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Effect of methanol extract of *Bryophyllum pinnatum* leaves on ethylene glycol-induced urolithiasis in adult male albino rats

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Abstract

Methanol extract of *Bryophyllum pinnatum* was used to evaluate its antilithic property in adult male albino rats. Experimental urolithiasis was induced by oral administration of ethylene glycol and ammonium chloride with drinking water for 7 days. The treatment with methanol extract of *Bryophyllum pinnatum* and cystone by oral administration was continued for 14 days. Fresh urine samples were collected daily for urine crystal counts. On 14th day fresh urine and serum samples were collected for creatinine estimation and rats were sacrificed for kidney histopathology. An increased serum creatinine and decreased urine creatinine level were observed in urolith group, where as the findings did not differ significantly in *Bryophyllum pinnatum* extract and cystone treated groups with the control group. Urine crystal counts were significantly reduced and histopathology of kidney showed apparently normal architecture with very less crystal deposition in kidneys of the *Bryophyllum pinnatum* extract and cystone treated groups.

Keywords: Ammonium chloride, *Bryophyllum pinnatum*, cystone, ethylene glycol, male albino rats, urolithiasis

Introduction

Urolithiasis is worldwide in distribution and affecting 2% of the world population ^[1]. In order to find herbal remedy for this disease, the present study was undertaken to evaluate the antilithic activity of methanolic extract of *Bryophyllum pinnatum*. Synonyms of this plant are *Bryophyllum calycinum*, *Kalanchoe pinnata*, *K. pinnata*; belonging to family Crassulaceae and genus *Bryophyllum* or *Kalanchoe* and are commonly known as acid plant, air plant, coirama, curtain plant, miracle leaf, life leaf etc. in different parts of the world. *Kalanchoe* is also a panacea to the indigenous people of the Amazon ^[2].

In Indian, traditionally this plant occupies a unique position for its important medicinal values in treating abdominal discomfort, boils, cuts, wounds, bruises, sores, indigestion, cholera, scabies, kidney stones, diabetes, urinary insufficiency, headaches, eye infection, insect bites, gastric ulcer, diarrhoea, dysentery, cholera, jaundice, epistaxis and anxiety related insomnia ^[3]. Documents revealed that traditionally *B. pinnatum* is used as antilithic ^[2,4,5], analgesic ^[6], anti-inflammatory ^[7], antitumorous ^[8], antiulcerous ^[9], antihistaminic ^[10], antimicrobial ^[11], insecticidal ^[8], muscle relaxant and sedative ^[12], tocolytic ^[13] and antiprotozoal against Cutaneous Leishmaniasis ^[14].

This plant contains alkaloids, glycosides, steroids and lipids. A group of pharmacologically very active chemicals were isolated from the leaves called bufadienolides ^[2]. Infrared spectroscopy and GLC results of the petroleum ether extract revealed the presence of n-alkenes, triterpenes (α - β amyrans), β -sitosterols and other sterols, p-hydroxy benzoic acid, fumeric acid, p-hydroxy cinnamic acid, syringic acid, quercetin, flavonoids, diarbinoside, kaempferol-3-monoglucoside and n-primary alcohol ^[15]. Thus, the current study aims to study the effect of methanol extract of *Bryophyllum pinnatum* leaves on ethylene glycol-induced urolithiasis in adult male albino rats.

Materials and Methods

Collection of the plant materials and preparation of the methanolic extract

The fresh leaves of *Bryophyllum pinnatum* plant were collected from the medicinal garden of CVSc, AAU, Khanapara for the experiment after subsequent identification and authentication of the plant by Botanical Survey of India, Shillong, Maghalaya, India.

After chopping, air drying and pulverization of Fresh leaves, the crude extract was obtained by soaking 500 g of air dried finely powdered leaves in 1 liter of methanol for 48 hours at room temperature. The extract was filtered through a buckner funnel using whatman no. 1 filter paper and 500 ml of extract was obtained. The methanol extract was concentrated up to 10 ml with the help of a rotary evaporator. The concentrated extract was again dried in a hot water bath at 40 °C. The dried extract was collected, weighed and stored in an air tight vial at 4 °C in the refrigerator.

Experimental animal

Adult male albino rats weighing 150 g to 230 g, kept under standard management with *ad lib.* food and water supply, were divided into 5 groups consisting of 5 animals in each group for this experiment. Urolithiasis was induced in all animals except the control group by oral administration of ethylene glycol and ammonium chloride solution (0.75 ml ethylene glycol & 0.75 g ammonium chloride in 100 ml of drinking water) @ 5 ml 100g⁻¹ body weight daily for 7 days.

Group-I : Urolith induced without any treatment

Group-II: Urolith induced and *B. pinnatum* extract orally @ 300 mgKg⁻¹ for 14 days

Group-III: Urolith induced and *B. pinnatum* extract orally @ 900 mgKg⁻¹ for 14 days

Group-IV: Urolith induced and cystone orally @ 10 mgKg⁻¹ for 14 days

Group-V: Control

Rats were sacrificed at 14th day of the experiment for histopathological studies.

Urine collection and analysis

Fresh urine samples were collected in the morning without any preservatives for crystal analysis. For microscopy, 0.5 ml of fresh urine sample was centrifuged at 3500 r.p.m for 10 minutes, and then 450 µl of the sediment was transferred to a Neubauer chamber. The types of the crystals were identified and counted using a compound microscope at 400X. The average of 4 WBC counting chambers was considered as one field. The per day mean urine crystal counts were calculated for each group including control group.

Urine samples were preserved at -20°C with thymol for further analysis. On 14th day urine samples were analyzed for creatinine level (mg%) by alkaline picrate method (creatinine kit) using UV- spectrophotometer at 520 nm.

Kidney histopathology

The rats were sacrificed on 14th day and the kidneys were removed and fixed in 10% formalin. Tissues from kidney were further processed for histological slide of 5 µm thickness before staining with hematoxylin and eosin [16]. The slides were examined under microscope for crystal deposits.

Collection of serum

Heart blood was collected on 14th day and serum was separated. Fresh serum samples were used for analysis of creatinine by alkaline picrate method (creatinine kit) using UV- spectrophotometer at 520 nm.

Results

Maximum crystals from the urine samples of group-I, II, III and IV were identified as oxalate crystals (Fig. 1). The mean urine crystal counts were found to be significantly lower in group-II, III, IV and V compared to group- I (Table 1). The crystal counts were found to be higher during last three days of experiment in Group I, whereas in other groups the counts were more or less similar throughout the experiment.

Serum creatinine concentration (mg %) was found to be significantly higher in group-I compared to other group (Table 2), whereas no significant differences were observed in between group-II, III, IV and V, indicating oral administration of methanol extract of *B. pinnatum* has similar antilithic effect as cystone.

Urine creatinine level (mg %) were found to be significantly lower in group-I compared to other groups (Table 2), whereas no significant differences were observed in between group-II, III, IV and V, indicating oral administration of methanol extract of *B. pinnatum* has equally good antilithic effect as cystone.

Kidney histopathology showed that there were deposition of crystals of various sizes and shapes in the lumen of the kidney tubules and tubular degeneration in group-I (Fig.2). However, mild to moderate glomerular damage in group-II and III and apparently normal architecture was observed in group-IV. There are very less to no crystal deposition in group-II, III and IV (Fig.3-6), indicating antilithic effect of methanol extract of *B. pinnatum* and cystone in adult male albino rats.

Discussion

The present study revealed that oral administration of ethylene glycol (0.75%) and ammonium chloride (0.75%) in adult male albino rats can produce significant crystalluria and crystal deposition in kidney [17]. Histology of kidney shows significant damage due to deposition of crystals in the kidney which were predominantly calcium oxalate type [18, 19, 20]. Oral administration of methanol extract of *B. pinnatum* significantly reduces crystalluria and kidney crystal depositions which may be due to presence of flavonoids and saponin fractions present in *B. Pinnatum* methanol extract [21, 22]. Kidney histology also supports the antilithic effect of methanol extract of *B. pinnatum* (@300 mgKg⁻¹ or 900 mgKg⁻¹) and cystone (@10 mgKg⁻¹) which may be due to anti-inflammatory and diuretic property of *B. pinnatum* [21, 23]. Significantly higher serum creatinine level (mg %) and significantly lower urine creatinine level (gL⁻¹) were observed in the urolith induced group compared to the treated and control groups. Thus, oral administration of methanol extract of *B. pinnatum* (@300 mgKg⁻¹ or 900 mgKg⁻¹) and cystone (@10 mgKg⁻¹) significantly reduces crystalluria. Previous study also reveals that aqueous & hydroalcoholic extract of the plant have inhibitory effects on urinary oxalate stones [24, 25]. Reduction of crystalluria and kidney crystal deposition by methanol extract of *B. pinnatum* could be corroborated with its antilithic property as mentioned in folklore medicine [2, 4, 5].

Table 1: Number of daily Urine crystal count (Mean±SE)

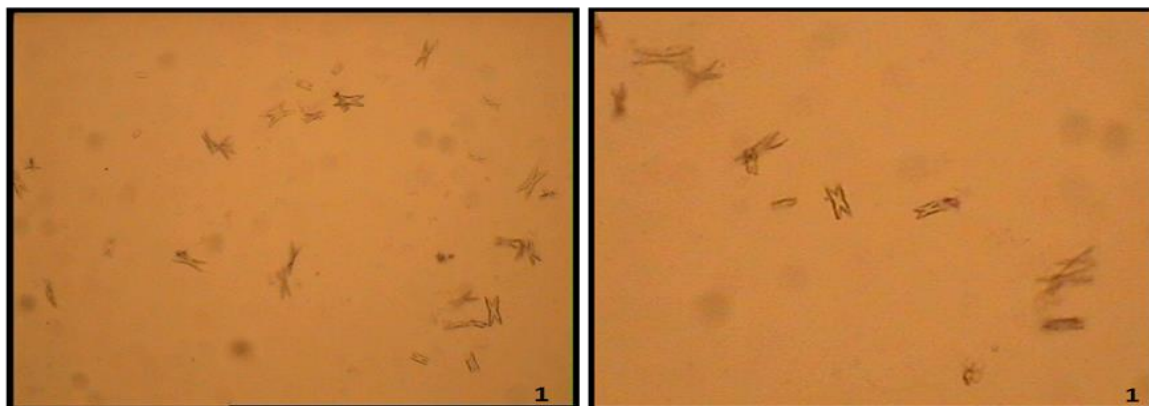
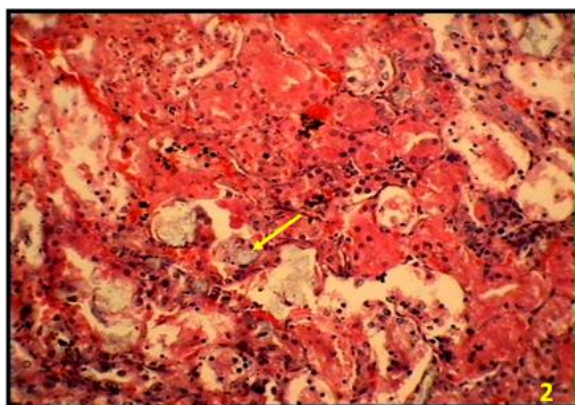
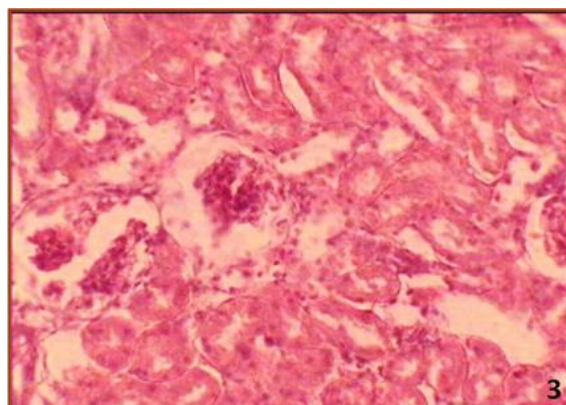
Days	Group-I	Group-II	Group-III	Group- V	Group-V
1	1.000	0.000	1.000	0.000	0.000
2	18.600	2.800	1.600	0.800	0.000
3	18.400	1.600	2.800	1.000	1.000
4	20.600	6.800	4.200	1.400	2.000
5	31.000	1.400	4.200	3.400	1.000
6	30.800	4.600	3.400	2.000	0.000
7	19.800	12.600	2.400	1.200	0.000
8	35.400	4.400	3.600	0.600	1.000
9	28.600	2.200	2.600	0.600	2.000
10	31.200	1.400	2.000	0.600	1.000
11	33.600	1.200	1.000	0.200	0.000
12	48.400	2.400	2.200	0.200	0.000
13	54.800	0.400	1.000	0.200	0.000
14	53.600	1.000	1.000	0.200	2.000
Mean	30.41 ± 3.96 ^b	3.057 ± 0.88 ^a	2.357 ± 0.31 ^a	0.8857 ± 0.24 ^a	0.7143±0.22 ^a

Mean having different superscripts differ significantly ($P<0.05$). N=5.

Table 2: Serum and urine creatinine level (Mean±SE)

Group	Group-I	Group-II	Group-III	Group-IV	Group-V
Serum creatinine level (mg %)	1.273± 0.099 ^b	0.666 ± 0.030 ^a	0.644 ± 0.021 ^a	0.641 ± 0.049 ^a	0.599 ± 0.038 ^a
Urine creatinine level (mg %)	0.3528± 0.048 ^b	1.102 ± 0.069 ^a	1.293 ± 0.119 ^a	1.393 ± 0.086 ^a	1.247± 0.098 ^a

Mean having different superscripts differ significantly ($P<0.05$). N=5.

**Fig 1:** Group-I. Oxalate crystals in urine. (400X)**Fig 2:** Group-I. Kidney showing deposition of crystals (arrow) and tubular degeneration. (HE 400X)**Fig 3:** Group-II. Kidney showing moderate glomerular damage. HE 400X

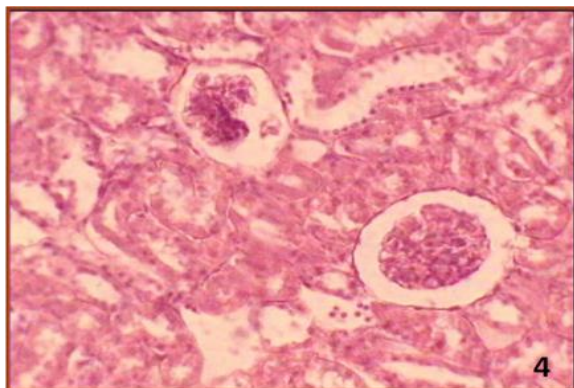


Fig 4: Group-III. Kidney showing mild glomerular damage. HE 400X

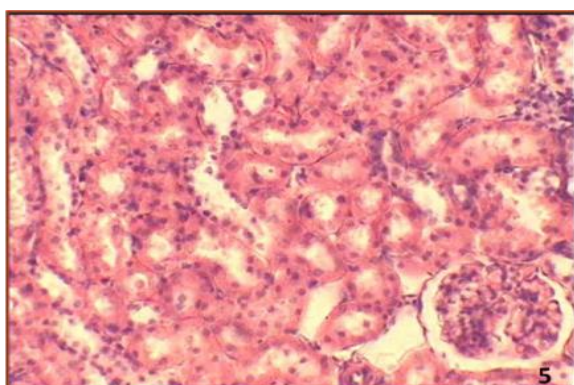


Fig 5: Group-IV. Kidney showing apparently normal architecture. HE 400X

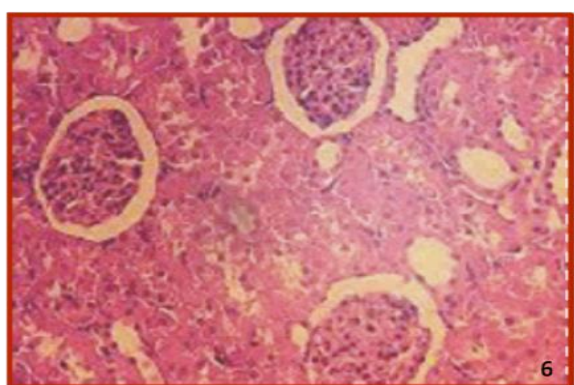


Fig 6: Group-V. Kidney showing no crystal deposition. HE 400X

Conclusion

From the present study it may be concluded that administration of ethylene glycol (0.75%) and ammonium chloride (0.75%) produces significant crystalluria and crystal deposition in rat kidney and oral administration of *Bryophyllum pinnatum* methanolic extract can significantly reduce kidney crystal deposition and crystalluria. The findings of this present study can be corroborated with the therapeutic usefulness of *Bryophyllum pinnatum*, as mentioned in traditional medicine.

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