**Bryophyllum pinnatum** Leaf Extracts Prevent Formation of Renal Calculi in Lithiatic Rats

**Abstract**

**Background:** *Bryophyllum pinnatum*, commonly known as *Pattharcaṭṭa*, is used traditionally in ethnomedicinal practices for the treatment of kidney stone and urinary insufficiency. **Aim:** The present study evaluated the effect of *Bryophyllum pinnatum* on ethylene glycol (EG)-induced renal calculi in rats. **Materials and Methods:** Renal calculi were induced in rats by administration of 0.75% EG in drinking water and co-treated orally with standard drug, Cystone (750 mg/kg), or alcoholic and hydro-alcoholic extracts in doses of 100, 200 and 400 mg/kg for 28 days. Weekly body weights were recorded. On day 29, urolithiasis was confirmed by assessing the urinary parameters (urine volume, pH, uric acid, calcium, phosphorus, oxalate, magnesium and creatinine clearance), serum biochemical parameters (creatinine, uric acid, urea, calcium, phosphorus and magnesium), oxidative stress parameters and histology of kidney. **Results:** Treatment with extracts attenuated the EG-induced decrease in body weight and elevation in urinary parameters (uric acid, calcium, phosphorus and oxalate) and serum biochemical parameters (creatinine, uric acid, urea, calcium, phosphorus and magnesium). Extract treatment also reversed EG-induced decrease in urine volume, pH, magnesium and creatinine clearance, oxidative and histological damages in kidneys. Results were comparable to standard drug, Cystone. Results indicated that EG administration caused renal calculi formation which is prevented by treatment with extracts. The observed antilithiatic effect may be attributed to the presence of high content of phenolics, flavonoids and saponins in the extracts. **Conclusion:** *Bryophyllum pinnatum* leaves showed preventive effect against renal calculi formation and validates its ethnomedicinal use in urinary disorders. It further supports its therapeutic potential for the treatment of urinary calculi.

**Keywords:** *Bryophyllum pinnatum*, ethnomedicine, ethylene glycol, kidney stone, lithiasis, urinary disorder

**Introduction**

Renal or urinary calculi also called urolithiasis, is a condition which involves the process of formation and retention of stone(s) in kidney, bladder and/or urethra that results in renal colic, urine retention and pain in the abdomen and flank.[1] It is estimated to occur in approximately 12% of world population and 50% of recurrence rate in 5-10 years of treatment.[2] The pathogenesis of urolithiasis involves the imbalance between promoters and inhibitors of crystallization in the kidneys.[3] The mechanism of calculi formation is a complex process concerned with supersaturation, nucleation, aggregation, growth and retention of crystals within the renal tubules.[4] Among urinary stones, majority of stones are calcium oxalate (CaOx).[5] The etiopathogenesis of renal calculi is multifactorial involving anatomic, environmental, infections, metabolic and dietary lifestyle habits.[2,6,7]

Medical or surgical stone removal and extracorporeal shock wave lithotripsy are the latest options to manage and treat such stone disorders. But they are expensive, pose various side effects and also do not prevent the recurrence of stone formation.[8,9] Hence, there is a growing interest towards the use of medicinal plants for correction of stone disorders and ailments. Ayurvedic system of medicine also advocates the use of various medicinal plants and their formulations for the treatment of urinary stones and kidney diseases.[10,11]

*Bryophyllum pinnatum* (Lam.) Oken (Crassulaceae) is a perennial herb commonly known as *Zakhm-e-hyat, patharcaṭṭa* and *panṇabīja*. The other synonyms are *Bryophyllum calycinum* and *Kalanchoe pinnata*. It is widely...
Yadav, et al.: Antilithiatic activity of Bryophyllum pinnatum

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medicinal garden of J. L. Chaturvedi College of Pharmacy, leaves of Bryophyllum pinnatum in Dibrugarh district[19] of Assam for treatment of kidney and gall bladder stones. Leaves are also used by tribes in Trinidad and Tobago, Nigeria[20] and Mianwali district of Pakistan[21] in ethnomedicinal practices for treatment of urinary calculi. Moreover, the leaves of this plant constituted an important part of the poly-herbal formulations which are used for antilithiatic purposes.[22,23] Leaves are known to possess neurosedative and muscle relaxant,[24] antimicrobial,[25] antiulcer,[26] uterine contractility,[27] antinociceptive, anti-inflammatory and antidiabetic,[28] antihypertensive[29] and nephroprotective[30] activities. The medicinal and pharmacological properties of Bryophyllum pinnatum are ascribed to the presence of alkalanes, alkanols, triterpenes and sterols,[31] triterpenoids and phenanthrenes,[32] flavonoids,[25] bufadienolides,[33] alkaloids, glycosides and lipids.[34] Previously, Yasir et al. reported in-vitro inhibitory activity of the leaves of Bryophyllum pinnatum on calcium oxalate crystallisation.[13] In view of the traditional and ethnomedicinal use of leaves of Bryophyllum pinnatum for the treatment of kidney and bladder stones, and urinary insufficiency, it was thought worthwhile to investigate its effect on experimentally induced in-vivo lithiatic model. Hence, the present study evaluates the effects of alcoholic and hydro-alcoholic extracts of Bryophyllum pinnatum leaves on formation of urinary calculi (urolithiasis) in ethylene glycol-induced lithiases in rats.

Materials and Methods

Drugs and chemicals

Ethylene glycol (Qualigens Fine Chemicals, Mumbai), Cystone (The Himalaya Drug Company, Bangalore), creatinine estimation kit (Merck Specialities Pvt. Ltd., Mumbai), urea, uric acid, calcium, phosphorous estimation kit (ERBA diagnostics Mannheim, GmbH, Germany) and magnesium estimation kit (Lab-Care Diagnostics (India) Pvt. Ltd., Valsad) were procured from local market. All other chemicals used in the experiment were of the highest grade commercially available.

Plant material

Leaves of Bryophyllum pinnatum were collected from the medicinal garden of J. L. Chaturvedi College of Pharmacy, Nagpur and authenticated by Dr. Vinayak Naik, Senior Taxonomist, Piramal Life Sciences Ltd., Mumbai. The voucher specimen no. MY/BPL/2012 was deposited in the herbarium.

Preparation of the extracts

About 500 g of fresh leaves were soaked separately in ethanol and water:Ethanol (30:70) in a stopper container for 3 days (maceration) with frequent agitation. The solutions were filtered to obtain alcoholic extract (AlcE) and hydro-alcoholic extract (HAlcE), respectively. The extracts were dried in a rotary evaporator and stored in desiccators for further use.

Preliminary and quantitative estimation of phytoconstituents

AlcE and HAlcE were screened for the presence of carbohydrates, proteins, phenols, flavonoids, saponins, glycosides, alkaloids, terpenoids and, steroids using standard procedures.[35] The total phenolic content of the extracts was determined spectrometrically by Folin–Ciocalteu method[36] and expressed as percentage of gallic acid equivalent. The total flavonoid content was determined by aluminium chloride colorimetric assay[37] and expressed as percentage of rutin equivalent in extracts. Total saponins were determined according to previously described method of Obadoni and Ochuko and expressed as percentage of saponins in extracts.[19]

Experimental animals and ethical approval

Male albino Wistar rats (150–200 g) were selected for the assessment of oral acute toxicity study and antilithiatic activity. The animals were acclimatized to standard laboratory conditions of temperature 25±2°C with relative humidity 50% under 12 h light: 12 h dark cycle. They were provided with regular standard feed and drinking water ad libitum. All the protocols and experiments were approved by the Institutional Animal Ethics Committee (Approval no. JLCCP/IAEC/2012–13/13) and conducted according to ethical guidelines provided by Committee for the Purpose of Control and Supervision of Experiments on Animals, New Delhi.

Acute toxicity study

Acute oral toxicity study (limit test) for AlcE and HAlcE were carried out as per Organization for Economic Co-operation and Development (OECD) 423 guidelines.[40] The overnight fasted rats (n = 3) were administered with AlcE and HAlcE in the limit dose of 2000 mg/kg, orally, and observed for a period of 72 h and thereafter up to 14 days for behavioral, neurological, autonomic profiles and for any lethality or moribund state.[41]

Induction of renal calculi

Renal calculi were induced by administration of 0.75%v/v ethylene glycol (EG) in drinking water ad libitum
for 28 consecutive days as per the methods described previously.[42]

Experimental design

Rats were divided into nine groups (n = 6) and EG (0.75% in drinking water) was administered to group II–IX for 28 days.

- Group I served as normal control and received drinking water only
- Group II served as lithiatic control and received vehicle of extracts
- Group III co-treated with standard drug, cystone (750 mg/kg)[11]
- Group IV to VI co-treated with AlcE in doses of 100, 200 and 400 mg/kg body weight
- Group VII to IX co-treated with HAlcE in doses of 100, 200 and 400 mg/kg body weight.

All the doses were prepared in distilled water using 1% tween 80 as suspending agent and administered daily, orally for 28 days. During the experimental period weekly body weight of each rat were recorded. On the 29th day, the biochemical estimations of urine and serum, and oxidative stress parameters and histology of kidney tissues were evaluated.

Body weight

The body weight of each rat was recorded during the experimental period, once before the treatment and every week during the treatment. The percentage change in body weight of each animal was calculated weekly as follow:

\[
\% \text{Change in BW} = \frac{\Delta \text{BW (g)}}{\text{IB (g)}} \times 100
\]

Where \( \Delta \text{BW} \) is the difference in body weight at one time interval and IB is the initial body weight of rat on the beginning of the treatment.

Urine biochemical parameters

On 28th day of experiment all animals were kept individually in metabolic cages with free access to drinking water. Urine samples of 24 h were collected. Urine volume and pH (by using Urodip pH strips, ERBA diagnostics Mannheim, GmbH, Germany) were determined. A drop of conc. HCl was added to the urine before being stored at -20°C. Urine was analysed for uric acid, calcium, phosphorus, magnesium and creatinine by using commercially available biochemical kits. Oxalate was determined spectrophotometrically.[43] Creatinine clearance (ml/min) was calculated by the following formula:[44]

\[
\text{Creatinine clearance (ml / min)} = \frac{(\text{mg creatinine} / \text{dl urine}) \times (\text{ml urine} / 24h)}{(\text{mg creatinine} / \text{dl serum}) \times 1440}
\]

Serum biochemical parameters

On the 29th day of experiment, rats were anaesthetised and blood samples were collected from the retro-orbital region. Serum was separated by centrifugation and analysed for creatinine, uric acid, urea, calcium, phosphorous and magnesium by using commercially available biochemical kits.

Kidney homogenate and histopathological analysis

The rats were sacrificed and kidneys were removed. Left kidney was taken and washed thoroughly with ice-cold 0.1 M phosphate buffered saline (pH 7.4). It was blotted dry and homogenized in 1.15% KCl to prepare a 10% w/v suspension. This suspension was centrifuged at 16000 \( \times g \) in a cooling centrifuge at 0°C. The supernatant obtained was further employed for estimation of malondialdehyde (MDA),[45] glutathione (GSH),[46] catalase (CAT)[47] and superoxide dismutase (SOD).[48] Protein content of the supernatant was estimated by Biuret method.[49] MDA and GSH were expressed as nmol/mg protein and SOD and CAT were expressed as U/mg protein. To confirm the incidence of lithiasis, right kidney of each animal was preserved in 10% neutralized formalin for further histopathological study. The isolated kidney was embedded in paraffin and cut into 5 \( \mu \)m thin sections and stained with hematoxylin-eosin dye. The slides were examined under binocular microscope for histopathological changes in kidney architecture, renal cellular and tubular necrosis and presence of calcium oxalate crystals.

Statistical analysis

The results were expressed as mean ± SEM (n = 6). Statistical analysis was performed by one way ANOVA followed by Tukey’s post hoc test. A value of \( P < 0.05 \) was considered significant in all cases.

Results

Preliminary and quantitative estimation of phytoconstituents

The phytochemical screening of the AlcE revealed the presence of carbohydrates, proteins, phenols, flavonoids, saponins, glycosides, alkaloids, terpenoids and steroids while HAlcE showed the presence of carbohydrates, proteins, phenols, flavonoids, saponins, glycosides and alkaloids. The total phenolic contents of AlcE and HAlcE were 51.07% and 46.71% gallic acid equivalent, respectively. Similarly, total flavonoids were 4.19% and 3.67% rutin equivalent and saponin contents were 2.32% and 3.93%, respectively for AlcE and HAlcE.

Acute oral toxicity

There were no toxic symptoms or mortality or moribund stage observed with single acute limit dose level of 2000 mg/kg in any animal during 14 consecutive days of observation. It implies that the approximate lethal dose (LD \(_{50}\) ) of the AlcE and HAlcE in Wistar rats was higher than 2500 mg/kg body weight.

Effect on body weight

EG administration showed significant (\( P < 0.001 \)) body weight loss in lithiatic control group when
compared to normal control group. Treatment with cystone or AlcE and HAlcE (100, 200 and 400 mg/kg) significantly ($p < 0.05 - p < 0.001$, where applicable) attenuated the loss in body weight of animals when compared to lithiatic control group [Table 1].

**Effect on urine parameters**

There was significant ($p < 0.001$) decrease in urine volume and pH in lithiatic control group when compared to normal control group [Table 2]. Cystone treated group showed a significant ($p < 0.001$) increase in urine volume and pH when compared to lithiatic control group. AlcE or HAlcE treatment in dose of 200 and 400 mg/kg showed significant ($p < 0.01 - p < 0.001$, wherever applicable) increase in urine volume and pH. AlcE (100 mg/kg) caused significant ($p < 0.001$) increase in urine volume without any significant change in pH whereas HAlcE (100 mg/kg) showed significant increase in urine volume ($p < 0.001$) and pH ($p < 0.01$) when compared to lithiatic control group.

Administration of EG significantly ($p < 0.001$) increased the uric acid, calcium, phosphorus and oxalate levels in urine whereas the creatinine clearance and magnesium levels were reduced when compared to normal control group [Table 2]. Administration of AlcE or HAlcE (100, 200 and 400 mg/kg) significantly ($p < 0.05 - p < 0.001$, wherever applicable) increased creatinine clearance level in dose dependent manner along with significant decrease in uric acid, phosphorus and oxalate levels ($p < 0.001$, in all cases) compared to lithiatic control group. Cystone or AlcE and HAlcE (200, 400 mg/kg) treatment significantly ($p < 0.05 - p < 0.001$, wherever applicable) decreased the urine calcium levels while the lower dose (100 mg/kg) was found insignificant. Treatment with cystone or AlcE and HAlcE (100, 200, 400 mg/kg) caused significant ($p < 0.05 - p < 0.001$, wherever applicable) increase in urinary magnesium when compared to lithiatic control group.

**Effect on serum parameters**

There was significant ($p < 0.001$) increase in serum creatinine, uric acid, urea, calcium, phosphorus and magnesium in lithiatic control group when compared

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**Table 1: Effect of Bryophyllum pinnatum leaf extracts on the body weight of rats against ethylene glycol-induced renal calculi**

| Group | Treatment | Dose (mg/kg) | Body weight (g) | Change in body weight (%) |
|-------|-----------|--------------|-----------------|--------------------------|
|       |           |              | Day 0 | Day 7 | Day 14 | Day 21 | Day 28 |
| I     | Vehicle   | -            | 162.00±2.07 | 21.49±1.62 | 42.21±2.33 | 56.28±1.90 | 67.81±2.15 |
| II    | EG 0.75%  | -            | 180.66±0.84 | −6.33±0.92* | −1.29±0.66* | 7.56±0.46* | 18.45±0.57* |
| III   | Cystone   | 750          | 167.33±0.84 | 20.72±0.76† | 36.46±0.97‡ | 48.21±1.47‡ | 59.97±1.01‡ |
| IV    | AlcE      | 100          | 193.00±1.50 | 3.62±0.23 (NS) | 10.46±0.91 (NS) | 25.48±1.12‡ | 36.58±0.56‡ |
| V     | AlcE      | 200          | 179.00±3.29 | 10.25±0.60* | 21.64±1.40* | 41.28±1.36² | 46.69±2.17² |
| VI    | AlcE      | 400          | 173.50±1.15 | 13.87±0.56* | 23.54±0.19* | 42.95±0.68³ | 53.15±1.10³ |
| VII   | HAlcE     | 100          | 154.17±1.17 | 8.10±0.18¹ | 16.55±0.81¹ | 25.87±1.22² | 38.84±1.28² |
| VIII  | HAlcE     | 200          | 163.33±1.67 | 13.97±1.07³ | 28.53±1.65³ | 41.01±1.58³ | 55.25±1.47³ |
| IX    | HAlcE     | 400          | 163.50±1.09 | 18.45±0.84* | 33.15±1.15* | 42.84±1.34* | 64.64±1.45* |

Values are expressed in mean±SEM (n=6); Significant values are *$p<0.001$ compared to normal control (Group I) and †$p<0.001$, §$p<0.001$ compared to lithiatic control (Group II). NS: Not significant, AlcE: Alcoholic extract, HAlcE: Hydro-alcoholic extract, SEM: Standard error of mean, EG: Ethylene glycol

**Table 2: Effect of Bryophyllum pinnatum leaf extracts on urine parameters against ethylene glycol induced renal calculi**

| Group | Treatment | Dose (mg/kg) | Volume (ml/min) | pH | Urine parameters | Calcium (mg/24 h) | Phosphorus (mg/24 h) | Oxalate (mg/24 h) | CC (ml/min) | Magnesium (mg/24 h) |
|-------|-----------|--------------|-----------------|----|-----------------|-------------------|-------------------|-------------------|-------------|-------------------|
| I     | Vehicle   | -            | 11.98±0.29      | 8.00±0.18 | 4.54±0.16 | 4.74±0.44 | 3.31±0.10 | 0.32±0.02 | 1.20±0.13 | 2.47±0.11 |
| II    | EG 0.75%  | -            | 5.07±0.14*      | 5.83±0.24* | 7.95±0.14* | 7.03±0.29* | 6.92±0.16* | 2.56±0.17* | 0.17±0.03* | 6.40±0.03* |
| III   | Cystone   | 750          | 11.65±0.41      | 7.75±0.17* | 4.70±0.08† | 4.50±0.36‡ | 3.56±0.17‡ | 0.42±0.01‡ | 1.09±0.14† | 2.34±0.08† |
| IV    | AlcE      | 100          | 7.32±0.15      | 6.00±0.25 (NS) | 6.19±0.12 | 6.47±0.16 (NS) | 5.84±0.11 | 1.80±0.07 | 0.79±0.11 | 0.96±0.02 |
| V     | AlcE      | 200          | 8.43±0.17      | 7.16±0.16 | 5.09±0.08 | 5.83±0.17 | 4.25±0.11 | 1.09±0.07 | 0.87±0.07 | 1.34±0.05 |
| VI    | AlcE      | 400          | 10.20±0.14     | 7.50±0.18 | 4.65±0.11 | 5.03±0.13 | 4.08±0.09 | 0.61±0.03 | 0.93±0.15 | 1.74±0.03 |
| VII   | HAlcE     | 100          | 8.50±0.23      | 6.75±0.21 | 6.09±0.09 | 6.09±0.16 (NS) | 5.14±0.08 | 1.55±0.08 | 0.79±0.12 | 1.20±0.04 |
| VIII  | HAlcE     | 200          | 9.55±0.16      | 7.25±0.28 | 4.82±0.08 | 5.61±0.18 | 4.13±0.06 | 0.93±0.04 | 0.92±0.07 | 1.60±0.04 |
| IX    | HAlcE     | 400          | 12.63±0.31     | 7.66±0.16 | 4.48±0.09 | 4.81±0.20 | 3.72±0.16 | 0.49±0.03 | 1.01±0.13 | 2.39±0.07 |

Values are expressed in mean±SEM (n=6); Significant values are *$p<0.001$ compared to normal control (Group I) and †$p<0.001$, §$p<0.001$ compared to lithiatic control (Group II). NS: Not significant, AlcE: Alcoholic extract, HAlcE: Hydro-alcoholic extract, CC: Creatinine clearance, SEM: Standard error of mean, EG: Ethylene glycol
to normal control group [Table 3]. The treatment with AlcE (200 and 400 mg/kg) or HAlcE (100, 200 and 400 mg/kg) significantly reduced serum creatinine levels ($p < 0.05$–$p < 0.001$, wherever applicable) compared to lithiatic control group while AlcE 100 mg/kg was found ineffective. Treatment with AlcE or HAlcE (100, 200 and 400 mg/kg) showed significant decrease ($p < 0.01$, in all cases) in serum uric acid, urea and magnesium levels compared to lithiatic control group. Treatment with AlcE or HAlcE (200 and 400 mg/kg) significantly ($p < 0.01$ – $p < 0.001$, wherever applicable) decreased serum calcium levels whereas the lower dose of extracts (100 mg/kg) did not show any significant change in serum calcium compared to lithiatic control group. AlcE or HAlcE (100, 200 and 400 mg/kg) treatment significantly ($p < 0.01$ – $p < 0.001$, wherever applicable) decreased serum phosphorus levels in a dose dependent manner compared to lithiatic control group. The standard drug, cystone showed ameliorative effect on serum creatinine, uric acid, urea, calcium, phosphorus and magnesium as compared to the lithiatic control group.

**Effect on oxidative stress parameters in kidney tissue**

Ethylene glycol administration significantly ($p < 0.001$) increased the MDA level and decreased GSH content and activities of CAT and SOD in lithiatic control group when compared to normal control group [Table 4]. The treatment with AlcE and HAlcE (200 and 400 mg/kg) significantly ($p < 0.001$, in all cases) reduced the levels of MDA and increased GSH content, CAT and SOD activities when compared to lithiatic group. However AlcE (100 mg/kg) did not show any significant effect on MDA level, GSH content and CAT activity whereas SOD activity was significantly ($p < 0.05$) increased. HAlcE (100 mg/kg) significantly ($p < 0.05$ – $p < 0.01$, wherever applicable) decreased MDA levels and increased GSH content and SOD activity, whereas CAT activity remained unaffected when compared to lithiatic control group. Treatment with cystone significantly ($p < 0.001$)

### Table 3: Effect of Bryophyllum pinnatum leaf extracts on serum parameters against ethylene glycol-induced renal calculi

| Group | Treatment | Dose (mg/kg) | Creatinine (mg/dl) | Uric acid (mg/dl) | Urea (mg/dl) | Calcium (mg/dl) | Phosphorus (mg/dl) | Magnesium (mg/dl) |
|-------|-----------|--------------|--------------------|-------------------|-------------|----------------|-----------------|-------------------|
| I     | Normal    | -            | 0.51±0.11          | 2.55±0.12         | 46.58±0.93  | 6.78±0.39      | 3.04±0.09       | 0.59±0.01         |
| II    | EG 0.75%  | -            | 1.51±0.13*         | 5.13±0.09*        | 76.42±0.70* | 14.47±1.05*    | 5.95±0.17*      | 1.77±0.02*        |
| III   | Cystone   | 750          | 0.48±0.06*         | 2.89±0.21†        | 43.63±0.74‡ | 7.30±0.52      | 3.14±0.06†      | 0.75±0.03‡        |
| IV    | AlcE      | 100          | 0.98±0.13 (NS)     | 4.49±0.11†       | 69.49±0.37‡ | 12.96±1.02 (NS)| 5.40±0.08§      | 1.49±0.02†        |
| V     | AlcE      | 200          | 0.83±0.13†         | 3.88±0.12†       | 59.03±0.64‡ | 10.33±0.35†    | 4.91±0.05†      | 1.26±0.02†        |
| VI    | AlcE      | 400          | 0.58±0.09§         | 3.11±0.11†       | 52.45±0.46‡ | 8.67±0.57‡     | 4.18±0.06‡      | 1.03±0.04‡        |
| VII   | HAlcE     | 100          | 0.93±0.21†         | 4.26±0.08§       | 64.27±0.67† | 11.45±0.62 (NS)| 5.41±0.06†      | 1.42±0.03‡        |
| VIII  | HAlcE     | 200          | 0.80±0.12†         | 3.52±0.12†       | 55.04±0.63† | 9.34±0.58‡     | 4.76±0.08§      | 1.11±0.03‡        |
| IX    | HAlcE     | 400          | 0.60±0.07†         | 3.02±0.12†       | 48.71±0.44‡ | 7.72±0.51†     | 3.82±0.07§      | 0.80±0.02†        |

Values are expressed in mean±SEM (n=6); Significant values are *$P<0.001$ compared to normal control (Group I) and †$P<0.001$, ‡$P<0.01$, §$P<0.05$ compared to lithiatic control (Group II). NS: Not significant, AlcE: Alcoholic extract, HAlcE: Hydro-alcoholic extract, SEM: Standard error of mean, EG: Ethylene glycol

### Table 4: Effect of Bryophyllum pinnatum leaf extracts on oxidative stress parameters in kidney against ethylene glycol-induced renal calculi

| Group | Treatment | Dose (mg/kg) | MDA (nmol/mg protein) | GSH (nmol/mg protein) | CAT (U/mg protein) | SOD (U/mg protein) |
|-------|-----------|--------------|-----------------------|-----------------------|-------------------|-------------------|
| I     | Normal    | -            | 3.75±0.08             | 3.54±0.16             | 3.28±0.05         | 9.11±0.18         |
| II    | EG 0.75%  | -            | 8.73±0.56*            | 0.43±0.05*            | 0.83±0.09*        | 4.11±0.08*        |
| III   | Cystone   | 750          | 3.94±0.27†            | 3.24±0.15‡            | 3.14±0.04‡        | 7.87±0.13†        |
| IV    | AlcE      | 100          | 7.65±0.18 (NS)        | 0.95±0.10 (NS)        | 1.06±0.09 (NS)    | 4.84±0.14†        |
| V     | AlcE      | 200          | 6.74±0.17†            | 1.68±0.12‡            | 2.06±0.12‡        | 5.52±0.08‡        |
| VI    | AlcE      | 400          | 4.89±0.16†            | 2.86±0.04§            | 2.93±0.08§        | 6.32±0.20†        |
| VII   | HAlcE     | 100          | 7.03±0.13†            | 0.98±0.06†            | 1.26±0.14 (NS)    | 5.03±0.12†        |
| VIII  | HAlcE     | 200          | 5.07±0.31†            | 2.02±0.12†            | 2.13±0.13†        | 6.12±0.16†        |
| IX    | HAlcE     | 400          | 4.39±0.18†            | 3.16±0.16             | 3.02±0.90†        | 7.04±0.06§        |

Values are expressed in mean±SEM (n=6); Significant values are *$P<0.001$ compared to normal control (Group I) and †$P<0.05$, ‡$P<0.01$, §$P<0.001$ compared to lithiatic control (Group II). NS: Not significant, AlcE: Alcoholic extract, HAlcE: Hydro-alcoholic extract, SEM: Standard error of mean, EG: Ethylene glycol, MDA: Malondialdehyde, GSH: Glutathione, CAT: Catalase, SOD: Superoxide dismutase
decreased MDA levels and increased GSH content, CAT and SOD activities when compared to lithiatic control group.

**Effect on histopathology of kidney**

Figure 1 depicts representative photomicrographs of microscopic observations of the kidney. There were no histopathological changes in renal tubules, glomeruli and blood vessels in normal group of rats [Figure 1a]. The EG induced lithiatic group showed the presence of CaOx crystals in lumen of tubules accompanied by inflammation and cast formation which causes dilation of tubules and blood vessels. The lithiatic group also revealed the presence of crystals in interstitial spaces with moderate to marked glomerular congestion and tubular degeneration [Figure 1b]. Very few or no crystal deposition and changes in the architecture of kidneys were found in cystone [Figure 1c], AlcE (400 mg/kg) [Figure 1d] and HAicE (400 mg/kg) [Figure 1e] treated groups when compared to lithiatic control group.

**Discussion**

*Bryophyllum pinnatum* is commonly known as *Pattharcaṭṭa* in Indian traditional systems of medicine which implies its stone breaking property. The leaves of the plant are widely used by tribal and other populations for treatment of stones. Despite its wide traditional use, the scientific studies are limited to delineate its antilithiatic effect. The present study was undertaken to substantiate the ethnomedicinal use of leaves of *Bryophyllum pinnatum* in kidney stones and urinary insufficiency in traditional practices. The present investigation showed ameliorative effect of alcoholic and hydro-alcoholic extracts of *Bryophyllum pinnatum* by preventing EG-induced alterations in body weight, urine and serum biochemical parameters, oxidative stress and histology of kidney.

The decreased body weight in lithiatic control group indicates EG toxicity which caused oxalate deposition in intracellular spaces resulting in metabolic disturbances and cellular injury.[50,51] Treatment with AlcE and HAicE prevented the loss of weight caused by EG.

The decreased urinary output in lithiatic control rats was due to the formation of CaOx crystals and its retention. Formation of CaOx crystals causes reduction in glomerular filtration rate, which further decreases excretion of Na+, Cl and Ca²⁺, and promotes stone formation.[52] Treatment with AlcE and HAicE caused increased urination and prevented the supersaturation of stone forming salts in urinary system. The increased urinary uric acid, phosphorus, calcium and oxalate levels were observed in lithiatic rats. The urine oxalate levels are relatively more important than those of calcium since it is accepted that hyperoxaluria is a higher risk factor in the formation of renal calculi than hypercalciuria.[53] Further, increased uric acid level in urine interferes with calcium oxalate solubility, and it binds and reduces the stone inhibitory activity of endogenous stone inhibitor, glycosaminoglycans.[54] AlcE and HAicE lowered the levels of these stone forming substances in urine. Previously aqueous extract of the plant showed inhibitory influence on urinary oxalate and supports the findings of the present study.[54] The decrease in creatinine clearance and urinary magnesium levels indicate their accumulation in blood which further increases the risk of urolithiasis. Treatment with AlcE and HAicE elevated the creatinine clearance and urinary magnesium level and reduced the tendency to crystallize.

EG increases the activity of glycolic acid oxidase resulting in the formation of glycolate and oxalate that increase free radicals, lipid peroxidation and nitrogenous substances (creatinine, urea and uric acid) which ultimately leads to acute tubular necrosis in the kidneys of rats.[50,55] The serum creatinine, urea and uric acid levels were elevated in lithiatic group indicating marked renal damages. The decreased levels of these nitrogenous substances due to extracts or cystone treatment suggest the prevention of renal damage. The effect of extract is in agreement with an earlier study[54] where the aqueous extract of *Bryophyllum pinnatum* exhibited inhibitory effect on serum creatinine and urea. Treatment with AlcE and HAicE restored calcium, phosphorus, oxalate and magnesium levels in a dose dependent manner, thus preventing and reducing the risk of lithiasis. This effect was more pronounced in HAicE compared with AlcE, possibly due to presence of saponins and other constituents.[56]

It was reported that EG administration contributes to increased production of reactive oxygen species (ROS) and oxidative stress in kidney.[55] It was observed that administration of EG increased MDA content and decreased activity of the antioxidant enzymes in the kidney of lithiatic control group. Cystone or AlcE and HAicE significantly attenuated the oxidative stress by reducing...
MDA level and restoring the changes in GSH content and CAT and SOD activity. The decreased lipid peroxidation and increased activity of antioxidant enzymes by cystone or extracts indicate their preventive effect against oxidative damage. Thus, extracts or cystone treatment prevented the renal tissue damage caused by EG.

EG treatment induces extensive CaOx crystal deposition and cellular damages in different parts of kidney of rats accompanied by oxidative damages. Microscopic study of kidney sections in lithiatic control rats showed CaOx crystals in tubular and interstitial spaces with glomerular congestion and tubular necrosis. Treatment with cystone or AlcE and HAlcE prevented the deposition of CaOx crystals in different parts of the renal tubules possibly by hastening the dissolution of preformed stone and/or preventing the formation of new crystals. Aqueous extract of the plant showed prevention of CaOx crystal depositions and supports the findings. The large crystals of CaOx might cause the obstruction of renal tubular flow leading to glomerular congestions and tubular degeneration. AlcE and HAlcE prevented the renal damages by inhibiting the accumulation and retention of CaOx crystals in renal tubules. These effects of extracts suggest their stone dissolving property. Previously, Bryophyllum pinnatum extract showed in-vitro anti-crystallisation effect thus supporting the current findings. It is reported that the saponin content of plants contributes to diuretic and stone dissolving ability. Previous studies reported that antioxidant effects of the plants also played important role in antilithic action. In present study, the quantitative studies of Bryophyllum pinnatum extracts show the presence of saponins and antioxidant phytochemicals such as flavonoids and polyphenols which might have contributed to antilithic activity through stone dissolving and antioxidant action. However, further studies are necessary to comment on the role of exact phytochemical constituents and mechanism of action of Bryophyllum pinnatum in prevention of urolithiasis.

Conclusion

Bryophyllum pinnatum leaf extracts exhibited preventive effect on ethylene glycol-induced formation of renal calculi. The antilithic effect of extracts may be through dissolution of preformed stones and/or prevention of the formation of CaOx crystals and antioxidant activity. This study validates the ethnomedical use of Bryophyllum pinnatum leaves in treatment of urinary stones.

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Conflicts of interest

There are no conflicts of interest.

References

1. Moe OW. Kidney stones: Pathophysiology and medical management. Lancet 2006;367:333-44.
2. Tselius HG. Epidemiology and medical management of stone disease. BJU Int 2003;91:758-67.
3. Fleisch H. Inhibitors and promoters of stone formation. Kidney Int 1978;13:361-71.
4. Jethi RK. Urolithiasis in man. Probe 1982;21:277-80.
5. Prien EL, Prien EL Jr. Composition and structure of urinary stone. Am J Med 1968;45:654-72.
6. Alessandra CP, Elvino JG. Dietary calcium intake among patients with urinary calculi. Nutr Res 2003;23:1651-60.
7. Obligado SH, Goldfarb DS. The association of nephrolithiasis with hypertension and obesity: A review. Am J Hypertens 2008;21:257-64.
8. Tombolini P, Ruoppolo M, Bellorofonte C, Zaatar C, Follini M. Lithotripsy in the treatment of urinary lithiasis. J Nephrol 2000;13 Suppl 3:S71-82.
9. Kishimoto T, Yamamoto K, Sugimoto T, Yoshihara H, Maekawa M. Side effects of extracorporeal shock-wave exposure in patients treated by extracorporeal shock-wave lithotripsy for upper urinary tract stone. Eur Urol 1986;12:308-13.
10. Mishra LC. Scientific Basis for Ayurvedic Therapies. Boca Raton, New York: CRC Press; 2004. p. 535-50.
11. Mitra SK, Gopumadhavan S, Venkataramananna MV, Sundaram R. Effect of cystone: A herbal formulation, on glycolic acid-induced urolithiasis in rats. Phytother Res 1998;12:372-4.
12. Kamboj A, Saluja AK. Bryophyllum pinnatum (Lam.) Kurz.: Phytochemical and pharmacological profile: A review. Pharmacogn Rev 2009;3:364-74.
13. Khare CP. Indian Medicinal Plants: An Illustrated Dictionary. 1st ed. Berlin: Springer Science + Business Media, LLC; 2007. p. 22.
14. Prachi, Chauhan N, Kumar D, Kasana MS. Medicinal plants of Muzzaffarnagar district used in treatment of urinary tract and kidney stones. Indian J Tradit Knowl 2009;8:191-5.
15. Mondal T, Samanta S. An ethnobotanical survey on medicinal plants of Ghatal block, West Midnapur district, West Bengal, India. Int J Curr Res Biosci Plant Biol 2014;1:35-7.
16. Mistry J. Traditional medicinal plants used by local people of Murshidabad district, West Bengal, India. World J Pharm Pharm Sci 2015;4:1225-35.
17. Zahid IH, Bawazir AS, Naser R. Plant based native therapy for the treatment of kidney stones in Aurangabad (M.S.). J Pharmacogn Phytochem 2013;1:189-93.
18. Sikdar M, Dutta U. Traditional phytotherapy among the Nath people of Assam. Ethno-Med 2008;2:39-45.
19. Moromi T. Ethnomedicinal plants used by the Sonowal Kacharis of Bhekulajian village in Dibrugarh district, Assam, NE India. Int J Environ Sci 2014;3:54-7.
20. Lans CA. Ethnomedicines used in Trinidad and Tobago for urinary problems and diabetes mellitus. J Ethnobiol Ethnomed 2006;2:45.
21. Ahmad M, Khan MR, Manzoor M, Zafar M, Sultana S. Check list of medicinal flora of tehsil Isakhel, district Mianwali-Pakistan. Ethnobot Leaflets 2006;10:41-8.
Yadav, et al.: Antilithiatic activity of Bryophyllum pinnatum

22. Barik LD, Rathia KK, Das M, Hazra J. HPTLC method for quantitative determination of quercetin in a polyherbal compound for urolithiasis. Int J Pharmcogn Phytochem Res 2016;8:1187-90.

23. Jain S, Argal A. Effect of a polyherbal formulation on glycolic acid-induced urolithiasis in rats. Bull Pharm Res 2013;3:40-3.

24. Yemitan OK, Salahdeen HM. Neurosedative and muscle relaxant activities of aqueous extract of Bryophyllum pinnatum. Fitoterapia 2005;76:187-93.

25. Okwu DE, Nnamdi FU. Two novel flavonoids from Bryophyllum pinnatum and their antimicrobial activity. J Chem Pharm Res 2011;3:1-10.

26. Pal S, Nag Chaudhuri AK. Studies on the anti-ulcer activity of a Bryophyllum pinnatum leaf extract in experimental animals. J Ethnopharmacol 1991;33:97-102.

27. Gwegenberger B, Rist L, Huch R, von Mandach U. Effect of Bryophyllum pinnatum versus fenoterol on uterine contractility. Eur J Obstet Gynecol Reprod Biol 2004;113:164-71.

28. Ojewole JA. Antinociceptive, anti-inflammatory and antidiabetic effects of Bryophyllum pinnatum (Crassulaceae) leaf aqueous extract. J Ethnopharmacol 2005;99:13-9.

29. Ojewole JA. Antihypertension properties of Bryophyllum pinnatum (Lami) (oken) leaf extracts. Am J Hypertens 2002;15:34-9.

30. Harlalka GV, Patil CR, Patil MR. Protective effect of Kalanchoe pinnata pers. on gentamycin induced nephrotoxicity in rats. Indian J Pharm 2007;39:201-5.

31. Gaind KN, Gupta RL. Alkanes, alkanols, triterpenes, and sterols of Kalanchoe pinnata. Phytochemistry 1972;11:1500-2.

32. Siddiqui S, Faizi S, Siddiqui BS, Sultana N. Triterpenoids and phenanthrenes from leaves of Bryophyllum pinnatum. Phytochemistry 1989;28:2433-8.

33. Yamagishi T, Haruna M, Yan XZ, Chang JJ, Lee KH. Antitumor agents, 110. Bryophyllin B, a novel potent cytotoxic bufadienolide from Bryophyllum pinnatum. J Nat Prod 1989;52:1071-9.

34. Devbhuhi D, Gupta JK, Devbhuhi P, Bose A. Phytochemical and acute toxicity study on Bryophyllum calycinum Salish. Acta Pol Pharm 2008;65:501-4.

35. Yasar F, Waqar MA. Effect of indigenous plant extracts on calcium oxalate crystallization having a role in urolithiasis. Urol Res 2011;39:345-50.

36. Khandelwal KR. Practical Pharmacognosy. 15th ed. Pune: Nirali Prakashan; 2006. p. 149-56.

37. Singleton VL, Orthofer R, Lamuela-Ravento’s RM. Analysis of total phenols and other oxidation substrates and antioxidants by means of Folin-Ciocalteu reagent. Methods Enzymol 1999;299:152-78.

38. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal 2002;10:178-82.

39. Obadoni BO, Ochuko PO. Phytochemical studies and comparative efficacy of the crude extracts of some homostatic plants in Edo and Delta states of Nigeria. Global J Pure Appl Sci 2001;8:203-8.

40. Organization for Economic Co-operation and Development (OECD), Guideline for the Testing of Chemicals. Revised Draft Guideline 423, Document on Acute Oral Toxicity and Acute Toxicity Class Method; 2001. Available from: http://www.oecd.org. [Last cited on 2013 Jun 21].

41. Dey YN, Mahor S, Kumar D, Wanjari M, Gaidhansi S, Jadhav A. Gastrokinetic activity of Amorphophallus paeoniifolius tuber in rats. J Intercult Ethnopharmacol 2015;5:36-42.

42. Atmani F, Slimani Y, Mimouni M, Aziz M, Hacht B, Ziyyat A. Effect of aqueous extract from Herniaria hirsuta L. on experimentally nephrolithiasis rats. J Ethnopharmacol 2004;95:87-93.

43. Hodgkinson A, Williams A. An improved colorimetric procedure for urine oxalate. Clin Chim Acta 1972;36:127-32.

44. Bashir S, Gilani AH. Antiurolithic effect of Bergenia ligulata rhizome: An explanation of the underlying mechanisms. J Ethnopharmacol 2009;122:106-16.

45. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351-8.

46. Ellman GL. Tissue sulfhydryl groups. Arch Biochem Biophys 1959;82:70-7.

47. Takahara S, Hamilton HB, Neel JV, Kobar Y, Ogu Y, Nishimura ET. Hypocatalasemia: A new genetic carrier state. J Clin Invest 1960;39:610-9.

48. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 1972;247:3170-5.

49. Gornall AG, Bardawill CJ, David MM. Determination of serum proteins by means of the biuret reaction. J Biol Chem 1949;177:751-66.

50. Brent J. Current management of ethylene glycol poisoning. Drugs 2001;61:979-88.

51. Bouanani S, Henchiri C, Migiana-Griffoni E, Aouf N, Lecouvey M. Pharmacological and toxicological effects of Paronychia argentea in experimental calcium oxalate nephrolithiasis in rats. J Ethnopharmacol 2010;129:38-45.

52. Karadi RV, Gadge NB, Alagawadi KR, Savadi RV. Effect of Moringa oleifera Lam. root-wood on ethylene glycol induced urolithiasis in rats. J Ethnopharmacol 2006;105:306-11.

53. Agarwal MM, Singh SK, Mavuduru R, Mandal AK. Preventive fluid and dietary therapy for urolithiasis: An appraisal of strength, controversies and lacunae of current literature. Indian J Urol 2011;27:310-9.

54. Shukla AB, Mandavia DR, Barvaliya MJ, Baxi SN, Tripathi CR. Evaluation of anti-urolithiatic effect of aqueous extract of Bryophyllum pinnatum (Lam.) leaves using ethylene glycol-induced renal calculi. Avicenna J Phytomed 2014;4:151-9.

55. Sumathi R, Jayanthi S, Kalpanadevi V, Varalakshmi P. Effect of DL alpha-lipoic acid on tissue lipid peroxidation and antioxidant systems in normal and glycolate treated rats. Pharmacol Res 1993;27:309-18.

56. Patel PK, Patel MA, Vyas BA, Shah DR, Gandhi TR. Antilithiatic activity of saponin rich fraction from the fruits of Solanum xanthocarpum Schrad. & Wendl. (Solanaceae) against ethylene glycol induced urolithiasis in rats. J Ethnopharmacol 2012;144:160-70.