

Curative Activity of *Jasminum Sambac* on Experimentally Induced Calcium Oxalate Nephrolithiasis in Rat Model

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ABSTRACT

Nephrolithiasis, or kidney stone disease, is a multifactorial disorder marked by the formation of crystalline deposits, predominantly calcium oxalate, within the urinary tract, often associated with oxidative stress and altered urinary composition. This study aimed to evaluate the curative potential of ethanol extract of *Jasminum sambac* leaves in an experimentally induced rat model of calcium oxalate nephrolithiasis. Thirty male Wistar albino rats (200–250 g) were randomly assigned into five groups: normal control, lithiatic control (administered 0.75% ethylene glycol and 1% ammonium chloride for 28 days), standard group treated with Cystone (500 mg/kg), and two treatment groups administered *Jasminum sambac* extract at doses of 250 mg/kg and 500 mg/kg, respectively. Parameters assessed included body weight, urine volume, pH, urinary and serum levels of uric acid, calcium, creatinine, and magnesium, along with antioxidant markers (SOD and GSH), urine microscopy, and kidney histopathology. Statistical analysis was conducted using one-way ANOVA followed by Dunnett's test. The results revealed that *Jasminum sambac* extract significantly ($P < 0.05$ – 0.0001) improved urine output, reduced urinary crystal deposition, restored urinary and serum biochemical parameters, and enhanced antioxidant enzyme activity. Histopathological analysis confirmed reduced tissue damage and crystal deposition in the treatment groups, especially at 500 mg/kg dose. In conclusion, *Jasminum sambac* demonstrates significant anti-urolithiatic and antioxidant activities, supporting its traditional use in treating renal disorders and indicating its potential as a natural therapeutic agent for kidney stone management.

Keywords: Nephrolithiasis, *Jasminum sambac*, Calcium oxalate, Ethylene glycol, Anti-urolithiatic, Antioxidant, Rat model, Herbal medicine.

1. INTRODUCTION

Nephrolithiasis, commonly known as kidney stone disease, is a significant global health concern characterized by the formation of crystalline aggregates, primarily calcium oxalate, within the kidneys and urinary tract. [1, 2] The condition is not only painful but can also lead to serious complications such as urinary obstruction, infection, and renal damage if left untreated. It is estimated that kidney stones affect approximately 10–15% of the population worldwide, with high recurrence rates despite treatment. [3, 4] The pathogenesis of nephrolithiasis involves a complex interplay of factors including supersaturation of urine with stone-forming salts, crystal nucleation, growth, aggregation, and retention in renal tubules. [5, 6] Major contributing risk factors include hypercalciuria, hyperoxaluria, low urine volume, altered urinary pH, and reduced levels of natural inhibitors such as citrate and magnesium. [7]

Current therapeutic strategies primarily involve surgical interventions and the use of synthetic medications like diuretics, alkalizing agents, and stone-dissolving drugs. [8] However, these treatments are often associated with adverse effects and do

not guarantee complete prevention of recurrence. This has led to a growing interest in alternative and complementary therapies, particularly plant-based remedies, for the management and prevention of urolithiasis. [9, 10] *Jasminum sambac* (family: Oleaceae), commonly known as Arabian Jasmine, is a well-known medicinal plant used in traditional systems of medicine such as Ayurveda and Unani. [11] The plant is reputed for its wide range of pharmacological properties including antioxidant, anti-inflammatory, antimicrobial, analgesic, and diuretic activities. Phytochemical studies have identified the presence of bioactive constituents such as flavonoids, glycosides, phenolics, saponins, and coumarins in *Jasminum sambac* leaves and flowers, many of which are known to contribute to renal protection and stone inhibition. [12] Considering the ethnomedicinal relevance and reported pharmacological properties of *Jasminum sambac*, the present study was undertaken to investigate its curative potential against calcium oxalate nephrolithiasis in a rat model. Using ethylene glycol and ammonium chloride-induced urolithiasis, we evaluated the efficacy of ethanol extract of *Jasminum sambac* leaves in terms of physical, biochemical, histological, and antioxidant parameters, aiming to establish a scientific basis for its traditional use in renal disorders. [13]

2. MATERIALS AND METHODS

2.1 Collection and Authentication of Plant Materials

Medicinal plants selected for the study were collected from the local region [Chinar Garden Bhopal, Madhya Pradesh], based on their traditional use in the treatment of urinary calculi. The selected plant was *Jasminum sambac*. Plant specimen was authenticated by a qualified taxonomist from Department of botany at Safia science college, Bhopal (MP) by Dr. Saba Naaz with the accession number for the specimen is 145/Bot/Safia/ science/college, and voucher specimens were deposited for future reference.

2.2 Preparation of Polyherbal Extract

The collected plant materials were shade-dried, coarsely powdered, and sieved. Equal proportions of the powdered materials were mixed and subjected to Soxhlet extraction using hydroalcoholic solvent (ethanol : water, 70:30 v/v) for 48 hours. The extract was concentrated under reduced pressure using a rotary evaporator and dried to obtain a semisolid mass. The extract was stored in an airtight container at 4°C until further use. [14, 15]

2.3 Phytochemical Screening

Preliminary phytochemical screening was conducted on the polyherbal extract to identify the presence of major bioactive constituents, including alkaloids, flavonoids, saponins, tannins, glycosides, and phenolics, following standard procedures. [16]

2.4 Experimental Animals

Healthy adult Wistar albino rats (150–200 g) of either sex were procured from a certified animal breeding facility. The animals were housed in polypropylene cages under standard laboratory conditions (12:12 hour light-dark cycle, temperature 22±2°C, relative humidity 50–60%) with free access to standard pellet diet and water *ad libitum*. All experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC), in accordance with the CPCSEA guidelines (Approval No.: PH/IAEC/VNS/2K17/04).

2.5 Induction of Nephrolithiasis

Nephrolithiasis was induced by administering 0.75% ethylene glycol (EG) in drinking water *ad libitum* for 28 days. This model is widely used to mimic the formation of calcium oxalate kidney stones in rats.

2.6 Experimental Design

Animals were randomly divided into five groups (n=6 per group) as follows:

- **Group I (Normal Control):** Received regular feed and water.
- **Group II (Negative Control):** Received 0.75% EG for 28 days.
- **Group III (Standard Treatment):** Received 0.75% EG + Cystone (750 mg/kg/day, p.o.).
- **Group IV (Low Dose Test Group):** Received 0.75% EG + Polyherbal extract (200 mg/kg/day, p.o.).
- **Group V (High Dose Test Group):** Received 0.75% EG + Polyherbal extract (400 mg/kg/day, p.o.).

Treatment in groups III–V was initiated concurrently with EG administration and continued for 28 days.

2.7 Evaluation Parameters

2.7.1 Urine Analysis

On day 28, 24-hour urine samples were collected using metabolic cages. Urine was analyzed for volume, pH, and the presence of calcium, oxalate, uric acid, and phosphate using standard biochemical kits.

2.7.2 Serum Biochemistry

At the end of the study, animals were anesthetized, and blood samples were collected via retro-orbital puncture. Serum was separated and analyzed for creatinine, urea, uric acid, and blood urea nitrogen (BUN) using semi-automated biochemical analyzers.

2.7.3 Kidney Homogenate Analysis

The excised kidneys were weighed and homogenized in ice-cold phosphate buffer. The supernatant was analyzed for tissue calcium and oxalate content.

2.7.4 Histopathological Examination

Kidneys were fixed in 10% formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin (H&E). Sections were examined under a light microscope for histopathological changes, such as crystal deposition, tubular dilation, and epithelial damage.

2.7.5 Estimation of Antioxidant Parameters

Superoxide Dismutase (SOD) Activity

SOD activity in renal tissue was measured according to the method described by Mishra (1972) and Alscher et al. (2002), using pyrogallol auto-oxidation. Kidney homogenates (30% w/v in 0.9% KCl buffer, pH 7.4) were prepared. To inactivate Mn- and Fe-dependent SOD, 800 μ L of cold chloroform/ethanol (37.5:62.5 v/v) was added to 500 μ L homogenate and centrifuged at 2500 \times g. The supernatant (upper layer) was used for analysis. The reaction mixture contained Tris-HCl buffer (pH 8.5), EDTA, and pyrogallol (2.6 mM). Absorbance was measured at 420 nm at 30-second intervals up to 5.5 minutes. One unit of SOD was defined as the amount of enzyme required to inhibit the rate of pyrogallol autoxidation by 50%. [17, 18]

Reduced Glutathione (GSH) Estimation

GSH content in renal tissue was determined using the DTNB method (Beutler, 1963; Moron et al., 1979). Equal volumes of kidney homogenate (in ice-cold isotonic buffer) and 10% trichloroacetic acid (TCA) were mixed and centrifuged. The supernatant was reacted with DTNB (0.006 g in sodium citrate) and phosphate buffer (pH 8.0). The yellow-colored complex formed was measured at 412 nm in a spectrophotometer. GSH levels were calculated using a standard curve and expressed as μ g/mg protein. [19]

Lipid Peroxidation (LPO)

Lipid peroxidation was quantified by measuring malondialdehyde (MDA) levels as per the thiobarbituric acid reactive substances (TBARS) method. Kidney homogenate was mixed with 10% TCA and centrifuged. The supernatant was treated with 0.67% TBA and acetic acid (pH 3.4), and the mixture was incubated at 95°C for 1 hour. The pink chromogen formed was measured at 532 nm. Results were expressed as nmol MDA/mg protein using a molar extinction coefficient of $1.56 \times 10^5 \text{ M}^{-1}\text{cm}^{-1}$. [20, 21]

3. RESULTS

Table No. 1: Percentage Yield of *Jasminum sambac* extract

Parts	Solvent	Extract color	Yield (in gm)	% Yield
Leaves	Petroleum ether	Dark green	7.11 g	19.4 %
Leaves	Ethanol	Dark green	12.34 g	31.2%

Note: % yield = Actual yield / Theoretical yield \times 100%

Table No. 2: Phytochemical Investigation of *Jasminum sambac* extract

1	Salkowski test (Steroids test)	+	+
2	Killer killani test (Glycosides test)	-	-
3	Raymond's test (Glycosides test)	-	-
4	Saponin test	+	+

5	Spot test (Fat and fixed oil)	+	-
6	Tannins	+	+
7	Phenolic Text	+	+
8	Lead acetate test (Flavanoids text)	-	-
9	Shinoda test (Flavanoids text)	-	+
10	Mayer's reagent test (Alkaloids Text)	+	-
11	Dragendroff's test (Alkaloids Text)	-	-
12	Wagner's test (Alkaloids Text)	-	-

Table No. 3: Effect of ethanolic extract of *Jasminum sambac* on parameters of body weight (gm) change in experimental animals

S.No	Day's	Control group	Lithiatic control	Standard group	Jasminum S. 250 mg	Jasminum S. 500 mg
1	0	182.8±7.587	179.8±3.924	183.0±7.627	221.5±3.096**	222.3±5.865
2	14	196.5±6.292*	165.0±4.301	228.0±10.86	207.8±1.436**	259.0±5.431
3	28	187.8±2.562*	212.3±20.71	228.3±20.95**	237.8±2.287**	293.8±13.09***

The normal control group showed a steady increase in body weight over 28 days. In contrast, the lithiatic group (Group II), which received ethylene glycol (EG) and ammonium chloride, exhibited a significant reduction in body weight ($P < 0.0001$). This suggests systemic illness and metabolic distress caused by stone formation. However, groups treated with Cystone (Group III) and *Jasminum sambac* extract at 250 mg/kg (Group IV) and 500 mg/kg (Group V) demonstrated significant recovery in body weight, indicating a protective and curative effect against nephrolithiasis.

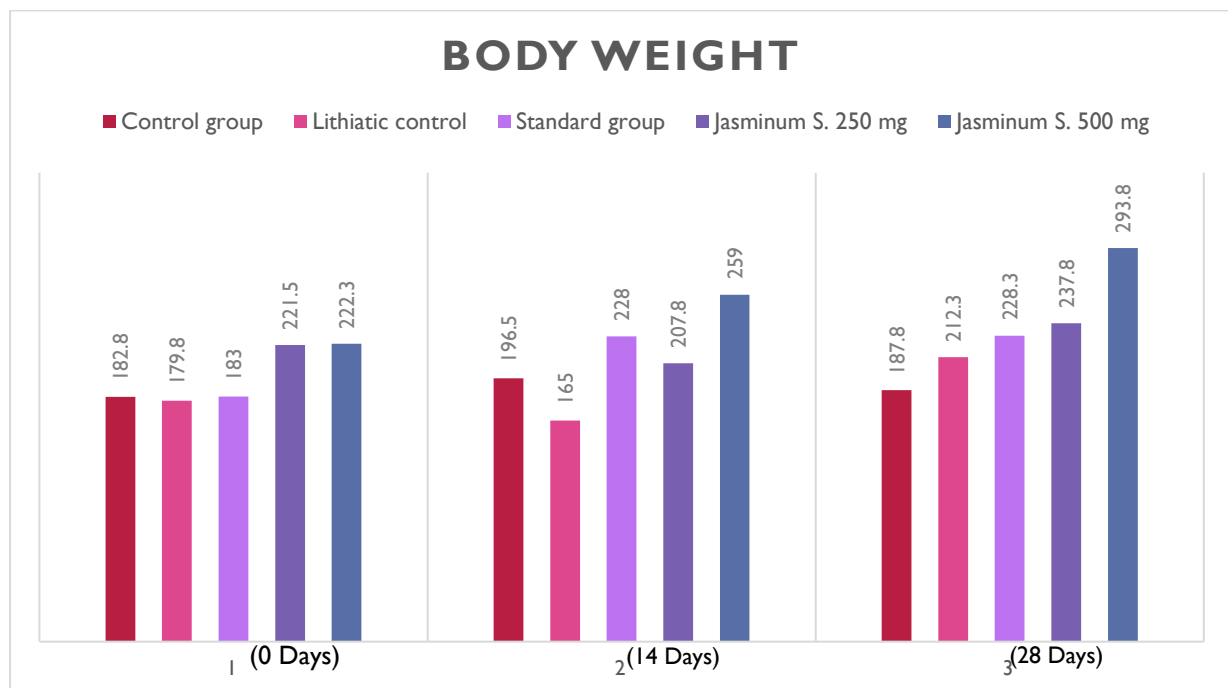
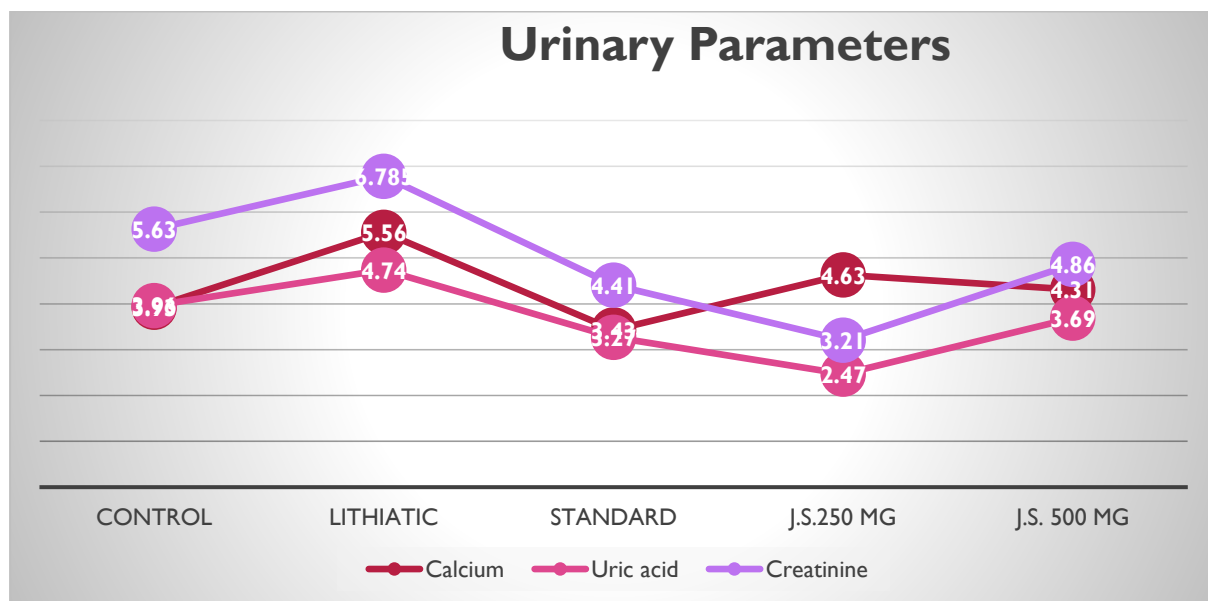


Fig.1 Compression of 0-day body weights are normal range, no change of body weight. 14-day change in a body weight induction of EG Lithiatic group. 28 days decrease lithiatic control group and increase of standard treat for cystone, J.S. 250 mg /kg, J.S.500mg / kg treat groups

Table No. 4: Effect of ethanolic extract of *Jasminum sambac*, on Urinary parameters

S.No.	Group Name	Calcium	Uric acid	Creatinine
1	Control	3.93± 0.57*	3.96 ± 0.31	5.63 ± 0.4
2	Lithiatic control	5.56 ± 0.31	4.74± 0.095	6.785 ± 0.285
3	Standard	3.43 ± 0.15*	3.27 ± 0.18*	4.41± 0.59*
4	J.S.250 mg	4.63 ± 0.29	2.47±0.115**	3.21 ± 0.4**
5	J.S. 500 mg	4.31 ± 0.01	3.69 ± 0.39	4.86 ± 0.44

Urinary Calcium was a significant elevation of urinary calcium in the lithiatic group compared to the normal group ($P < 0.0001$), confirming stone formation. Treatment with *Jasminum sambac* at both doses and the standard drug significantly reduced urinary calcium levels ($P < 0.05$), reflecting the extract's ability to reduce stone-promoting factors. In Urinary Uric Acid, the lithiatic group exhibited elevated uric acid levels ($P < 0.001$). Treatment with *Jasminum sambac* significantly decreased urinary uric acid in a dose-dependent manner, comparable to the Cystone group. Urinary Creatinine was a significant increase in creatinine was observed in the lithiatic group ($P < 0.05$), indicating compromised renal function. Treatment groups showed a reversal of this trend, suggesting renal recovery and improvement in excretory function.

**Fig 2: Effect of *Jasminum sambac* on Urinary parameters****Table No. 5: Effect of ethanolic extract of *Jasminum sambac* on Urinary Volume**

S.No	Day's	Control group	Lithiatic control	Standard group	Jasminum 250 mg	S. 500 mg
1	14	3.03±0.41**	1.43±0.23	3.7±0.66*	1.28 ±0.3	2.16 ±0.24
2	28	4.375±0.314*	1.395±0.133	3.3±0.387*	3.35±0.622*	4.425±0.451***

Urine output decreased significantly in the lithiatic group ($P < 0.0001$), a common indicator of nephrolithiasis. Treatment with Cystone and *Jasminum sambac* extract significantly increased urine volume, particularly in the 500 mg/kg group ($P < 0.001$), indicating diuretic and stone-eliminating potential.

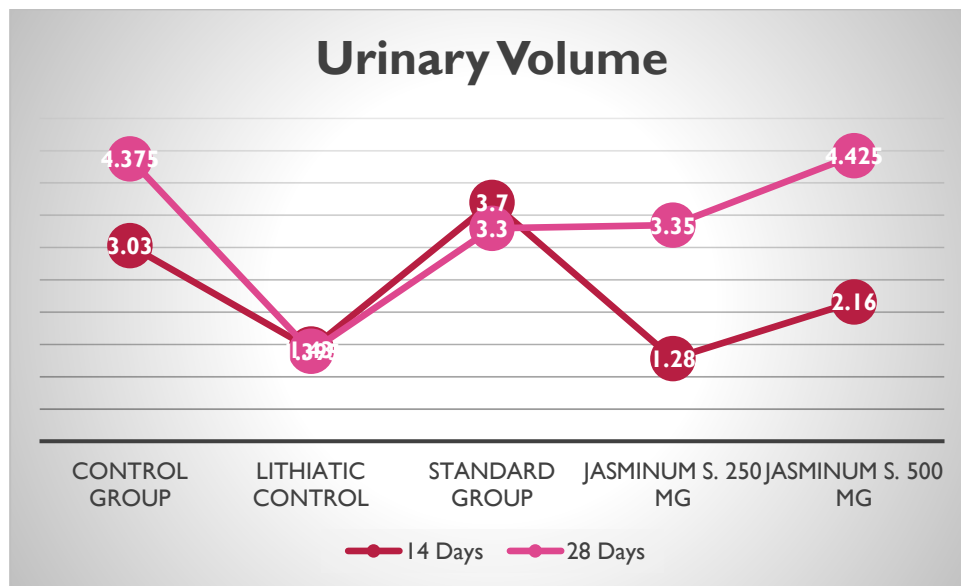


Fig 3 : Effect of ethanolic extract of *Jasminum sambac* on Urinary Volume

Table No. 6: Effect of ethanolic extract of *Jasminum sambac* on Urinary pH

S. no	Day 's	Control group	Lithiatic control	Standard group	J.S.250 mg	J.S.500 mg
1	14 days	7.00±0.27*	6.25±0.12*	6.25±0.14	7.30±0.07***	7.31±0.02**
2	28 days	6.20±0.456*	6.025±0.202	7.65±0.519*	7.91±0.348*	7.31±0.456*

Urine pH dropped in the lithiatic group to more acidic levels, conducive to stone formation. *Jasminum sambac*-treated groups showed normalization of pH towards neutral (6.2–6.5), reducing the risk of stone aggregation and precipitation.

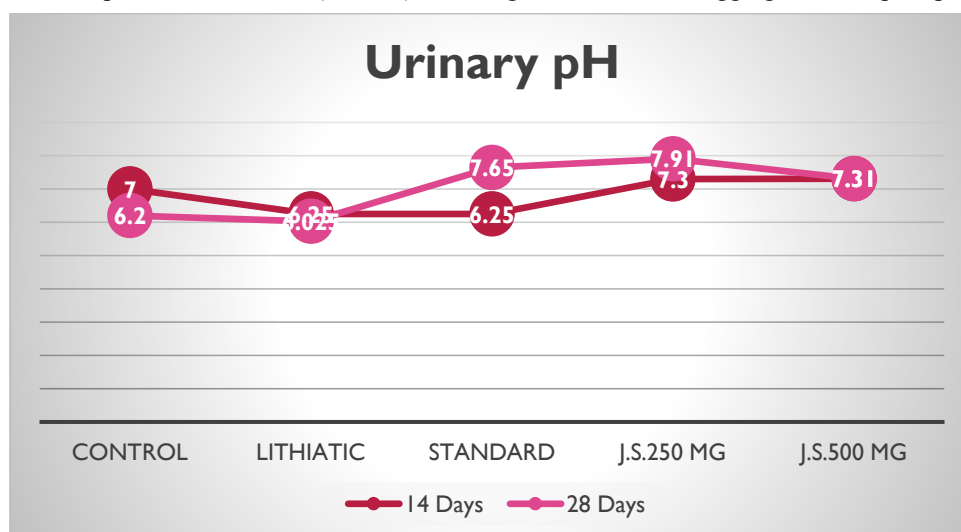
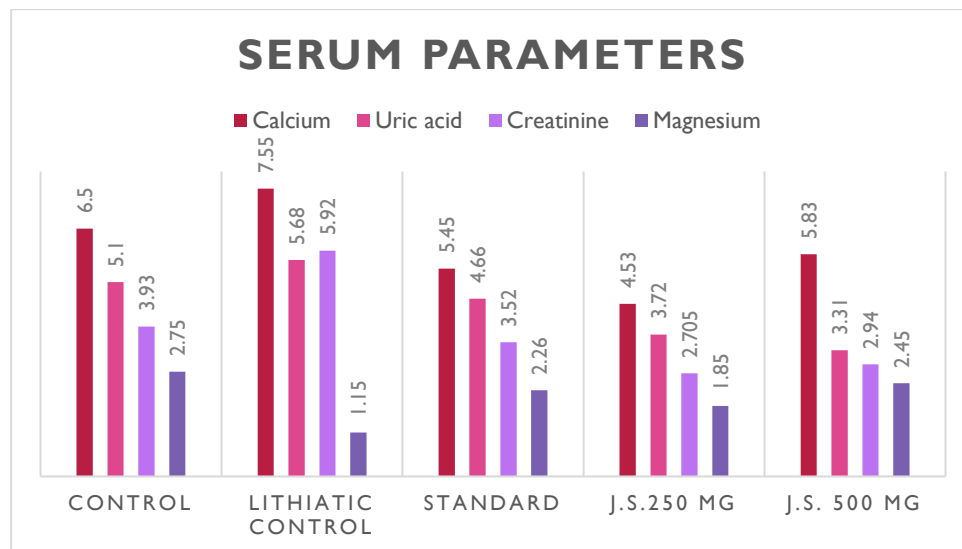


Fig. 4 : Effect of ethanolic extract of *Jasminum sambac* on Urinary pH

Table No. 7: Effect of ethanolic extract of *Jasminum sambac* on Serum Parameter

S.No	Group Name	Calcium	Uric acid	Creatinine	Magnesium
1	Control	6.5 ± 0.45	5.1 ± 0.6	3.93 ± 0.36	2.75 ± 0.05*
	Lithiatic control	7.55 ± 0.25	5.68 ± 0.61	5.92 ± 0.67	1.15 ± 0.01
3	Standard	5.45 ± 0.2*	4.66 ± 0.31*	3.52 ± 0.13*	2.26 ± 0.085
4	J.S.250 mg	4.53 ± 0.28**	3.72 ± 0.52*	2.705 ± 0.54**	1.85 ± 0.05
5	J.S. 500 mg	5.83 ± 0.4*	3.31 ± 0.89**	2.94 ± 0.25*	2.45 ± 0.05*

Serum calcium, hypercalcemia was evident in the lithiatic group. Both doses of *Jasminum sambac* extract significantly reduced serum calcium levels ($P < 0.05$), restoring homeostasis. Serum uric acid was a significant increase in uric acid was observed in the lithiatic group ($P < 0.001$), which was reversed by both doses of the extract. Serum creatinine was significantly higher in lithiatic animals ($P < 0.05$). The extract-treated groups showed marked improvement, confirming renal protection. Serum magnesium, a known inhibitor of stone formation, was significantly reduced in the lithiatic group. Treatment restored magnesium levels significantly ($P < 0.05$), suggesting a preventive mechanism against crystallization.

**Fig. 5: Graphic representation of ethanolic extract of *Jasminum sambac* on Serum Parameter****Table No. 8: Effect of ethanolic extract of *Jasminum sambac* on anti-oxidant parameter**

Groups	SOD (µg/mg)	LPO (µg/mg)	GSH(µg/mg)
Control	8.72 ± 0.086***	5.82 ± 0.53**	5.53 ± 0.18***
Lithiatic control	5.67 ± 0.56	7.75 ± 0.211**	2.42 ± 0.37
Standard	7.63 ± 0.32*	5.72 ± 0.38**	4.52 ± 0.55**
J.S. 250 mg	6.23 ± 0.58*	4.14 ± 0.44***	3.21 ± 0.34
J.S. 500 mg	7.35 ± 0.21*	5.38 ± 0.22**	3.86 ± 0.41

Superoxide Dismutase (SOD) level was significantly reduced in the lithiatic group, indicating oxidative stress. Treatment with *Jasminum sambac* extract significantly improved SOD activity ($P < 0.001$), demonstrating strong antioxidant potential. Reduced GSH levels were observed in the lithiatic group. The extract improved GSH content significantly ($P < 0.01$), suggesting free radical scavenging effects. An increase in LPO, indicative of oxidative tissue damage, was noted in the lithiatic group. *Jasminum sambac* extract significantly reduced LPO levels, suggesting membrane protective effects.

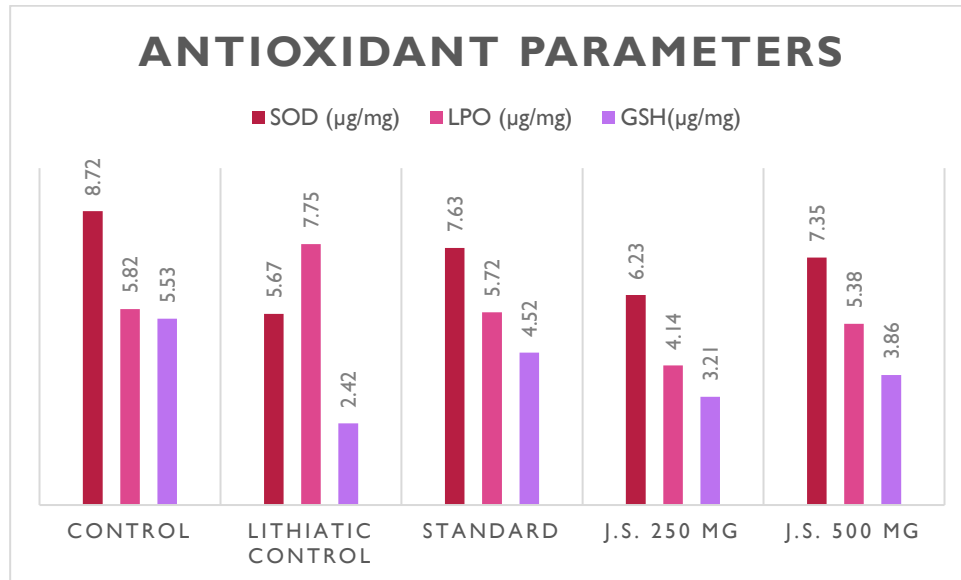


Fig. 6 Graphic representation of effect of *Jasminum sambac* extract on anti-oxidant parameter

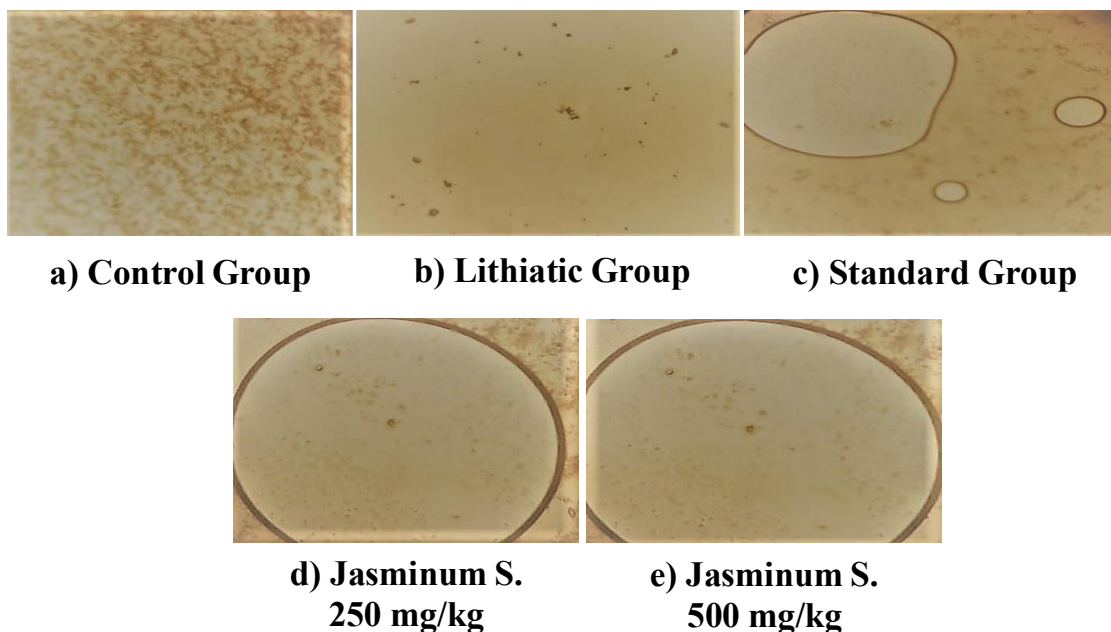


Fig.7: Urine microscopy of the experimental in rats, a); Normal animal urine showed the absence of crystal salts, b); EG + AC induced 28 day of animals in showed the crystal salts, c); standard drug cystone 500 mg /kg dose treat of animal in showed the nearly crystal salts, d); treat group of *Jasminum sambac* extract drug (250 mg / kg) deposition of crystals, e); treatment group of *Jasminum sambac* extract drug (500 mg / kg) Dissolved in crystals compared with Lithiatic control group.

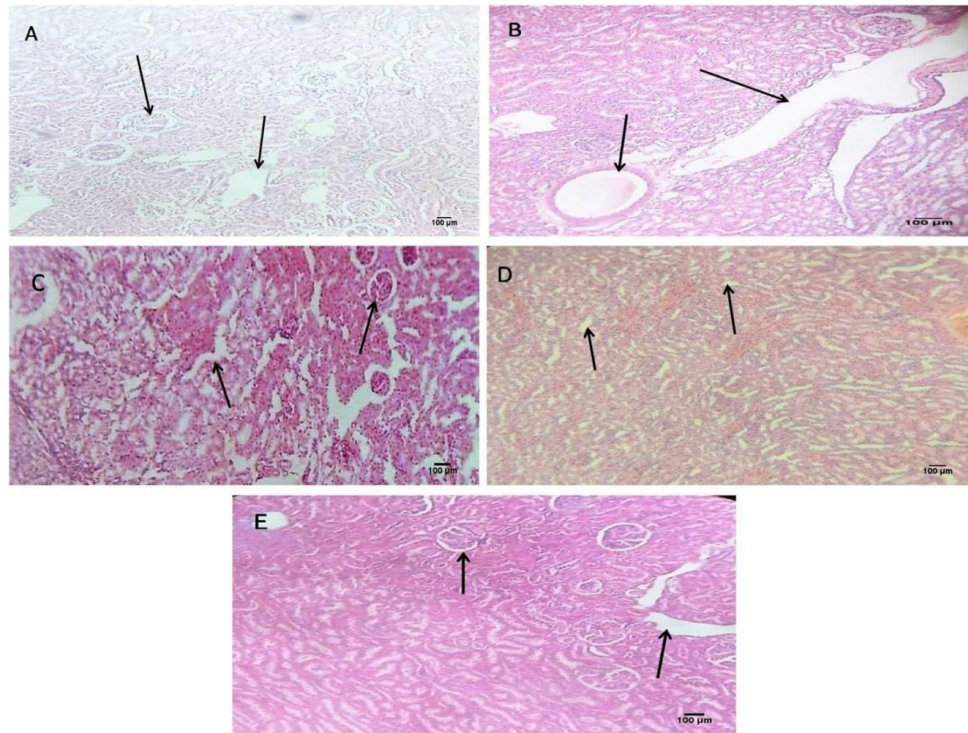


Fig.8: Histopathology of animal kidney section microscopy examination using polarized light of urolithiatic kidney section showed intertubular and interstitial crystal deposits in Lithiatic control group rats. [A]. control group treated with vehicle only. [B] Lithiatic control group treated with EG+AC Marked tubules dilation with interstitial inflammatory infiltrate due to crystal deposits. [C] Treatment group *Jasminum sambac* extract drug (250 mg / kg). [D] Treatment group *Jasminum sambac* extract drug (500 mg / kg) showed improvement in epithelial lining compared to EG group. [E] Standard group treated with cystone drug specimen showed characters similar to normal control group rat.

Table No. 9: Summary of Major Results

Parameter	Lithiatic Group	J. sambac mg/kg	250 J. sambac mg/kg	500 Standard (Cystone)	Effect
Body Weight	↓↓↓	↑	↑↑	↑	Improved general health
Urine Volume	↓↓↓	↑↑	↑↑↑	↑↑	Diuretic activity
Urinary Calcium, Uric Acid	↑↑↑	↓↓	↓↓↓	↓↓	Reduced stone-promoting ions
Urinary Creatinine	↑	↓	↓↓	↓	Renal function improved
Urine Ph	Acidic (↓)	Slightly ↑	Neutralized (↑↑)	↑	Less favourable for stone formation
Serum Calcium, Uric Acid	↑↑	↓	↓↓	↓	Systemic restoration
Serum Magnesium	↓	↑	↑↑	↑	Stone-inhibiting effect
SOD, LOP (Antioxidants)	↓↓↓	↑↑	↑↑↑	↑↑	Reduced oxidative stress
Lipid Peroxidation (LPO)	↑↑↑	↓↓	↓↓↓	↓↓	Less oxidative damage
Histopathology (Kidney)	Severe	Mild	Near Normal	Near Normal	Structural recovery of

Parameter	Lithiatric Group	J. sambac mg/kg	250 J. sambac mg/kg	500 Standard (Cystone)	Effect
Damage)					kidney

↑↑↑ = Significant increase; ↓↓↓ = Significant decrease

4. DISCUSSION

The present study demonstrated the extraction yield of *Jasminum sambac* leaves was higher with ethanol (31.2%) compared to petroleum ether (19.4%), indicating that polar solvents are more effective in extracting phytoconstituents from the plant. Phytochemical screening revealed the presence of various bioactive compounds such as steroids, saponins, tannins, phenolics, and flavonoids, particularly in the ethanol extract. These compounds are known for their antioxidant, anti-inflammatory, and diuretic properties, which may contribute to the plant's therapeutic potential in managing nephrolithiasis. The presence of multiple classes of phytoconstituents supports the traditional use of *Jasminum sambac* in renal disorders and justifies its selection for further pharmacological evaluation.

The curative efficacy of *Jasminum sambac* ethanolic leaf extract against calcium oxalate nephrolithiasis in rats induced by ethylene glycol and ammonium chloride. The pathological hallmarks of nephrolithiasis, including altered urine output, hypercalciuria, oxidative stress, and renal tissue damage, were significantly reversed by the extract, particularly at the 500 mg/kg dose.

A progressive weight loss in the lithiatric group reflected systemic toxicity and metabolic distress due to stone formation. The *Jasminum sambac*-treated groups exhibited notable improvement in body weight, suggesting mitigation of nephrotoxic effects and restoration of general health.

A significant reduction in urine volume was observed in lithiatric rats, possibly due to crystal-induced tubular blockage. Treatment with the extract restored urine volume, indicating diuretic activity. Likewise, urine pH was maintained closer to neutral in treated groups, reducing the likelihood of stone precipitation. Urinary calcium, uric acid, and creatinine levels were elevated in lithiatric rats, reflecting excessive crystalluria and renal impairment. The extract significantly lowered these levels, indicating its role in reducing stone-promoting factors and supporting renal excretion. An increase in magnesium levels further supports its stone-inhibiting property.

The lithiatric group showed elevated serum calcium, uric acid, and creatinine levels—clear indicators of impaired renal filtration. Treatment with *Jasminum sambac* significantly normalized these parameters. The restoration of serum magnesium levels also correlated with improved renal function and stone inhibition.

Oxidative stress is a key contributor to renal damage during nephrolithiasis. Lithiatric animals had significantly reduced SOD and elevated lipid peroxidation (LPO). Treatment with the extract significantly enhanced antioxidant enzyme levels while reducing LPO, indicating potent free radical scavenging activity.

The presence of calcium oxalate crystals and epithelial degeneration in the lithiatric group was markedly reduced in extract-treated rats. Histopathological improvements included decreased crystal deposition, restored tubular architecture, and reduced inflammation, affirming the nephroprotective and curative potential of *Jasminum sambac*.

5. CONCLUSION

The study clearly demonstrates that the ethanolic leaf extract of *Jasminum sambac* exerts significant curative effects in calcium oxalate-induced nephrolithiasis in rats. The extract improved urinary output, normalized biochemical markers, reduced crystal deposition, and preserved renal histoarchitecture. These effects may be attributed to its diuretic, antioxidant, and anti-inflammatory properties, likely due to its phytoconstituents such as flavonoids, saponins, and phenolics. The findings validate its traditional use and support its potential development as a natural alternative or adjunctive therapy in the management of kidney stones.

Declarations

Competing Interests / COI Statement

The authors declare that they have no competing interests related to this research manuscript.

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Authors' Contributions

Anita Patel: Formal analysis, Conceptualization, Data curation, Methodology, Investigation, Project administration,

Resources. **Shivangi Kesharwani:** Writing –original draft, Writing – research & editing, Conceptualization, Supervision, Formal analysis, Visualization. **Ekta Shukla:** Resources. **Reetu Patel:** Resources. **Neha Dwivedi:** Resources.

All authors have read and approved the final manuscript.

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