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Phytochemical screening and diuretic activity of *Euphorbia granulata*

Hammad Saleem[1,2], Irshad Ahmad[2] and M. Shoaib Ali Gill[1]

[1]*Institute of Pharmaceutical Sciences (IPS), University of Veterinary and Animal Sciences, Lahore, Pakistan;*
[2]*Department of Pharmacy, The Islamia University of Bahawalpur, Pakistan.*

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Abstract

The aim of this study was to evaluate diuretic activity of aqueous methanolic extract of *Euphorbia granulate* in rats. Albino rats were divided into five groups. Group I served as reference, Group II as standard and Group III, IV and V served as test. The three doses of extract (30, 50 and 100 mg/kg) were given to rats (i.p) in acute diuretic model. Furosemide (10 mg/kg i.p) was used as standard drug. The extract induced diuretic effects and induced electrolytes excretion in a dose-dependent manner when compared with control. The extract (100 and 50 mg/kg) significantly ($p<0.01$) increased the volume of urine in comparison to control group. Similarly, the excretion of potassium and sodium were also significantly ($p<0.05$) increased following extract administration. However, there was no significant change in the pH of urine samples of the extract-treated group compared with control. The result of this study thus offers support to the traditional folker use of this plant as a diuretic agent.

Introduction

Diuretics are the drugs which are used to enhance urinary output and electrolyte excretion. They show their action mostly on different parts of nephrons and increases urine outflow. Diuretics can also increase the electrolytes elimination (Bhavna and Rani, 2006). Several adverse effects are associated with the use of high-ceiling and thiazides diuretics like ototoxicity, potassium depletion, hyperuricemia, acute hypovolemia, hypomagnesemia, hyponatremia, hypercalcemia, hyperlipidemia, hyperglycemia and hypersensitivity (Howland and Mycek, 2006).

Euphorbia granulata belonging to family Euphorbiaceae is native to north and topical Africa, Iran, Pakistan, Palestine, Sinai, North India, Afghanistan, Japan and China and is found to grow in plains and lower hills (Jan et al., 2009). The latex of *E. granulata* is internally used to expel intestinal worms. Its latex is externally applied to snake bites and scorpion stings. Its latex is used as a purgative, anthelmintic and diuretic, as well as for its blood purifying characteristics in Saudi Arabia (Schmelzer and Gurib-Fakim, 2008). It shows inhibitory effects against Human immunodeficiency virus (HIV-1) protease (Hussein et al., 1999). As in herbal practice, it is used as a diuretic agent.

However, no scientific evidence has been reported related to its diuretic activity. Therefore, this study was designed to provide scientific evidence to the ethnobotanical uses of diuretic activity of crude aqueous methanol extract of *E. granulata* in normal albino rat model, and determine the toxicity and effects of *E. granulate* on excretion of urinary electrolytes.

Materials and Methods

Collection of plant material

Whole plant of *E. granulate* was collected from the

surroundings of Khanewal, District Punjab, Pakistan. Plant specimen was identified by Taxonomist, Cholistan Institute of Desert Studies (CIDS), The Islamia University of Bahawalpur, Pakistan and a specimen was deposited there in the herbarium with voucher number 3730/CIDS/IUB.

Preparation of crude extract

The plant was washed with distilled water after extraneous substances had been removed. Thereafter, plant material was dried under shade at temperature between 21-30ºC for 30 days.

The dried material was mechanically reduced to coarse powder and stored in an air tight container (1.5 kg) of powdered material of *E. granulate* was taken in beaker having 8 liter capacities and 3.5 liter of 80 % dichloromethane (DCM) was added, macerated for 72 hours and then with methanol again for 72 hours with occasional shaking and stirring. The residues were extracted thrice with the same fresh solvent and extract combined. The macerated plant material was filtered through several layers of muslin cloth for coarse filtration. The filtrate was filtered through Whatman No. 1 filter paper. The filtered extract was concentrated under reduced pressure (-760 mmHg) at 40ºC in a rotary evaporator (Heidolph Laborota 4000-efficient, Germany). The semisolid mass obtained indicate a yield of 19.3%, and was dried in an oven at 40ºC (Jabeen et al., 2009; Patel et al., 2009).

Preliminary phytochemical analysis

Phytochemical tests were performed on the extract to determine different phytochemical constituents using the methods which are variously described elsewhere (Evans, 2009; Sofowara, 2006). The presence of alkaloid content was determined by performing Mayer's test; white precipitate (ppt) indicated the presence of alkaloids. Flavonoids were determined when on addition of few drops of sodium hydroxide solution, formed intense yellow coloration that became colorless after addition of dilute acetic acid. Saponins were identified by formation of froth upon simple shaking (frothing test). Tannins and phenols were identified on addition of ferric chloride to the extract solution; the appearance of blue or green ppt indicated the presence of tannins. Sterols and triterpenoids were identified on addition of few drops of acetic anhydride to the extract solution, boiled, cooled and then add concentrated sulfuric acid, producing brown ring at the junction of two layers, the turning of upper layer to green indicated sterols and deep red color indicated triterpenoids (Evans, 2009; Sofowara, 2006).

Dose preparation

The aqueous methanol extract of *E. granulate* was dissolved in normal saline in a caped test tube. After shacking it was subjected to the vortex mixture (My Lab®, Korea) and then the mixture was placed in Ultrasonic (Lc 30/H, Germany). Final dose was filtered by Filter paper (Grade 1) and its pH was measured at calibrated pH meter (Ino Lab WTW, Ph 720, Germany).

Animal handling

Adult albino rats of either sex, weighing 220–240 g, were obtained from the animal house of the Department of Microbiology, University of veterinary and animal sciences, Lahore, Pakistan. The animals were kept in polycarbonate cages (47 × 34 × 18 cm) and housed under standard conditions of temperature (24 ± 1ºC), humidity and dark light cycle (12 hours/12 hours). They were fed with standard animal feed, containing chokar, chicken feed and dry milk in the ratio of 2:2:1. Study protocols and ethical issues were approved by Ethical committee of University of veterinary and animal sciences, Lahore, Pakistan.

Diuretic activity

All animals were divided into five groups of six animals each. The control, group "I" received normal saline (10 mL/kg, i.p.), group "II" (reference group) was given furosemide (10 mg/kg, i.p.) as reference diuretic while group III, IV and V (test groups) were treated with the different doses of *E. granulata* crude extract (30, 50 and 100 mg/kg, i.p.), respectively. Immediately after injecting, the animals were placed separately in metabolic cages (Techniplast, Italy) which are specially designed to separate urine and feces. Urine was collected in graduated vials and total volume was measured after 6 and 10 hours interval and was closely monitored on hourly basis. The mean volume of urine expressed as mL/100 g of body weight was calculated (Gilani et al., 2008; Ratnasooriya et al., 2004).

Measurement of urine output and electrolyte analysis

Electrolyte (sodium and potassium) concentrations and pH were calculated from the urine sample of each rat at the end of the experimental period and expressed as mEq/100 g body weight. Sodium and potassium concentrations were measured using a Sherwood Flame Photometer 410 Classical, UK (Abdala et al., 2008). The instrument was calibrated with standard solutions containing different concentrations of sodium and potassium. The pH was measured with a pH meter (Ino Lab WTW, Ph 720, Germany) on urine sample (Jesupillai et al., 2008; Overman and Davis, 1947).

Statistical analysis

SPSS (version 17.0) program was used to carry out t-test on the data and the results expressed as mean ± SEM. The results were considered statistically significant when $p<0.05$.

Table I

Effect of *Euphorbia granulate* extract on urine volume

Treatment	Urine volume (mL/100 mg)					
	1st hour	2nd hour	4th hour	6th hour	8th hour	10th hour
Saline 10 mL/kg	0 ± 0	0.4 ± 0.2	0.8 ± 0.2	1.4 ± 0.2	2.2 ± 0.2	3.0 ± 0.2
Euphorbia granulate 30 mg/kg	0.2 ± 0	0.4 ± 0.2	1.0 ± 0.4	1.9 ± 0.2	3.1 ± 0.4[a]	4.0 ± 0.4[b]
Euphorbia granulate 50 mg/kg	0.7 ± 0	1.5 ± 0.2[a]	1.9 ± 0.4	3.4 ± 0.5[a]	4.1 ± 0.6[b]	4.5 ± 0.7[b]
Euphorbia granulate 100 mg/kg	0.9 ± 0	1.1 ± 0.2	2.6 ± 0.3	3.7 ± 0.4[a]	4.4 ± 0.6[b]	4.5 ± 0.7[b]
Furosemide 10 mg/kg	1.9 ± 0	2.0 ± 0.2	3.3 ± 0.1[a]	4.1 ± 0.4[b]	5.3 ± 0.4[b]	6.1 ± 0.5[b]

n=6; $p<0.05$ significantly ([a]) and $p<0.01$ significantly ([b]) different from the control

Results

The results of phytochemical tests revealed that the extract contains certain pharmacologically active secondary metabolites such as saponins, flavonoids and tannins. Alkaloids and glycosides were absent.

The aqueous methanol extract of the whole plant of *E. granulate* (100 mg/kg) showed marked diuresis (3.7 ± 0.4 mL) during the 6th hour versus control (1.4 ± 0.2 mL), compared with reference standard furosemide (4.1 ± 0.4 mL) ($p<0.05$). The lower dose (50 mg/kg) of *E. granulate* extract also showed significant ($p<0.05$) diuretic activity (3.4 ± 0.5 mL) versus control (1.4 ± 0.2 mL). The urine output at 8th hour and 10th hour was also significant ($p<0.01$) (Table I).

The diuretic index of *E. granulate* (30, 50 and 100 mg/kg) was recorded as 1.4, 2.7 and 2.9 at 6th hour while it was 1.4, 1.7 and 1.8 at the 10th hour, respectively (Table II).

The effect of furosemide (10 mg/kg) and the extract of *E. granulate* (100 mg/kg) on electrolyte (Na+ and K+) excretion in the 6th hour urine are presented in Table III. The plant extract significantly enhanced the excretion of the electrolytes ($p<0.05$) which was comparable to that of furosemide.

The pH of urine samples following treatment with *E. granulate* extract doses of 30, 50 and 100 mg/kg was 6.3, 6.7 and 6.8 respectively. The change in mean pH of urine after administration of *E. granulate* extract at 30, 50 and 100 mg/kg was 6.9, 6.7 and 6.9, respectively. The change in urinary pH was non-significant.

Table II

Diuretic index of *Euphorbia granulata* extract in rats

Treatment	Diuretic effect	
	6 hours	10 hours
Euphorbia granulate 30 mg/kg	1.4	1.4
Euphorbia granulate 50 mg/kg	2.7	1.7
Euphorbia granulate 100 mg/kg	2.9	1.8
Furosemide 10 mg/kg	3.1	1.9

Discussion

The results showed that *E. granulate* aqueous methanol extract exhibited a significant but dose dependent diuretic effects when compared with the control (furosemide). Similarly, the extract also showed an increase outflow of sodium and potassium when compared with control. The major active compound which shows the diuretic activity of the extract has not yet been determined, but the results of phytochemical evaluation divulge that the plant contains secondary metabolites such as flavonoids, tannins and saponins. There can be number of mechanisms which might be responsible for the diuretic activity of the plant extract. Previous experimental data revealed that flavonoids and tannins are subsidized with both diuretic and vasodilator effects (Martin-Herrera et al., 2008) .

The diuretic effect of the compounds such as saponins in association with vitamin D and steroids has also been documented. Similarly, the flavonoids are also reported to show diuretic, anti-inflammatory and vasoprotective effects by inhibition of arachidonic acid metabolism (Mythreyi et al., 2008). Therefore, it can be suggested that the diuretic activity of the aqueous methanolic extract of *E. granulate* can be due to the presence of these secondary metabolites which may act individually or in combination. Previously it has been well demonstrated that increase in regional blood flow, vasodilation or an inhibition of tubular secretion contributes to an increased urinary excretion (Pantoja et al., 1991; Stanic and Samarzija, 1993). Hence, any of these processes could be associated with diuretic effect of the extract.

Conclusion

In the light of the above findings and results, it is concluded that *E. granulate* exhibits significant diuretic

Table III

Concentration of sodium and potassium in urine of rats treated with *Euphorbia granulata* extract

Treatment	Excretion in urine			
	Sodium (ppm)	Potassium (ppm)	Lipschitz value	
			6 hour	10 hour
Saline 10 mL/kg	497.6 ± 0.4	29.2 ± 0.2	0.8 ± 0.2	1.4 ± 0.2
Euphorbia granulate 30 mg/kg	523.3 ± 0.5	36.3 ± 0.2	1.0 ± 0.4	1.9 ± 0.2
Euphorbia granulate 50 mg/kg	541.2 ± 0.4[a]	47.1 ± 0.3[a]	1.9 ± 0.4	3.4 ± 0.5[a]
Euphorbia granulate 100 mg/kg	549.7 ± 0.3[b]	48.9 ± 0.1[b]	2.6 ± 0.3	3.7 ± 0.4[a]
Furosemide 10 mg/kg	571.8 ± 0.0	51.0 ± 0.1	3.3 ± 0.1[a]	4.1 ± 0.4[b]

([a])$p < 0.05$ and ([b])$p < 0.01$ when compared with control

activity when compared to that of furosemide, and thus this offers some support for the use of the plant in traditional medicine as a diuretic.

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Author Info
Hammad Saleem (Principal contact)
e-mail: hammad.saleem@uvas.edu.pk; Tel: +92-3357885316