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Antilithiatic and diuretic activity of *Cucurbita moschata* on ethylene glycol induced lithiasis in male albino Wistar rats

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ABSTRACT

A detailed investigation on the antilithiatic and diuretic activity of ethanolic extract of seeds and fruit of *Cucurbita moschata* was undertaken to find the pharmacological basis for the ethnomedicinal use. The ethanolic extract was evaluated for its inhibitory potential on lithiasis (stone formation), induced by oral administration of 0.75% ethylene glycolated water to adult male albino Wistar rats for 28 days. The ethanolic extract significantly reduced the elevated levels of crucial urinary ions, viz. calcium, oxalate, and phosphate and also significantly reduced the increased deposition of stone forming constituents in the kidney of calculogenic rats. The high serum creatinine level observed in ethylene glycol-treated rats was also reduced, following treatment with the extract. The diuretic activity of *Cucurbita moschata* extract at the dosage of 200 mg/kg and 400 mg/kg was compared with standard drug Furosemide at the dosage of 20 mg/kg by determination of urine volume, its sodium and potassium concentrations. The extract exhibited significant diuretic activity in rats. All the observations under study provided the basis for the conclusion that seed and fruit extract of *Cucurbita moschata* inhibits stone formation induced by ethylene glycol treatment and also have significant diuretic activity.

Key words: Antilithiatic activity, Aqueous extract, *Cucurbita moschata*, Diuretic activity, Rats

Kidneys play an important role in water and electrolyte conservation and it forms an integral part of body homeostatic mechanism. Diuretics are drugs used to treat several conditions including cardiac failure, nephrotic syndrome, cirrhosis of liver and treatment of hypertension. Naturally occurring diuretics include caffeine in coffee, tea, and cola, which inhibit Na reabsorption and also alcohol in beer, wine and mixed drinks, which inhibit secretion of ADH (anti diuretic hormones). Most diuretic drugs have the adverse effect on quality of life including impotence, fatigue and weakness (Vanamala *et al*. 2012).

Urinary stone disease has afflicted humankind since antiquity and can persist, with serious medical consequences, throughout a patient's lifetime. Urinary calculi are the third most prevalent disorder of the urinary system. Kidney stone formation or urolithiasis is a complex process that is a consequence of an imbalance between promoters and inhibitors in the kidneys and it may cause obstruction, hydronephrosis, infection and hemorrhage in the urinary tract system (Morgan and Pearle 2016). Between 1% and 15% of people globally are affected by kidney stones at some point in their life. Not all standard pharmaceutical drugs used to prevent urolithiasis are effective in all patients, and many have adverse effects that compromise their long term use. Traumatic effect, chances of infection, acute renal injury, recurrence of urolithiasis and high cost of treatment make people to move toward indigenous treatments to get rid of the problem (Dinesh *et al*. 2013). Therefore, it is worthwhile to look for an alternative to these means by, using medicinal plants or phytotherapy. Several pharmacological investigations on the medicinal plants used in traditional urolithic therapy have revealed their therapeutic potential in the *in vitro* or *in vivo* models (Atmani *et al*. 2003). In spite of substantial progress in the pathophysiology and treatment of urolithiasis, there is no satisfactory drug being used in clinical therapy. Thus a drug for the prevention of this disease or its recurrence would be of great interest.

Cucurbita moschata, commonly known as pumpkin belongs to family cucurbitaceae. It is widely used like food and in folk medicine around the world and is a popular folk remedy for treatment of urolithiasis. For this purpose, it is use as a decoction made from the seeds and fruits. Traditionally it is used in most countries as anti-diabetic, antitumor, antihypertensive, anti-inflammatory, immunomodulatory and antibacterial agents (Caili *et al*. 2006). Pumpkin seed oil has a strong antioxidant property, and has been recognized for several health benefits such as prevention of the growth and reduction of the size of prostate, reduction of bladder and urethral pressure and

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improving bladder compliance, alleviation of diabetes by promoting hypoglycemic activity, and lowering level of gastric, breast, lung, and colorectal cancer (Mitra *et al*. 2009). Survey of literature showed that no previous pharmacological or clinical study was carried out to test the antilithiatic and diuretic activity of this plant. The main aim of the present investigation was to evaluate the antilithiatic and diuretic activity of *Cucurbita moschata* on ethylene glycol induced lithiasis in male albino Wistar rats.

MATERIALS AND METHODS

Plant material and extraction: Plants of *Cucurbita moschata* were collected from the rural area around North Eastern Himalayan (NEH) region of India. Air-dried powdered material of *Cucurbita moschata* (both seed and fruit) was exhaustively extracted with ethanol and extract (EPF) was filtered, concentrated on rotavapour (Büchi, USA) and then freeze-dried (Freezone® 4.5, Labconco, USA) under high vacuum (1.33 Pa) and at temperature of –40±2°C.

Animals: Albino rats, Wistar strain, of the both sex were randomly distributed into groups and housed in cages (6 per cage) and maintained under standard conditions at 26±2°C and relative humidity 44–56% and 10 h light 14 h dark cycles each day for one week before and during the experiments. Albino rats weighing between 150 and 200 g were selected for the study. All animals were fed the standard rodent pellet diet (Amrut, India) and drank water *ad libitum*. All studies were performed in accordance with the guidelines for the care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Care Committee, CPCSEA, India. All animal experiments were conducted with the permission from Institutional Animal Ethics Committee of University Institute of Pharmacy, C.S.J.M. University, Kanpur (1589/PO/ a/12/ CPCSEA).

Ethylene glycol induced urolithiasis model: Ethylene glycol induced hyperoxaluria model (Atmani *et al*. 2003) was used to assess the antilithiatic activity in albino rats. Animals were divided into five groups containing six animals in each. Group 1 served as control and received regular rat food and drinking water *ad lib.* Ethylene glycol (0.75%) in drinking water was fed to Groups 2 to 5 for induction of calculi till 28th day. Group 3 received standard antiurolithiatic drug, cystone (750 mg/kg body weight) from 15th day till 28th day (Mitra *et al*. 2009). Groups 4 served as curative regimen (CR) and received EPF (200 mg/kg body weight) from 15th day till 28th day, Group 5 received EPF (200 mg/kg body weight) from 1st day till 28th day and served as preventive regimen (PR). All extracts were given once daily by oral route.

Analysis of urine for assessment of antiurolithiatic activity: All animals were kept in individual metabolic cages and urine samples of 24 h were collected on 28th day. Animals had free access to drinking water during the urine collection period. A drop of concentrated hydrochloric acid was added to the urine before being stored at 4°C. Urine was analyzed for calcium (Mustafa and Medeiros 1985), phosphate (Fiske and Subbarow 1925) and oxalate (Hodgkinson and Williams 1972) content.

Serum analysis for assessment of antiurolithiatic activity: After the experimental period, blood was collected from the retro-orbital under anesthetic conditions and serum was separated by centrifugation at 10,000 × *g* for 10 min and analyzed for creatinine (Raghuramulu *et al*. 1983), uric acid (Caraway 1963) and urea nitrogen (Raghuramulu *et al*. 1983).

Diuretic activity assessment: The method of Lipschitz *et al.* (1947) was employed for the assessment of diuretic activity. Male albino rats weighing between 150–200 g, deprived of food and water for 18 h prior to the experiment, were divided in four groups of six rats in each. Diuretic activity was measured by collecting total excreted urine (0–5 h) of rat kept in metabolic cage. The urine sample was collected for a total period of 5 h. The Group 1 animals served as control, received 5 ml of 0.9% NaCl solution per kg body weight; Group 2 received furosemide (20 mg/kg p.o.) in 5 ml of 0.9% NaCl solution; Group-3 received *C. moschata* extract (200 mg/kg body weight in 5 ml of 0.9% NaCl solution suspended in 1% carboxy methyl cellulose); Group 4 received *C. moschata* extract (400 mg/kg body weight in 5 ml of 0.9% NaCl solution suspended in 1% carboxymethyl cellulose). Immediately after administration, the animals were placed in metabolic cages (2 per cages), specially designed to separate urine and faeces at 20°C±0.5°C. The volume of urine collected was measured at the end of 5 h. During this period, no food and water was made available to animals. The parameters taken were total urine volume, concentration of Na^+, K^+ and Cl^- in the urine. Na^+ and K^+ concentration were determined by flame photometer and Cl^- concentration was determined by titration with silver nitrate solution (N/50) using 3 drops of 5% potassium chromate solution as indicator (Yar and Ansari 2009).

Statistical analysis: All the data are presented as a mean±SEM and one-way analysis of variance (ANOVA) and Newman-Keuls Comparison Test were applied for determining the statistical significance between different groups.

RESULTS AND DISCUSSION

In the present study, chronic administration of 0.75% (v/v) ethylene glycol aqueous solution to male Wistar rats resulted in hyperoxaluria. Oxalate, calcium and phosphate excretion were grossly increased in calculi-induced animals (Table 1, Group 2). However, supplementation with ethanolic extract of *Cucurbita moschata* significantly ($P<0.001$) lowered the elevated levels of oxalate, calcium and phosphate in urine in CR and PR as compared to cystone-treated animals (Table 1, Group 3).

The serum uric acid and BUN were remarkably increased in calculi-induced animals (Table 1, Group 2) while serum creatinine was only slightly elevated in Group 2, indicating marked renal damage. However, *Cucurbita moschata*

Table 1. Effect of *C. moschata* extracts on urinary and serum parameters in control and in rat

Parameter (unit)	Group 1 (Control)	Group 2 (Calculi-induced)	Group 3 (Cystone-treated)	Group 4 (Curative regimen)	Group 5 (Preventive regimen)
Urine (mg/dl)					
Oxalate	0.37±0.03	3.64±0.11*	0.53±0.04*	1.29±0.06*	1.10±0.08*
Calcium	1.27±0.07	4.51±0.10*	1.50±0.06*	2.08±0.06#	1.96±0.08#
Phosphate	3.64±0.04	7.29±0.06*	3.81±0.09*	4.25±0.10#	4.14±0.09#
Serum (mg/dl)					
BUN	37.61±0.15	49.97±0.48*	39.30±0.48#	42.88±0.38*	41.83±0.10*
Creatinine	0.75±0.01	0.94±0.03*	0.81±0.02*	0.92±0.01#	0.91±0.02#
Uric acid	1.49±0.07	3.64±0.11*	1.71±0.04*	2.10±0.06*	2.06±0.05*

Values for urine parameters were assessed in 24 h urine sample. All values are expressed as mean±S.D. for six animals in each group. *Statistically signifcant at P<0.001, #Statistically significant at P<0.05.

extract treatment in curative (Table 1, Groups 4) and prophylactic (Table 1, Groups 5) regimen significantly (P<0.001) lowered the elevated serum levels of creatinine, uric acid and BUN.

In the present study, oxalate and calcium excretion progressively increased in calculi-induced animals (Group 2). Since it is accepted that hyperoxaluria is a far more significant risk factor in the pathogenesis of renal stones than hypercalciuria (Kumar *et al*. 2016), the changes in urinary oxalate levels are relatively much more important than those of calcium (Cho *et al*. 2014). Increased urinary calcium is a factor favouring the nucleation and precipitation of calcium oxalate or apatite (calcium phosphate) from urine and subsequent crystal growth (Donna 2015). However, ethanolic extract of *Cucurbita moschata* lower the levels of oxalate as well as calcium excretion.

An increase in urinary phosphate is observed in calculi induced rats (Group 2). Increased urinary phosphate excretion along with oxalate stress seems to provide an environment appropriate for stone formation by forming calcium phosphate crystals, which epitaxially induces calcium oxalate deposition (Gadge and Jalalpure 2012). Treatment of ethanolic extract of *Cucurbita moschata* restores phosphate level, thus reducing the risk of stone formation.

In the present study, aqueous extract of *C. moschata* at a dose level of 200 and 400 mg/kg was used to measure the diuretic activity of the Hydro-alcoholic extract. Urine volume and electrolyte were measured from the rat model in order to evaluate diuretic potential. Hydro-alcoholic extract of *Cucurbita moschata* at 200 mg/kg showed less significant (P<0.05) diuretic activity and 400 mg/kg showed more significant (P<0.01) diuretic activity when compared with control (Table 2). The *Cucurbita moschata* extract at 400 mg/kg doses produce a significant electrolyte excretion efficiency and dose dependent increase in urine volume shows more excretion of Na^+, Cl^- and K^+ in urine when compared to the control group (Table 3). *Cucurbita moschata* extract dose of 200 mg/kg also showed less excretion (Table 3). Furosemide, a loop diuretic agent was used as a standard drug. The urinary output after 5 h of study had been presented in Table 2. Result shows that *Cucurbita moschata* extract increased diuresis and Na^+, $K^+$and Cl^-electrolyte in urinary excretion. In all cases, the excretion of electrolytes and the volume of urine increase was less than the standard diuretic, furosemide (20 mg/kg) (Table 2).

From the study, it was found that diuretic action of the extract at 400 mg/kg was as potent as the standard drug Furosemide. The increase in urinary electrolytes may be probably due to inhibition of renal Na^+K^+pump that would lead to reduction in Na^+ and K^+ reabsorption leading thus

Table 2. Effect of *C. moschata* extract on urinary output in rats

Treatment group	Dose	Total urinary output	Normal saline intake	% Urinary excretion	Diuresis	
					Diuretic action	Diuretic activity
Control	1 ml/kg body weight in 5 ml of 0.9% NaCl	3.98±0.25	4.65±0.21	98.21	1.0	0.48
Furosemide	20 mg/kg body weight in 5 ml of 0.9% of NaCl	8.97±0.12[b]	4.51±0.11	173.78	2.09	1.0
C. moschata	200 mg/kg body weight in 5 ml of 0.9% of NaCl	5.78±0.24[a]	4.98±0.12	119.02	1.77	0.72
C. moschata	400 mg/kg in 5 ml of 0.9% of NaCl	9.21±0.14[b]	4.76±0.18	182.87	2.12	0.96

Values are in mean±SEM; [a]P<0.05 vs control; [b]P<0.01 vs control; Urinary excretion = Total urinary output/total liquid intake×100; Diuretic action = Urinary excretion of treated group/urinary excretion of control group; Diuretic activity = Diuretic action of treated group/diuretic action of standard group.

Table 3. Effect of *C. moschata* extract on urinary electrolytes excretion in rats

Treatment group	Dose	Electrolytes (meq/L)			Na^+/K^+
		Na^+	K^+	Cl^-	
Control	1 ml/kg body weight in 5 ml of 0.9% NaCl	189.77±15	324.41±0.11	109.18±2.98	41.78
Furosemide	20 mg/kg body weight in 5 ml of 0.9% NaCl	244.12±4.32[b]	5.98±0.21[b]	151.23±4.89[b]	39.67
C. moschata	200 mg/kg body weight in 5 ml of 0.9% NaCl	219.38±4.29[a]	4.76±0.14	121.21±3.12[a]	29.23
C. moschata	400 mg/kg body weight in 5 ml of 0.9% NaCl	249.23±9.78[b]	5.82±0.11[b]	141.14±2.52[b]	38.21

Values are in mean±SEM; [a]P<0.05 Vs control; [b]P<0.01 vs control

to an osmotic water flow in to the lumen and dieresis (Parial *et al*. 2009). The mechanism of action by which diuresis was induced by the aqueous extract of *Cucurbita moschata* was assessed by comparing the effect with that of furosemide, a high ceiling loop diuretics. Diuresis has two components: increase in urine volume (water excretion) and net loss of solutes (i.e. electrolytes) in the urine. Diuretic relieve pulmonary congestion, kidney troubles particularly stone and peripheral edema. In case of *Cucurbita moschata* extract, the urine output started after 2 h of administration of test drug, while in case of furosemide, it started just after 30 minutes of administration. The differences in the time of the onset of the diuretic action of test and reference drug may be related to gastrointestinal absorption characteristics of the active principle(s). This activity of *Cucurbita moschata* hydro-alcholic extract was may be due to the presence of glycosides, flavonoids, polyphenols and alkaloids (Chandra *et al*. 2011). From this study, it was found that aqueous extract of *Cucurbita moschata* has significant effects on urinary excretion of electrolytes and support the claim of diuretic efficacy. Similar results had also been reported by Syeda *et al*. (2016) working with *Cucurbita maxima*.

The present data from the study indicated that administration of the ethanolic extract of *Cucurbita moschata* to rat model with ethylene glycol induced lithiasis, reduced and prevented the growth of urinary stones, supporting folk information regarding antiurolithiatic activity of the plant. *Cucurbita moschata* produced a significant increase in urinary volume, urinary and serum electrolytes excretion when compared to control. The diuretic activity of *Cucurbita moschata* extract was significant at 400 mg/kg body when compared to control and it was also comparable to standard diuretic drug, Furosemide. Further isolation and characterization of compounds needs to be explored for the antiurolithiatic and diuretic potency of hydro-alcoholic extract of *Cucurbita moschata*.

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