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Original Article

Evaluation of *Pyracantha crenulata* Roem for Antiurolithogenic Activity in Albino Rats

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ABSTRACT

Objective: The aim of the present study was to investigate the effect of juice extract and alcohol extract of fruit of *Pyracantha crenulata* (D.Don) Roem (Rosaceae) against ethylene glycol-induced urolithiasis in male albino rats.
Patients and Methods: Lithiasis was induced in rats by administering 0.75% ethylene glycol in drinking water for 28 days and was manifested by hyperoxaluria as well as increased renal excretion of calcium, phosphate and a low urinary magnesium content. Curative and preventive treatment was then tried by supplementation with juice and alcohol extracts (250 mg/kg b.w., p.o.) of *P. crenulata* fruit.
Results: The increased deposition of stone forming constituents in the kidneys of calculogenic rats was significantly lowered by curative and preventive treatment using juice and alcohol extracts (250 mg/kg b.w., p.o.) of *P. crenulata* fruit which showed a regulatory action on endogenous oxalate synthesis.
Conclusion: From this study, we conclude that both prophylactic and therapeutic treatment with juice and alcohol extract of fruit of *P. crenulata* may reduce precipitation of calcium oxalate, with improvement of kidney function as well as cytoprotective effect

Keywords : Ethylene glycol, hyperoxaluria, nephrolithiasis, *Pyracantha crenulata*

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INTRODUCTION

Despite technological and conceptual developments in the practice of medicine, the formation and growth of renal calculi continue to afflict mankind. The incidence of kidney stones has increased in western societies in the last five decades, in association with economic development. Most calculi in the urinary system arise from a common component of urine, calcium oxalate (CaOx), representing up to 80% of all analyzed stones[1].

A large number of plant drugs have been used in India since ancient times which claim efficient cure of urinary stones[2]. Amongst the medicinal plants used in the treatment of urolithiasis are 'patharphor' (*Didymocarpus*

Fig. 1: Fruits and leaves of P. crenulata

pedicellata), several Bergenia species, three species of Tribulus *(T. systoides, T. terrestris and T. alatus*), 'manjit' *(Rubia cordifolia and Rubia tinctorum)*, 'varuna' *(Crataeva nurvala)* and 'imli' *(Tamarindus indica)*[3], *Costus spiralis*[4], *Raphanus sativus*[5], *Moringa oleifera*[6], *Crataeva adansonii*[7], *Melia azedarach*[8] *and Eleusine coracana*[9].

"The plant *Pyracantha crenulata* Roem, syn. *Crataegus crenulata* Roxb (Rosaceae), locally known as Ghingaaru, is found in the Himalayas from Sutlaj to Bhutan, at altitudes of 800-2500 m. In Ayurvedic medicine it is reported to be useful in the treatment of a number of ailments, including hepatic, stomach and skin diseases due to its diuretic, depurative, tonic, antirheumatic, cardiotonic, hypoglycemic, hypotensive, anti-inflammatory and lithontripic properties[10-14]. However, there are no records of systematic pharmacological studies that support its antiurolithogenic effect. Due to the wide distribution in Uttarakhand, India, and in continuation of our research work on *P. crenulata*[15], the present investigation was carried out to determinate the antiurolithogenic property of the alcohol and juice extracts of the fruit of *P. crenulata* using an ethylene glycol induced hyperoxaluria model in albino rats and to confirm the traditional medicinal use of the plant.

PATIENTS AND METHODS

Fresh *Pyracantha crenulata* fruit were collected from local areas of Dehradun, Uttarakhand, India during May 2008 and authenticated at the Botanical Survey of India (BSI), Dehradun, India. A voucher specimen of the plant was deposited in the Botanical Survey of India herbarium under the number BSD 112215 for further reference.

The fresh and semi-ripe fruit were cut into small pieces and fed through a juicer. The juice was filtered and vacuum dried to obtain *P. crenulata* fruit juice extract (PCJE, yield 17% w/w). In addition, the fruit were sliced using a home slicer, then shade-dried, pulverized and passed through a 20-mesh sieve. The dried, coarsely powdered plant material was extracted with 70% (v/v) alcohol by the hot continuous extraction method using a soxhlet apparatus at a temperature of 60-70ºC. The solvent was evaporated under vacuum yielding a semisolid mass (PCAE, yield 22% w/w with respect to the dried powder). Both extracts were stored in tight containers in a desiccator for further use[16].

For acute toxicity studies, albino rats of either sex weighing 150-200 g were selected, and healthy adult male albino rats weighing 150-200 g were selected for assessing the antiurolithogenic activity. The animals were acclimatized to standard laboratory conditions (temperature: 25 ± 2ºC) and maintained on a 12-h light: 12-h dark cycle. They were housed in polypropylene cages and provided with regular rat chow (Lipton India Ltd., Mumbai, India) and drinking water *ad libitum*. The animal care and experimental protocols were approved by the Institutional Animal Ethical Committee (IAEC).

The acute oral toxicity study[17] was carried out as per the guidelines set by the Organization for Economic Co-Operation and Development (OECD) received from the Committee for the Purpose of Control and Supervision of Experiments on Animals

Table 1: Urinary excretion of stone forming constituents in control and experimental animals

Groups	Dose (mg/kg)	Urine parameters (mg/dl)		
		Oxalate	Calcium	Phosphate
Normal (N)	Vehicle	0.28 ± 0.01	1.18 ± 0.03	2.96 ± 0.04
Calculi induced (PR)	Vehicle	2.61 ± 0.17*	3.85 ± 0.18*	6.82 ± 0.13*
Cystone treated (CR)	750	0.46 ± 0.10***	1.34 ± 0.10***	3.36 ± 0.03***
Alcohol extract (CR I)	250	0.86 ± 0.01***	2.03 ± 0.24***	3.40 ± 0.26***
Juice extract (CR II)	250	2.25 ± 0.24**	3.52 ± 0.31**	5.98 ± 0.28**
Alcohol extract (PR I)	250	1.28 ± 0.13***	2.27 ± 0.13***	4.52 ± 0.22***
Juice extract (PR II)	250	2.36 ± 0.16**	3.41 ± 0.23**	6.48 ± 0.32**

Values are expressed as mean ± SEM of 6 observations; Statistical comparisons are made between Group N vs Group PR and CR (*P<0.001); Group PR vs PR I and PR II (**P<0.01); Group CR vs Group CR I and CR II (***P<0.01)

(CPCSEA). One tenth of the median lethal dose (LD50) was taken as an effective dose[18]. From the acute toxicity study, the LD50 cut-off dose was found to be 2500 mg/kg body weight for both extracts. Hence, the therapeutic dose was taken as 250 mg/kg body weight for both extracts.

An ethylene glycol induced hyperoxaluria model[19] was used to assess the antiurolithogenic activity in albino rats. The animals were divided into seven groups containing six animals each.

- Group N served as a normal control group and received regular rat food and drinking water ad libitum.
- Groups PR, PR I and PR II for the preventive regimen
- Groups CR, CR I and CR II for the curative regimen

Starting from day 1, ethylene glycol (0.75% v/v) in the drinking water was given to all groups except the control group for the induction of renal calculi.

From day 1 - 28, group PR I received alcohol extract (250 mg/kg body weight) and group PR II juice extract (250 mg/kg body weight). Group PR did not receive any extract.

From day 15 – 28, group CR received a standard antiurolithogenic drug (cystone; 750 mg/kg body weight[20-23]), while groups CR I and CR II received alcohol extract (250 mg/kg body weight) and juice extract (250 mg/kg body weight), respectively.

All extracts were given orally once daily. All animals were kept in individual metabolic cages, and 24-h urine samples were collected on day 28. The animals had free access to drinking water during the urine collection period. A drop of concentrated hydrochloric acid was added to the urine before it was stored at 4ºC. The urine was analyzed for calcium[24], phosphate[25] and oxalate[26].

After the experimental period, blood was collected from the retro-orbital area under

Table 2: Kidney retention of stone forming constituents in control and experimental animals

Groups	Dose (mg/kg)	Kidney parameters (mg/g)		
		Oxalate	Calcium	Phosphate
Normal (N)	Vehicle	1.36 ± 0.02	3.02 ± 0.03	2.58 ± 0.04
Calculi induced (PR)	Vehicle	4.85 ± 0.11*	4.94 ± 0.21*	3.88 ± 0.11*
Cystone treated (CR)	750	2.15 ± 0.03***	1.82 ± 0.27***	1.62 ± 0.03***
Alcohol extract (CR I)	250	2.18 ± 0.03***	2.82 ± 0.21***	1.80 ± 0.06***
Juice extract (CR II)	250	3.42 ± 0.06**	4.26 ± 0.34**	3.47 ± 0.16**
Alcohol extract (PR I)	250	4.28 ± 0.36***	3.46 ± 0.22***	3.02 ± 0.07***
Juice extract (PR II)	250	4.11 ± 0.23**	4.49 ± 0.32**	3.50 ± 0.16**

Values are expressed as mean ± SEM of 6 observations; Statistical comparisons are made between Group N vs Group PR and CR (*P<0.001); Group PR vs PR I and PR II (**P<0.01); Group CR vs Group CR I and CR II (***P<0.01)

anesthetic conditions, and the animals were sacrificed by cervical decapitation. The serum was separated by centrifugation at 10,000 rpm for 10 min and analyzed for creatinine, urea nitrogen[27] and uric acid[28].

The abdomen was cut open to remove both kidneys, which were dissected from extraneous tissue and preserved in 10% neutral formalin. They were then dried at 80ºC in a hot-air oven. A sample of 100 mg of the dried kidney was boiled in 10 ml of 1N hydrochloric acid for 30 min and homogenized. The homogenate was centrifuged at 2,000 rpm for 10 min and the supernatant was separated[29]. The calcium[24], phosphate[25] and oxalate[26] contents in the kidney homogenate were determined.

The results were expressed as mean ± SD. Differences among the data were statistically analyzed using one-way ANOVA followed by Student Newman Keul's test (GraphPad Prism software for Windows, Version 5.01.2007). Statistical significance was set accordingly[30].

RESULTS

In the present study, chronic administration of 0.75% (v/v) ethylene glycol aqueous solution to male albino rats resulted in hyperoxaluria. Oxalate, calcium and phosphate excretion were grossly increased in calculi-induced animals (Table 1). However, supplementation with juice and alcohol extracts of *P. crenulata* fruit significantly (p<0.001) lowered the elevated levels of oxalate, calcium and phosphate in urine and kidney in the animals of groups CR I and CR II (curative regimen) and those of groups PR I and PR II (preventive regimen) as compared to group PR (calculi-induced) (Table 1).

The deposition of crystalline components in the renal tissues, namely oxalate, phosphate and calcium, was increased in the stone-forming rats (Table 2). Treatment with juice and alcohol extracts of *P. crenulata* fruit significantly (p<0.001) reduced the renal content of these stone-forming constituents in both regimens (Table 2, CR I, CR II, PR I, PR II).

Table 3: Serum parameters in control and experimental animals

Groups	Dose (mg/kg)	Serum parameters (mg/dl)		
		BUN	Creatinine	Uric acid
Normal (N)	Vehicle	38.21 ± 0.16	0.56 ± 0.03	1.26 ± 0.04
Calculi induced (PR)	Vehicle	52.67 ± 0.46*	0.98 ± 0.08*	3.48 ± 0.11*
Cystone treated (CR)	750	42.60 ± 0.38***	0.58 ± 0.01***	1.88 ± 0.03***
Alcohol extract (CR I)	250	40.31 ± 0.22***	0.60 ± 0.03***	2.06 ± 0.04***
Juice extract (CR II)	250	52.90 ± 0.42**	0.78 ± 0.07**	3.18 ± 0.08**
Alcohol extract (PR I)	250	46.23 ± 0.23***	0.61 ± 0.06***	2.46 ± 0.06***
Juice extract (PR II)	250	51.87 ± 0.36**	0.84 ± 0.10**	3.12 ± 0.16**

Values are expressed as mean ± SEM of 6 observations; Statistical comparisons are made between Group N vs Group PR and CR (*P<0.001); Group PR vs PR I and PR II (**P<0.01); Group CR vs Group CR I and CR II (***P<0.01)

Although the extent of reduction was insignificant on inter-regimen comparison (curative versus preventive regimens), the differences were significant (p<0.001) when compared with the cystone-treated animals (Table 2).

Serum uric acid and blood urea nitrogen (BUN) were remarkably increased in the stone-forming animals (Table 3), while serum creatinine was only slightly elevated in group PR indicating marked renal damage. However, *P. crenulata* extracts significantly (p<0.001) lowered the elevated serum levels of creatinine, uric acid and BUN in the groups of curative and preventive regimen (Table 3).

DISCUSSION

In the present study, male rats were selected to induce urolithiasis because the urinary system of male rats resembles that of humans and earlier studies showed that the amount of stone deposition in female rats was significantly less[31,32].

Urinary supersaturation with stone-forming constituents is generally considered one of the causative factors in calculogenesis. Previous studies indicate that in response to the administration of ethylene glycol (0.75%, v/v) over a 14-day period, young male albino rats form renal calculi composed mainly of calcium oxalate[18,33,34]. The biochemical mechanisms of this process are related to an increase in the urinary concentration of oxalate. Stone formation in ethylene glycol-fed animals is caused by hyperoxaluria, which causes increased urinary retention and excretion of oxalate[33]. Similar results have been obtained when rats were treated with ethylene glycol and ammonium oxalate[35,36].

In the present study, oxalate and calcium excretion progressively increased in stone-forming animals (PR). Since it is accepted that hyperoxaluria is a more significant risk

factor in the pathogenesis of renal stones than hypercalciuria, the changes in urinary oxalate levels are relatively more important than those of calcium[37,38]. Increased urinary calcium is a factor favoring the nucleation and precipitation of calcium oxalate or apatite (calcium phosphate) from urine and subsequent crystal growth[39]. However, juice and alcohol extracts of *P. crenulata* fruit lowered the levels of oxalate as well as calcium excretion.

An increase in urinary phosphate is observed in stone-forming rats (PR). 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[40]. Treatment with *P. crenulata* fruit extract restored the phosphate level, thus reducing the risk of stone formation.

In urolithiasis, the glomerular filtration rate (GFR) decreases due to obstruction of urine outflow by stones in the urinary system. Due to this, the waste products, particularly nitrogenous substances such as urea, creatinine, and uric acid accumulate in the blood[41]. Also, increased lipid peroxidation and decreased levels of antioxidant potential have been reported in the kidneys of rats supplemented with a calculi-producing diet[42,43]. In this context, oxalate has been reported to induce lipid peroxidation and to cause renal tissue damage by reacting with polyunsaturated fatty acids in the cell membrane[44]. In the stone-forming rats (PR) marked renal damage was seen by the elevated serum levels of creatinine, uric acid and BUN. However, curative and prophylactic treatment with juice and alcohol extracts of *P. crenulata* fruit caused diuresis[15] and hastened the process of dissolving the preformed stones and preventing new stone formation in the urinary system.

In conclusion, the presented data indicate that administration of juice and alcohol extracts of *P. crenulata* fruit to rats with ethylene glycol-induced lithiasis reduced and prevented the growth of urinary stones, thus supporting folk information regarding the antiurolithogenic activity of the plant. The mechanism underlying this effect is still unknown, but is apparently related to increased diuresis and lowering of urinary concentrations of stone forming constituents. These effects could explain the antiurolithogenic property of *P. crenulata*.

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EDITORIAL COMMENT

This study is interesting and should be published, as based on the investigations and results presented by the authors *Pyracantha crenulata* may gain clinical importance for the inhibition of urinary calculi. However, clinical studies on patients with urinary calculi are still necessary to confirm the said effect in humans

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