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PHYTOCHEMICAL, ANTIOXIDANT, AND *IN-VIVO* ANTIUROLITHIATIC EVALUATION OF *SYZYGIUM CUMINI* BARK EXTRACT IN ETHYLENE GLYCOL-INDUCED UROLITHIASIS IN RATS

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ABSTRACT

Background: *Syzygium cumini* (L.) Skeels is traditionally used for its medicinal properties, including management of kidney disorders. This study aimed to evaluate the phytochemical profile, antioxidant potential, and antiurolithiatic activity of its methanolic bark extract. **Methods:** Bark of *S. cumini* was collected, authenticated, shade- and oven-dried, and extracted successively with petroleum ether and methanol. Preliminary phytochemical screening and quantitative estimation of total phenolics (TPC) and flavonoids (TFC) were performed. Antioxidant activity was assessed by the DPPH assay. Acute oral toxicity studies were conducted following OECD guidelines. Antiurolithiatic activity was evaluated in ethylene glycol-induced urolithiasis in albino rats at doses of 100 and 200 mg/kg, with cystone as standard. Biochemical parameters including serum creatinine, calcium, urea, and phosphorus were measured, Committee (IAEC Approval No: 1650/PO/Re/S/11/CCSEA). **Results:** Methanolic extract yielded 2.20% w/w and contained significant amounts of phenolics (54.66 mg GAE/g) and flavonoids (18.5 mg RE/g). The extract exhibited dose-dependent DPPH radical scavenging activity (IC_{50} = 54.09 µg/mL), indicating antioxidant potential. In vivo, the extract significantly reduced elevated serum creatinine, calcium, urea, and phosphorus levels in a dose-dependent manner, with the 200 mg/kg dose showing effects comparable to cystone. **Conclusion:** The methanolic bark extract of S. cumini possesses significant antioxidant and antiurolithiatic activities, likely mediated by its phenolic and flavonoid constituents. These findings support its potential use as a natural therapeutic agent for the prevention and management of urolithiasis.

KEYWORDS: Syzygium cumini, urolithiasis, antioxidant, phenolics, flavonoids, DPPH assay, nephroprotective.

INTRODUCTION

Urolithiasis, commonly referred to as kidney stone disease, is a chronic and recurrent disorder of the urinary system characterized by the formation of insoluble mineral concretions within the kidneys or urinary tract. Its prevalence has been increasing worldwide due to sedentary lifestyle, dietary habits, and metabolic imbalances, affecting a significant proportion of the population, with a higher recurrence rate in males than females. Calcium oxalate stones constitute the majority of urinary calculi, and their pathogenesis is associated with hyperoxaluria, hypercalciuria, urinary tract infections, and oxidative stress, leading to renal tubular injury and crystal deposition. Current therapeutic options such as extracorporeal shockwave lithotripsy, surgical removal, and pharmacotherapy provide only symptomatic relief, often accompanied by high recurrence rates and undesirable side effects. This highlights the need for safer, cost-effective, and efficacious alternatives from natural sources. Medicinal plants have long been a cornerstone in the management of urolithiasis in traditional systems such as Ayurveda, Siddha, and Unani. Phytochemicals including flavonoids, phenolics, saponins, tannins, and terpenoids are known to reduce lithogenic risk factors through antioxidant, anti-inflammatory, diuretic, and crystal-inhibitory mechanisms. Among such plants, *Syzygium cumini* (L.) Skeels, commonly known as Jamun or black plum (Figure 1) and belonging to the family Myrtaceae, is widely distributed in tropical and subtropical regions of Asia. Its bark, seeds, and leaves are rich in tannins, flavonoids, alkaloids, and phenolic compounds, which have been reported to possess gastroprotective, hypoglycemic, hypolipidemic, and antioxidant activities. Despite its wide ethnomedicinal use, its potential in the prevention and management of urolithiasis has not been comprehensively investigated. The present study was designed to evaluate the antiurolithiatic activity of the methanolic bark extract of *Syzygium cumini* in an ethylene glycol-induced urolithiasis rat model. This

model closely mimics human calcium oxalate stone formation and provides insights into biochemical, antioxidant, and renal functional alterations. The investigation also included phytochemical screening, quantitative estimation of phenolic and flavonoid content, and antioxidant assessment to establish a correlation between bioactive phytoconstituents and the observed pharmacological effect. The findings are expected to validate traditional claims and provide a scientific basis for the development of novel phytopharmaceuticals for the prevention and management of kidney stone disease.

Figure 1: *Syzygium cumini* (Jamun).

MATERIALS AND METHODS

Plant Material and Extraction

Bark of *Syzygium cumini* was collected, authenticated by a plant taxonomist (Authentication No:202/Saif./Sci./Clg/Bpl.), and shade-dried followed by oven-drying at 45 °C. The dried material (300 g) was coarsely powdered and extracted successively using Soxhlet apparatus. Methanol was used as the primary solvent after petroleum ether defatting. The obtained extracts were concentrated under reduced pressure using a rotary evaporator at 40 °C, weighed, and stored in airtight containers until further use.

Phytochemical Screening

The methanolic extract was subjected to standard qualitative tests to detect the presence of alkaloids, flavonoids, phenolics, tannins, saponins, glycosides, carbohydrates, and steroids.

Quantitative Phytochemical Estimation

Total Phenolic Content (TPC): Determined using the Folin–Ciocalteu method with gallic acid as standard. Results were expressed as mg gallic acid equivalents (GAE) per g of extract.

Total Flavonoid Content (TFC): Determined using the aluminum chloride colorimetric method with rutin as standard. Results were expressed as mg rutin equivalents (RE) per g of extract.

Antioxidant Activity (DPPH Assay)

The free radical scavenging activity was evaluated using the DPPH assay. Different concentrations of extract (20–100 µg/mL) were mixed with 0.1 mM DPPH solution and incubated for 30 minutes in the dark. Absorbance was measured at 517 nm using a UV spectrophotometer. The percentage inhibition was calculated, and IC_{50} values were determined.

Acute Toxicity Study

Acute oral toxicity was assessed according to OECD guidelines. The extract was administered to rats in stepwise doses (5, 50, 300, and 2000 mg/kg body weight) with observation for mortality or behavioral changes. No signs of toxicity were observed up to 2000 mg/kg, and doses of 100 mg/kg and 200 mg/kg were selected for the pharmacological study.

Animals and Experimental Design

Healthy male albino rats (200–250 g) were housed under standard laboratory conditions (12 h light/dark cycle, controlled temperature and humidity) with free access to food and water. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC Approval No: 1650/PO/Re/S/11/CCSEA). Animals were divided into five groups (n = 6 each):

- **Group I:** Normal control, received standard diet and water.
- **Group II:** Lithiatic control, received 1% ethylene glycol in drinking water for 28 days.
- **Group III:** Lithiatic rats treated with extract (100 mg/kg, p.o.) for 28 days.
- **Group IV:** Lithiatic rats treated with extract (200 mg/kg, p.o.) for 28 days.
- **Group V:** Standard group, lithiatic rats treated with cystone (600 mg/kg, p.o.) for 28 days.

Biochemical Estimations

At the end of the study, urine samples were collected in metabolic cages, acidified, and analyzed for calcium,

oxalate, and phosphate. Blood samples were obtained via retro-orbital plexus under anesthesia, and serum was separated for the estimation of creatinine, urea, calcium, and phosphorus. Kidneys were excised, weighed, and preserved in 10% neutral formalin for further biochemical and histological analysis.

RESULTS AND DISCUSSION

Phytochemical Analysis

Preliminary phytochemical screening of the methanolic bark extract of *Syzygium cumini* revealed the presence of alkaloids, flavonoids, phenolics, tannins, saponins, carbohydrates, and steroids, whereas proteins, amino acids, and glycosides were absent. These findings indicate that the extract contains multiple classes of bioactive compounds that may contribute to its pharmacological activity (Table 1).

Table 1: Phytochemical Screening of Methanolic Extract of *Syzygium cumini* Bark.

Phytochemical Class	Test Performed	Result
Alkaloids	Dragendorff's, Mayer's, Wagner's, Hager's	Present
Glycosides	Borntrager's, Keller–Killiani	Absent
Carbohydrates	Molisch's, Fehling's, Benedict's, Barfoed's	Present
Proteins & Amino acids	Biuret test	Absent
Flavonoids	Shinoda's test	Present
Tannins & Phenolics	Ferric chloride, Gelatin, Lead acetate	Present
Saponins	Froth test	Present
Steroids/Triterpenoids	Salkowski, Libermann–Burchard	Present

Percentage Yield

Soxhlet extraction of the bark powder produced different yields depending on the solvent used. Methanol extraction gave a higher yield (2.20% w/w) compared to petroleum ether (0.52% w/w), indicating that phytoconstituents of *Syzygium cumini* bark are more soluble in polar solvents.

Table 2: Percentage Yield of Crude Extracts of* Syzygium cumini *Bark.

Solvent	Theoretical Weight (g)	Yield (g)	% Yield (w/w)
Petroleum ether	297.00	1.56	0.52%
Methanol	299.15	6.61	2.20%

Quantitative Phytochemical Estimation

The methanolic extract of Syzygium cumini was analyzed for total phenolic content (TPC) and total flavonoid content (TFC). Phenolic and flavonoid compounds are known for their antioxidant, anti-inflammatory, and crystal inhibitory activities, suggesting a potential role in preventing kidney stone formation. The total phenolic content and total flavonoid content of the methanolic extract were found to be 54.66 mg GAE/g extract and 18.5 mg RE/g extract, respectively (Table 3). The TPC and TFC were calculated using standard calibration curves of Gallic acid and Rutin, respectively (Figure 2 for Gallic acid and Figure 3 for Rutin).

Table 3: Total Phenolic and Flavonoid Content of Methanolic Extract.

Extract	Total Phenolic Content (mg GAE/g)	Total Flavonoid Content (mg RE/g)
Methanol	54.66	18.5

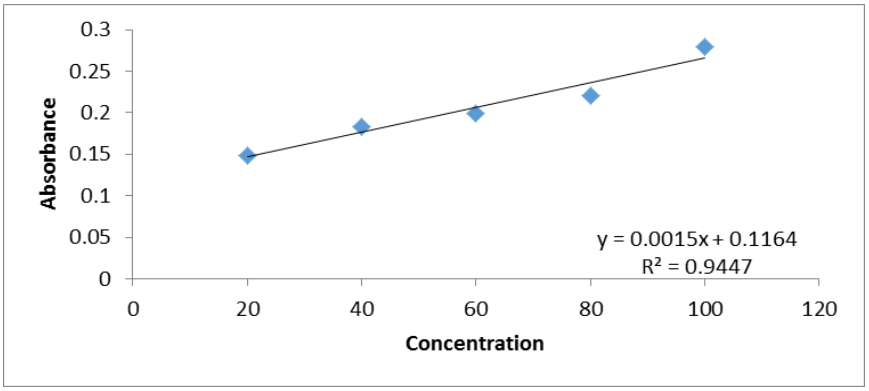

Figure 2: Standard curve of Gallic acid for TPC estimation.

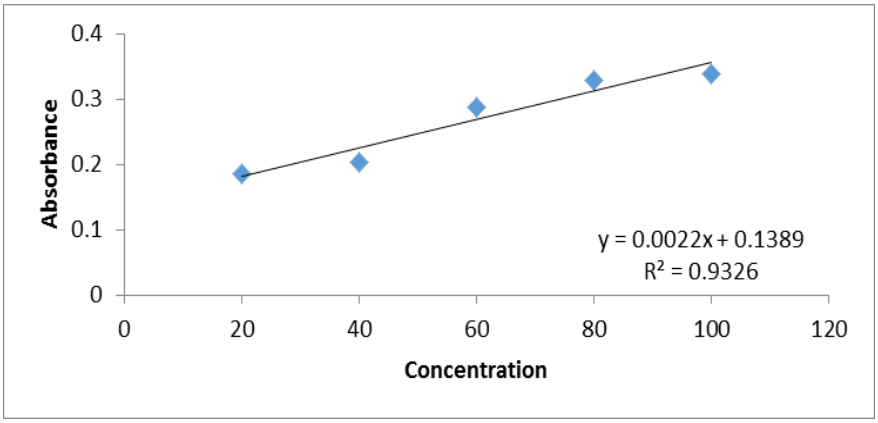

Figure 3: Standard curve of Rutin for TFC estimation.

Antioxidant Activity

The antioxidant potential of the methanolic extract of Syzygium cumini was evaluated using the DPPH radical scavenging assay. The extract exhibited dose-dependent free radical scavenging activity, indicating its capacity to neutralize reactive oxygen species. The IC_{50} value of the methanolic extract was 54.09µg/mL, whereas the standard antioxidant, ascorbic acid, showed a lower IC_{50} of 21.53 µg/mL (Table 4). Although the extract was less potent than ascorbic acid, it demonstrated substantial antioxidant activity, supporting its potential role in mitigating oxidative stress, which is a key factor in urolithiasis pathogenesis (Figure 4 and 5).

Table 4: DPPH Radical Scavenging Activity of Methanolic Extract and Standard (Ascorbic Acid).

Sample	Concentration (µg/mL)	% Inhibition	IC_{50} (µg/mL)
Ascorbic acid	20–100	51.16–84.99	21.53
Methanolic extract	20–100	43.29–59.87	54.09

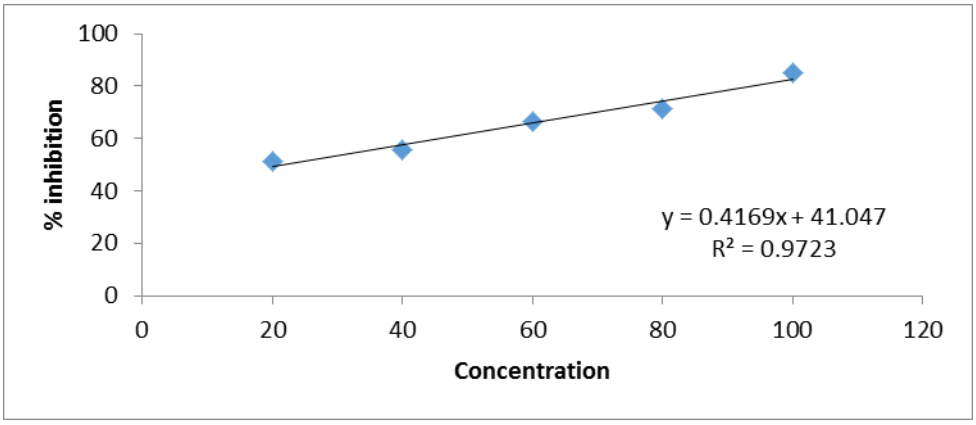

Figure 4: % Inhibition vs. Concentration of Ascorbic acid.

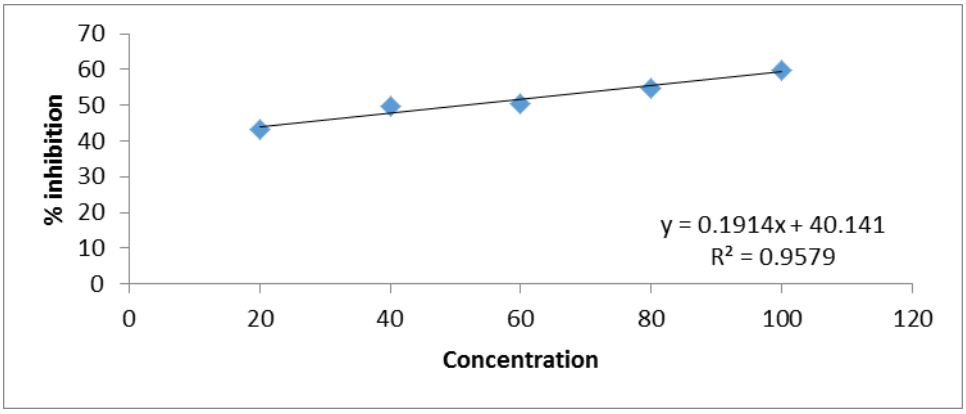

Figure 5: % Inhibition vs. Concentration of Methanolic extract.

ANTIUROLITHIATIC ACTIVITY

Serum Creatinine

Administration of ethylene glycol significantly increased serum creatinine levels (0.70 ± 0.01 mg/dL) compared to the control group (0.39 ± 0.02 mg/dL, Table 5), indicating renal impairment. Treatment with Syzygium cumini extract resulted in a dose-dependent reduction of creatinine levels, with the 200 mg/kg extract group (0.45 ± 0.02 mg/dL) approaching the values observed in the standard cystone-treated group (0.41 ± 0.01 mg/dL, Figure 6).

Table 5: Effect of* Syzygium cumini *Extract on Serum Creatinine Levels.

Study Group	Serum Creatinine (mg/dL)
Group I (Normal Saline)	0.39 ± 0.02
Group II (Inducer: Ethylene Glycol)	0.70 ± 0.01
Group III (100 mg/kg S. cumini)	0.62 ± 0.03
Group IV (200 mg/kg S. cumini)	0.45 ± 0.02
Group V (Standard: Cystone)	0.41 ± 0.01

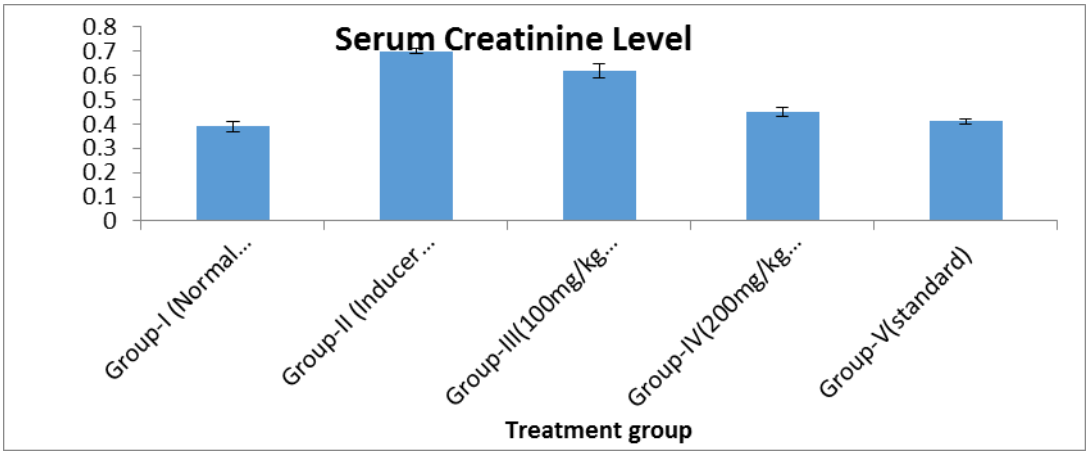

Figure 6: Serum Creatinine Levels Across Different Study Groups.

Serum Calcium

Lithiatic control group exhibited a marked increase in serum calcium levels (13.01 ± 0.10 mg/dL) compared to normal rats (7.52 ± 0.08 mg/dL, Table 6). Administration of S. cumini extract significantly reduced calcium levels in a dose-dependent manner, with the 200 mg/kg extract group (10.12 ± 0.09 mg/dL) showing superior efficacy over the 100 mg/kg dose (12.73 ± 0.06 mg/dL, Figure 7).

Table 6: Effect of *Syzygium cumini* Extract on Serum Calcium Levels.

Study Group	Serum Calcium (mg/dL)
Group I (Normal Saline)	7.52 ± 0.08
Group II (Inducer: Ethylene Glycol)	13.01 ± 0.10
Group III (100 mg/kg S. cumini)	12.73 ± 0.06
Group IV (200 mg/kg S. cumini)	10.12 ± 0.09
Group V (Standard: Cystone)	8.17± 0.07

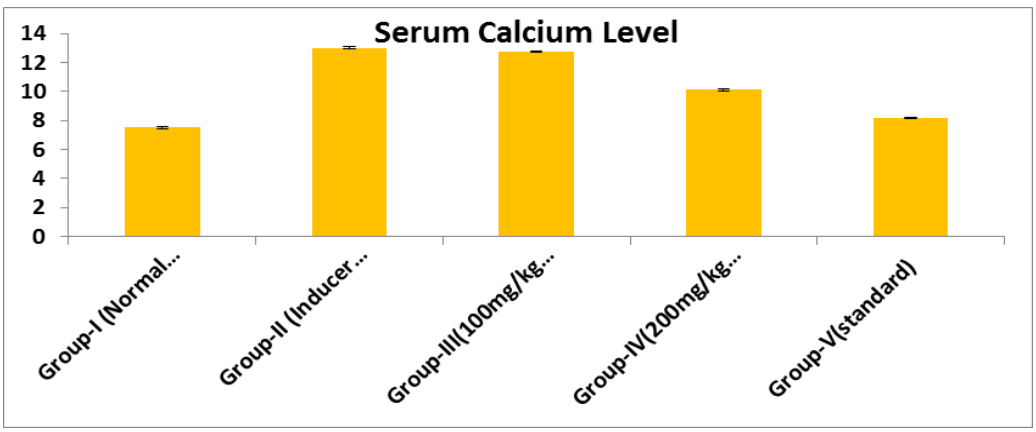

Figure 7: Serum calcium levels across study groups.

Serum Urea

Serum urea levels were significantly elevated in lithiatic rats (33.15 ± 0.08 mg/dL) compared to controls (4.21 ± 0.07 mg/dL, Table 7). Treatment with S. cumini extract resulted in a dose-dependent reduction, with the 200 mg/kg group (21.32 ± 0.09 mg/dL) showing greater improvement than the 100 mg/kg group (30.11 ± 0.06 mg/dL). The standard cystone-treated group exhibited the most significant reduction (15.34 ± 0.03 mg/dL, Figure 8).

Table 7: Effect of* Syzygium cumini *Extract on Serum Urea Levels.

Study Group	Serum Urea (mg/dL)
Group I (Normal Saline)	4.21 ± 0.07
Group II (Inducer: Ethylene Glycol)	33.15 ± 0.08
Group III (100 mg/kg *S. cumini*)	30.11 ± 0.06
Group IV (200 mg/kg *S. cumini*)	21.32 ± 0.09
Group V (Standard: Cystone)	15.34 ± 0.03

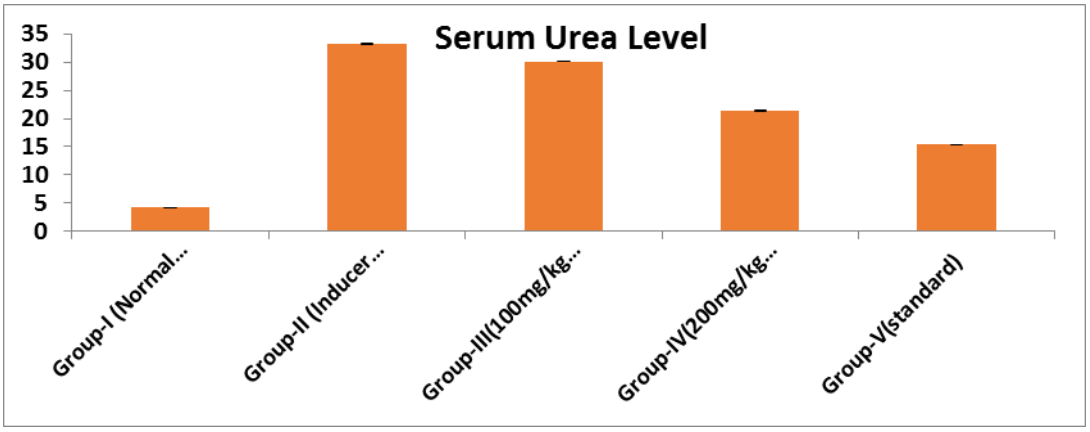

Figure 8. Serum urea levels across study groups.

Serum Phosphorus

Lithiatic rats showed elevated serum phosphorus levels (9.11 ± 0.11 mg/dL) compared to normal controls (7.50 ± 0.04 mg/dL, Table 8). Treatment with S. cumini extract lowered phosphorus levels, with the 200 mg/kg dose (8.76 ± 0.09 mg/dL) demonstrating better efficacy than the 100 mg/kg dose (8.85 ± 0.06 mg/dL, Figure 9).

Table 8: Effect of* Syzygium cumini *Extract on Serum Phosphorus Levels.

Study Group	Serum Phosphorus (mg/dL)
Group I (Normal Saline)	7.50 ± 0.04
Group II (Inducer: Ethylene Glycol)	9.11 ± 0.11
Group III (100 mg/kg S. cumini)	8.85 ± 0.06
Group IV (200 mg/kg S. cumini)	8.76 ± 0.09
Group V (Standard: Cystone)	7.98 ± 0.05

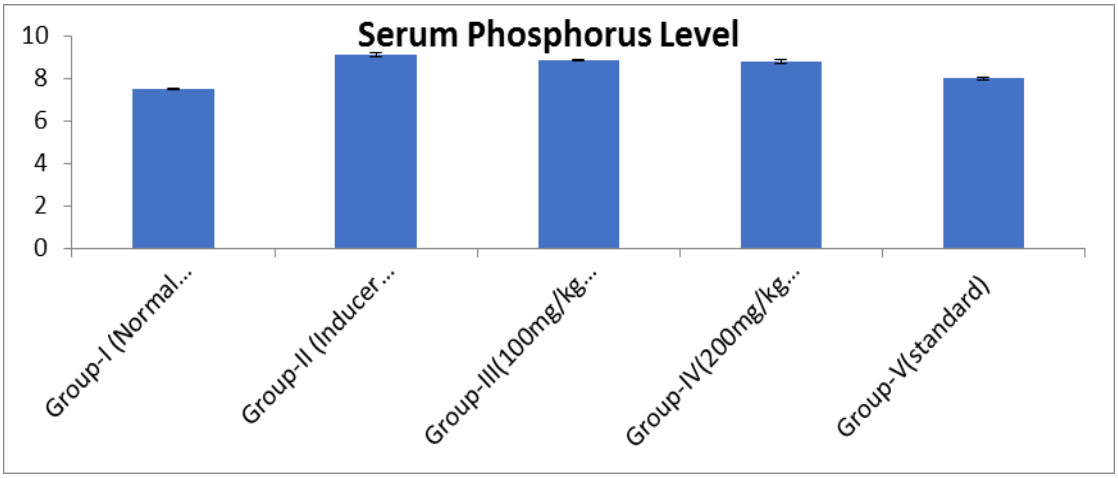

Figure 9: Serum Phosphorus levels across study groups.

CONCLUSION

The present study demonstrated that the methanolic extract of Syzygium cumini bark possesses significant phytochemical, antioxidant, and antiurolithiatic activities. The extraction yield was higher in methanol (2.20%) compared to petroleum ether (0.52%), indicating better solubility of bioactive compounds in polar solvents. Preliminary phytochemical screening confirmed the presence of alkaloids, flavonoids, phenolics, tannins, saponins, carbohydrates, and steroids, which are known to contribute to various pharmacological effects. Quantitative analysis revealed a total phenolic content of 54.66 mg GAE/g extract and a total flavonoid content of 18.5 mg RE/g extract, supporting the observed antioxidant activity. The DPPH assay demonstrated dose-dependent free radical scavenging, with an IC_{50} of 54.09 µg/mL, indicating the extract's potential to mitigate oxidative stress, a key factor in urolithiasis pathogenesis. In the ethylene glycol-induced urolithiasis model, the methanolic extract significantly reduced serum creatinine, calcium, urea, and phosphorus levels in a dose-dependent manner. The 200 mg/kg dose exhibited superior efficacy, approaching the effects of the standard drug cystone, suggesting strong antiurolithiatic potential. The study supports that Syzygium cumini bark extract is a promising natural agent for the prevention and management of urolithiasis, likely due to its antioxidant, crystal inhibitory, and nephroprotective properties. Further studies on mechanistic pathways and active constituents are warranted to validate its therapeutic potential.

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