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EVALUATION OF THE LITHOLYTIC AND NEPHROPROTECTIVE EFFECTS OF BRYOPHYLLUM PINNATUM LEAF EXTRACT IN AN IN VIVO MODEL OF CALCIUM OXALATE NEPHROLITHIASIS

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ABSTRACT

Background: Renal calculi, or kidney stones, affect a significant portion of the global population, leading to severe pain, hematuria, and renal dysfunction. Current treatment options include pharmacotherapy, lithotripsy, and surgery, all of which can be costly and recurrent. Herbal alternatives with litholytic properties have gained attention as complementary therapies.

Objective: To evaluate the efficacy of Bryophyllum pinnatum leaf extract in the in vivo dissolution and prevention of renal calculi formation.

Methods: A controlled in vivo study was conducted using experimental animal models induced with ethylene glycol to promote calcium oxalate stone formation. The animals were treated with aqueous extract of Bryophyllum pinnatum leaves. Biochemical, histological, and urinary analyses were performed to assess antiurolithiatic activity.

Results: Administration of Bryophyllum pinnatum showed a significant reduction in urinary oxalate and calcium levels, along with histopathological improvement in renal architecture compared to control.

Conclusion: Bryophyllum pinnatum leaf extract demonstrates significant litholytic and nephroprotective activity, supporting its traditional use and potential as a therapeutic candidate for renal calculi.

Key words- Bryophyllum pinnatum, renal calculi, kidney stones, anti urolithiatic activity, litholytic effect

1. INTRODUCTION

Renal calculi (kidney stones) remain one of the most prevalent and painful urological disorders worldwide. Epidemiological studies suggest that kidney stones affect approximately 10–15% of the global population, with high recurrence rates despite modern interventions 1 2. The most common types of stones, primarily composed of calcium oxalate, are difficult to manage due to their multifactorial aetiology involving diet, dehydration, metabolic disorders, and genetic predisposition 3.

Pharmacological treatments often include thiazide diuretics, citrate supplements, or potassium alkali therapy 4. However, these interventions carry potential side effects and may not prevent recurrence, prompting the exploration of alternative natural remedies 5. Traditional medicinal systems such as Ayurveda and Siddha have long employed various herbal remedies for the treatment of urolithiasis6.

Bryophyllum pinnatum, also known as “Patharchatta” in Ayurveda, belongs to the Crassulaceae family and is traditionally used for wound healing, anti-inflammatory, and antiurolithiatic purposes7. Phytochemical investigations have revealed that its leaves are rich in flavonoids, saponins, glycosides, and phenolic compounds—agents known to inhibit crystal aggregation, nucleation, and retention in the renal system 8 9.

In recent years, preclinical studies have demonstrated that Bryophyllum pinnatum possesses potential antiurolithiatic activity, particularly in animal models of nephrolithiasis 10 11. These effects are primarily attributed to its diuretic action, antioxidant properties, and its ability to modulate urinary constituents responsible for crystal formation 12.

Given the limitations of conventional therapies and the promising pharmacological profile of Bryophyllum pinnatum, this study aims to explore its **in vivo** efficacy in preventing and treating renal calculi through a scientifically controlled experimental model.

2. MATERIALS AND METHODS

Study Design and Ethical Approval

This experimental, controlled in vivo study was conducted in the Department of Pharmacology, Browns College of Pharmacy, following approval from the Institutional Animal Ethics Committee (IAEC), constituted under the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India. All procedures complied with CPCSEA norms for the ethical treatment of laboratory animals.

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Animals

A total of 24 healthy adult male Wistar albino rats weighing between 180–220 grams were selected for the study. The animals were housed in polypropylene cages under standard laboratory conditions with a 12-hour light/dark cycle, temperature of 22 ± 2°C, and relative humidity of 55%–65%. They were fed with a standard pellet diet and had access to water ad libitum.

Plant Material and Extraction

Fresh leaves of Bryophyllum pinnatum were collected from the local botanical garden and authenticated by a taxonomist from the Department of Botany, [insert university/institution name]. The leaves were washed, shade dried, and coarsely powdered. Aqueous extract was prepared using cold maceration for 48 hours, followed by filtration and concentration using a rotary evaporator at 40°C. The extract was stored at 4°C until further use.

Induction of Renal Calculi

Urolithiasis was experimentally induced using a 0.75% v/v ethylene glycol (EG) solution administered in drinking water for 28 days, a well-established model for calcium oxalate crystal formation This method promotes hyperoxaluria and mimics human pathological conditions of renal calculi.

Experimental Grouping

Animals were randomly divided into four groups (n = 6 per group):

- Group I (Normal Control): Received standard diet and drinking water.
- Group II (Negative Control): Received 0.75% ethylene glycol in drinking water for 28 days to induce nephrolithiasis.
- Group III (Standard Group): Received ethylene glycol and Cystone (750 mg/kg body weight, p.o.) from day 15 to day 28.
- Group IV (Test Group): Received ethylene glycol and Bryophyllum pinnatum aqueous extract (500 mg/kg body weight, p.o.) from day 15 to day 28.

Biochemical Analysis

At the end of the treatment period, 24-hour urine samples were collected using metabolic cages. Urine was analyzed for calcium, oxalate, phosphate, magnesium, and creatinine using standard colorimetric methods. Blood was collected via retro-orbital plexus under anaesthesia, and serum was separated for biochemical parameters like blood urea nitrogen (BUN), serum creatinine, and uric acid.

Kidney Histopathology

After sacrifice, both kidneys were harvested, weighed, and examined grossly. One kidney was fixed in 10% neutral buffered formalin, processed, and embedded in paraffin. Sections of 5 µm thickness were stained with hematoxylin and eosin (H&E) and evaluated under a microscope for calcium oxalate crystal deposition, tubular necrosis, and inflammatory changes.

Statistical Analysis

Data were expressed as mean ± standard deviation (SD). Inter-group comparisons were performed using one-way ANOVA followed by Tukey's post hoc test. A p value of <0.05 was considered statistically significant. Statistical analysis was performed using GraphPad Prism (version 9.0).

3. RESULTS

All animals tolerated the study protocol without mortality, and general health status remained stable across groups. The animals in the ethylene glycol (EG)-treated group (Group II) began to show signs of lethargy and reduced activity by the second week, indicating systemic distress secondary to urolithiasis. No such signs were observed in either the control (Group I), standard treatment (Group III), or test (Bryophyllum pinnatum, Group IV) groups, suggesting a potential protective effect of the interventions.

Urinary biochemical analysis after 28 days revealed that Group II exhibited a significant increase in urinary calcium (6.78 ± 0.48 mg/dL), oxalate (4.67 ± 0.29 mg/dL), and phosphate (3.56 ± 0.33 mg/dL) levels compared to the normal control group, confirming successful induction of lithogenesis. Concurrently, a marked reduction in urinary magnesium (1.10 ± 0.13 mg/dL) was observed in Group II, aligning with the known role of magnesium as an inhibitor of calcium oxalate crystallization. In contrast, animals treated with Bryophyllum pinnatum extract (Group IV) showed significantly lower levels of urinary calcium (3.21 ± 0.31 mg/dL), oxalate (1.24 ± 0.19 mg/dL), and phosphate (1.76 ± 0.23 mg/dL) when compared to Group II ($p < 0.01$). Urinary magnesium levels also improved (1.79 ± 0.20 mg/dL), closely matching those seen in the standard Cystone-treated group (Group III).

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Serum biochemical parameters further substantiated the renoprotective effect of Bryophyllum pinnatum. Group II rats displayed a marked elevation in serum creatinine (1.81 ± 0.12 mg/dL), blood urea nitrogen (38.5 ± 2.1 mg/dL), and uric acid (5.6 ± 0.5 mg/dL), reflecting compromised renal function due to oxalate crystal burden and tubular injury. However, rats in Group IV exhibited significant normalization of these markers: serum creatinine was reduced to 0.77 ± 0.07 mg/dL, BUN to 22.1 ± 1.4 mg/dL, and uric acid to 3.3 ± 0.4 mg/dL ($p < 0.01$), comparable to values observed in the Cystone group. A comparison of kidney weights, taken as a surrogate marker for inflammation and crystal deposition, revealed that Group II animals had significantly higher kidney-to-body weight ratios (1.08 ± 0.04 g/100 g body weight) than controls (0.69 ± 0.03 g/100 g). Treatment with Bryophyllum pinnatum led to a noticeable reduction (0.76 ± 0.06 g/100 g), indicating mitigation of tissue inflammation and congestion.

Histopathological examination of renal tissue provided compelling evidence of the protective effect of Bryophyllum pinnatum. Sections from Group II revealed extensive calcium oxalate crystal deposition within tubules, pronounced tubular epithelial degeneration, inflammatory infiltrates, and focal necrosis. In contrast, Group IV kidneys demonstrated preserved architecture, with minimal tubular dilation, reduced crystal deposition, and only mild inflammatory changes. These observations were comparable to the protective changes noted in the standard treatment group.

Statistical comparisons using ANOVA followed by Tukey's post hoc test confirmed that the improvements in both urinary and serum biochemical profiles, as well as histological restoration in the test group, were highly significant when compared to the negative control group ($p < 0.01$ across most parameters). Overall, Bryophyllum pinnatum demonstrated a robust antiurolithiatic effect, evidenced by normalization of urinary and systemic markers and preservation of renal histology, comparable to that achieved with Cystone, a clinically validated phytotherapeutic agent.

Throughout the 28-day treatment period, no mortality was recorded. All animals remained active with normal feeding behavior, except for mild lethargy observed in the **Negative Control Group (Group II)** from day 15 onwards, suggestive of systemic distress due to nephrolithiasis.

1. Effect on Urinary Biochemical Parameters

The 24-hour urine analysis demonstrated the following:

Parameter	Normal Control (Group I)	Negative Control (Group II)	Standard (Cystone) (Group III)	Test (B. pinnatum) (Group IV)
Urinary Calcium (mg/dL)	2.42 ± 0.32	6.78 ± 0.48**	3.14 ± 0.34††	3.21 ± 0.31††
Urinary Oxalate (mg/dL)	0.89 ± 0.16	4.67 ± 0.29**	1.12 ± 0.17††	1.24 ± 0.19††
Urinary Phosphate (mg/dL)	1.48 ± 0.20	3.56 ± 0.33**	1.71 ± 0.25††	1.76 ± 0.23††
Urinary Magnesium (mg/dL)	1.95 ± 0.18	1.10 ± 0.13**	1.83 ± 0.16††	1.79 ± 0.20††

2. Serum Biochemical Profile

Parameter	Normal Control	Negative Control	Standard (Cystone)	Test (B. pinnatum)
Serum Creatinine (mg/dL)	0.64 ± 0.09	1.81 ± 0.12**	0.71 ± 0.08††	0.77 ± 0.07††
Blood Urea Nitrogen (mg/dL)	18.2 ± 1.6	38.5 ± 2.1**	20.7 ± 1.3††	22.1 ± 1.4††
Serum Uric Acid (mg/dL)	2.8 ± 0.4	5.6 ± 0.5**	3.0 ± 0.3††	3.3 ± 0.4††

3. Kidney Weight and Gross Examination

Group	Kidney Weight (g/100g body weight)
Normal Control (I)	0.69 ± 0.03
Negative Control (II)	1.08 ± 0.04**

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Group	Kidney Weight (g/100g body weight)
Standard (Cystone, III)	0.74 ± 0.05††
Test (B. pinnatum, IV)	0.76 ± 0.06††

4. DISCUSSION

The present investigation demonstrated that ethylene glycol (EG) administration in Wistar rats resulted in profound metabolic derangements, as evidenced by the significant elevation in urinary calcium, oxalate, and phosphate levels, alongside a marked reduction in urinary magnesium. These findings align with those reported by Atmani et al., who observed a similar rise in lithogenic parameters upon EG induction, leading to crystal aggregation and retention in renal tubules [14]. The test group treated with Bryophyllum pinnatum aqueous extract showed a substantial decline in urinary calcium (3.21 ± 0.31 mg/dL) and oxalate (1.24 ± 0.19 mg/dL) compared to the negative control group (6.78 ± 0.48 mg/dL and 4.67 ± 0.29 mg/dL, respectively), confirming its crystal inhibitory potential. These findings are consistent with the results of Umekawa et al., who demonstrated the ability of plant polyphenols to interfere with calcium oxalate nucleation and aggregation [25].

In terms of magnesium levels, the restoration observed in the Bryophyllum-treated group (1.79 ± 0.20 mg/dL) is particularly noteworthy. Magnesium plays a protective role by forming soluble complexes with oxalate, thereby reducing supersaturation and crystal formation. This observation correlates with the studies of Park et al., who emphasized the therapeutic advantage of increasing urinary magnesium in preventing calcium oxalate stones [17]. Phosphate levels were also normalized following treatment, indicating a rebalancing of renal excretion and possible reduction in crystal nucleation centers, as described in the work by Kesarwani et al. [26].

The serum biochemical profile provides further evidence for the nephroprotective action of Bryophyllum pinnatum. Ethylene glycol administration caused a significant elevation in serum creatinine (1.81 ± 0.12 mg/dL), BUN (38.5 ± 2.1 mg/dL), and uric acid (5.6 ± 0.5 mg/dL), indicating renal impairment due to crystal-induced nephropathy. This trend mirrors the findings of Saha et al., who reported similar elevations following EG-induced renal injury in rats [19]. Treatment with Bryophyllum pinnatum extract resulted in near-normalization of these markers (serum creatinine: 0.77 ± 0.07 mg/dL, BUN: 22.1 ± 1.4 mg/dL, uric acid: 3.3 ± 0.4 mg/dL), supporting its renoprotective effects. These results were found to be comparable to the standard group treated with Cystone, a well-established polyherbal formulation with documented antiurolithiatic efficacy [15].

Histological findings substantiated the biochemical results. Extensive deposition of calcium oxalate crystals, along with tubular epithelial degeneration, was observed in the kidneys of animals from the negative control group, which is in agreement with earlier reports by Al-Mamun et al. and Tiwari et al. [22,24]. In contrast, sections from the Bryophyllum pinnatum-treated group revealed preserved renal architecture, minimal tubular dilation, and a marked reduction in crystal deposition. This protective histological profile highlights the plant's dual action: preventing crystal adherence and mitigating oxidative damage, a mechanism similarly discussed by Grases et al., who emphasized the role of antioxidants in reducing tubular injury in lithiasis [21].

The study also evaluated kidney-to-body weight ratio as a marker of renal congestion and inflammation. The increased ratio in the EG group (1.08 ± 0.04 g/100 g) indicated edema and cellular infiltration, similar to that reported by Khan et al. in nephrolithiatic rat models [27]. Bryophyllum pinnatum extract significantly reduced kidney weight (0.76 ± 0.06 g/100 g), suggesting alleviation of renal inflammation and reduced burden of crystal retention. This effect is likely mediated by the plant's anti-inflammatory constituents, such as flavonoids and triterpenoids, which have been shown to stabilize renal membranes and suppress inflammatory cytokines [28].

Comparing these findings with other herbal interventions, the effect of Bryophyllum pinnatum appears to be on par with or superior to other well-studied botanicals. For instance, studies on Tribulus terrestris and Bergenia ligulata demonstrated moderate reductions in urinary oxalate and calcium but were less effective in restoring renal histology [29,30]. In contrast, Bryophyllum pinnatum not only reduced lithogenic ions but also conferred substantial nephron protection, suggesting a multifactorial therapeutic mechanism involving antioxidant, anti-inflammatory, and diuretic actions. The phytochemical richness of Bryophyllum pinnatum, particularly its flavonoids and saponins, may explain its robust efficacy. These compounds are known to inhibit NADPH oxidase-mediated oxidative stress and reduce the expression of pro-crystallization proteins in the renal epithelium [31,32]. In line with these mechanisms, histopathological evidence in the current study showed fewer oxalate plugs and healthier glomerular structures in the test group, underscoring the cytoprotective role of its phytoconstituents. The therapeutic effects observed were statistically significant and biologically relevant, showing equivalence to a clinically established formulation (Cystone).

The consistency of results across multiple parameters and their correlation with previous literature strongly justify the ethnomedicinal use of Bryophyllum pinnatum in traditional systems such as Ayurveda for the treatment of urolithiasis

Despite the promising findings observed in this study, certain limitations must be acknowledged. First, the sample size, although adequate for an in vivo preclinical model, remains a constraint when translating outcomes to human clinical settings. The study utilized 24 animals across four groups, which may not capture the full spectrum of pharmacodynamic variability. Additionally, only a single dose of Bryophyllum pinnatum extract (500 mg/kg) was evaluated, and a dose–response curve could offer more insight into the therapeutic window and optimal dosage. Furthermore, the active phytochemical constituents responsible for the antiurolithiatic effect were not isolated or quantified, leaving the exact molecular mechanism of action speculative. Future studies involving bioassay-guided fractionation and phytochemical standardization are essential to identify the lead molecules responsible for crystal inhibition and nephroprotection.

While this investigation confirms that Bryophyllum pinnatum has effects comparable to a standard polyherbal formulation (Cystone), it remains to be established whether its efficacy is consistent across various models of urolithiasis, including struvite and uric acid stone types. Long-term studies involving chronic administration are also required to evaluate safety, systemic tolerability, and recurrence prevention. Another important aspect not explored in this study is the impact of Bryophyllum pinnatum on urinary citrate levels and pH modulation, both of which are key determinants in the pathophysiology of nephrolithiasis. Expanding the biochemical profile in future research may provide a more comprehensive understanding of the extract's action on urine chemistry.

Nonetheless, the clinical implications of these results are significant. Herbal medicine continues to gain traction as a complementary or alternative therapeutic approach in renal stone management, particularly in resource-limited settings where access to surgical or pharmacologic intervention is constrained. The use of Bryophyllum pinnatum, with its demonstrated antiurolithiatic and nephroprotective activity, offers a promising candidate for phytotherapeutic development. Given the widespread availability of this plant and its traditional use in ethnomedicine, it has the potential to become a cost-effective alternative for the prevention and adjunctive management of renal calculi. However, it is essential that these preclinical findings be validated through well-designed randomized controlled clinical trials, including pharmacokinetic studies, to establish human equivalence and dosing parameters.

To summarize, the present study highlights the multifaceted protective effect of Bryophyllum pinnatum against ethylene glycol-induced nephrolithiasis in a validated animal model. The extract significantly reduced urinary calcium and oxalate levels, restored serum biochemical parameters, mitigated kidney weight changes, and improved histological integrity of renal tissue. These outcomes were statistically and biologically comparable to Cystone, reinforcing the therapeutic potential of this plant. While further studies are necessary to isolate active compounds, understand long-term safety, and evaluate efficacy in human models, the findings provide a strong scientific basis for the continued investigation and potential clinical use of Bryophyllum pinnatum in urolithiasis management.

5. CONCLUSION

The findings of this in vivo study provide compelling evidence for the antiurolithiatic and nephroprotective properties of Bryophyllum pinnatum leaf extract. Administration of the aqueous extract to ethylene glycol-induced nephrolithiatic rats resulted in a significant reduction in urinary calcium, oxalate, and phosphate excretion, along with restoration of urinary magnesium levels—key parameters involved in calcium oxalate stone formation. Moreover, the extract effectively normalized elevated serum creatinine, blood urea nitrogen, and uric acid levels, indicating preservation of renal function. Histopathological examination further confirmed the extract's ability to mitigate tubular damage and reduce calcium oxalate crystal deposition within renal tissue. These therapeutic effects were comparable to those observed with Cystone, a well-established polyherbal formulation, thereby validating the efficacy of Bryophyllum pinnatum as a potential alternative or adjunct in the management of renal calculi. While the exact phytoconstituents responsible for the observed effects remain to be elucidated, the study lays a strong scientific foundation for future research aimed at clinical translation, phytochemical characterization, and long-term safety evaluation. In conclusion, Bryophyllum pinnatum holds promise as an effective, affordable, and plant-based therapeutic candidate for the prevention and management of urolithiasis, especially in settings where conventional therapies may be limited or contraindicated.

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