

Original Research Article

Diuretic Activity of *Trianthema portulacastrum* Crude Extract in Albino Rats

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Received: 5 October 2012

Revised accepted: 13 October 2013

Abstract

Purpose: To evaluate the diuretic effect and acute toxicity of the crude aqueous extract of *Trianthema portulacastrum* in a rat model.

Method: Albino rats were divided into five groups. Control group received normal saline (10 mg/kg), reference group received furosemide (10 mg/kg) and test groups were given different doses of crude extract (10, 30 and 50 mg/kg) by intraperitoneal route. Urine was collected and the total volume of urine excreted was expressed as ml/6 hr/100 g body weight. Diuretic index and Lipschitz values were also calculated to make comparison with normal saline and furosemide treated groups, respectively.

Results: Significant diuretic ($p < 0.001$), kaliuretic ($p < 0.001$) and natriuretic ($p < 0.001$) effects were observed in treated groups in a dose-dependent manner. Urinary pH remained mostly unchanged during the course of the study. Diuretic index showed good diuretic activity of the crude extract. Lipschitz values indicated that the crude extract at the dose of 50 mg/kg exhibited 79 % diuretic activity compared with that of the reference, furosemide. No lethal effects were observed among albino mice even at the high dose of 3000 mg/kg.

Conclusion: The extract of *Trianthema portulacastrum*, particularly, at the dose of 50 mg/kg significantly increased the urinary volume and concentration of urinary electrolytes with no signs of toxicity and therefore, is a potential diuretic. Further studies, however, are required to isolate the active constituents.

Keywords: *Trianthema portulacastrum*, Saliuretic, Natriuretic, Lipschitz value, Diuretic index, Na^+/K^+ ratio.

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Medicinal plants are important source of unknown chemical substances with potential therapeutic effects. The World Health Organization (WHO) has estimated that over 75 % of the world's population still relies on plant-derived medicines for their basic healthcare needs [1]. At present, thousands of plant

metabolites are being successfully used in the treatment of a variety of diseases.

Cardiovascular diseases are responsible for approximately one-third of all deaths throughout the world [2]. Conditions such as hypertension lead to other types of diseases, such as stroke, and kidney and other heart diseases. Common clinical strategies to achieve lowering of blood pressure include the use of angiotensin

converting enzyme inhibitors, beta blockers, calcium-channel blockers and diuretics.

Diuretics mostly work by stimulating urine output together with urinary excretion of sodium from the body. Diuretics such as the high-ceiling loop and thiazides diuretic have been associated with several side effects, such as electrolyte and metabolic changes, new-onset diabetes development, renin-angiotensin system activation and weakening of sexual function [3]. This fact necessitate that there is a strong need for novel diuretics which are relatively safe with better or equivalent diuretic activity.

Trianthema portulacastrum (Tp) is a green leafy plant commonly growing as a weed in cultivated and wastelands. In Pakistan, it is widely distributed in Punjab and Sindh regions. It is traditionally used in gastrointestinal, cardiovascular, hormonal, respiratory, skin and various other disorders [4].

Despite the herbal use of Tp as a diuretic agent, to date existing literature fails to evidence its diuretic activity. Therefore, the aim of the present study was to evaluate the diuretic effects of aqueous extract of this plant in normal albino rats. We also aimed to study the effect of the crude extract on urinary excretion of electrolytes. Furthermore, acute toxicity of the plant extract was carried out in albino mice.

EXPERIMENTAL

Plant material and extraction

Dried aerial parts of Tp were collected in the month of May (2011) from cultivated areas of district Bahawalpur, Punjab, Pakistan. After identification by the taxonomist (Dr. Irshad Ahmad, Assistant Professor, The Islamia University of Bahawalpur, Punjab, Pakistan), the sample was submitted to the herbarium in the Pharmacology research laboratory at Department of Pharmacy, The Islamia University of Bahawalpur, Pakistan and labelled as TP-AP-06-10-007 for future reference. After removing the extraneous material, the aerial parts were crushed into a coarse powder with an electric grinder (National, Model MJ-176NR, China). Approximately, 500 g of the crushed material was soaked in one liter of hot water at room temperature (23 – 25 °C) for 3 days\ with occasional shaking. The material was then filtered and the residue was again soaked in hot water for 3 days and this procedure was repeated thrice (total 9 days) and finally, the filtrate was evaporated in a rotary evaporator (Heidolph Laborota 4000-efficient, Germany)

under reduced pressure (-760 mmHg) to a thick, semi-solid pasty mass of dark brown color. Crude extract of *Trianthema portulacastrum* (Tp.Cr.) was dissolved in distilled water and normal saline for use in *in-vitro* and *in-vivo* experimentation, respectively [5].

Reference and control drugs

Furosemide (Lasix, Aventis Pharma, Pakistan), a high-ceiling loop diuretic, was used as the reference drug (positive control). Normal saline (Merck, Germany) was used as control drug.

Animals and treatment

Adult albino rats and mice were kept in polycarbonate cages (Techniplast, Italy) and were housed under the standard conditions of temperature, humidity and dark light cycle. Animals were also given pelleted food and drinking water *ad libitum*. The bedding of the animal cages was changed after every 48 hr. Tp.Cr. was given by intraperitoneal (IP) route for diuretic activity and by oral route for acute toxicity [6].

Phytochemical screening of crude plant extract

Preliminary screening of the crude aqueous extract for a variety of secondary metabolites (alkaloids, flavonoids, tannins, saponins, coumarins and anthraquinones) was carried out using standard methods reported in literature [7].

Assessment of diuretic activity

Adult albino rats of either sex having weights in the range of 200-220 g were divided into five groups of six animals each. Animals were screened for any visible signs of disease and only the healthy animals were selected for the study. Seven days prior to experimentation animals were placed in metabolic cages for 2-3 hr daily to acclimatize them to the experimental conditions. The whole experiment was carried out in same environmental conditions. Temperature of the room was also kept constant to 25±5 °C.

Before experimentation, the bladder of rats were emptied by gentle compression of the pelvic area and by the pull of their tails [8]. Group I (control group) was given normal saline 10 ml/kg, Group II (reference group) was given 10 mg/kg of furosemide and test groups (III, IV and V) were given 10, 30 and 50mg/kg of Tp.Cr., respectively. All the doses were made in same volume of normal saline in order to administer same volume

in each group. IP was used for the administration of drugs because of its benefits over other routes i.e. ease of administration and freedom to administer large volume of fluids compared with other routes. Immediately following administration, animals were placed in metabolic cages (one animal per cage), specially designed to separate urine and faeces. The urine collected in graduated vials was measured at the end of 6 hr and expressed as ml/100g of body weight per 6 hr [6].

Determination of electrolyte levels

Levels of sodium and potassium in fresh urine samples were estimated using calibrated Flame Photometer (Coring 410, UK). Before estimating urinary sodium and potassium levels, samples were filtered to remove debris and shedding. Concentration of electrolytes was expressed in mEq/L [9].

Determination of urine pH

pH of the fresh urine samples from all the five groups was measured with the help of a calibrated pH meter (Model: WTW-Series pH-720) [10].

Assessment of acute toxicity

Acute toxicity test of Tp.Cr. was performed on albino mice of 18-25 g body weight. Animals were divided in different groups of five mice each. The control group of mice was given normal saline (10 ml/kg), while other groups received increasing doses of extracts up to 3000 mg/kg. All the treatments were administered by oral gavage. Animals were observed closely for 2 hr, then at 30 minute intervals for 6 hr for any visible sign of toxicity (salivation, lacrimation, ptosis, squinted eyes, writhing, convulsions, tremors, yellowing of fur, loss of hair), stress (erection of fur and exophthalmia), behavioural abnormalities (such as impairment of spontaneous movement, climbing, cleaning of face and ataxia, and other postural changes) and aggressive behaviour (biting and scratching behaviour, licking of tail, paw and penis, intense grooming behaviour and vocalization) and diarrhea [6] and then mortality was noted at end of 24 hr [11].

Computation of diuretic parameters

Diuretic parameters were determined as in Eqs 1 - 4 [9,10].

$$\text{Diuretic index} = V_t/V_c \dots\dots\dots (1)$$

where V_t is mean urine volume of test group and V_c is mean urine volume of control group.

$$\text{Lipschitz value} = V_t/V_r \dots\dots\dots (2)$$

where V_t is mean urine volume of test group and V_r is mean urine volume of reference group.

$$\text{Saliuretic index} = C_t/C_c \dots\dots\dots (3)$$

where C_t is the concentration of electrolyte in urine of test group and C_c is the concentration of electrolyte in urine of control group.

$$\text{Na}^+/\text{K}^+ \text{ ratio} = C_n/C_k \dots\dots\dots (4)$$

where C_n is the concentration of Na^+ in urine of a group and C_k is the concentration of K^+ in urine of same group.

Ethical approval

The study was approved by the Board of Advance Studies at The Islamia University of Bahawalpur as a part of MPhil research project with registration number 125/IUB MPhil/2009 [12].

Statistical analysis

Data collected were analyzed and expressed as mean \pm standard error of mean (SEM, $n = 6$ with 95 % confidence interval (CI). Student t -test was applied to test difference among groups and $p < 0.05$ was considered significant. All the data were compared with control group. Graph Pad Prism (Graph PAD, San Diego, USA) was used for statistical analysis.

RESULTS

Phytochemical analysis

The phytochemical analysis of crude aqueous extract of Tp was positive for saponins, alkaloids, flavonoids and anthraquinones, while negative for coumarins and tannins.

Effect of plant extract on urinary output in rats

IP administration of the Tp.Cr. increased the urinary flow in a dose-dependent manner ($p < 0.001$) as shown in Figure 1. When compared with group I, approximately 60, 130 and 258 times increase in urine output was observed in test groups, respectively.

The diuretic index values of test groups (group III, IV and V) were 1.95, 3.06 and 5.09, respectively indicating a good diuretic activity while maximum diuretic effect was observed at the dose of 50 mg/kg of extract (Table 1). The Lipschitz values showed that at the doses of 10, 30 and 50 mg/Kg, Tp.Cr. was having 30 %, 47 % and 79 % of diuretic activity, respectively compared with furosemide (Table 1)

Table 1: Effect of *Trinathema portulacastrum* extract on urinary volume and electrolyte concentration

Group	Dose (mg/kg)	Volume of urine (mL/6 hr)	pH	Diuretic Index	Lipschitz value	Saliuretic Index	Na ⁺ /K ⁺
1	Normal saline 10 (mL/kg)	0.63±0.07	7.0	-----	-----	-----	1.26
2	Furosemide 10	4.06±0.07	7.0	6.34	-----	1.19 1.52	0.99
3	Tp.Cr. 10	1.23±0.05*	6.96	1.95	0.30	1.0 1.03	1.20
4	Tp.Cr. 30	1.93±0.12*	6.91	3.06	0.47	1.30 1.14	1.40
5	Tp.Cr. 50	3.21±0.15*	7.01	5.09	0.79	1.43 1.28	1.40

Values given are mean ± S.E.M of six observations. All the values are compared with control group (normal saline treated) and considered significant at * $p < 0.001$.

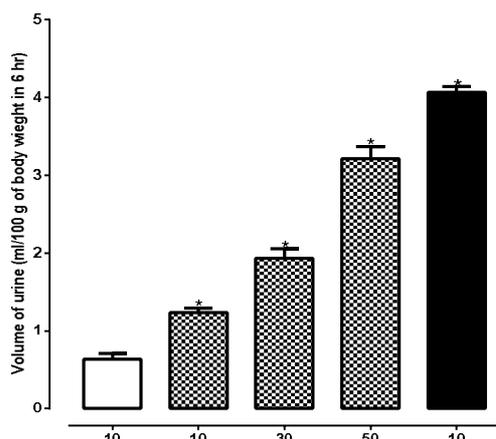


Figure 1: The effect of the Tp.Cr. on urination in albino rats. Values shown are mean ± S.E.M (n = 6)

Effect of plant extract on urinary electrolyte excretion and urinary pH

Tp.Cr. produces natriuretic effects in a dose-dependent manner as shown in Figure 2. The concentration of sodium excreted in test group V was greater than that of reference standard showing that at higher doses, Tp.Cr. has significant natriuretic effects.

The IP administration of Tp.Cr. also produced kaliuretic effects in a dose-dependent manner (Figure 3) which was also evident through saliuretic index values.

The pH of fresh urine samples in treated groups was not significantly different from the pH of control group (Table 1).

Acute toxicity

Acute toxicity study in albino mice proved that the crude extract of Tp.Cr. was safe even at the dose of 3000 mg/kg. The plant extract did not provoke any visible signs of toxicity, stress or adverse behaviours. In addition, there was also no sign of diarrhea and none of the treated animals died in 24 hr.

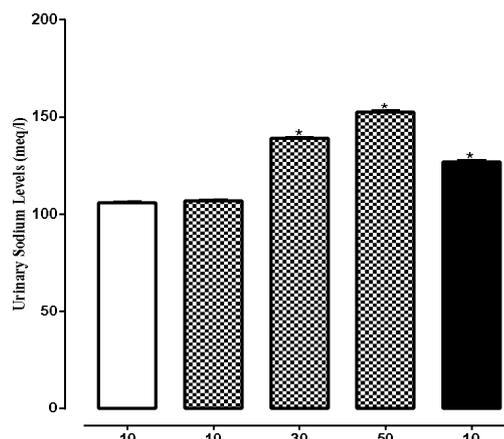


Figure 2: The effect of the Tp.Cr. on urinary sodium levels in urine excreted during 6 hrs. Values shown are mean ± S.E.M (n = 6) and the values are compared with control group and considered significant at * $p < 0.001$. □ = Normal saline (mg/kg); ▨ = Tp.Cr. (mg/kg); ■ = Furosemide (mg/kg).

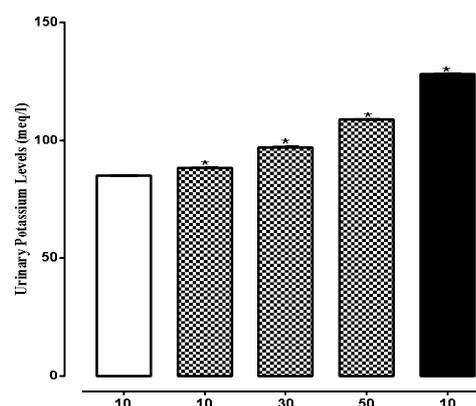


Figure 3: The effect of the Tp.Cr. on urinary potassium levels in urine excreted during 6 hrs. Values shown are mean ± SEM (n = 6) and the values are compared with control group and considered significant at * $p < 0.001$. □ = Normal saline (mg/kg); ▨ = Tp.Cr. (mg/kg); ■ = Furosemide (mg/kg)

DISCUSSION

The previous studies on phytochemical constituents of Tp showed that it contains

alkaloids, saponins, flavonoids, tannins, phenolic compounds, sterols, proteins and reducing sugars [4]. The preliminary phytochemical investigation revealed the presence of saponins, anthraquinones, flavonoids and alkaloids in the Tp.Cr. Studies have established that there are numerous compounds which could be accountable for the plant's diuretic effects such (eg., flavonoids, saponins or organic acids) which could be accountable for the plant's diuretic effects [5]. Studies have shown that diuretic activity of flavonoids may be due to their binding with adenosine A₁ receptors [13]. Some studies have also shown that diuretic activity might be consequence of alkaloids [14]. The precise site, molecule and cellular mechanisms still remain to be elucidated.

Clinical conditions like excessive accumulation of fluids (nephritic syndrome, cirrhosis of liver and congestive cardiac failure) and high blood pressure mostly necessitates the administration of diuretics [15]. Progression of renal diseases is evident in uncontrolled hypertension and control of blood pressure is an effective strategy in preventing this progression [16]. Tp has also been proven to have antioxidant effect [4] believed to play a key role in controlling cell damage.

Our findings indicated that Tp.Cr. showed a dose-dependent increase in urine excretion while, maximum diuretic effects were observed at the dose of 50 mg/kg. The diuretic activity is considered to be good if the diuretic index values are greater than 1.50, moderate if the values are in between 1.00 and 1.50, mild if the values lie in between 0.72 and 1.00 and there is no diuretic activity if the value is < 0.72 [17]. The diuretic index values of treated groups were 1.95, 3.06 and 5.09, respectively indicating that crude extract exhibits very good diuretic activity especially at the dose of 50 mg/kg. Lipschitz values indicate that at the maximal dose of crude extract (50 mg/kg), the plant showed 79% diuretic activity when compared with furosemide.

In primary hypertension, sodium is considered an important external factor. Numerous studies have shown the adverse effects of increased sodium uptake on arterial blood pressure [18]. Increased excretion of urinary sodium in our experimental animals after the administration of Tp.Cr. showed that plant is a potential candidate to be used as an antihypertensive agent [19]. Interestingly, at the dose of 50 mg/kg the Tp.Cr. showed more natriuretic effect compared with furosemide. This finding is similar to the one earlier reported [20]. Also, the excretion of potassium in the urine was also significantly increased in a dose-dependent

pattern but it is noteworthy that excretion of potassium in the treated groups was less than that of reference standard treated group suggesting that the Tp.Cr. has potassium-sparing properties. Based on these observations it is suggested that the diuretic action of Tp.Cr. may be attributed to inhibition of aldosterone action or inhibition of epithelial sodium channels. However further studies are required to test this hypothesis.

In the toxicological evaluation, no lethal effects were observed in all animals even with the higher doses. It is possible to consider that the plant is free from toxic effects. However, it is necessary to study eventual advance adverse effects by observing neural, metabolic and hormonal parameters.

CONCLUSION

The present study indicates that Tp.Cr. is a potential candidate as a diuretic agent that could potentially be used to prevent commonly encountered side effects associated with available diuretics. However, further studies are encouraged to isolate the active phytochemical constituent for exploring exact mechanism of diuresis.

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