

## Research Article

# Evaluation of Diuretic Activity of Aqueous and Methanol Extracts of *Lepidium sativum* Garden Cress (Cruciferae) in Rats

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## Abstract

**Purpose:** The present study was undertaken to investigate diuretic effect of aqueous and methanol extracts of the dried seeds of *Lepidium sativum* in normal rats.

**Method:** Aqueous and methanol extracts of *L. sativum* seeds were administered to experimental rats orally at doses of 50 and 100 mg/kg p.o. Hydrochlorothiazide (10 mg/kg) was used as positive control in study. The diuretic effect of the extracts was evaluated by measuring urine volume, sodium and potassium content, conductivity and pH.

**Result:** Urine volume was significantly increased by the two doses of aqueous and methanol extracts in comparison to control group. While the excretion of sodium was also increased by both extracts, potassium excretion was only increased by the aqueous extract at a dose of 100 mg/kg. There was no significant change in the conductivity and pH of urine after administration of the *L. sativum* extracts. The diuretic effect of the extracts was comparable to that of the reference standard (hydrochlorothiazide) and the methanol had the additional advantage of a potassium-conserving effect.

**Conclusion:** We can conclude that aqueous and methanol extracts of *L. sativum* produced notable diuretic effect which appeared to be comparable to that produced by the reference diuretic HCTZ. The present study provides a quantitative basis for explaining the folkloric use of *L. sativum* as a diuretic agent in Moroccan population.

**Keywords:** Diuretic activity, *Lepidium sativum*, Herbal medicine, Medicinal plants.

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## INTRODUCTION

Medicinal plants can be important sources of unknown chemical substances with potential therapeutic effects. Besides, the World Health Organization has estimated that over 75% of the world's population still relies on plant-derived medicines, usually obtained from traditional healers, for basic health-care needs<sup>1</sup>. *Lepidium sativum* Garden Cress of the family Cruciferae is an annual erect herbaceous plant, growing up to 30 cm. It is a well known culinary herb and the leaves are widely used as a garnish and are consumed raw in salads. The plant is known to possess varied medicinal properties. The seeds are aperient, diuretic, tonic, demulcent, aphrodisiac, carminative, galactagogue and emmenagogue<sup>2</sup>. The seeds are rubefacient and are applied as a poultice for hurts and sprains<sup>3</sup>. The plant also shows teratogenic effect and antiovolatory properties<sup>4-7</sup>. The root is used in the treatment of secondary syphilis and tenesmus<sup>3</sup>. A preliminary pharmacological study of the seeds indicate the presence of cardioactive substance and is shown to have probable action through adrenergic mechanisms<sup>8</sup>. The aqueous extract of *L. sativum* seeds has been reported to exhibit a potent hypoglycaemic activity in normal and streptozotocin induced diabetic rats<sup>9</sup> as well as an antihypertensive effect when studied in both normotensive and spontaneously hypertensive rats. The effectiveness of this plant in the treatment of bronchial asthma, hiccups, cough with expectoration and bleeding piles has been reported<sup>2</sup>. Preliminary phytochemical study of *L. sativum* with standard procedures, showed that it contains flavonoids, coumarins, sulphur glycosides, triterpenes, sterols and various imidazole alkaloids<sup>10</sup>. According to previous ethnopharmacological survey carried out in the north central region of Morocco, seeds of *L. sativum* were largely used for the treatment of hypertension and renal disease<sup>11</sup>, but no previous pharmacological or clinical study was carried out to test the diuretic activity of this plant. Since the diuretic effect of *L. sativum* has never been

experimentally confirmed, the main aim of the present investigation was to evaluate the claimed diuretic activity of *L. sativum* in rats. Hydrochlorthiazide, was selected as the reference drug, since it is used clinically in some pathologies.

## MATERIALS AND METHODS

### Plant material and extraction procedure

*L. sativum* seeds were purchased from a local market in Saswad, Pune, India, and authenticated by Dr AS Upadhyay, a taxonomist at Agharkar Research Institute, Pune. A voucher specimen no. L6721 was deposited in the herbarium of the institute. The aqueous extract was prepared by boiling 1 g of dried powdered seeds of *L. sativum* in 100 ml of distilled water for 10 min and left for 15 min to infuse. Thereafter, the extract was cooled and filtered to remove particulate matter. The filtrate was lyophilized by first freezing at -22°C in a deep freezer for 8 h. The frozen material was then dried in a freeze-dryer at -40°C for 12 h at 0.01 MPa pressure. The required doses were taken and reconstituted in 10 ml of distilled water just before oral administration. For the methanol extract, the seeds were powdered and defatted with petroleum ether at 60-70°C. The powdered material was then air-dried and subjected to Soxhlet extraction for 18 h at 50-55°C. The extract was thereafter concentrated under vacuum and air-dried. The yield was 10%.

### Animals

Adult male Wistar rats, each in the weight range of 180 - 200g, were obtained from the Animal House, SGRS College of Pharmacy, Saswad, Pune, India. The animals were randomly allocated to six treatment groups of 6 animals each and kept in polypropylene cages and housed under standard conditions of temperature, humidity and dark light cycle (12h – 12h).

## Experimental protocol

All experimental protocols were approved by the Institutional Animal Ethical Committee of SGRS College of Pharmacy, Saswad, Pune. Diuretic activity was determined following the methods of Kau *et al*<sup>12</sup>, with minor modifications. The rats were randomly divided into six groups of six animals each as follows: (1) Control – given 5 ml/kg body weight of de-ionized water; (2) aqueous extract – 50 mg/kg body weight; (3) aqueous extract – 100 mg/kg body weight; (4) methanol extract – 50 mg/kg body weight; (5) methanol extract – 100 mg/kg body weight; and (6) hydrochlorothiazide – 10 mg/kg body weight<sup>13-14</sup>. In all cases, the volume of the dose was administered 5 ml/kg body weight. The animals were fasted overnight (18 h) prior to the test but with free access to tap water only and then were given an oral loading of normal saline (0.9%) of 0.05 ml per g body weight. Immediately after administration, the rats were paired and placed in metabolism cages. Urine was collected in a graduated cylinder and its volume was recorded at 2 h intervals for 8 h. Cumulative urine excretion was calculated in relation to body weight and expressed as ml/100 g b.w. Electrolyte (Na<sup>+</sup> and K<sup>+</sup>) concentrations, pH and conductivity were estimated (as described below) from the urine sample of each pair of rats at the end of the experimental period (8 h) and expressed as mequiv./100 g b.w.

## Measurement of Urine Output and Analysis of electrolytes

Na<sup>+</sup> and K<sup>+</sup> concentrations were measured using a Toshniwal group model TCM-35 flame photometer. The instrument was calibrated with standard solutions containing different concentrations of Na<sup>+</sup> and K<sup>+</sup>. The conductivity was directly determined on fresh urine samples using a conductometer (Toshniwal group model TCM-15). pH was measured with a pH meter (Lab India) on fresh urine sample.

## Statistical analysis

The results are expressed as mean values  $\pm$  S.E.M. (standard error of mean) of six pairs of rats. Statistical comparison was carried out by analysis of variance (ANOVA). The difference between the means of treated groups and the non-treated control group was evaluated by the Bonferroni Multiple Comparisons Test. The statistical analysis was carried out with software, SigmaPlot<sup>®</sup>, version 2.03. The results were considered statistically significant when was  $P < 0.05$ .

## RESULTS

The results of the evaluations carried out on the extracts are listed in Tables 1 and 2. Table 1 shows the urinary volume (ml/100g/8h) and other parameters related to excretion such as the conductivity, pH while Table 2 shows the electrolyte (Na<sup>+</sup> and K<sup>+</sup>) content (mequiv./100g/8 h) of the urine of the animals.

### Urine volume

Table 1 shows that the reference diuretic, HCTZ, increased urine volume by 54%. The extracts also caused an increase in urine volume. For the aqueous extract, the increase at doses of 50 mg/kg body weight and 100 mg/kg body weight was 29 % ( $P < 0.001$ ) and 49 % ( $P < 0.001$ ), respectively, compared to the control group while for the methanol extract, is the corresponding values are 18 % ( $P < 0.01$ ) and 41 % ( $P < 0.001$ ), respectively.

### Electrolyte excretion

Table 2 shows the urinary electrolyte content following the administration of the extracts. The dose of 50 mg/kg aqueous extract produced a significant increase in Na<sup>+</sup> excretion, compared with the control group ( $P < 0.01$ ). The dose of 100 mg/kg aqueous extract also produced a significant increase in the Na<sup>+</sup> excretion ( $P < 0.001$ ). However, only

**Table 1:** Effect of oral administration of aqueous and methanol extracts of *L. sativum* and HCTZ on urine volume, diuretic index, conductivity and pH.

Treatment	Dose (mg/kg p.o.)	Sodium (meq./100g/8 hr) x10 <sup>-2</sup>	Potassium (meq./100g/8 hr) x 10 <sup>-2</sup>
Control	-	54.16 ± 1.72	17.00 ± 1.37
HCTZ	10	91.50 ± 1.12***	29.66 ± 1.75***
<i>L. sativum</i> (Aq)	50	62.50 ± 1.76**	16.83 ± 1.45
<i>L. sativum</i> (Aq)	100	87.61 ± 1.25***	25.33 ± 1.16**
<i>L. sativum</i> (Me OH)	50	60.00 ± 1.37	17.83 ± 1.70
<i>L. sativum</i> (Me OH)	100	78.66 ± 1.76***	18.83 ± 1.07

\*\**p* < 0.01 and \*\*\**p* < 0.001 compared with the control group (Bonferroni Multiple Comparisons Test). Diuretic index = volume treated group / volume control group.

**Table 2:** Effect of oral administration of aqueous and methanol extracts of *L. Sativum* and HCTZ on sodium and potassium excretion in urine.

Treatment	Dose (mg/kg b)	Urine volume ml/100gm/hr	Diuretic Index	Conductivity	pH
Control	-----	4.75 ± 0.13	-----	12.80 ± 0.54	7.43 ± 0.18
HCTZ	10	7.48 ± 0.18***	1.5747	14.73 ± 0.73	7.40 ± 0.32
<i>L. sativum</i> (Aq)	50	6.15 ± 0.18***	1.2947	13.77 ± 1.36	7.23 ± 0.24
<i>L. sativum</i> (Aq)	100	7.12 ± 0.12***	1.4989	14.68 ± 1.14	7.22 ± 0.21
<i>L. sativum</i> (Me OH)	50	5.61 ± 0.13**	1.1810	13.89 ± 0.99	6.88 ± 0.22
<i>L. sativum</i> (Me OH)	100	6.70 ± 0.134***	1.4105	14.43 ± 0.77	6.50 ± 0.27

\*\**p* < 0.01 and \*\*\**p* < 0.001 compared with the control group. (Bonferroni Multiple Comparisons Test).

the 100 mg/kg of the methanol extract produced a significant increase in Na<sup>+</sup> excretion (*P* < 0.001) when compared to control group. Only HCTZ and the 100 mg/kg dose of the aqueous extract produced significant increases in potassium excretion. Changes in other parameters – conductivity and pH - were not significant when compared to control group.

## DISCUSSION

According to previous ethnopharmacological survey carried out in the north central region of Morocco, the seeds of *L. sativum* are

largely used for the treatment of hypertension and renal disease<sup>11</sup>, but to the best of our knowledge, no previous pharmacological or clinical study has been carried out to test the diuretic activity of this plant.

Both the aqueous and methanol extract of *L. sativum* showed a dose-dependent increase in urine excretion. With respect to the aqueous extract, the maximum increase in urinary excretion was produced at 100 mg/kg with a value of 49.89 % compared while the methanol extract (100mg/kg) showed an increase of 41.05 % grouping urine volume. The specific conductivity, which is an indirect

measure of the ionic content of the urine, was increased in a dose-dependent manner in all the extract-treated groups. Thus the diuretic effect of both extracts are indicated by increase in both water excretion and excretion of sodium and potassium. The active principles responsible for the diuretic effects of the extracts of this plant have not yet been elucidated but preliminary phytochemical analysis of the extracts revealed the presence of polar compounds such as flavonoids and steroids. A previous investigation of the composition of *L. sativum* has suggested the presence of flavanoids and steroidal compounds<sup>10</sup>. It may be suggested that these substances might be responsible, at least in part, for the observed diuretic activity and that they may act individually or synergistically. Previous studies have demonstrated also that there are several compounds which could be responsible for the plants diuretic effects such as flavonoids, saponins or organic acids<sup>16</sup>. The effect may be produced by stimulation of regional blood flow or initial vasodilation<sup>17</sup>, or by producing inhibition of tubular reabsorption of water and anions<sup>18</sup>, the result in both cases being diuresis. The increased sodium and water excretion activity also provides strong basis for its proved anti-hypertensive action<sup>11</sup>.

## CONCLUSION

The results obtained in this study provide a quantitative basis to explain the traditional folkloric use of *L. sativum* as a diuretic agent in Moroccan population.

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