DIURETIC ACTIVITY OF METHANOLIC EXTRACT OF LEAVES OF *SALVADORA PERSICA* L.

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The present study was undertaken to investigate the diuretic effect of methanolic extract of the dried leaves of *Salvadora persica* in normal rats. Methanolic extract of *Salvadora persica* leaves was administered to experimental rats orally at the doses of 50 and 100 mg/kg p.o. Hydrochlorothiazide (10 mg/kg) was used as positive control in the study. The diuretic effect of the extract was evaluated by measuring urine volume & sodium and potassium content. Urine volume was significantly increased by methanolic extract in comparison to the control group, while the excretion of sodium was also increased by extract. The methanolic extract had the additional advantage of a potassium-conserving effect. We can conclude that methanolic extract of *Salvadora persica* produced notable diuretic effect which appeared to be comparable to that produced by the reference diuretic HCTZ (Hydrochlorothiazide). The present study provides a quantitative basis for explaining the folkloric use of *Salvadora persica* as a diuretic agent.

Key words: Diuretic activity, *Salvadora persica*, Hydrochlorothiazide, medicinal plants.

INTRODUCTION

Salvadora persica is an evergreen small tree belonging to family Salvadoraceae, commonly known as 'Pilu', 'Jal' and 'Tooth brush tree' and is widely distributed in India, Africa, Saudi Arabia, Iran, Israel and Pakistan. It has been claimed in traditional literature to be valuable against a wide variety of diseases (*e.g*., Khare, 2007; Bhandari, 1990; Wealth of India 1972).

The leaves are used in the treatment of nose trouble, piles, scabies, leucoderma, inflammation, scurvy, gonorrhea and pain. The bark is useful in the treatment of low fever and amenorrhea. The root is useful in the treatment of toothache and chest disease. Miswak is a chewing stick prepared from the roots, twigs, or stems of *Salvadora persica*. Miswak extract showed a high content of sodium chloride and potassium chloride as well as salvadourea and salvadorine, saponins, tannins, vitamin C, silica, and resin in addition to cyanogenic glycoside and benzylisothio-cyanate (Darout *et al.*, 2000). There is

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no report on the diuretic studies of the methanolic extract of dried leaves of *Salvadora persica,* so far, though it is used in folk medicine. Thus it was considered worthwhile to take up such an investigation in detail.

The present study was therefore aimed to explore the diuretic effects of methanolic extract of the leaves of *Salvadora persica.*

MATERIALS AND METHODS

Collection of leaves of the *Salvadora persica* plant was done from the campus of Central Arid Zone Research Institute (CAZRI), Jodhpur, India, in the month of May 2008. Taxonomic identification of the plant has been done by the Department of Botany, University of Pune, India. Leaves of the *Salvadora persica* plant were dried in shade for 10–12 days. After complete drying, the leaves were pulverized to a coarse powder of 40 mesh size in a mechanical grinder.

Extraction Procedure. The leaves were powdered to obtain a methanolic extract and then defatted with petroleum ether at 60–70^{o}C. The powdered material was then air-dried and subjected to sohxlet extraction for 18 h at 50–55^{o}C. The extract was thereafter concentrated under vacuum and air-dried (Mukherjee, 2004; Kokate, 2004; Evans, 2002).

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

Experimental protocol. Diuretic activity was determined by the following methods of Kau *et al.*, with minor modifications. The rats were randomly divided into four groups of six animals each as follows: (1) Control – given 5 ml/kg body weight of de-ionized water; (2) methanol extract – 50 mg/kg body weight; (3) methanol extract – 100 mg/kg body weight; and (4) hydrochlorothiazide – 10 mg/kg body weight (Abdala, 2008; Martín-Herrera, 2008). 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^+ and K^+) concentrations 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 mequ/100 g b.w.

Measurement of Urine Output and Analysis of Electrolytes. Na^+ and K^+ concentrations were measured using flame photometer (Toshniwal, Model TCM-35). The instrument was calibrated with standard solutions containing different concentrations of Na^+ and K^+.

Statistical Analysis. The results are expressed as mean values ± S.E.M. (standard error of mean) for 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. The results were considered statistically significant when $P < 0.05$.

RESULTS AND DISCUSSION

The results of the evaluations carried out on the extracts are listed in Table 1 and Table 2. Table 1 shows the urinary volume (ml/100g/8h) while Table 2 shows the electrolyte (Na^+ and K^+) content (mequiv/100g/8h) of the urine of the animals.

Urine volume. Table 1 shows that the reference diuretic, HCTZ, increased urine volume by 54%. The extract also caused an increase in urine volume. For the methanolic extract, the increase at doses of 50 mg/kg body weight and 100 mg/kg body weight was 18 % ($P < 0.01$) and 41 % ($P < 0.001$), respectively, compared to the control group.

Table 1

Effect of oral administration of methanol extract of *Salvadora persica* and HCTZ on urine volume, diuretic index

Treatment	Dose (mg/kg b)	Urine volume (ml/100g/hr)	Diuretic Index
Control	–	4.75 ± 0.13	–
HCTZ	10	7.48 ± 0.18***	1.5747
***S. persica* (MeOH)**	50	5.61± 0.13**	1.181
***S. persica* (MeOH)**	100	6.70 ± 0.134***	1.4105

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

Electrolyte excretion. Only 100 mg/kg of the methanol extract produced a significant increase in Na^+ excretion ($P < 0.001$) when compared to the control group. Only HCTZ produced significant increases in potassium excretion.

According to a previous survey carried out, the leaves of *Salvadora persica* largely used for the treatment of hypertension and renal disease, 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. Methanolic extract of *Salvadora persica* shows a dose-dependent increase in urine excretion. The methanol extract (100 mg/kg) shows an increase of 41.05 % grouping urine volume. Thus, the diuretic effect of extract indicates an increase in both water excretion and excretion of sodium. Methanolic extract (100 mg/kg) shows a significant result in excretion of water & sodium, which proves as a strong diuretic agent, but active constitute responsible for the diuretic effect cannot be concluded on the basis of this study. The preliminary phytochemical investigation reveals the presence of phytosterol, alkaloids in methanol extract which can be responsible for diuretic activity but need to be confirmed by a further study.

Table 2

Effect of oral administration of methanol extract of *Salvadora persica* and HCTZ on sodium and potassium excretion in urine

Treatment	**Dose (mg/kg b)**	**Sodium (meq./100g/8 hr) ×10-2**	**Potassium (meq./100g/8 hr) ×10-2**
Control	–	54.16 + 1.72	17.00 + 1.37
HCTZ	10	91.50 + 1.12***	29.66 + 1.75***
***S. persica* (MeOH)**	50	60.00 + 1.37	17.83 + 1.70
***S. persica* (MeOH)**	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). *HCTZ (Hydrochlorothiazide).*

CONCLUSION

The results obtained in this study provide a quantitative basis to explain the traditional folkloric use of *Salvadora persica* as a diuretic agent.

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