

Evaluation of Anti-Urolithiatic and Nephroprotective Potential of Bacopa Monnieri Extract in Ethylene Glycol-Induced Urolithiasis in Experimental Rats

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ABSTRACT

Urolithiasis is a recurrent and multifactorial renal disorder characterized by the formation of urinary calculi, predominantly calcium oxalate stones, due to metabolic imbalance, oxidative stress, and crystal aggregation within the urinary tract. The present study was designed to investigate the anti-urolithiatic and nephroprotective potential of Bacopa monnieri extract in an ethylene glycol-induced experimental model of urolithiasis in Wistar albino rats. The study involved hydroalcoholic extraction of authenticated plant material, phytochemical screening, acute toxicity evaluation, induction of hyperoxaluria, biochemical analysis of urinary and serum parameters, antioxidant assessment, and histopathological examination of renal tissues. The methodology focused on evaluating the protective role of Bacopa monnieri against stone formation through its phytoconstituent-mediated antioxidant and anti-inflammatory properties. The research framework suggests that Bacopa monnieri may serve as a promising herbal therapeutic candidate for the prevention and management of kidney stone disease.

Keywords: Bacopa monnieri, Urolithiasis, Ethylene glycol, Nephroprotection, Calcium oxalate crystals

INTRODUCTION

Urolithiasis, commonly known as kidney stone disease, is one of the most prevalent renal disorders worldwide and is associated with significant

morbidity, recurrence, and healthcare burden. The disease is characterized by the formation of crystalline calculi in the urinary tract due to supersaturation of lithogenic substances such as calcium, oxalate,

phosphate, and uric acid. Calcium oxalate stones represent the most common type of renal calculi. Several contributing factors, including dehydration, metabolic abnormalities, dietary habits, oxidative stress, inflammation, and genetic predisposition, are involved in the pathogenesis of stone formation¹⁻³.

Experimental evidence indicates that hyperoxaluria-induced oxidative stress and inflammatory damage to renal tubular epithelium significantly contribute to crystal adhesion, nucleation, and retention within renal tissues. Ethylene glycol-induced hyperoxaluria in rats is a well-established and reproducible model that closely mimics human calcium oxalate nephrolithiasis.

Current therapeutic strategies for urolithiasis, including pharmacological interventions and surgical procedures, often present limitations such as adverse effects, recurrence, and high cost. Consequently, increasing attention has shifted toward medicinal plants possessing antioxidant, anti-inflammatory, and nephroprotective properties^{3,4}.

Bacopa monnieri (Brahmi), a well-known medicinal herb used in traditional systems of medicine, contains bioactive phytoconstituents such as bacosides, flavonoids, alkaloids, and phenolic compounds. These compounds have demonstrated significant antioxidant and anti-inflammatory effects, providing scientific rationale for investigating *Bacopa monnieri* as a potential anti-urolithiatic agent^{5,6}.

Objective of the Study

To evaluate the anti-urolithiatic and nephroprotective potential of *Bacopa monnieri* extract against ethylene glycol-induced urolithiasis in experimental rats. To assess its effects on urinary biochemical parameters, serum renal markers, antioxidant status, and renal histopathological changes associated with kidney stone formation.

Materials and Methods

3.1 Plant Material, Extraction, and Phytochemical Screening:

Fresh whole plants of *Bacopa monnieri* were collected from a reliable source and authenticated by a qualified botanist. The plant material was thoroughly washed, shade dried for 10–14 days, mechanically powdered, and stored in airtight containers. The dried powder was subjected to hydroalcoholic extraction using ethanol:water (70:30) through Soxhlet apparatus for 48–72 hours^{7,8}. The obtained extract was filtered, concentrated under reduced pressure, dried, and preserved at 4°C for further studies. Preliminary phytochemical screening of the extract was carried out using standard qualitative methods to identify major bioactive constituents including alkaloids, flavonoids, saponins, phenolic compounds, triterpenoids, and glycosides⁷⁻⁹.

3.2 Experimental Animals and Acute Toxicity Study:

Healthy adult Wistar albino rats weighing 150–200 g were procured and maintained under standard laboratory conditions with controlled temperature, humidity, and a 12-hour light/dark cycle in accordance with CPCSEA guidelines. Animals were provided standard pellet diet and water ad libitum and acclimatized before experimentation. Acute oral toxicity of *Bacopa monnieri* extract was evaluated according to OECD guideline 423 to determine the safety profile and establish suitable therapeutic dose levels for anti-urolithiatic evaluation⁸⁻¹⁰.

3.3 Induction of Urolithiasis and Experimental Design:

Experimental urolithiasis was induced by administering 0.75% ethylene glycol in drinking water continuously for 28 days to produce hyperoxaluria and calcium oxalate crystal deposition^{11,12}. Animals were randomly divided into five groups consisting of normal control, disease control, standard treatment, low-dose *Bacopa monnieri* treatment, and high-dose

Bacopa monnieri treatment groups. Treatments were administered orally throughout the study duration¹⁰⁻¹⁴.

3.4 Biochemical, Antioxidant, and Histopathological Evaluation: At the end of the experimental period, 24-hour urine samples were collected using metabolic cages and analyzed for urine volume, calcium, oxalate, phosphate, magnesium, uric acid, and pH. Blood samples were collected for serum biochemical analysis of creatinine, blood urea nitrogen, uric acid, calcium, and phosphorus to evaluate renal function^{15,16}. Kidneys were excised, homogenized, and assessed for antioxidant parameters including superoxide dismutase, catalase, reduced glutathione, and malondialdehyde to determine oxidative stress levels. Additionally, kidney tissues were subjected to histopathological examination to evaluate calcium oxalate crystal deposition, tubular degeneration, inflammation, glomerular changes, and overall renal tissue architecture¹⁵⁻¹⁸.

Results

4.1 Plant Material, Extraction, and Phytochemical Screening

The collected *Bacopa monnieri* plant material was successfully authenticated by a qualified botanist, confirming the correct botanical identity of the medicinal herb. The plant material was properly shade dried, powdered, and subjected to hydroalcoholic Soxhlet extraction, yielding a stable semisolid extract with satisfactory recovery. Preliminary phytochemical analysis revealed the presence of multiple therapeutically important phytoconstituents, which may contribute to its pharmacological activity against urolithiasis.

Table 4.1: Preliminary Phytochemical Constituents Identified in *Bacopa monnieri* Extract

Sr. No.	Phytochemical Constituent	Observation
1	Alkaloids	Present
2	Flavonoids	Present
3	Saponins	Present
4	Phenolic compounds	Present
5	Triterpenoids	Present
6	Glycosides	Present

The presence of flavonoids, phenolics, and saponins particularly supports the antioxidant and nephroprotective potential of the extract, thereby justifying its use for anti-urolithiatic evaluation.

4.2 Experimental Animals and Acute Toxicity Study

All experimental animals remained healthy under standard laboratory conditions throughout the study period. Acute oral toxicity testing conducted as per OECD guideline 423 demonstrated that *Bacopa monnieri* extract was well tolerated and did not produce any mortality or toxic symptoms at tested doses.

Table 4.2: Acute Oral Toxicity Observation of *Bacopa monnieri* Extract

Sr. No.	Parameter Evaluated	Observation
1	Mortality	None observed
2	Behavioral abnormalities	None observed
3	Food intake	Normal
4	Water intake	Normal
5	Neurological symptoms	Absent

These results indicate that the extract was safe and suitable for further therapeutic studies at selected dose levels.

4.3 Induction of Urolithiasis and Experimental Design

Administration of 0.75% ethylene glycol for 28 consecutive days successfully induced experimental urolithiasis in disease control rats, characterized by hyperoxaluria and calcium oxalate crystal deposition.

Treatment with *Bacopa monnieri* extract demonstrated dose-dependent protective effects.

Table 4.3: Experimental Groups and General Outcomes

Sr. No.	Experimental Group	Treatment	Outcome
1	Group I	Normal Control	Normal renal function
2	Group II	Disease Control	Severe urolithiasis
3	Group III	Standard Treatment	Significant protection
4	Group IV	Low-Dose <i>Bacopa monnieri</i>	Moderate protection
5	Group V	High-Dose <i>Bacopa monnieri</i>	Marked protection

The high-dose treatment group exhibited superior protective effects, closely comparable to standard anti-urolithiatic therapy.

4.4 Biochemical, Antioxidant, and Histopathological Evaluation

A. Urinary Biochemical Parameters: Ethylene glycol administration significantly altered urinary biochemical parameters, promoting lithogenesis, whereas *Bacopa monnieri* treatment improved urinary composition.

Table 4.4: Effect of *Bacopa monnieri* on Urinary Biochemical Parameters

Sr. No.	Parameter	Disease Control	<i>Bacopa monnieri</i> Treatment
1	Urine volume	Decreased	Increased
2	Urinary calcium	Increased	Reduced
3	Urinary oxalate	Increased	Reduced

Sr. No.	Parameter	Disease Control	<i>Bacopa monnieri</i> Treatment
4	Phosphate	Increased	Reduced
5	Uric acid	Increased	Reduced
6	Magnesium	Decreased	Improved
7	Urinary pH	Altered	Normalized

These findings indicate that *Bacopa monnieri* significantly reduced lithogenic risk factors and improved urinary health.

B. Serum Biochemical Parameters: Serum biochemical analysis revealed significant renal dysfunction in disease control animals, while treatment improved kidney function markers.

Table 4.5: Effect of *Bacopa monnieri* on Serum Biochemical Parameters

Sr. No.	Parameter	Disease Control	<i>Bacopa monnieri</i> Treatment
1	Serum creatinine	Elevated	Reduced
2	Blood urea nitrogen	Elevated	Reduced
3	Uric acid	Elevated	Reduced
4	Serum calcium	Elevated	Improved
5	Serum phosphorus	Elevated	Improved

Improvement in serum biochemical markers confirms the nephroprotective action of the extract.

C. Antioxidant Parameters: Disease control animals exhibited significant oxidative stress, whereas treatment restored antioxidant defense.

Table 4.6: Effect of *Bacopa monnieri* on Renal Antioxidant Status

Sr. No.	Antioxidant Marker	Disease Control	<i>Bacopa monnieri</i> Treatment
1	Superoxide dismutase (SOD)	Decreased	Increased
2	Catalase (CAT)	Decreased	Increased
3	Reduced glutathione (GSH)	Decreased	Increased
4	Malondialdehyde (MDA)	Increased	Reduced

These results suggest strong antioxidant potential of *Bacopa monnieri* against oxalate-induced oxidative damage.

D. Histopathological results: Histological examination confirmed severe renal damage in untreated animals and substantial tissue protection in treated groups.

Table 4.7: Histopathological Evaluation of Kidney Tissue

Sr. No.	Histological Parameter	Disease Control	<i>Bacopa monnieri</i> Treatment
1	Calcium oxalate crystal deposition	Extensive	Markedly reduced
2	Tubular degeneration	Severe	Mild/minimal
3	Inflammation	Significant	Reduced
4	Glomerular damage	Present	Improved
5	Renal tissue architecture	Distorted	Preserved

Histopathological improvement strongly validates the anti-urolithiatic and nephroprotective efficacy of *Bacopa monnieri*.

4.5 Biochemical, Antioxidant, and Histopathological Evaluation

Table 4.8 summarizes the effects of *Bacopa monnieri* extract on urinary biochemical parameters in

ethylene glycol-induced urolithiasis rats. Parameters including urine volume, pH, calcium, oxalate, phosphate, magnesium, and uric acid were evaluated to assess the antiurolithiatic potential of different dose levels.

Table 4.8: Effect of *Bacopa monnieri* Extract on Urinary Biochemical Parameters

Group	Urine Volume (ml)	pH	Calcium (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)	Magnesium (mg/dl)	Uric Acid (mg/dl)
Group I – Normal Control	18.5	7.1	2.8	0.9	3.1	1.9	1.2
Group II – Disease Control	10.2	5.8	6.9	3.8	6.4	0.8	2.9
Group III – Standard (Cystone)	16.7	6.9	3.4	1.5	3.8	1.6	1.4

Group	Urine Volume (ml)	pH	Calcium (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)	Magnesium (mg/dl)	Uric Acid (mg/dl)
Group IV – Test I (Low Dose)	13.9	6.4	5.1	2.6	5.2	1.1	2.1
Group V – Test II (Medium Dose)	15.2	6.6	4.3	2.1	4.6	1.3	1.8
Group VI – Test III (High Dose)	16.1	6.8	3.7	1.7	4.1	1.5	1.5

The disease control group showed significant alterations in urinary parameters, indicating stone formation and renal dysfunction. Treatment with *Bacopa monnieri* extract, particularly at medium and

high doses, markedly improved these parameters toward normal values, demonstrating dose-dependent protective effects comparable to the standard drug Cystone.

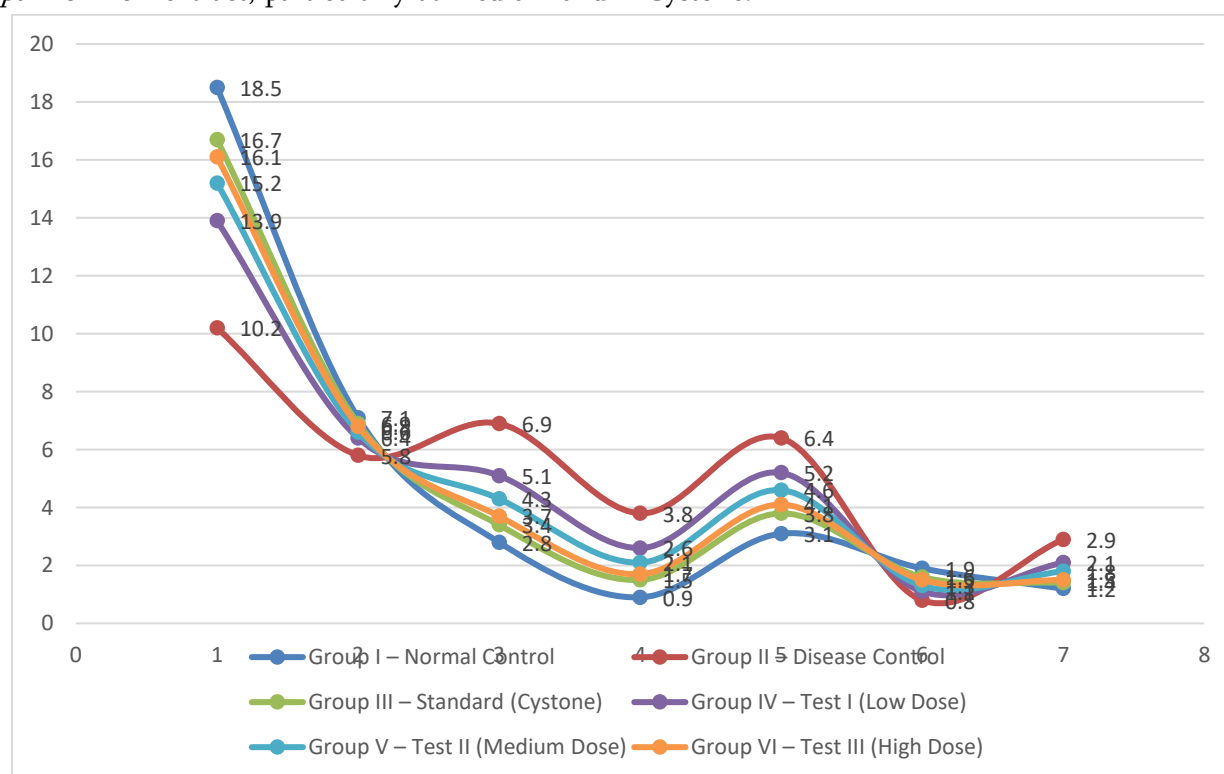


Chart 4.1: Figure of *Bacopa monnieri* Extract on Urinary Biochemical Parameters

4.6 Serum Biochemical Parameters in Ethylene Glycol-Induced Urolithiatic Rats

Table 4.9 presents the effect of *Bacopa monnieri* extract on serum biochemical parameters, specifically

blood urea nitrogen (BUN) and creatinine levels, in experimental urolithiatic rats. These markers were analyzed to evaluate renal function and the protective efficacy of the extract against kidney damage.

Table 4.9: Effect of *Bacopa monnieri* Extract on Serum Biochemical Parameters in Ethylene Glycol-Induced Urolithiatic Rats

Sr. No.	Group	BUN (mg/dl)	Creatinine (mg/dl)
1	Group I – Normal Control	18.6	0.62
2	Group II – Disease Control	46.3	1.58
3	Group III – Standard (Cystone)	22.7	0.78
4	Group IV – Test I (Low Dose)	35.4	1.21
5	Group V – Test II (Medium Dose)	29.6	1.02
6	Group VI – Test III (High Dose)	24.8	0.86

The disease control group exhibited significantly elevated BUN and creatinine levels, indicating impaired renal function due to urolithiasis induction. Administration of *Bacopa monnieri* extract produced a dose-dependent reduction in these serum parameters, with the high-dose group showing results closer to the standard treatment, suggesting notable nephroprotective and antiurolithiatic activity.

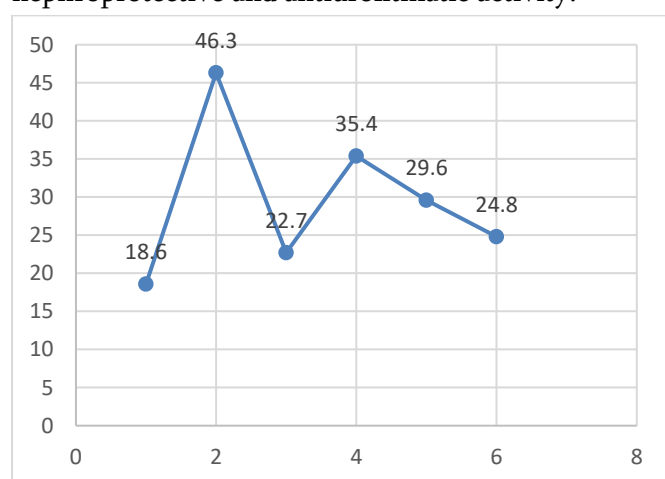


Chart 4.6: *Bacopa monnieri* Extract on Urinary Biochemical Parameters

Discussion

Ethylene glycol administration successfully induced urolithiasis in rats, as evidenced by decreased urine volume (10.2 ml), acidic urinary pH (5.8), and elevated calcium (6.9 mg/dl), oxalate (3.8 mg/dl), phosphate (6.4 mg/dl), and uric acid (2.9 mg/dl). Serum biochemical markers also confirmed renal dysfunction, with blood urea nitrogen (46.3 mg/dl) and creatinine (1.58 mg/dl) significantly elevated. Treatment with *Bacopa monnieri* extract produced dose-dependent improvements. High-dose therapy restored urine volume (16.1 ml), normalized pH (6.8), and reduced calcium (3.7 mg/dl) and oxalate (1.7 mg/dl), approaching values seen in the standard Cystone group. Serum creatinine (0.86 mg/dl) and BUN (24.8 mg/dl) were markedly reduced, confirming nephroprotection. Antioxidant markers such as SOD, CAT, and GSH increased, while MDA decreased, indicating strong oxidative stress mitigation. Histopathological analysis further validated these findings, showing reduced calcium oxalate deposition, minimal tubular degeneration, and preserved renal tissue architecture. These results highlight *Bacopa monnieri*'s phytoconstituents as key contributors to its protective efficacy.

Conclusion

This study highlights how *Bacopa monnieri* extract can genuinely protect the kidneys against stone formation and damage. In untreated rats, ethylene glycol led to sharp declines in urine volume (10.2 ml) and dangerously high levels of calcium (6.9 mg/dl), oxalate (3.8 mg/dl), and serum creatinine (1.58 mg/dl), all clear signs of urolithiasis. By contrast, rats receiving *Bacopa monnieri* showed remarkable recovery. At high doses, urine volume rose to 16.1 ml, calcium dropped to 3.7 mg/dl, oxalate to 1.7 mg/dl, and creatinine fell to 0.86 mg/dl—values close to those achieved with the standard drug Cystone. Antioxidant defenses were restored, and kidney tissue appeared healthier, with fewer crystals and less

inflammation. These improvements are not just numbers; they reflect real protection of renal function. Taken together, the findings suggest *Bacopa monnieri* could be a safe, effective herbal option for preventing kidney stones, offering hope for patients seeking natural therapies.

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