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ORIGINAL ARTICLE

Evaluation of the diuretic and urinary electrolyte effects of methanolic extract of *Peganum harmala* L. in Wistar albino rats

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Abstract The use of traditional medicines as a diuretic agent has been increasing in recent years. The diuretic activity of a number of plant extracts used as diuretic agents in ethnomedicine has been confirmed in experimental animals. However, despite the widespread use of *Peganum harmala* in traditional medicine, there is a paucity of data supporting its use as a diuretic agent. Therefore, the present study aimed to envisage the true effect and magnitude of diuresis of methanolic extract of *P. harmala* (MEPH) in comparison with a well-known diuretic drug furosemide using Wistar albino rats. MEPH was administered orally in three different doses (150, 300 and 450 mg/kg) to experimentally dehydrated rats. Furosemide (10 mg/kg orally) was used as a reference drug. The diuretic effect of the MEPH was evaluated by measuring urine volume, urine pH, urinary electrolyte levels, natriuretic and saliuretic effects. The urine volume (in mL) measured at 5 h and 24 h and electrolyte excretion (Na^+, K^+, and Cl^-) at 24 h duration were measured. The urine output and urinary electrolyte excretion were found to be significantly higher in rats treated with MEPH as compared to normal rats in a dose dependent manner ($P < 0.05$). The results of our study were comparable to furosemide drug. Based on observed results, we can recommend that *P. harmala* may be an effective diuretic, however, toxicity studies should be conducted before administration.

1. Introduction

Medicinal plants play an important role in traditional medicine for therapeutic purposes. *Peganum harmala* L. (Zygophyllaceae), also known as Assyrian Rue, used in traditional medicine from ancient times, is considered an important medicinal plant for the treatment of a variety of human ailments (Chopra et al., 1957). It is rich in alkaloids and contains up to 4% total alkaloids (Muhi-eldeen et al., 2008;

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Asgarpanah and Ramezanloo, 2012; Chopra et al., 1957; Berrougui et al., 2006; Asghari and Lockwood, 2002; Arshad et al., 2008).

The diuretic activity of a number of plant extracts used as diuretic agents in ethnomedicine has been confirmed in experimental animals (Berrougui et al., 2006; Asgarpanah and Ramezanloo, 2012). However, in spite of the widespread use of *P. harmala* in traditional medicine, there is a paucity of data supporting its use as a diuretic.

Diuretics, either alone or in combination with other drugs, are effective in the treatment of hypertension, congestive heart failure, ascites, and pulmonary edema (Gupta and Neyses, 2005). Plant extracts are commonly used in traditional medicine for the treatment of some renal diseases, because of their significant diuretic activity (Berrougui et al., 2006). There is uncertainty about the efficacy of *P. harmala* as diuretic agent. Hence, this study aimed at evaluating the acute diuretic, saliuretic, and natriuretic effects of orally administered methanolic extract of *P. harmala* seeds in normal rats.

2. Materials and methods

2.1. Preparation of plant extract

The seeds of *P. harmala* were purchased from the local market of Al-Kharj, Kingdom of Saudi Arabia. The seeds were then examined and authenticated.

The seeds were cleaned and a fine powder (100 g) was extracted with methanol (700 mL) by Soxhlet extraction. The extract was filtered and dried at room temperature.

2.2. Animals

Adult Wistar albino rats of either sex weighing 150–180 g were used. The study protocol was approved by the Institutional Animal Ethics Committee of the College of Pharmacy, Prince Sattam Bin Abdulaziz University, KSA. The animals were kept under standard laboratory conditions, with food and water *ad libitum*, under a 12 h light/12 h dark cycle.

2.3. Chemicals

Methanol was purchased from Scharlab, S.L. Spain, Furosemide was obtained from Sigma–Aldrich, USA. All other chemicals used were of reagent grade.

2.4. Diuretic activity

Wistar albino rats were randomly divided into five groups of six rats each. Control group, furosemide treated group, and *P. harmala* treated group. Control group was treated with 2 ml/100 g of body weight methanol vehicle, furosemide treated group was treated with furosemide 10 mg/kg orally (Da Silva et al., 2015; Hailu and Engidawork, 2014). Herbal treated group was subdivided into three subgroups, they were treated with 150, 300 and 450 mg/kg doses of methanolic extracts of *P. harmala* (MEPH), respectively. All administered drugs were given orally.

Each animal was placed in isolation in metabolic cages, 24 h prior to commencement of the experiment for adaptation and then fasted overnight with free access to water. Urine samples were collected after 5 h and 24 h of the last dose. The urine samples were filtered and finally stored at −20 °C for electrolyte analyses.

2.5. Measurement of urine parameters

Total urine volume was measured after 5 h and 24 h for all rats. Urine pH and conductivity of fresh urine samples were measured with a digital pH meter and conductivity meter, respectively. The total urine output samples (24 h) were then diluted (1:1000 in deionized water) to estimate the total concentrations of electrolytes (sodium, potassium, and chloride ions) in urine (Hailu and Engidawork, 2014).

2.6. Diuretic action and activity

The diuretic action and diuretic activity were derived from the ratio of urine volume in the test group and that in the control and positive control groups, respectively. It was decided prior to the start of the experiment that diuretic activity will be considered "nil", "little", "moderate", and "good", if the values were <0.72, 0.72–1.00, 1.00–1.5, and >1.5, respectively (Hailu and Engidawork, 2014).

2.7. Saliuretic, natriuretic and carbonic anhydrase inhibition

The sum of Na^+ and Cl^- urinary excretion was calculated as a parameter of saliuretic activity. The ratio Na^+/K^+ was calculated for natriuretic activity. The ratio $Cl/(Na + K)$ was calculated to estimate carbonic anhydrase inhibition (Somova et al., 2003).

2.8. Statistical analysis

All values were expressed as mean values ± SEM (standard error of mean) and data were analyzed by applying an analysis of variance (ANOVA) followed by Student's *t*-test. The results were considered statistically significant if $P < 0.05$.

3. Results

3.1. Effects on urine output and diuretic activity

The details of urine volume, diuretic action, and diuretic activity are presented in Table 1. From the result, it appears that *P. harmala* exhibited diuretic activity at all doses like furosemide at 5 h and 24 h, and its effect was dose dependent.

The total urine volume over the period of 5 h and 24 h was measured for the *P. harmala* methanolic extracts, (150, 300 and 450 mg/kg), reference diuretic (furosemide) and normal control. Furosemide and *P. harmala* increased the urine flow significantly at 5 h and 24 h ($P < 0.05$) when compared with control rats. The high dose excreted more than two fold the volume of urine as compared to control.

The diuretic activity of a drug is considered nil if it is less than 0.72, little if it is between 0.72 and 1.00, moderate if it is within 1.00–1.50, and good if it is above 1.50. In this respect, *P. harmala* exhibits good diuretic activity.

Table 1 Effect of methanolic extract of *P. harmala* L. on urine volume in Wistar albino rats at 5 h and 24 h interval.

Groups	At 5 h after drug administration			At 24 h after drug administration		
	Urine volume (mL)	Diuretic action[a]	Diuretic activity[b]	Urine volume (mL)	Diuretic action[a]	Diuretic activity[b]
Control (2 ml/kg)	1.53 ± 0.12	1.00	–	4.67 ± 0.51	1.00	–
Furosemide (10 mg/kg)	3.85 ± 0.25*	2.52	1.00	8.90 ± 0.45*	1.91	1.00
MEPH (150 mg/kg)	2.87 ± 0.11*	1.87	0.75	6.67 ± 0.19*	1.43	0.75
MEPH (300 mg/kg)	3.17 ± 0.28*	2.07	0.82	8.03 ± 0.45*	1.72	0.90
MEPH (450 mg/kg)	3.57 ± 0.19*	2.33	0.93	10.17 ± 0.95*	2.18	1.14

Values are expressed as mean ± SEM.
[a] Diuretic action = urine volume of test group/urine volume of control group.
[b] Diuretic activity = urine volume of test group/urine volume of furosemide group.
* Significant change at $P < 0.05$ with respect to control rats.

3.2. Effects on urine pH and conductivity

Urinary pH and conductivity were measured at 24 h (Fig. 1). The urinary pH of control rats was 5.79 ± 0.07. The urine pH after administration of *P. harmala* extract, at doses of 150, 300 and 450 mg/kg body weight were 7.83 ± 0.10, 7.97 ± 0.16 and 8.47 ± 0.18, respectively at 24 h urine sample. Furosemide increased the urine pH 8.17 ± 0.35, thus making the urine more alkaline.

Conductivity of control rats was reported 12.62 ± 0.33 at 24 h collected urine sample. Conductivity of urine of furosemide treated rats was significantly increased to 20.09 ± 0.13 ($P < 0.01$). *P. harmala* extract treated rats' urine conductivity, at doses of 150, 300 and 450 mg/kg was 15.44 ± 1.42, 18.55 ± 1.35 and 19.60 ± 0.55, respectively.

3.3. Effects on electrolyte excretion

The diuretic responses with its electrolyte excretion potency of the methanolic *P. harmala* extract were highly moderate in comparison to normal control rats except lowest dose (150 mg/kg). The *P. harmala* extract at doses of 300 and 450 mg/kg showed a significant increase in Na^+, K^+ and Cl^- excretion. The results of urinary electrolyte excretion after treatment of *P. harmala* extract were comparable to the furosemide group (Table 2).

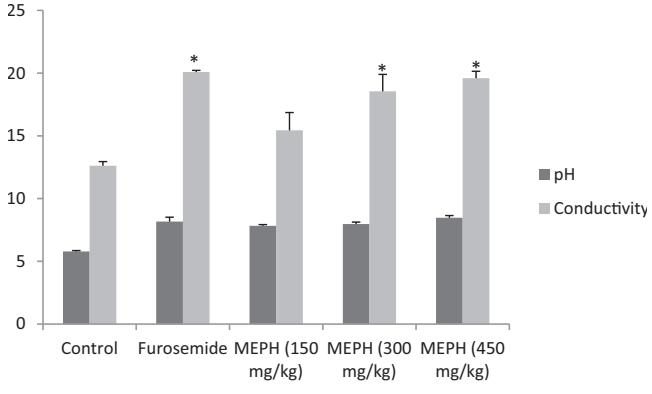

Figure 1 Effect of methanolic extract of *P. harmala* L. on urinary pH and conductivity of Wistar albino rats at 24 h of sample collection. Values are mean ± SEM. *$P < 0.05$, significant against the control group.

3.4. Effects on natriuretic, saliuretic and carbonic anhydrase inhibition

Table 3 depicts the results for natriuretic, saliuretic activity and carbonic anhydrase inhibition. The furosemide (10 mg/kg) and methanolic extract of *P. harmala* at doses (300 and 450 mg/kg) showed potent natriuretic and saliuretic activity as compared to normal control. Methanolic extract of *P. harmala* didn't show carbonic anhydrase inhibition in our study.

4. Discussion

In the present study, the diuretic effect of three doses of methanolic extract of *P. harmala* was evaluated in Wistar albino rats. The results indicate that *P. harmala* at all dose levels (150, 300 and 450 mg/kg) significantly increased urine output in a dose-dependent manner over a period of 5 h and 24 h. Therefore, good diuretic activity was observed among rats treated with furosemide and *P. harmala* extracts at all doses, except 150 mg/kg dose, wherein moderate activity was observed. The diuretic activities of the extracts were found to be highly potent when compared to the normal control group. However, significant differences in urinary excretion followed by diuretic action and diuretic activity were observed. It showed that the extract action was time and dose dependent. This can be explained by kinetic differences of the active principle presence in the extracts. Nilveses et al. (1989), reported that an increment of the urine output in rats might result from high potassium content in the plant extract. The pH values were also alkaline as compared with control.

P. harmala extract increased the urinary excretion of sodium, potassium and chloride ions significantly. Therefore, *P. harmala* has been shown to possess significant saliuretic and natriuretic effects (Asgarpanah and Ramezanloo, 2012).

All doses except 150 mg/kg of the methanolic extract of *P. harmala* resulted in a significant increase in urine volume, Na^+, K^+, and Cl^- ion excretion as compared to the normal control group. This effect may be due to the synergistic mechanism of the $[HCO_3^-/Cl^-]$, $[HCO_3^+/H^+]$ and the $[Na^+/H^+]$ antiporter, leading to diuresis (Dubois and Geiling, 1959). This is a characteristic of high ceiling diuretic. Furosemide acts by

Table 2 Effect of methanolic extract of *P. harmala* L. on urinary electrolyte excretion of Wistar albino rats at 24 h urine sample collection.

Groups	Urinary Na^+[a] (mmol/L)	Urinary K^+[a] (mmol/L)	Urinary Cl^-[a] (mmol/L)	Na^+ index[b]	K^+ index[b]	Cl^- index[b]
Control (2 ml/kg)	110.14 ± 9.12	58.49 ± 4.74	78.46 ± 4.29	1.00	1.00	1.00
Furosemide (10 mg/kg)	196.48 ± 12.53*	119.84 ± 14.27*	141.75 ± 7.97*	1.78	2.05	1.81
MEPH (150 mg/kg)	141.29 ± 5.47*	75.38 ± 3.10	87.11 ± 6.72	1.28	1.29	1.11
MEPH (300 mg/kg)	160.25 ± 6.12*	86.73 ± 3.70*	98.97 ± 4.99*	1.45	1.48	1.26
MEPH (450 mg/kg)	185.78 ± 17.12*	104.91 ± 6.23*	114.89 ± 5.43*	1.69	1.79	1.46

[a] Values are expressed as mean ± SEM.
[b] Index = excretion in test group/excretion in control group.
* Significant change at $P < 0.05$ with respect to control rats.

Table 3 Effect of methanolic extract of *P. harmala* L. on natriuretic effect, saluretic effect and carbonic anhydrase inhibition of Wistar albino rats at 24 h of urine sample collection.

Groups	Saluretic effect (Na + Cl)[a]	Natriuretic effect (Na/K)[a]	CAI ($Cl/[Na^+ + K^+]$)[a]	Saluretic index[b]	Natriuretic index[b]	CAI index[c]
Control (2 ml/kg)	188.60 ± 10.75	1.98 ± 0.28	0.47 ± 0.03	1.00	1.00	1.00
Furosemide (10 mg/kg)	338.23 ± 13.15*	1.79 ± 0.25	0.46 ± 0.03	1.79	0.90	0.97
MEPH (150 mg/kg)	228.40 ± 10.09	1.91 ± 0.15	0.40 ± 0.03	1.21	0.96	0.85
MEPH (300 mg/kg)	259.22 ± 1.31*	1.85 ± 0.04	0.41 ± 0.04	1.37	0.93	0.86
MEPH (450 mg/kg)	300.68 ± 16.15*	1.81 ± 0.20	0.41 ± 0.03	1.59	0.91	0.86

[a] Values are expressed as mean ± SEM.
[b] Index = excretion in test group/excretion in control group.
[c] CAI, carbonic anhydrase inhibition.
* Significant change at $P < 0.05$ with respect to control rats.

inhibiting electrolyte re-absorption in the thick ascending loop of Henle (Shinkawa et al., 1993).

The natural compounds present in *P. harmala* are harmine and harmaline, which are responsible for the various properties, (Berrougui et al., 2006). However, it is still not clear whether harmine or harmaline is responsible for the diuretic effects of *P. harmala*. Therefore, there is a need of further research to find out the active principles responsible for the diuretic activities.

In the light of the above mentioned study, we can report that the methanol extract of *P. harmala* is an effective diuretic and also resulted in increased sodium, potassium and chloride ions in urine; which correlate well with the traditional use of the plant as a diuretic. The observations showed, *P. harmala* had a diuretic spectrum similar to that of furosemide.

5. Conclusion

This study confirms the significant diuretic activity of the methanolic extract of *P. harmala*. during the measurement period of the study (24 h). However, further studies are recommended for explaining the mechanism of diuretic activity and chronic toxicity.

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