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Original Article

Anti-urolithiatic activity of *Bryophyllum pinnatum* Lam. hydroalcoholic extract in sodium oxalate-induced urolithiasis in rats



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ABSTRACT

Background and Aim: Despite advances in modern medicine, the development and growth of calculi continues to be a source of concern for mankind, as there is no effective treatment for kidney stones. In the present study we investigated antiurolithiatic activity of *Bryophyllum pinnatum* Lam against sodium oxalate (NaOx) induced urolithiasis in rats.

Experimental procedure: In rats with renal calculi caused by sodium oxalate (NaOx, 70 mg/kg, i.p.); the antiurolithiatic action of *Bryophyllum pinnatum* hydroalcoholic extract (BPHE) was studied. BPHE was given every day orally at doses of 50, 200 mg/kg for 14 days to rats to examine activity against sodium oxalate (NaOx) mediated urolithiasis, with Cystone (500 mg/kg, p.o.) as a reference standard. The effect of the extract on urine oxalate, creatinine and phosphate retention and excretion in the kidney, as well as serum and biochemical analysis of kidney homogenate and histopathological examinations were studied. **Results and conclusion:** Oral administration of BPHE at doses of 50, 100, and 200 mg/kg to rats with sodium oxalate-mediated renal calculi showed dose-dependent substantial ($P < 0.05$) antiurolithiatic potential, with notable reversal of NaOx-induced ion excretion and urinary CaOx concentration. These findings justify the traditional use of *Bryophyllum pinnatum* hydroalcoholic extract (BPHE) in the treatment of renal calculi.

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1. Introduction

Kidney stone disease is a multifactorial condition caused by the interaction of epidemiological, biochemical, and genetic risk factors. It affects both men and women, but the risk is higher in men, and it is becoming more prevalent among young women.¹ According to studies, calcium oxalate and calcium phosphate make up 80% of renal stones, while struvite, which contains magnesium

ammonium phosphate, makes up 10%. Uric acid 9% and cystine or ammonium acid urate are the causes of drug-related renal stones 1%.^{2,3}

The formation of stone is thought to be caused by an abnormal increase in urinary levels of calcium, oxalate, and uric acid, which reduces urinary citrate levels.⁴ Citrate and magnesium are the main inhibitors of stone formation in the urinary tract, and decreased levels or a lack of these inhibitors in the urine causes stone formation.⁵

Urolithiasis affects both men and women between the ages of 20 and 49 years.⁶ Surgical procedures, lithotripsy, and local calculus destruction with a high-powered laser are all common methods for removing the calculi. However, these treatments are costly, and recurrence is common.⁷ Without preventive care, the recurrence rate is about 10% after one year, 33% after five years, and 50% after

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ten years.⁸ Various drugs, such as thiazide diuretics and alkali-citrate, are used to avoid recurrence, but clinical evidence for their effectiveness is lacking.⁹

Except for some composite herbal drugs and plants, most remedies in traditional systems of medicine, including Ayurveda, were derived from plants and were found to be effective, even though the rationale for their use was not well established through systematic pharmacological and clinical studies. These plant products are said to reduce the recurrence rate of renal calculi while having no side effects.

Bryophyllum pinnatum (Lam.) (*Crassulaceae*) *Kalanchoe pinnata* (Lam.) Pers., *Kalancho* in tropical America, China, India, Australia, and tropical Africa, it is commonly known as life leaf and air plant and is widely cultivated as a garden ornamental. *Bryophyllum pinnatum* leaves are commonly used in traditional and ethnomedical practice to treat urinary insufficiency and stone disorders.

Various tribes of Muzaffarnagar (Uttar Pradesh), Midnapur and Murshidabad districts of West Bengal, Aurangabad (Maharashtra), Nath people and Sonowal kacharis of Bhekulajan village in Dibrugarh district of Assam use fresh leaf juice or powder of 2–3 black peppers (*Piper nigrum* Linn.) as folklore medicine for kidney and gall bladder stones.^{10–15} Tribes in Trinidad and Tobago, Nigeria, and Pakistan's Mianwali district use 10–15 leaves in ethnomedical activities to treat urinary calculi.^{16,17} Furthermore, the leaves of this plant were an essential component of poly-herbal formulations used for antilithiatic purposes.^{18,19}

Leaves have antimicrobial, antiulcer, uterine contractility, antinociceptive, anti-inflammatory, and antidiabetic properties, as well as antihypertensive and nephroprotective properties.^{20–26} The existence of alkanes, alkanols, triterpenes and sterols, triterpenoids and phenanthrenes, flavonoids, bufadienolides, alkaloids, glycosides, and lipids is attributed to the medicinal and pharmacological properties of *Bryophyllum pinnatum*.^{27–29}

In-vitro inhibitory activity of *Bryophyllum pinnatum* leaves on calcium oxalate crystallisation is well documented.^{30,31} It was thought worthwhile to investigate the effect of *Bryophyllum pinnatum* leaves on an experimentally induced in-vivo lithiasis model because of the traditional and ethno medicinal uses of the leaves for the treatment of kidney and bladder stones, as well as urinary insufficiency. As a result, we tested the anti-urolithiatic activity of *Bryophyllum pinnatum* Lam. against sodium oxalate (NaOx)-induced urolithiasis in rats in the current study.

2. Materials and methods

2.1. Plant material and preparation of extract

Fresh leaves of *Bryophyllum pinnatum* Lam. (family: Crassulaceae), were collected from Mahatma Phule Krishi Vidyapeeth's medicinal and aromatic herb garden in Rahuri, Maharashtra. It was then authenticated by Dr. J. Jayanti, Botanist, Botanical Survey of India (BSI), Pune (BSI/WRC/Tech./2013/462). The dried leaves were coarsely powdered (passed through sieve no. 40) and immersed in a stopper cup for maceration with 70% v/v ethanol in water for 3 days. This extract of plant was then evaporated at 45 °C and then dried in oven. The dried extract was stored in airtight container.

2.2. Chemicals and apparatus

Sodium oxalate (Fine Chemicals, Mumbai), Cystone (Himalaya Herbal Healthcare, Bangalore, India) were used for the study. All other chemicals and reagents used were of analytical grade and procured from approved suppliers. Apparatus such as the metabolic cage (Dolphin, Mumbai), the biochemistry analyzer (Benspera Clinical Chemistry Analyzer C-61), UV-spectrophotometer (Jasco

model V- 630) and centrifuge (Remi) were used in the study.

2.3. Animals

Adult male Wistar rats (*Rattus norvegicus*) weighing 150–200 g were acquired from MES's College of Pharmacy, Sonai's animal house. Rats were housed in polypropylene cages lined with husk in standard environmental conditions (temperature 25±2 °C; relative humidity 55 ± 10%; and 12:12 light: dark cycle). The rats were fed on a standard pellet diet (Amrut rat and mice feed, Sangli, India) *ad libitum* and had free access to water. The animals were acclimatised to the laboratory conditions for at least one week prior to the experiment. The experimental protocol was approved by Institutional Animal Ethical Committee (MES/COP/IAEC/02/2013-14) of MES's College of Pharmacy, Sonai and studies were carried out accordance with current CPCSEA regulations.

2.4. NaOx -induced urolithiasis in rats

Sodium oxalate-induced urolithiasis model was used to evaluate antiurolithiatic effect in rats.³² The Wistar albino rats were divided into six groups, each consisting of six animals (n = 6): Group I served as Vehicle Control, received 0.5% (w/v) gum acacia solution (5 ml/kg p.o.), Group II served as lithiatic control, received Sodium oxalate 70 mg/kg, i.p., Group III served as positive control, received standard drug Cystone (500 mg/kg, p.o.) + Sodium oxalate 70 mg/kg, i.p., and Groups IV, V and VI received Sodium oxalate 70 mg/kg, i.p., + BPHE 50, 100 and 200 mg/kg, p.o. respectively. The above treatment repeated up to 14 days.³²

2.5. Collection and analysis of urine sample

Animals were kept in separate metabolic cages on the 14th day, and urine samples were taken after 24 h. The amount and pH of the urine is determined. After collection, urine samples were preserved by adding 2 drops thymol as a preservative before being tested for oxalate, calcium, uric acid, magnesium and phosphate content in a clinical semi autoanalyzer using a diagnostic kit (Span Diagnostics Pvt. Ltd., India). Various urine parameters such as calcium, oxalate, magnesium, phosphate and uric acid, were tested according to the instructions included with the various kits.^{33–39}

2.6. Serum analysis

Blood samples were withdrawn from the retro-orbital plexus using a capillary procedure under mild ether anaesthesia. Blood samples were centrifuged at 10,000 g for 10 min and serum was separated for sodium, potassium, uric acid and creatinine analysis using diagnostic kits (Span Diagnostics Pvt. Ltd., India) according to the instructions included with the kits.

2.7. Kidney homogenate and histopathological study

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 16,000×g in a cooling centrifuge at 0 °C. The supernatant obtained was further employed for estimation of malondialdehyde (MDA),⁴⁰ glutathione (GSH),⁴¹ catalase (CAT)⁴² and superoxide dismutase (SOD).⁴³ Protein content of the supernatant was estimated by Biuret method.⁴⁴ 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 µm 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.

2.8. Statistical analysis

The results are expressed as mean ± SEM. The statistical significance was assessed using One-way Analysis of Variance (ANOVA) followed by Dunnett's multiple comparison test and value of p < 0.05 was considered statistically significant.

3. Results

3.1. Urine analysis

There was a significant (p < 0.01) decrease in urine volume with no any significant changes in pH in NaOx treated control rats in comparison to normal rats. Cystone treated rats showed a significant (p < 0.05) increase in urine volume when compared to lithiatic control group. BPHE treated rats at doses of 50, 100 and 200 mg/kg body weight caused a significant (p < 0.05 to 0.01) increase in urine volume without any significant change in pH when compared to NaOx treated control rats [Table 1].

In the present experiment, administration of Sodium oxalate 70 mg/kg, i.p as a renal stone inducer caused hyperoxaluria, which was identified by an increase in urinary excretion of oxalate, uric acid, creatinine, phosphorous and decrease in magnesium. However, repeated administration of Cystone 500 mg/kg and BPHE at doses of 50, 100 and 200 mg/kg body weight for 14 days effectively avoided these increase in urinary oxalate, uric acid, creatinine, phosphorous and decrease in magnesium in a dose-dependent manner when compared with NaOx-treated control group and recovered it to normal levels [Table 2].

3.2. Serum analysis

The induction of renal stone by NaOx treatment resulted in impaired renal function as evidenced by elevated serum sodium, potassium, creatinine and uric acid, which are markers of glomerular and tubular injury. However, repeated administration of Cystone 500 mg/kg and BPHE at doses of 50, 100 and 200 mg/kg body weight for 14 days effectively avoided these changes when compared to the NaOx-treated control group [Table 3].

3.3. Kidney homogenate analysis

Sodium oxalate administration significantly (p < 0.01) increased the MDA level and decreased GSH content and activities of CAT and SOD in NaOx treated lithiatic control rats when compared to

normal control rats. The treatment with Cystone 500 mg/kg significantly (p < 0.01) reduced the levels of MDA and increased GSH content, CAT and SOD activities when compared to lithiatic control rats. However, repetitive administration of BPHE at doses of 50, 100 and 200 mg/kg body weight for 14 days significantly (p < 0.01) reduced MDA and increased GSH content, CAT and SOD activities in a dose dependent manner when compared to NaOx lithiatic control rats.

In the NaOx-treated urolithiatic control rats, calcium deposition in the renal tissue was also increased. In the prophylactic types, however, BPHE at doses of 50, 100, and 200 mg/kg body weight for 14 days slightly (p < 0.01) decreased the increase in calcium in renal tissue [Table 4].

3.4. Kidney histopathology

The induction of renal stone by NaOx treatment caused marked histological changes such as tubular dilatation and initial cystic changes, tubular atrophy, calcium oxalate crystal deposits and interstitial mononuclear cell infiltration in the kidneys. However, repeated administration of Cystone 500 mg/kg and BPHE at doses of 50, 100 and 200 mg/kg body weight for 14 days substantially reduced the amount of calcium oxalate deposits and other anomalies in the renal tubules in a dose-dependent manner [Fig. 1].

4. Discussion

Epidemiological studies have shown that the majority of stones are commonly composed of calcium oxalate (CaOx). A number of animal models using rats have been used to induce calcium oxalate urolithiasis. Out of these models, sodium oxalate induced hyperoxaluria rat model causes rapid formation calcium oxalate crystals in renal tubules of experimental animals and hence commonly employed for rapid screening of antiurolithiatic drugs. The intraperitoneal injection of NaOx into rats may cause fast crystal formation in kidneys. The injection dose and interval will affect the size, number and location of crystals. A high dose of NaOx (10 mg/kg) may cause tubular necrosis and dilatation of the rat's kidney.^{45,46}

Bryophyllum pinnatum is generally known as Pattharcaṭṭa in Indian traditional systems of medicine which suggests its stone dissolving power. The leaves of the plant are often used by tribal and other communities for treatment of stones. Despite its widespread traditional usage, scientific research on its antilithiatic efficacy is limited. The purpose of this study was to verify the ethno medicinal usage of *Bryophyllum pinnatum* leaves in the treatment of kidney stones and urine insufficiency in traditional approaches. The current study found that hydro-alcoholic extracts of *Bryophyllum pinnatum* had a beneficial impact in reducing NaOx-induced changes in urine and serum biochemical parameters, oxidative stress and histology of kidney.

Since conventional drugs are usually taken orally, the anti-

Table 1
Effect of BPHE against sodium oxalate induced urolithiasis in rats - urine parameters.

Sr. no	Groups	Treatment	Urine output ml/24hrs	Urine pH
1	Normal	0.5% (w/v) gum acacia solution (5 ml/kg p.o.)	12.50 ± 0.42	6.50 ± 0.03
2	Control	Sodium oxalate 70 mg/kg, i.p.	10.33 ± 0.55	7.75 ± 0.042
3	Standard	500 mg/kg, p.o. Cystone suspended in gum acacia 0.5% + Sodium oxalate 70 mg/kg	11.66 ± 0.42*	6.74 ± 0.043 ^{ns}
4	BPHE	50 mg/kg, b.wt.p.o suspended in gum acacia 0.5% + Sodium oxalate 70 mg/kg	13.50 ± 0.76*	7.15 ± 0.042 ^{ns}
5	BPHE	100 mg/kg, b.wt.p.o suspended in gum acacia 0.5% + Sodium oxalate 70 mg/kg	14.66 ± 0.71**	7.04 ± 0.046 ^{ns}
6	BPHE	200 mg/kg, b.wt.p.o suspended in gum acacia 0.5% + Sodium oxalate 70 mg/kg	16.00 ± 0.73**	6.85 ± 0.048 ^{ns}

ns- Nonsignificant, *P < 0.05, **P < 0.01 values are mean ± SEM, n = 6, when compared with control by using one-way ANOVA followed by Dunnett's multiple comparison test. ANOVA: Analysis of variance, SEM: Standard error of mean, BPHE: *Bryophyllum pinnatum* hydroalcoholic extracts.

Table 2
Effect of BPHE against sodium oxalate induced urolithiasis in rats - Urine parameters.

Sr. no	Groups	Treatment	Oxalate (mg/dL)	Uric acid (mg/dL)	Magnesium (mg/dL)	Creatinine (mg/dL)	Phosphorus (mg/dl)
1	Normal	0.5% (w/v) gum acacia solution (5 ml/kg p.o.)	0.73 ± 0.004	0.16 ± 0.006	0.83 ± 0.005	1.59 ± 0.03	0.87 ± 0.01
2	Control	Sodium oxalate 70 mg/kg, i.p.	1.93 ± 0.004	2.32 ± 0.02	0.34 ± 0.006	3.61 ± 0.14	1.43 ± 0.01
3	Standard	500 mg/kg, p.o. Cystone suspended in gum acacia 0.5% + Sodium oxalate 70 mg/kg	0.87 ± 0.005**	1.60 ± 0.02**	0.69 ± 0.005**	1.65 ± 0.04**	0.82 ± 0.02**
4	BPHE	50 mg/kg, b.wt.p.o suspended in gum acacia 0.5% + Sodium oxalate 70 mg/kg	1.31 ± 0.04*	1.93 ± 0.01*	0.45 ± 0.007*	2.60 ± 0.05*	1.02 ± 0.01*
5	BPHE	100 mg/kg, b.wt.p.o suspended in gum acacia 0.5% + Sodium oxalate 70 mg/kg	0.95 ± 0.004**	1.80 ± 0.01*	0.55 ± 0.008*	1.90 ± 0.02*	0.94 ± 0.01*
6	BPHE	200 mg/kg, b.wt.p.o suspended in gum acacia 0.5% + Sodium oxalate 70 mg/kg	0.91 ± 0.006**	1.63 ± 0.01**	0.64 ± 0.008**	1.79 ± 0.02**	0.94 ± 0.01*

*P < 0.05, **P < 0.01 values are mean ± SEM, n = 6, when compared with control by using one way ANOVA followed by Dunnett's multiple comparison test. ANOVA: Analysis of variance, SEM: Standard error of mean, BPHE: *Bryophyllum pinnatum* hydroalcoholic extracts.

Table 3
Effect of BPHE against sodium oxalate induced urolithiasis in rats - serum parameters.

Sr. no	Groups	Treatment	Sodium mEq/l	Potassium mEq/l	Creatinine mg/dl	Uric acid mg/dl
1	Normal	0.5% (w/v) gum acacia solution (5 ml/kg p.o.)	3606.7 ± 25.77	25.08 ± 0.32	0.57 ± 0.01	2.52 ± 0.01
2	Control	Sodium oxalate 70 mg/kg, i.p.	5496.7 ± 26.16	54.00 ± 0.57	1.46 ± 0.01	4.73 ± 0.01
3	Standard	500 mg/kg, p.o. Cystone suspended in gum acacia 0.5% + Sodium oxalate 70 mg/kg	4145 ± 16.88**	30.50 ± 0.42*	0.59 ± 0.01**	2.57 ± 0.01**
4	BPHE	50 mg/kg, b.wt.p.o suspended in gum acacia 0.5% + Sodium oxalate 70 mg/kg	4371.7 ± 23.58**	34.50 ± 0.42*	0.63 ± 0.01**	2.67 ± 0.01**
5	BPHE	100 mg/kg, b.wt.p.o suspended in gum acacia 0.5% + Sodium oxalate 70 mg/kg	4283.3 ± 17.06**	32.00 ± 0.36*	0.65 ± 0.01**	2.64 ± 0.01**
6	BPHE	200 mg/kg, b.wt.p.o suspended in gum acacia 0.5% + Sodium oxalate 70 mg/kg	4201.7 ± 13.01**	30.83 ± 0.30*	0.65 ± 0.01**	2.64 ± 0.01**

*P < 0.05, **P < 0.01 values are mean ± SEM, n = 6, when compared with control by using one-way ANOVA followed by Dunnett's multiple comparison test. ANOVA: Analysis of variance, SEM: Standard error of mean, BPHE: *Bryophyllum pinnatum* hydroalcoholic extracts.

Table 4
Effect of BPHE against sodium oxalate induced urolithiasis in rats – kidney homogenate parameters.

Sr. no	Groups	Treatment	Kidney Calcium (mg/g)	MDA (nmol/mg of protein)	GSH (nmol/mg of protein)	CAT (U/mg protein)	SOD (U/mg protein)
1	Normal	0.5% (w/v) gum acacia solution (5 ml/kg p.o.)	0.12 ± 0.003	3.85 ± 0.07	3.50 ± 0.05	3.30 ± 0.05	9.18 ± 0.18
2	Control	Sodium oxalate 70 mg/kg, i.p.	0.65 ± 0.009	9.00 ± 0.05	0.84 ± 0.02	0.86 ± 0.04	4.15 ± 0.11
3	Standard	500 mg/kg, p.o. Cystone suspended in gum acacia 0.5% + Sodium oxalate 70 mg/kg	0.16 ± 0.008**	4.15 ± 0.07**	3.25 ± 0.04**	3.12 ± 0.02**	7.51 ± 0.06**
4	BPHE	50 mg/kg, b.wt.p.o suspended in gum acacia 0.5% + Sodium oxalate 70 mg/kg	0.33 ± 0.006*	7.06 ± 0.08*	1.05 ± 0.06*	1.24 ± 0.02*	5.05 ± 0.07*
5	BPHE	100 mg/kg, b.wt.p.o suspended in gum acacia 0.5% + Sodium oxalate 70 mg/kg	0.23 ± 0.009**	5.98 ± 0.06*	1.99 ± 0.05*	2.11 ± 0.06*	6.01 ± 0.09*
6	BPHE	200 mg/kg, b.wt.p.o suspended in gum acacia 0.5% + Sodium oxalate 70 mg/kg	0.19 ± 0.006**	4.88 ± 0.10**	3.00 ± 0.05**	2.99 ± 0.04**	7.01 ± 0.06**

*P < 0.05, **P < 0.01 values are mean ± SEM, n = 6, when compared with control by using one way ANOVA followed by Dunnett's multiple comparison test. ANOVA: Analysis of variance, SEM: Standard error of mean, BPHE: *Bryophyllum pinnatum* hydroalcoholic extracts.

urolithiatic action of *Bryophyllum pinnatum* Lam. against sodium oxalate (NaOx) mediated urolithiasis in rats was tested using the same method. Male rats were chosen to induce urolithiasis because their urinary systems are similar to humans and previous experiments have shown that female rats develop kidney stones at a lower rate than male rats.³⁷

Result of preliminary phytochemical analysis of *Bryophyllum pinnatum* Lam. hydroalcoholic extract showed presence of Alkaloids, glycosides, flavonoids, tannins, anthraquinones, fixed oils and fats, saponins, sugars, terpenoids, and steroids.

In the present study, Sodium oxalate -induced hyperoxaluria model was used to assess the antilithiatic activity in wistar rats.⁴⁷ Chronic administration of Sodium oxalate (70 mg/kg, i.p.) solution to male wistar rats resulted in hyperoxaluria. Oxalate, calcium and phosphate excretion were increased in the calculi-induced (Group II) animals. The biochemical mechanism involved in this process is associated with a raise in the urinary concentration of oxalate. Stone formation in Sodium oxalate -injected animals is

caused by hyperoxaluria, which causes increased renal retention and excretion of oxalate.⁴⁸

The formation of CaOx crystals and their retention resulted in a reduction in urine volume in lithiatic control rats. CaOx crystal formation reduces glomerular filtration rate, which further reduces Na⁺, Cl⁻, and Ca⁺² excretion and increases stone formation.^{50–49} Treatment with BPHE increased urination and prevented stone formation salts from becoming supersaturated in the urinary tract. In lithiatic control rats, urine uric acid, phosphorus, calcium, and oxalate levels were all elevated.

Because it is well believed that hypercalciuria is a greater risk factor for the development of renal calculi than hyperoxaluria, urine oxalate levels are more essential than calcium levels.⁵¹ Furthermore, elevated uric acid levels in the urine interfere with calcium oxalate solubility and binds to natural stone inhibitors, glycosaminoglycans, reducing their stone-inhibitory action. The amounts of these stone-forming compounds in urine were reduced by BPHE. Aqueous extract of the plant has previously been shown to

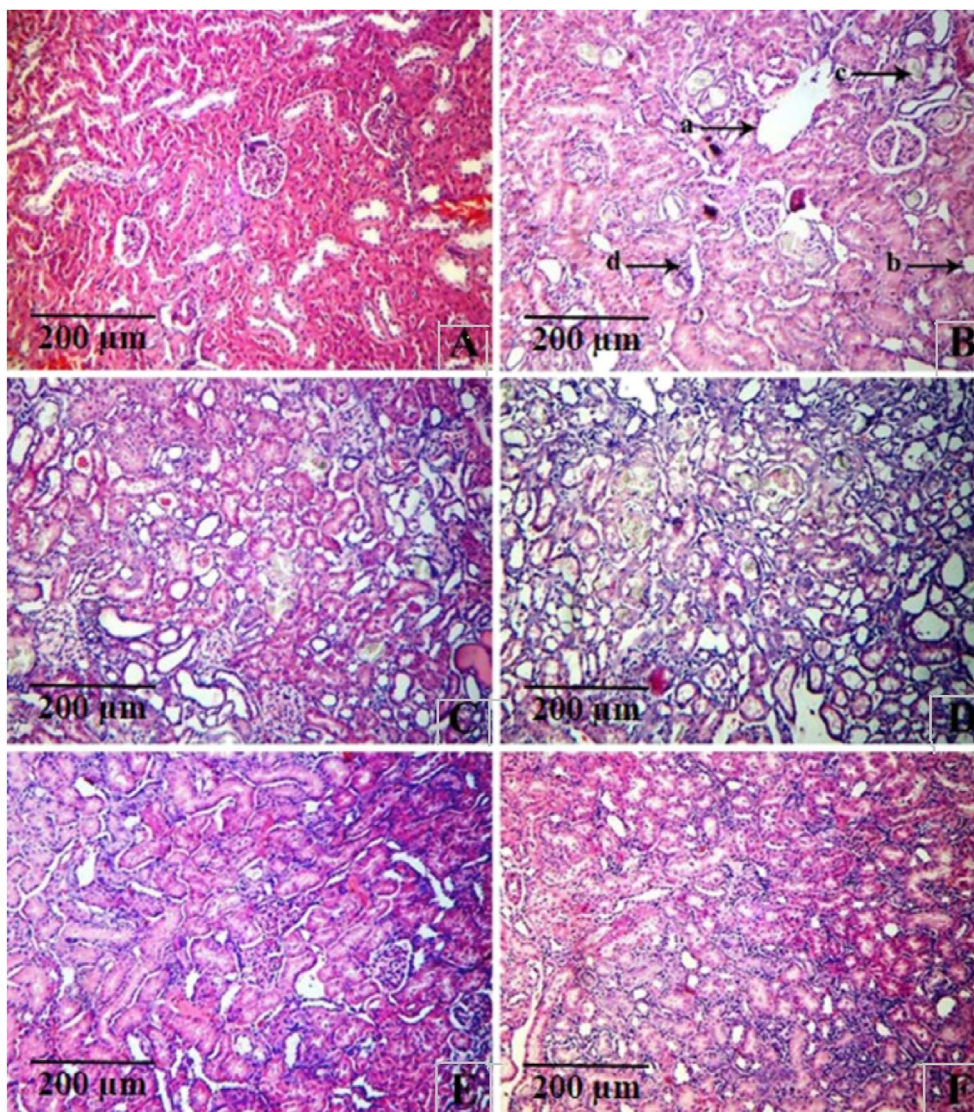


Fig. 1. Histopathology of kidney tissue of (A) vehicle control, (B) Na oxalate induced calculi group, showing severe histological changes including tubular dilatation and initial cystic changes marked as (a), tubular atrophy (b), calcium oxalate crystals deposits (c) and interstitial mononuclear cell infiltration (d); crystal of calcium oxalate shown in the box (C) Cystone, standard drug treated and (D, E, and F) prophylactic treatment with BPHE at the dose 50,100 and 200 mg/kg showing moderate histological changes respectively; Microscopic magnification: 40 × .

have an inhibitory effect on urinary oxalate, which validates the findings of the current investigation.⁵²

Creatinine clearance and urine magnesium levels are decreasing, indicating that they are accumulating in the blood, increasing the risk of urolithiasis. Treatment with BPHE increased creatinine clearance and urine magnesium levels while reducing crystallisation. Glycolic acid oxidase activity is increased by NaOx, resulting in the generation of glycolate and oxalate, which increases free radicals, lipid peroxidation, and nitrogenous compounds (creatinine, urea, and uric acid), finally leading to acute tubular necrosis in rats' kidneys.⁵³ In lithiatic control rats, serum creatinine, urea, and uric acid levels were all increased, indicating severe kidney injury. Reduced levels of these nitrogenous compounds after treatment with *Bryophyllum pinnatum* extract or Cystone indicates that kidney injury can be avoided. The effectiveness of the extract is consistent with a previous research in which an aqueous extract of *Bryophyllum pinnatum* decreased serum creatinine and urea levels. In a dose-dependent way, BPHE treatment restored calcium, phosphorus, oxalate, and magnesium levels, minimizing

and lowering the risk of lithiasis.

NaOx administration has been linked to an increase in the formation of reactive oxygen species (ROS) and oxidative stress in the kidney. In the kidneys of the lithiatic control group, injection of NaOx raised MDA concentration and lowered antioxidant enzyme activity. By lowering MDA levels and correcting alterations in GSH content, CAT, and SOD activity, Cystone or BPHE dramatically reduced oxidative stress. Cystone or extracts reduced lipid peroxidation and enhanced antioxidant enzyme activity, indicating a protective action against oxidative damage. Thus, therapy with *Bryophyllum pinnatum* extract or Cystone protected renal tissue damage.

In diverse areas of the kidney of rats, NaOx therapy caused significant CaOx crystal deposition and cellular damage, as well as oxidative damage. CaOx crystals were seen in tubular and interstitial areas in lithiatic control rats' kidney parts, along with glomerular congestion and tubular necrosis. Treatment with Cystone or BPHE inhibited the production of CaOx crystals in several sections of the renal tubules, probably by speeding up the

breakdown of preformed stone and/or inhibiting the production of new crystals. The findings are supported by the fact that extract of the plant prevented CaOx crystal depositions.

CaOx crystals can obstruct renal tubular flow, resulting in glomerular congestion and tubular degeneration. By preventing the accumulation and retention of CaOx crystals in renal tubules, BPHE was able to protect the kidneys from damage. The fact that extracts have these effects suggests that they can dissolve stones. *Bryophyllum pinnatum* extract had previously been shown to have an anti-crystallisation effect in vitro, which supports the current findings.⁵⁴ Plant saponin is said to help with diuretic and stone dissolving properties.⁵⁵ Previous research has found that the plants' antioxidant properties are important in their antilithiatic properties.⁵⁶

The presence of saponins and antioxidant phytochemicals such as flavonoids and polyphenols were found in quantitative investigations of *Bryophyllum pinnatum* extracts, which may have contributed to antilithiatic activity through stone dissolving and antioxidant action. However, more research is needed to determine the specific phytochemical ingredients and mode of action of *Bryophyllum pinnatum* in urolithiasis prevention.

5. Conclusion

The anti-urolithiatic effect of hydroalcoholic extracts of *Bryophyllum pinnatum* leaves on the development of urinary calculi (urolithiasis) in Na oxalate-induced lithiasis in rats was demonstrated in this research. As a result, BPHE can help to avoid calcium oxalate crystal deposition in the kidney by preventing hyperoxaluria-induced peroxidative damage to the renal tubular membrane surface, which can lead to calcium oxalate crystal attachment and kidney stone formation. These findings also showed that administering *Bryophyllum pinnatum* leaf hydroalcoholic extracts decreased and avoided the formation of urinary stones. As a result of its action on early stages of stone growth, *Bryophyllum pinnatum* leaves hydroalcoholic extracts are useful in preventing disease recurrence.

The mechanism behind this effect may be due to antioxidant nephroprotective properties as well as a reduction in urinary stone-forming constituent concentrations. However, further research is needed to determine the precise mechanism of this behaviour.

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Nil.

Declaration of competing interest

There are no conflicts of interest.

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