjected to necropsy. Histopathological examination is necessary only if gross pathological changes are observed in the test animal organ(s) (OECD GLP n.d.).

Our **results** demonstrated that the hydro-alcoholic extract of *Leptadenia pyrotechnica* possesses the lowest toxicity effects, as indicated in our rat model. No deaths or signs of toxicity were observed in the rats that received the extract up to an oral acute limit dose of 5000 mg/kg. Based on the above study, the fixed doses were 100, 200, and 400 mg/kg for diuretic action.



Figure 4 Doses prepared

2.5. Screening for Diuretic Activity (Lipschitz Test)

- Test System Details:
 - Species: *Rattus norvegicus*.
 - Strain: Wistar
 - Age: 6–10 weeks
 - Body Weight: 150–200g
 - Sex: male or female
 - of animals: 5 per group

- Allocation of Groups

Table 2 Allocation of Group

Groups	No. of Animals	Dosing	No. of Days
Group I (Control)	05	0.5CMC	6
Group II (standard)	05	10mg/kg	6
Group III (Test)	05	100mg/kg	6
Group IV (Test)	05	200mg/kg	6
Group V (Test)	05	400mg/kg	6

The route of drug administration is oral.

- Method

- The diuretic activity of the hydroalcoholic extract of *Leptadenia pyrethrina* albino rats was studied by the Lipschitz test.
- Wistar rats were divided into five groups of five rats each.
- The group I serves as the normal control vehicle (0.5 CMC); the group II vehicle (0.5 CMC); the group II received furosemide (10 mg/kg) in the vehicle; other groups III, IV, and V (100, 200, and 400 mg/kg b.w.) were treated with low, medium, and high doses of hydroalcoholic extract of *Leptadenia pyrotechnica* in the vehicle; and immediately after the extract treatment, all the rats were hydrated with saline (15 ml/kg) and placed in the metabolic cages (3 per cage), specially designed to separate urine and faeces, and kept at 21°C±0.5°C. The total volume of urine collected for 6 hours, 12 hours, and 18 hours was measured at the end. During this period, no food was available to animals.
- The parameters of total urine volume are measured.

The results obtained after the evaluation of the diuretic activity of the hydroalcoholic extract of the whole plant of *Leptadenia pyrethrina* are shown in Table 1. From the result, it can be observed that the hydroalcoholic extract of the whole plant of *Leptadenia pyrethrina* has shown significant diuretic activity by increasing urinary output when compared to the standard drug. The hydro-alcoholic effect of the extract of the whole plant of *Leptadenia pyrotechnica* was found to be dose-dependent, i.e., among the three doses studied, the higher dose produced more effect. A comparison was made with the standard diuretic drug furosemide (Lasix), and the diuretic effect observed after treatment with a hydroalcoholic extract of the whole plant of *Leptadenia pyrethrina* was found to be significant in terms of urinary output.

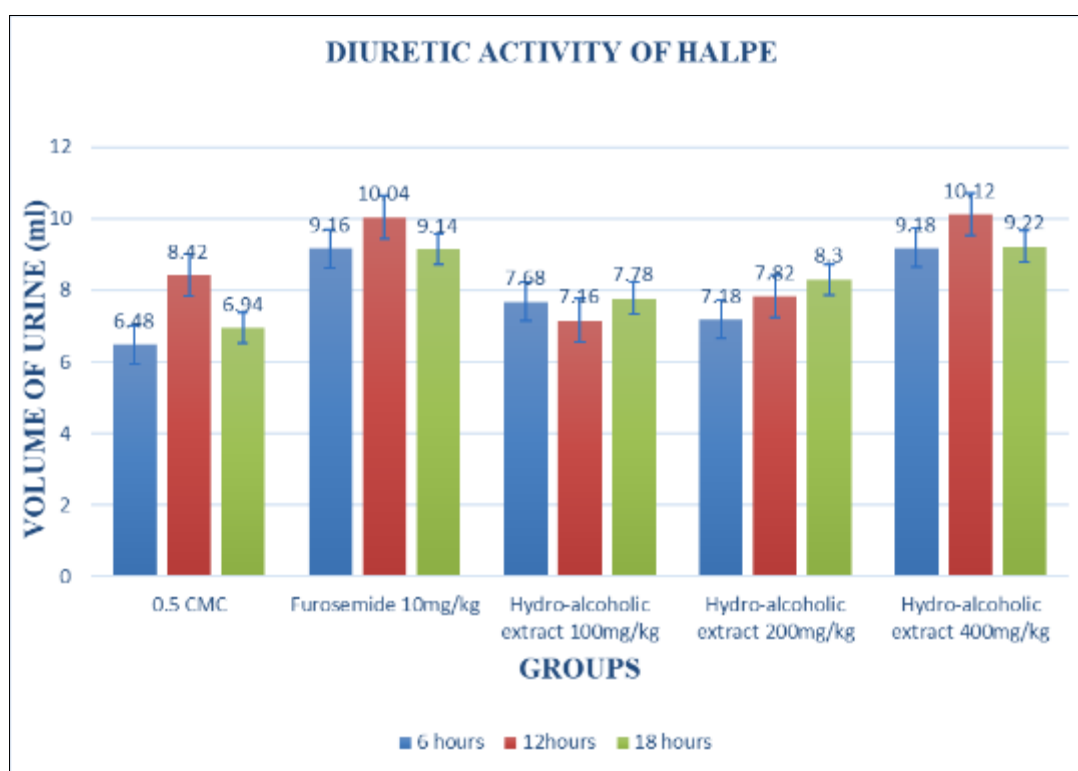


Figure 5 Collection of total urine volume of group I, II, III, IV and V

Table 3 Effect of Furosemide and Hydro-alcoholic extract of whole plant of *Leptadenia pyrotechnica* on Urine Volume in Rats

Group	6 hours	12hours	18 hours
0.5 CMC	6.48±0.2596 ^{ns}	8.42 ± 0.2353 ^{ns}	6.94 ±0.2942 ^{ns}
Furosemide 10mg/kg	9.16±0.2619 ^{**}	10.04±0.2111 ^{**}	9.14±0.2821 ^{**}
Hydro-alcoholic extract 100mg/kg	7.68±0.4994 ^{ns}	7.16± 0.5546 ^{ns}	7.78±0.4913 ^{ns}
Hydro-alcoholic extract 200mg/kg	7.18±0.2905 ^{ns}	7.82±0.3512 ^{ns}	8.3±0.3082 ^{ns}
Hydro-alcoholic extract 400mg/kg	9.18±0.3967 ^{**}	10.12±0.3527 ^{**}	9.22±0.2782 ^{**}

Values (ng/ml) are expressed as mean ± SEM (n=5). Evaluated by two way anova followed by Tukey's multiple comparisons test (****p <0.0001, ***p <0.001, **p<0.01, *p<0.1, ns- Non Significant).

**Figure 6** Effect of Frusemide and hydro-alcoholic extract of whole plant of *Leptadenia pyrotechnica* on Urine Volume in male Albino Rats

3. Result

The results obtained with the evaluation of the diuretic activity of the hydro-alcoholic extract of *Leptadenia pyrotechnica* are shown in the table and figure (graph). As a result, the extract of the plant *Leptadenia pyrotechnica* significantly increased the urine output when compared to the control group. A comparison was made with the standard drug furosemide, and the excretion of urine output significantly increased in the extract at the dose level of 400 mg/kg hydro-alcoholic extract of *Leptadenia pyrotechnica*.

4. Conclusion

The hydro-alcoholic extract of the whole plant of *Leptadenia pyrotechnica* at doses of 100, 200, 200 and 400 mg/kg and standard furosemide (10 mg/kg) have increased the urinary output in Wistar rats. The hydro-alcoholic extract of the whole plant of *Leptadenia pyrotechnica* at 400 mg/kg produced a greater diuretic activity that is comparable to that of

standard furosemide (10 mg/kg). In traditional medicine, *Leptadenia pyrotechnica* is used for its diuretic activity. This study supports and justifies the rationale behind the folklore use of the whole plant *Leptadenia pyrotechnica* for its diuretic activity. However, the chemical constituents responsible for the diuretic activity need to be identified and isolated in further studies.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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