

Anti-Urolithiatic Effect of Ethanolic Extract of *Pedaliium Murex* Linn. Fruits on Ethylene Glycol-Induced Renal Calculi

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Purpose: To evaluate effect of ethanolic extract of *Pedaliium murex* Linn. fruits on experimental model of calcium oxalate nephrolithiasis.

Materials and Methods: Thirty-six male *Wistar* albino rats were randomly divided in 6 groups. Normal controls received distilled water for 28 days. Other five groups received ethylene glycol (1% v/v) in distilled water for 28 days. *Pedaliium murex* ethanolic extract was given 200 mg/kg and 400 mg/kg orally in distilled water for 28 days in prophylactic groups (III and IV) and from 15th to 28th days in treatment groups (V and VI). The urea, creatinine, random blood sugar, aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, bilirubin and calcium were measured on 28th day. 24 hr urinary oxalate and volume were measured on day 0 and 28. On day 28, kidneys were removed, weighed and subjected to histopathological examination. Calcium oxalate crystallization was evaluated by renal histopathology and *in-vitro* method of mineralization. All parameters were analyzed by Kruskal-Wallis or one-way ANOVA with post-hoc test.

Results: *Pedaliium murex* showed significant improvement in renal function and kidney weight in prophylactic groups as compared to ethylene glycol controls. It did not show any effect on urinary oxalate, urine volume and any other serological parameters. Calcium oxalate crystallization was significantly reduced in all the *Pedaliium murex* treated groups ($P < .05$). Calcium oxalate and phosphate mineralization were also inhibited by 33% and 57%.

Conclusion: Ethanolic extract of *Pedaliium murex* fruits possess significant activity for prevention of renal calculi.

Keywords: calcium oxalate; nephrolithiasis; polyethylene glycols; *Pedaliium murex* Linn.; kidney calculi; pathology

INTRODUCTION

Renal calculi are the third leading cause among urinary diseases. In the United States, incidence of renal calculi was 1116 per 1,000,000 population in the year 2000 among adults.^(1,2) Recurrence of calculi is a serious problem. Recurrence rate is 30 to 40 % at 5 years as seen in observational study.⁽³⁾ Studies have shown that effective treatment like dietary modification or medication can reduce the recurrence rate significantly.⁽⁴⁻⁶⁾ So recurrence of renal stone is partially preventable. Seventy to eighty percent of calculi are made up of calcium oxalate and phosphate. The most common abnormality among stone formers is hyperoxaluria. Consumption of high dietary oxalate is a major risk factor for stone formation.^(7,8) At present, management of stone depends mainly on surgical treatment i.e. extracorporeal shock wave lithotripsy, percutaneous lithotripsy, and transureteral lithotripsy.⁽⁹⁾ These surgeries are expensive with higher complication rate than medical management, and do not affect the recurrence of stones. So there is need for medical treatment of renal stone that has curative as well as preventive action on stone formation. Medicinal plants are used for various chronic disorders worldwide. *Pedalium murex* (*P. murex*) Linn. (Name in vernacular language – Hindi, is Bada Gokhru) is a member of Pedaliaceae. It has been used as an important medicinal herb in India. According to Indian medicinal literature, it has aphrodisiac, antitussive, appetizer properties and useful in vesical calculi, urinary discharge, gonorrhoea etc.⁽¹⁰⁻¹⁴⁾ Fruits of *P. murex* Linn. are used traditionally for treatment of genito-urinary disorders, infertility, impotency, intestinal colic, diabetes. A few studies had been done for its antimicrobial, aphrodisiac, nephroprotective, hypolipidemic and anti-inflammatory activities.⁽¹⁵⁻¹⁹⁾ Though it is used as a folk medicine for renal calculi, its effectiveness and mechanism as anti-urolithiatic agent is still unknown. We have done this study to explore its effect and mechanism for prevention and treatment of renal calculi.

MATERIAL AND METHODS

Animals

All the experiments were performed after prior approval from Institutional Animal Ethics Committee (IAEC), Government Medical College, Bhavnagar, Gujarat, India. Male

Wistar albino rats were procured from the central animal house of the institute. They were housed in standard transparent polycarbonate cages and kept in a 12 hr light-dark cycle under controlled room temperature ($24 \pm 2^\circ \text{C}$) and humidity. Animals were given standard laboratory diet and allowed to acclimatize at least three days before starting experiments. The animal handling was performed according to the Good Laboratory Practice (GLP) guidelines.

Drugs and Chemicals

P. murex Linn. 10% ethanolic fruit extract (Tulsi Amrit Pvt. Ltd., Indore, Madhya Pradesh, India), ethylene glycol (Fisher Scientific Co., Mumbai, India), oxalate kit (Trinity Biotech, Ireland), sodium phosphate (Aldrich, India), sodium oxalate and calcium acetate (Alfa Aesar, Hyderabad, India) were used in this study.

Acute toxicity studies of ethanolic fruit extract of *P. murex* Linn. showed that it was safe to administer it up to 2000 mg/kg in rat.⁽¹⁷⁾ Based on these studies, we have taken 1/10th of the highest safe dose for the present study. We have done the study with two incremental doses of 200 and 400 mg/kg of *P. murex* Linn. ethanolic extract to see its dose dependent action.

Study Design

Thirty six male Wistar albino rat (250 - 350 g) were randomly divided into six equal groups. Group I received distilled water instead of tap water and served as normal control. Group II to VI received ethylene glycol 1% v/v in distilled water for 28 days. Group II served as ethylene glycol control. Group III and IV animals received *P. murex* ethanolic extract in 200 mg/kg and 400 mg/kg orally in distilled water for 28 days, respectively and served as prophylactic groups. Group V and VI animals did not receive *P. murex* for first 14 days. These groups received *P. murex* ethanolic extract in 200 mg/kg and 400 mg/kg orally in distilled water from 15th to 28th day, respectively and served as treatment groups.

Outcome measures

Biochemical parameters

Blood samples were collected in plain vacuates from the retro-orbital plexus under ketamine (50 mg/kg intra peritoneally) and xylazine (10 mg/kg intra peritoneally) anaesthesia

Table 1. Effect of *Pedalium murex* Linn. on various biochemical parameters

Parameter	Random blood sugar (mg/dL)	Blood urea (mg/dL)	Serum creatinine (mg/dL)	Alanine aminotransferase (IU/L)	Aspartate aminotransferase (IU/L)	Alkaline phosphatase (IU/L)	Serum bilirubin (mg/dL)	Serum calcium (mmol/L)
Group I (Normal control)	110.17 ±21.04	34.83 ± 4.51	0.65 ± 0.15	75.17 ± 14.93	247.83 ± 20.05	280.33 ± 42.45	0.68 ± 0.22	1.08 ± 0.05
Group II (EG control)	82.67 ± 11.27	258.50 ± 85.75*	2.90 ± 0.63*	154.33 ± 94.94	272.33 ± 112.00	343.67 ± 95.36	0.80 ± 0.27	1.21 ± 0.07
Group III (PM 200 mg/kg – 28 days)	176.33 ± 21.43	48.00 ± 1.57	0.78 ± 0.17 **	74.50 ± 7.62	148.17 ± 39.70	300.33 ± 50.72	0.32 ± 0.03	1.20 ± 0.03
Group IV (PM 400 mg/kg – 28 days)	58.00 ± 11.08	35.83 ± 11.60 **	0.82 ± 0.27	49.00 ± 3.52	141.00 ± 32.76	186.33 ± 17.05	0.22 ± 0.02	1.28 ± 0.04
Group V (PM 200 mg/kg – 15- 28 days)	151.83 ± 38.81	82.00 ± 22.22	0.98 ± 0.25	89.50 ± 4.71	103.83 ± 45.11	282.33 ± 44.29	0.27 ± 0.03	1.19 ± 0.02
Group VI (PM 400 mg/kg – 15 - 28 days)	241.17 ± 51.63	53.00 ± 3.82	0.77 ± 0.09	46.67 ± 8.66	225.50 ± 80.34	132.17 ± 15.18*	0.32 ± 0.10	1.14 ± 0.08

All values are expressed as mean ± SEM. n=6 for all groups. **P* < .05 as compared to group I, ***P* < .05 as compared to group II by Kruskal-Wallis test followed by Dunn's multiple comparisons.

Keys: PM, *Pedalium murex* Linn. EG, Ethylene glycol.

24 hr after the last dose of drug. Serological parameters for renal and hepatic functions were measured.

Urinary parameters

Twenty-four hr. urine specimens were collected on day 0 and 28 of the study by keeping each rat in separate metabolic cage (B.I.K. Industries, Mumbai, India). Urine volume was measured. It was acidified and kept under refrigeration (2 - 8° C). Urinary oxalate was measured by oxalate kit within 7 days of collection of sample by spectrophotometer.⁽²⁰⁾

Histopathological parameters

The animals were sacrificed soon after blood collection under the continued effect of anesthesia. Both kidneys were removed and kept in formaldehyde (10% v/v) for at least 24 hours. Then 5 mm thick sections were taken and enclosed in paraffin. They were cut into 5 µm thin sections, stained with hematoxylin-eosin (H & E) and evaluated under optical light binocular microscope. Calcium oxalate crystal depositions were calculated in 10 microscopic fields (159 × 10⁻⁹ m² each) and other changes e.g. necrosis, leukocyte infiltration and tubular dilatations were also noted.

In-vitro method of mineralization

To evaluate the inhibition of calcium oxalate and phosphate mineralization by *P. murex* Linn.; we used simultaneous flow static model (S.S.M.) described by Farook et al.⁽²¹⁾ We used *P. murex* Linn. 200 mg/ml, calcium acetate (0.1 M) and sodium oxalate (0.1 M) (for calcium oxalate) or sodium phosphate (for calcium phosphate) in three separate burettes (25 ml) and were allowed to fall simultaneously into a 250 ml beaker with a slow and steady pace. After 30-40 min, the mixture was kept in a hot water bath for 10 min, cooled to room temperature and the precipitate was collected into a pre-weighed centrifuge tube. Supernatant fluid was discarded. Then, these tubes were dried in a hot air oven at 120° C, cooled to room temperature and weighed till constant weight is achieved. Weight of the precipitates (ppt.) was calculated. Then percentage inhibition was calculated by following formula:

$$\text{Inhibition (\%)} = \frac{(\text{Weight of ppt. in blank set} - \text{Weight of ppt. in experimental set} \times 100)}{\text{Weight of ppt. in blank set}}$$

Statistical analysis

Table 2. Effect of *Pedalium murex* Linn. on urinary parameters

Urine oxalate (mmol/l)						
Day	Group I (Normal control)	Group II (EG control)	Group III (PM 200 mg/kg – 28 days)	Group IV (PM 400 mg/kg – 28 days)	Group V (PM 200 mg/kg – 15- 28 days)	Group VI (PM 400 mg/kg – 15 - 28 days)
Day 0	0.48 ± 0.14	0.35 ± 0.15	0.28 ± 0.07	0.56 ± 0.32	0.85 ± 0.39	1.68 ± 0.26
Day 28	0.18 ± 0.05	1.53 ± 0.19 *	1.74 ± 0.41*	1.58 ± 0.46	1.52 ± 0.23	1.86 ± 0.42 *
Urine Volume (ml)						
Day	Group I (Normal control)	Group II (EG control)	Group III (PM 200 mg/kg – 28 days)	Group IV (PM 400 mg/kg – 28 days)	Group V (PM 200 mg/kg – 15-28 days)	Group VI (PM 400 mg/kg – 15 - 28 days)
Day 0	17.58 ± 4.34	11.75 ± 1.82	14.50 ± 3.62	13.67 ± 2.54	16.17 ± 3.0	13.83 ± 2.52
Day 28	33.33 ± 4.22	24.00 ± 5.13	21.83 ± 5.7	30.67 ± 5.87	32.00 ± 8.21	12.83 ± 7.44

All values are expressed as mean ± SEM. n=6 for all groups. * $P < .05$ as compared to group I by Kruskal-Wallis test followed by Dunn's multiple comparisons. Keys: PM, *Pedalium murex* Linn. EG, Ethylene glycol.

Data were expressed as Mean ± Standard Error of Mean (SEM). All the quantitative variables (urinary parameters for day 0 and 28, biochemical parameters, kidney weight and calcium oxalate crystal depositions) were compared by one-way ANOVA or Kruskal-Wallis test followed by post hoc test as per Gaussian distribution of the data. All the statistical analysis was done using GraphPad InStat 3.0 (Demo version). $P < .05$ was considered as the significant difference.

RESULTS

Biochemical parameters

Ethylene glycol administration increased the values of blood urea and serum creatinine significantly as compared to normal control. *P. murex* Linn. showed the significant improvement in renal parameters in prophylactic groups ($P < 0.05$). Renal parameters were non-significantly improved in treatment groups. Random blood sugar, ALT, AST, ALP, serum bilirubin and serum calcium were not significantly affected by either ethylene glycol or *P. murex* Linn. (Table 1).

Urinary parameters

There was no statistically significant difference in urinary volume and oxalate in all the groups on day 0. Urinary oxalate was significantly increased after ethylene glycol administration. *P. murex* Linn. did not show significant effect on urinary oxalate. Urine volume was not significantly affected by ethylene glycol or *P. murex* Linn. (Table 2).

Kidney weight

Ethylene glycol significantly increased the kidney weight as compared to normal control. *P. murex* Linn. restored kidney weight in prophylactic group in lower dose. ($P < .05$ for group II vs. III) In other groups, kidney weights were improved but they were not statistically significant (Table 3).

Histopathological examination

Ethylene glycol caused significant calcium oxalate crystal depositions in the renal tubules. These crystals were found in proximal tubules, loop of Henle, distal tubules and associated with significant leukocyte infiltration, necrosis, hemorrhage and tubular dilatation. These crystals were large polygonal in shape, heterogeneous in distribution and pattern (Figure). Treatment with *P. murex* showed significant reduction in number of crystal depositions all the groups ($P < .05$). There was no significant difference between the *P. murex* treated groups. Calcium oxalate crystals were small and found in lesser area in group III, IV, VI as compared to group II. Tubular dilatation and leukocyte infiltration were found to lesser extent in these groups but necrosis was not detected (Table 3).

In vitro mineralization

P. murex Linn. 200 mg/ml showed 33% inhibition of calcium oxalate and 57% inhibition of calcium phosphate crystallization.

DISCUSSION

Table 3. Effect of *Pedalium murex* Linn. on body weight, kidney weight, calcium oxalate crystal depositions

Groups	Body Weight (g)	Kidney weight (g)	No. of calcium oxalate crystals in 10 microscopic fields
Group I (Normal control)	286.67 ± 12.56	1.19 ± 0.05	0.333 ± 0.211
Group II (EG control)	300.00 ± 8.16	2.03 ± 0.09*	17.667 ± 1.961 [#]
Group III (PM 200 mg/kg – 28 days)	263.33 ± 15.42	1.32 ± 0.14**	4.833 ± 1.887 ^{##}
Group IV (PM 400 mg/kg – 28 days)	263.33 ± 17.26	1.50 ± 0.24	6.833 ± 2.915**
Group V (PM 200 mg/kg – 15- 28 days)	255.00 ± 15.86	1.65 ± 0.1*	6.500 ± 2.579**
Group VI (PM 400 mg/kg – 15 - 28 days)	293.33 ± 17.26	1.64 ± 0.24	1.833 ± 0.872 ^{##}
One way ANOVA	<i>P</i> = 0.199	<i>P</i> = 0.0166	<i>P</i> < 0.0001
F (df)	1.601 (5, 30)	3.322 (5, 30)	9.619 (5, 30)

All values are expressed as mean ± SEM. n=6 for all groups. **P* < .05 as compared to group I, ***P* < .05 as compared to group II, #*P* < .001 as compared to group I, ##*P* < .001 as compared to group II by one - way ANOVA followed by Tukey-Kramer multiple comparison tests.

Keys: PM, *Pedalium murex* Linn. EG, Ethylene glycol.

Urolithiasis is a multifactorial, urological disorder in which super saturation of urine with oxalate plays a key role in pathogenesis. Presently, medical management of renal stone consists of lifestyle modification, calcium channel blocker, diuretics, citrate and magnesium-rich diet.⁽²²⁾ This therapy does not affect recurrence of stone, so traditional medicinal plants have been tried for the prevention of recurrence.

We have found increased level of urinary oxalate and calcium oxalate crystal depositions in ethylene glycol control group (*P* < .05). Ethylene glycol administration leads to development of nephrolithiasis by producing hyperoxaluria. It also increases urinary calcium and phosphate. Studies have shown that hyperoxaluria causes proximal renal tubular damage and shedding of brush border cells. It would become a site for calcium oxalate monohydrate (COM) crystal attachment with renal papilla.^(23,24) Urinary calcium and super saturation of oxalate develop into nucleation, aggregation and formation of renal stone. So rat model of ethylene glycol is useful for evaluation of renal papillary stone. In present study, all the *P. murex* treated groups showed significant reduction in calcium oxalate crystal depositions as compared to ethylene glycol control but urinary oxalate was not affected (Table 3). This may be due to lack of diuretic activity of the extract (Table 2). This suggests direct action of *P. murex* extract on calcium oxalate crystallization in kidney. In addition, *P. murex* extract has shown significant inhibition of calcium oxalate

and phosphate mineralization as seen by in vitro model.

We also found significant improvement in renal function (urea and creatinine) in prophylactic groups (*P* < .05). It suggests that *P. murex* extract can prevent renal damage caused by hyperoxaluria. Ethylene glycol administration also leads to hypertrophy of renal papilla and increased kidney weight probably by inflammation and fluid accumulation.⁽²⁵⁾ Restoration of renal function is also associated with improvement in kidney weight in *P. murex* treated rats in prophylactic group in low dose (*P* < .05). Previous study of *P. murex* fruit extract showed significant improvement in acidic phosphatase (ACP), ALT, ALP, and lactate dehydrogenase (LDH) in urine, serum and kidney homogenate in ethylene glycol-induced renal damage.⁽²⁶⁾ These enzymes are non-specific and may increase in many other pathological conditions of liver, kidney and muscles. We have considered more specific parameters for renal calculi like renal function test, kidney weight, urinary oxalate, volume and calcium oxalate crystal depositions in kidney.

Calcium oxalate crystal deposition is associated with severe oxidative stress to renal tissue. It leads to lipid peroxidation of membranes by generation of reactive oxygen species like hydroxyl, superoxide ions.⁽²⁷⁾ It causes accumulation of non protein nitrogenous (NPN) compounds like urea and creatinine in blood as found in our study. Several antioxidants like vitamin E, vitamin C, flavonoids and phenolic compounds

are found to be effective in prevention of oxidative damage and deterioration of renal function.^(28,29) Studies have shown that *P. murex* fruit extract possess strong nitric oxide, 2,2-diphenyl-1-picrylhydrazyl (DPPH), hydrogen peroxide, and hydroxyl radical scavenging activities.⁽³⁰⁾ Phytochemical analysis of ethanolic extract *P. murex* Linn. fruits show that it contains high levels of flavonoids, glycosides, steroids, phenols, terpenoids, saponins and tannins.⁽³¹⁾ Among these components, flavonoids and tannins possess significant antioxidant property.⁽³²⁾ Saponins and steroids possess antibacterial and antioxidant properties.⁽³³⁾

In present study, inhibition of crystallization may have decreased oxidative stress to the tissue. Antioxidants present in the extract also may have decreased lipid peroxidation-induced renal tubular damage and may contribute to its anti-urolithiatic action. Overall, *P. murex* fruit extract shows significant improvement of renal function, kidney weight, and calcium oxalate crystal depositions in prophylactic groups (III and IV) more than treatment groups (V and VI). Reason for this finding can be hypothesized as crystallization is ongoing process associated with continued oxidative damage. *P. murex* fruit extract may not reverse oxidative damage that as already occurred in 14 days treatment with ethylene glycol in group V and VI. So, renal function and kidney weight have not improved significantly in treatment groups. In addition, group VI had lowest calcium oxalate crystal depositions and group V had also similar rate of crystal depositions with prophylactic groups which is suggestive of solubilizing effect of the *P. murex* on already formed crystals. It suggests that *P. murex* fruit extract may have substantial action on process of crystallization. Further studies are needed to confirm above hypothesis.

Nowadays, there is increased concern about toxicity profile of phytotherapy. In present study, we have found that *P. murex* fruit extract does not affect hepatic function and blood sugar significantly even in ethylene glycol treated rats. It is safe to use for prevention of renal stone. Further clinical studies are required to evaluate efficacy and safety in human beings.

CONCLUSION

Pedaliium murex Linn. ethanolic fruit extract has significant anti-urolithiatic activity for prevention of renal calculi prob-

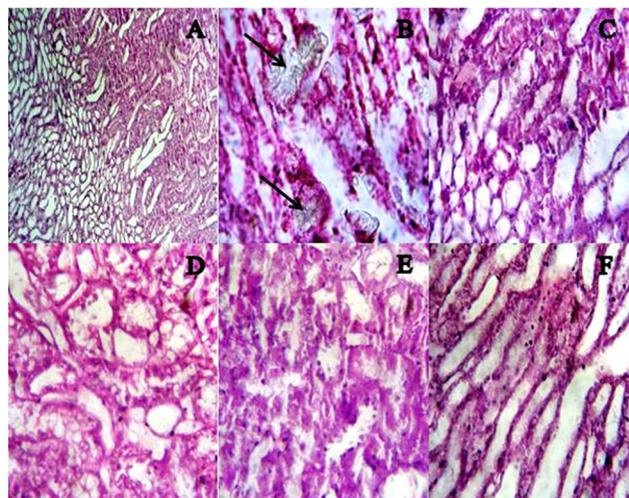


Figure 5. Histopathological images of kidney sections after hematoxylin & eosin staining under light microscope from (A) normal control showing normal renal tubules and glomeruli (10 × magnification), (B) ethylene glycol control showing calcium oxalate crystals (arrows), (C) group treated with *P. murex* 200 mg/kg for 28 days, (D) group treated with *P. murex* 400 mg/kg for 28 days, (E) group treated with *P. murex* 200 mg/kg from 15th – 28th days, (F) group treated with *P. murex* 400 mg/kg from 15th – 28th days (Images from B to F are of 40 × magnification).

ably by affecting calcium oxalate crystallization. There was no dose dependent increment in the effects.

CONFLICT OF INTEREST

None declared.

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