

## **Evaluation of diuretic activity of aqueous and ethanolic extracts of *Lawsonia inermis* leaves in rats**

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### **ABSTRACT**

*Lawsonia inermis* commonly known as henna is a perennial herbaceous plant belonging to family Lythraceae. Traditionally it has been used to treat skin diseases, dysentery, bronchitis, anemia and inflammation. The aim of present study was to investigate the diuretic activity of aqueous and ethanolic extracts of *Lawsonia inermis* leaves in rats. Aqueous and Ethanolic extracts were administered orally at a dose of 250mg/kg and 500mg/kg. The results were analysed by One Way Analysis Of Variance (ANOVA). The results showed that both the extracts of leaves showed significant diuresis relatively ethanolic extract has shown more activity than aqueous extract. The findings concluded that *Lawsonia inermis* leaves exhibit diuresis and further studies are suggested to isolate the active principles responsible for the activity.

**Keywords:** *Lawsonia inermis*, Aqueous extract, Ethanolic extract, Diuretic, Urine volume.

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### **INTRODUCTION**

Since the time immemorial our traditional system of medicine and folklore claiming that medicinal plants as a whole or their parts are being used in all types of diseases successfully [1]. About 65% of world populations have access to local medicinal plant knowledge system [2]. Traditional systems of medicine are popular in developing countries and upto 80% of population relies on traditional medicines or folk remedies for their primary health care needs [3]. India has about 45000 plant species and among them, several thousands have been claimed to possess medicinal properties [4]. Diuretics are 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 is beneficial in many life threatening disease conditions such as congestive heart failure, nephritic syndrome, cirrhosis, renal failure, hypertension and pregnancy toxemia [5]. Most diuretic drugs have the adverse effects on quality of life including impotence, fatigue and weakness [6]. Herbal medicines are in great demand in the developed as well as developing countries for primary health care because of their wide biological and medicinal activities, higher safety margins and lesser costs [7]. Indian ayurvedic system is rich in treating renal problems [8]. *Lawsonia inermis* (Lythraceae) is a perennial plant commonly called

as henna, having different vernacular names in India viz., mehendi in Hindi, mendika in Sanskrit, mailanchi in Malayalam, muruthani in tamil, benjati in oriya, mayilanchi in kannada and mehendi in Bengali [9]. It is native to North Africa and South East Asia and often cultivated as an ornamental plant throughout India, Persia and along the African coast of the Mediterranean sea [10]. *Lawsonia inermis* is a glabrous branched shrub or small tree (2 to 6 m in height). Leaves are small, opposite, entire margin elliptical to broadly lanceolate, sub-sessile, about 1.5 to 5cm long, 0.5 to 2cm wide, greenish brown to dull green, petiole short and glabrous acute or obtuse apex with tapering base [11,12,13]. The ethnomedical uses include the use of leaves as expectorant, anti-inflammatory, depurative, liver tonic, haematinic, styptic and febrifuge [14,15,16,17]. Literature review also indicated that diuretic activity of this species has not been clinically evaluated so far. So the present study aimed to evaluate the diuretic activity of aqueous and ethanolic extracts of *Lawsonia inermis* leaves in rats.

## MATERIALS AND METHODS

### Plant material

The fresh leaves of *Lawsonia inermis* belonging to the family Lythraceae were collected in January 2011 at the local areas of Anantapur district, Andhra Pradesh, India. The plant material was identified and authenticated by Dr. J. Raveendra Reddy, M.Pharm, Ph.D, Department of Pharmacognosy, Raghavendra Institute of Pharmaceutical Education and Research, Anantapur and voucher specimen riper-11/11 is preserved in department of pharmacology, Raghavendra institute of pharmaceutical education and research, Anantapur.

### Processing of sample

The fresh leaves of *Lawsonia inermis* plant were dried under shade for 20-25 days. The dried leaves were pulverized into fine powder and used for extraction.

### Preparation of extracts

#### Preparation of ethanolic extract of *Lawsonia inermis* leaves

The powdered drug of leaves was loaded into the soxhlet extractor and subjected to extraction with ethanol. After extraction, the solvent was distilled off and the extracts were concentrated to dryness at room temperature. The percentage yield of ethanolic extract of *Lawsonia inermis* was found to be 12% (Table.1).

#### Preparation of aqueous extract of *Lawsonia inermis* leaves

The powdered crude drug of leaves was subjected for extraction process by maceration with water at room temperature for 48 hours. The extract was filtered and concentrated to dryness at room temperature. The percentage yield of aqueous extract of *Lawsonia inermis* was found to be 10% (Table.1).

### Phytochemical analysis

The aqueous and ethanolic extracts of *Lawsonia inermis* leaves were subjected to preliminary phytochemical screening.

### Drugs and chemicals

Deionised water, methanol (Merck specialities pvt. Ltd.), furosemide (Aventis pharma Ltd).

### Experimental animals

Male wistar rats of 200-300gm were used to carry out the diuretic activity. The animals had free access to standard commercial diet and water *ad libitum* and were housed in cages under standard laboratory conditions i.e., 12:12 hour light/dark cycle at  $25\pm 2^{\circ}\text{C}$ . The Institutional Animal Ethics Committee (878/ac/05/CPCSEA/012/2011) has approved the experimental protocol at post graduate department of pharmacology, Raghavendra institute of Pharmaceutical education research, Anantapur, Andhra Pradesh, India.

### Acute toxicity studies

Acute toxicity studies for aqueous and ethanolic extracts of *Lawsonia inermis* was carried out in rats at different doses (500–3000 mg/kg, orally), showed no gross evidence of any abnormalities in the rats up to the end of 72hr of the observation period. This indicates the safety of extract.. Acute toxicity study was done as per OECD,2006 Guidelines. Hence we selected 250mg/kg and 500mg/kg as low and high doses.

### Diuretic activity

The method of Lipschitz et al was employed for the assessment of diuretic activity. According to this method, the animal should be deprived of food and water for 18 hours prior to the experiment, and were randomly divided into six groups of six animals each as follows:

- Group 1 (Control) - received saline 25ml/kg p.o.
- Group 2 (Standard) - received furosemide 20mg/kg p.o.
- Group 3 (Test ) - received aqueous extract of *Lawsonia inermis* 250 mg/kg p.o.
- Group 4 (Test ) - received aqueous extract of *Lawsonia inermis* 500 mg/kg p.o.
- Group 5 (Test ) - received ethanolic extract of *Lawsonia inermis* 250mg/kg p.o.
- Group 6 (Test ) - received ethanolic extract of *Lawsonia inermis* 500mg/kg p.o.

Immediately after administration, animals were placed in metabolic cages specially designed to separate urine faecal matter. During the period of study no food, water was made available to the animals. The total volume of urine was collected and measured from control, standard and extract treated groups up to 5hours of administration. The parameters monitored for the each individual rat were total urine volume and urine concentration of  $\text{Na}^+$ ,  $\text{K}^+$  and  $\text{Cl}^-$ . Concentration of  $\text{Na}^+$  and  $\text{K}^+$  were determined using flame photometer while  $\text{Cl}^-$  concentration was estimated titrimetrically using 0.02N  $\text{AgNO}_3$  with 5% potassium chromate as an indicator. Appearance of brick red precipitate was taken as the end point.

### Statistical Analysis

The results were expressed as a mean  $\pm$  S.E.M. The differences were compared using One Way Analysis of Variance (ANOVA) and subsequently followed by Bonferroni's test.

## RESULTS

**Table 1: %Yield, texture and colour of aqueous and ethanolic extracts of leaves of *Lawsonia inermis*.**

Plant part	Type of Extract	% Yield	Texture	Colour
Leaves of <i>Lawsonia inermis</i>	Aqueous extract	10	Gummy	Greenish black
	Ethanolic extract	12	Gummy	Greenish black

### Phytochemical analysis

Both aqueous and ethanolic extracts of *Lawsonia inermis* revealed the presence of alkaloids, tannins, flavonoids, phenolic compounds and triterpenoids (Table-2).

Table 2: Phytochemical analysis of aqueous and ethanolic extracts of *Lawsonia inermis*

Chemical constituents	<i>Lawsonia inermis</i>	
	Aqueous extract	Ethanolic extract
Alkaloids	+	+
Tannins	+	+
Flavonoids	+	+
Phenolic compounds	+	+
Triterpenoids	+	+

+ = Present

**Effect of aqueous and ethanolic extracts on urine output in rats:**

Urine volume in rats treated with aqueous extract of *Lawsonia inermis* at low and high doses were 4.6ml and 6.1ml respectively.

Urine volume in rats treated with ethanolic extract of *Lawsonia inermis* at low and high dose were 7.3ml and 9.0ml respectively.

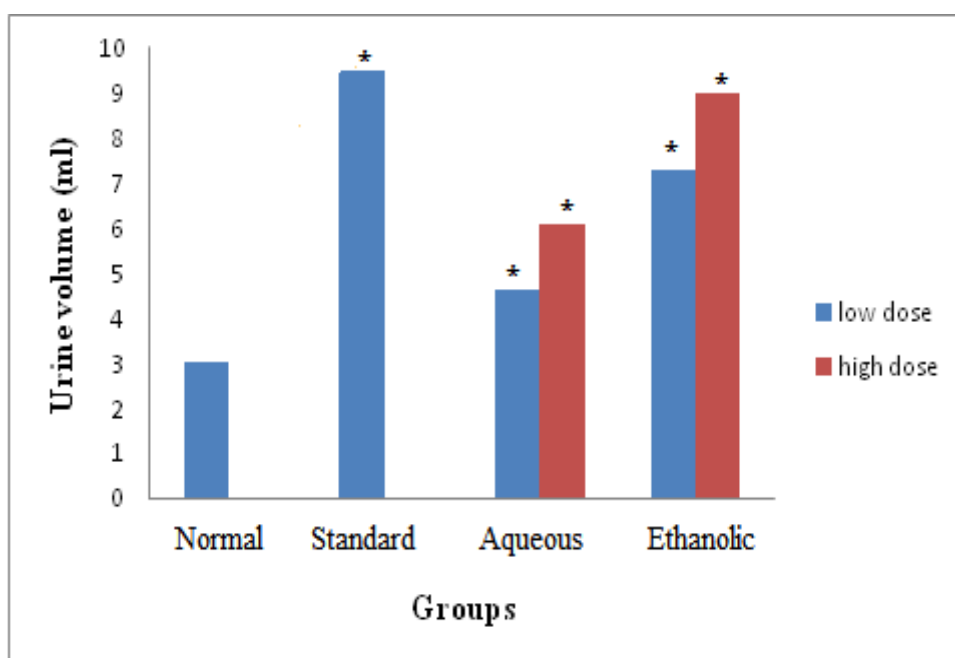
\* $p < 0.001$  when compared to normal

Figure 1: Effect of aqueous and ethanolic extracts on urine output in rats

Table3: Effect of aqueous and ethanolic extracts on electrolyte excretion in rats

Treatment	Dose & Route	Electrolyte levels		
		Sodium (m.Eq/L)	Potassium (m.Eq/L)	Chloride (m.Eq/L)
Control	25 ml/kg p.o	85.10 ± 2.892	59.03 ± 1.302	97.83 ± 1.126
Furosemide	20 mg/kg p.o	145.2 ± 2.470**	87.67 ± 1.782**	174.3 ± 2.634**
Aqueous extract	250 mg/kg p.o	113.8 ± 2.042**	66.60 ± 0.6429*	127.3 ± 1.868**
Aqueous extract	500 mg/kg p.o	127.8 ± 0.9849**	73.60 ± 0.5196**	155.6 ± 2.218**
Ethanolic extract	250mg/kg p.o	120.5 ± 0.5196**	71.20 ± 0.5033**	147.5 ± 1.637**
Ethanolic extract	500mg/kg p.o	136.2 ± 1.222**	89.13 ± 0.2906**	170.5 ± 1.947**

\* $p < 0.05$ , \*\* $p < 0.001$  when compared to normal

**Effect of aqueous and ethanolic extracts on electrolyte excretion in rats:**

The concentrations of Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup> in rats treated with aqueous extracts of *Lawsonia inermis* at low dose and high dose were 113.8mEq/L, 66.60mEq/L, 127.3mEq/L and 127.8mEq/L, 73.60mEq/L and 155.6mEq/L respectively. The concentrations of Na<sup>+</sup>, K<sup>+</sup> and Cl<sup>-</sup> in rats treated with ethanolic extracts of *Lawsonia inermis* at low dose and high dose were 120.5mEq/L, 71.20mEq/L, 147.5mEq/L and 136.2mEq/L, 89.13mEq/L and 170.5mEq/L respectively.

**DISCUSSION**

The phytochemical tests revealed the presence of alkaloids, tannins, flavonoids, phenolic compounds and triterpenoids in both ethanolic and aqueous extracts. In acute toxicity studies no mortality was found upto 3000mg/kg. Both the aqueous and ethanolic extracts showed a dose-dependent increase in urine excretion. Relatively ethanolic extract produced significant diuresis than aqueous extract. Thus the diuretic effects of both extracts are indicated by increase in both water and excretion of sodium, potassium and chloride. The active principles responsible for the diuretic effect of the extracts of *Lawsonia inermis* have not been elucidated but preliminary phytochemical analysis revealed the presence of polar compounds such as flavonoids. It may be suggested that these substances might be responsible, at least in part, for the observed diuretic activity. The effect may be produced by stimulation of regional blood flow or inhibition of vasopressin or by producing inhibition of tubular reabsorption of water and anions, the result in all cases being diuresis. From the above results it was concluded that both the extracts of *Lawsonia inermis* leaves showed significant diuretic activity relatively ethanolic extract showed more activity than aqueous extract. However, further investigations are needed to explore the exact active constituents and mechanisms responsible for the diuretic activity.

**CONCLUSION**

Aqueous and ethanolic extracts of *Lawsonia inermis* leaves have showed dose dependent increase in urine and electrolyte excretion. Relatively ethanolic extract showed more activity when compared to aqueous extract.

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