

# Evaluation of Polyherbal Preparation Containing *Aegle marmelos* and *Saraca asoca* Plants for Screening of Urolithiasis Disease

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## ABSTRACT

**Aim:** To evaluate the efficacy of a polyherbal formulation in alleviating urolithiasis induced by ethylene glycol in a rat model and to assess its effects on biochemical and histopathological parameters. **Background:** Urolithiasis is a condition characterized by the formation of stones in the urinary tract, often leading to significant health issues. This study investigates the anti-urolithiatic effects of a polyherbal formulation containing *Aegle marmelos* and *Saraca asoca* in albino Wistar rats. To investigate the anti-urolithiatic effects of a polyherbal formulation containing *Aegle marmelos* and *Saraca asoca* in albino Wistar rats. **Materials and Methods:** 36 albino Wistar rats were divided into six groups. Groups II, III, IV, V, and VI received drinking water containing 0.75% Ethylene Glycol (EG) to induce urolithiasis, while Group I served as the negative control. Groups IV and V were administered methanolic extracts of *Saraca asoca* and *Aegle marmelos* at a dose of 400 mg/kg body weight, while Group VI received the polyherbal formulation at the same dosage. After 28 days, various parameters, including urine volume, urinary oxalate and calcium levels, serum biochemical markers (blood urea, creatinine, uric acid), and histopathological changes in renal tissues, were assessed. **Results:** The positive control group exhibited significant decreases in urine volume, increases in urinary oxalate and calcium levels, and notable calcium oxalate deposits in the kidneys compared to the negative control group. Serum analysis indicated elevated levels of blood urea, creatinine, and uric acid in the urolithiasis control group. Conversely, treatment with the polyherbal formulation significantly reversed these biochemical alterations and reduced calcium oxalate deposits. Histopathological evaluations revealed reduced tissue damage and inflammation in the treatment groups compared to the control group. **Conclusion:** The polyherbal formulation demonstrated significant anti-urolithiatic effects in the rat model, reversing biochemical changes and alleviating kidney damage associated with urolithiasis. Further studies are warranted to explore the underlying mechanisms and potential clinical applications of this formulation.

**Keywords:** Urolithiasis, Urinary parameters, Serum parameters, Ethylene glycol, *Aegle marmelos*, *Saraca asoca*.

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**Received:** 17-04-2025;

**Revised:** 09-06-2025;

**Accepted:** 29-08-2025.

## INTRODUCTION

Kidney stones, sometimes referred to as urolithiasis, are a disorder in which the urinary tract gets clogged with hard deposits of salts and minerals. The four most widespread forms of urinary stones are cystine, struvite, uric acid, and calcium stones.<sup>1</sup> Urolithiasis is triggered by the supersaturation of urine with chemical substances that crystallize, leading to crystals to form and aggregate. Conditions like hypercalciuria and other metabolic diseases frequently have an impact on this process.<sup>2</sup> Studies indicate that men are more susceptible to stones in

their kidneys, which is a significant factor in the incidence of urolithiasis. Men have been affected to a greater extent than women (19% vs 9%).<sup>3</sup> This variation is linked to a number of variables, such as hormonal variations, dietary practices, and the existence of specific medical disorders like diabetes and obesity, which are more strongly associated with the development of stones in men. Severe flank difficulty, haematuria, and nausea tend to be indications. About 50% of patients will experience another episode of this illness within five years.<sup>4</sup> Medical treatments for urolithiasis include pain management and dietary modifications and Surgical treatments include ureteroscopy and extracorporeal shockwave lithotripsy. A rise in curiosity has been seen recently in the use of polyherbal formulations as an adjunctive method of treating urolithiasis. Due to their synergistic effects, which include diuretic and anti-inflammatory qualities, these formulations, which include a variety of medicinal herbs



DOI: 10.5530/ijper.20261403

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like *Boerhaavia diffusa*, *Moringa oleifera*, and *Aerva lanata*, have demonstrated promise for their antilithiatic properties.<sup>5,6</sup> Due to nephroprotective and therapeutic qualities of *Aegle marmelos* (L.) Corr. and *Saraca asoca* (Roxb.) De Wilde are combined in polyherbal formulations to increase the preparation's overall efficacy. *Aegle marmelos* is particularly included because of its well-established antioxidant qualities, which help avoid oxidative stress and renal damage (Prasad *et al.*, 2013).<sup>7</sup> Its capacity to lessen renal inflammation and avoid nephrotoxicity supports its use in polyherbal formulations intended to promote kidney health and prevent urolithiasis (Sathya *et al.*, 2018).<sup>8</sup> The strong nephroprotective qualities of *Saraca asoca*, which are ascribed to its anti-inflammatory and antioxidant components, make it a component of polyherbal compositions. Research conducted by Agrawal *et al.*, (2014) has indicated that it is a useful tool for treating kidney stones and associated disorders because it can effectively reduce renal damage and improve renal function. *Aegle marmelos* and *Saraca asoca* work in concert in these formulations to maximize their complementary benefits, offering a holistic strategy for kidney preservation and urolithiasis control.<sup>9</sup>

## MATERIALS AND METHODS

### Procurement and authentication

Fresh leaves of *Aegle marmelos* and bark from *Saraca asoca* were collected from the serene surroundings of the Botanical Garden. The taxonomic identification was made by Dr. Priyanka Ingle, scientist C, Botanic Garden of Indian Republic GB Nagar Noida, UP, India with specimen voucher no. BSI/BGIR/1/TECH./2024/87 on 15/02/2024.

### Collection, identification and extraction

Prior to further procedures, the leaves and bark of both *Aegle marmelos* and *Saraca asoca* were meticulously collected. The collected plant materials were then thoroughly cleaned to remove any foreign substances and left to dry in the shade for several days. After drying, each plant material was finely crushed into a coarse powder. The powder was used for extraction. Extraction was then carried out separately for *Aegle marmelos* leaves and *Saraca asoca* bark with 200 mL of methanol using Soxhlet apparatus by maintaining the temperature between 40°C to 50°C for 24 hr. The obtained extracts were condensed with a vacuum rotary evaporator. Subsequently, the condensed remains were placed in pre-weighed sample vials and stored in desiccators for future analysis. By applying the correct formulas, the extract yields were calculated to evaluate the efficiency of the extractions.<sup>10,11</sup>

### Preliminary phytochemical screening of methanolic extract of *Aegle marmelos* (MEAM) and Methanolic Extract of *Saraca asoca* (MESA)

A comprehensive investigation was conducted to explore the diverse phytochemical constituents present in the MEAM and

MESA. Different solvents were used to extract these components, which were then examined for essential metabolites including steroids, proteins, alkaloids, carbohydrates, flavonoids, and glycosides. The plant extracts exhibited notable physiological effects and demonstrated potential benefits. The detailed examination of the extracts showed clear signs of metabolites. Conducting preliminary phytochemical research is crucial for the isolation of the pharmacologically active compounds in these plants.<sup>12,13</sup> Phytochemical screening of *Aegle marmelos* and *Saraca asoca* revealed the presence of several key bioactive compounds. *A. marmelos* exhibited the presence of alkaloids, flavonoids, tannins, polyphenols, saponins, terpenoids, cardiac glycosides, and steroids, while lacking phlobatannins and carbonyls. *S. asoca* was found to contain flavonoids, tannins, cardiac glycosides, steroids, polyphenols, saponins, triterpenoids, and alkaloids. These findings provide a basis for further investigation into the potential therapeutic properties of these plants (Tables 1 and 2).

### Preparation of Polyherbal Suspension

The polyherbal formulation was prepared by combining the Methanolic Extract of *Aegle marmelos* (MEAM) with the Methanolic Extract of *Saraca asoca* (MESA) equal parts. This mixture was then dissolved in water. To improve the stability and effectiveness of the formulation, several stabilizers and preservatives were incorporated. Tween-80 served as a surfactant to ensure even distribution of the extracts, sodium CBC was used as a thickening agent to achieve the desired consistency, and methyl paraben was added to prevent microbial contamination. The final mixture was thoroughly blended to ensure uniformity and stored under suitable conditions for future experimental use.

### Chemical and apparatus

Ethylene glycol, HCl, Ethanol, Ketamine was obtained from Noida Institute of Engineering and Technology (Pharmacy Institute), U.P., India.

### Route of Administration (ROA)

The above polyherbal formulation and standard drug were administered by oral route to respective groups using oral catheter.

### Investigation of Acute oral toxicity in rat

(OECD 423, 2001) the Organization for Economic Co-operation and Development's recommendations were adhered to in the assessing acute toxicity. The toxicity experiments were conducted using Wistar rats as the subjects. From the animals, three groups of three rats each were formed. Before the experimental process started, the animal was fasted for the whole night. The acute toxicity study was designed to monitor any possible pharmacological or behavioural changes brought on by the extract's administration, as well as to establish safe dose limits for further pharmacological research. Rats were given the extract

orally at doses ranging from 500 to 2000 (500, 1000, 2000) mg per kilogram of body weight. After the treatment, the animal was examined every day for 14 days, including a 24-hr observation period after the first 4 hr to identify any behavioural changes. After that, the animal's mortality was evaluated. The results showed that a single dosage of 2000 mg/kg body weight of the extracts was safe. As an outcome, it was decided that providing experimental animals 1/10<sup>th</sup> of this dosage, or 400 mg/kg b.w., would be suitable.<sup>14</sup>

### Experimental animal

For both the anti-urolithiatic and anti-hypertensive activity studies, albino Wistar rats weighing 180-200 g were used. They came from the Noida Institute of Engineering and Technology (Pharmacy Institute), Central Animal House in Greater Noida. IAEC/NIET/2022/02/23 is the IAEC approval number for this study. The animals were adapted to typical conditions, which comprised a room temperature ranging from 18 to 25±2°C, humidity levels between 45 and 55%, a 12-hr light/dark cycle, and an unrestricted supply of food and water in the form of mouse feed. Under the CCSEA oversight, every inquiry complied to moral standards.

They were randomly divided into positive control, negative control, plant A, plant B and polyherbal groups and treated according to the experimental protocols for 28 days.<sup>15,16</sup>

### Ethylene Glycol (EG) induced urolithiasis model

Development of kidney calculus (uroliths) due to the ingestion of ethylene glycol is referred to as ethylene glycol-induced urolithiasis. The ethylene glycol-induced urolithiasis model in rats was chosen for this study due to its established use in evaluating the efficacy of potential anti-urolithiatic agents.<sup>17</sup>

Ethylene glycol, a toxic compound commonly found in antifreeze, poses serious health risks including kidney stone formation. Upon ingestion, ethylene glycol is rapidly absorbed from the gastrointestinal tract. In the liver, it undergoes metabolism by alcohol dehydrogenase and aldehyde dehydrogenase, resulting in the generation of harmful substances such as glyoxylic acid, glycolic acid and oxalic acid, with oxalic acid specifically associated with the pathogenesis of urolithiasis.

### Antiuro lithiatic activity of polyherbal extract procedure

Using an Ethylene Glycol (EG)-induced urolithiasis model, the antiuro lithiatic efficacy of polyherbal preparation was assessed. The selected animals were split into six groups, each having six animals. As the control group, Group I was given access to unlimited water and ordinary rat food. Groups II-VII were provided with a solution containing 0.75 % ethylene glycol with the aim of causing kidney stone formation until the 28<sup>th</sup> day. Group III was received cystone (750 mg/kg b.w.), a typical

antiuro lithiatic medication, between day 15 and day 28, Group VI was administered a polyherbal extract regularly at a dosage of 400 mg/kg, MESA was given to Group V at a dose of 400 mg per kilogram of total body weight, and MEAM was delivered to Group IV at exactly the same dosage.

Group I: Negative control: Animals were treated with regular rat food and water *ad libitum*.

Group II: Positive control: Ethylene glycol (0.75%) drinking water.

Group III: Standard: EG (0.75% v/v)+Cystone (750 mg/kg) p.o.

Group IV: Plant A: EG (0.75% v/v)+MEAM (400 mg/kg) p.o.

Group V: Plant B: EG (0.75% v/v)+MESA (400 mg/kg) p.o.

Group VI: Polyherbal: EG (0.75% v/v)+methanolic extract of *Aegle marmelos*+*Saraca asoca* 400 mg/kg p.o.

### Assessment of urolithiasis

#### Examination of urine

Every animal was housed in a separate metabolic cage, and on the 28<sup>th</sup> day, samples of pee from the previous 24 hr were taken. Water to drink was readily available to the animals during the pee collection time. Before the urine was kept at 4°C, one drop of strong HCl was added. Calcium, magnesium, and phosphorus, uric acid, urea, oxalate levels in urine were measured.<sup>18</sup>

#### Serum analysis

Samples of blood have been collected under sedation from the retro-orbital sinus once the patient had been unconscious. After centrifuging the serum at 10,000×g for 10 min, the quantity of urea nitrogen, uric acid, and creatinine were assessed.

#### Histopathological Studies

Rats were given ether anaesthesia and then thrown to death at the completion of the study the kidneys, A surgical opening was made in the abdominal region to retrieve and prepare the kidney which were then cleaned and stored in 10% neutral formalin. One kidney was fixed in formalin and then encased in paraffin. Slices of about 5 µm in thickness were subsequently derived from these kidney samples and stained using hematoxylin-eosin solution. These stained slices were then scrutinized under both regular and polarized light microscopy to document and assess any histopathological alterations.

#### Statistical investigation

GraphPad software Version 6.0 was employed for examining the results. One-way analysis of variance, or ANOVA, and Dunnett's correction were the methods used. The findings are shown as Mean±SEM. With significance levels established at  $p<0.05$ ,  $p<0.01$ , and  $p<0.001$ ,  $p<0.0001$  comparisons were carried out between groups receiving standard therapies, different

extracts, and extract combinations in relation to the positive control group.<sup>18</sup>

## RESULTS AND DISCUSSION

A key limitation of this study is its reliance on the ethylene glycol-induced urolithiasis rat model. While this model is widely used and accepted for studying various aspects of kidney stone formation,<sup>19</sup> it does not fully mimic the complex pathophysiology of human urolithiasis. Human stone formation is often multifactorial, involving factors such as genetics, diet, urinary pH, and underlying medical conditions, which are not always fully captured in the EG model.

### Impact of polyherbal preparation on urinary markers and urine output

In the study, treatments were evaluated for their ability to normalize biochemical parameters and increase urine volume in ethylene glycol-induced urolithiasis. The standard treatment with cystone (750 mg/kg) was the most effective, significantly increasing urine volume to  $11.6 \pm 1.27$  mL and reducing levels of calcium, urea, uric acid, oxalate, and phosphate (all  $p < 0.0001$ ). The polyherbal preparation also showed effectiveness, increasing urine volume to  $7.11 \pm 0.47$  mL and decreasing biochemical markers (all  $p < 0.001$ ), though slightly less effective than cystone. Herbal plants A (*Aegle marmelos*) and B (*Saraca asoca*) showed moderate improvements but were less effective compared to the polyherbal and standard treatments. Overall, cystone was the most effective, followed by the polyherbal preparation, with the individual herbal plants being the least effective (Table 3).

### Comparative Analysis of Urinary Biomarkers: Standard vs. Polyherbal Treatment

The bar graph represents urinary biochemical markers, including calcium, urea, uric acid, urine volume, oxalate, and phosphate,

across six experimental groups. Data are expressed as mean  $\pm$  SEM ( $n=6$ ), with statistical significance denoted as \*\*\*\* $p < 0.0001$ , \*\*\* $p < 0.001$ , \*\* $p < 0.01$ , and \* $p < 0.05$ .

Group II exhibited a substantial increase in urinary urea, calcium, oxalate, and uric acid levels, indicating renal dysfunction and stone formation. Treatment groups (III-VI) showed a significant reduction in these parameters, demonstrating nephroprotective effects. Notably, the polyherbal formulation (Group VI) exhibited the most pronounced improvement, restoring urinary biomarkers toward physiological levels and enhancing urine output, suggesting superior anti-urolithiatic efficacy.

### Impact of polyherbal preparation on serum parameter

In the ethylene glycol-induced urolithiasis model, the control group maintained normal BUN, creatinine, and uric acid levels, while the ethylene glycol group had significantly elevated levels, indicating kidney damage. The standard treatment with cystone (750 mg/kg) significantly reduced these markers, with BUN at  $39.1 \pm 0.36$  mg/dL, creatinine at  $0.76 \pm 0.23$  mg/dL, and uric acid at  $1.61 \pm 0.23$  mg/dL (all  $p < 0.0001$ ). The polyherbal treatment (400 mg/kg) also showed significant reductions, with BUN at  $43.4 \pm 5.31$  mg/dL, creatinine at  $0.79 \pm 0.07$  mg/dL, and uric acid at  $1.73 \pm 0.16$  mg/dL (all  $p < 0.001$ ), but it was slightly less effective than the standard treatment (Figure 1). The individual extracts from *Aegle marmelos* and *Saraca asoca* were less effective, showing moderate improvements in the serum parameters (Table 4). Overall, Cystone was the most effective treatment, followed by the polyherbal preparation (Figure 2).

The synergistic mechanism of *Aegle marmelos* and *Saraca asoca* in a polyherbal formulation is attributed to the complementary pharmacological actions of their bioactive compounds. Their combined anti-urolithiatic action is facilitated by the presence of flavonoids, tannins, and polyphenols in *Aegle marmelos*,

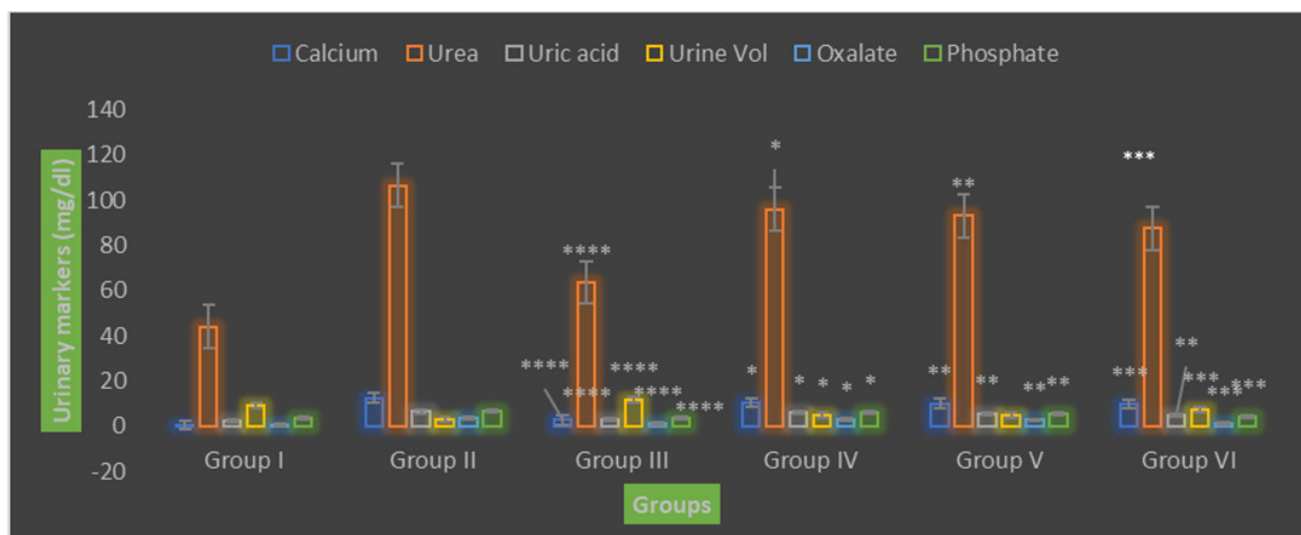
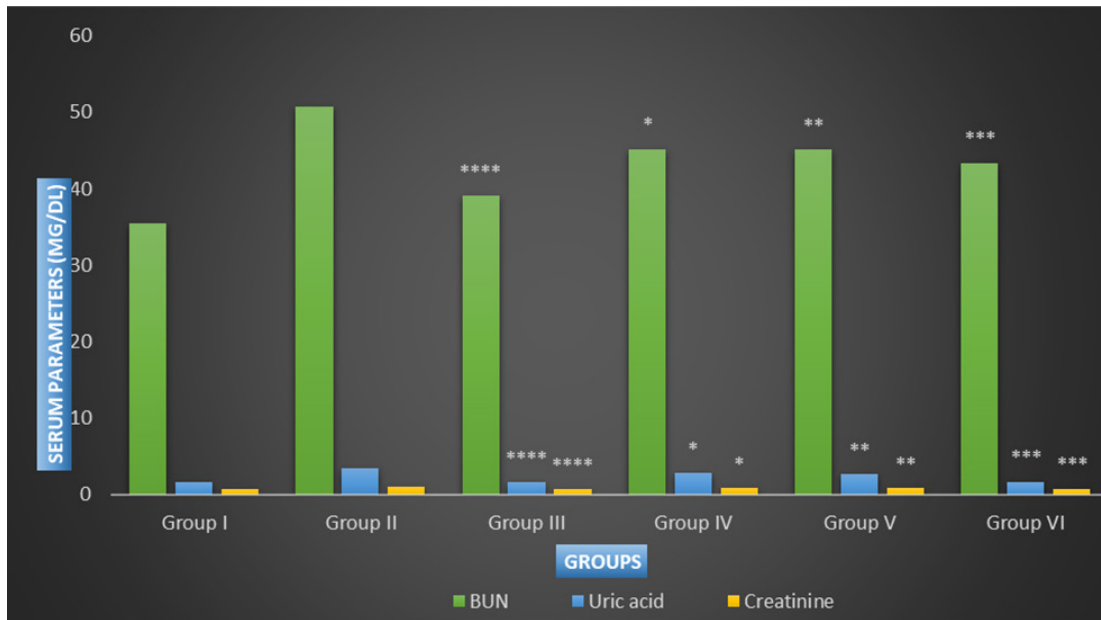
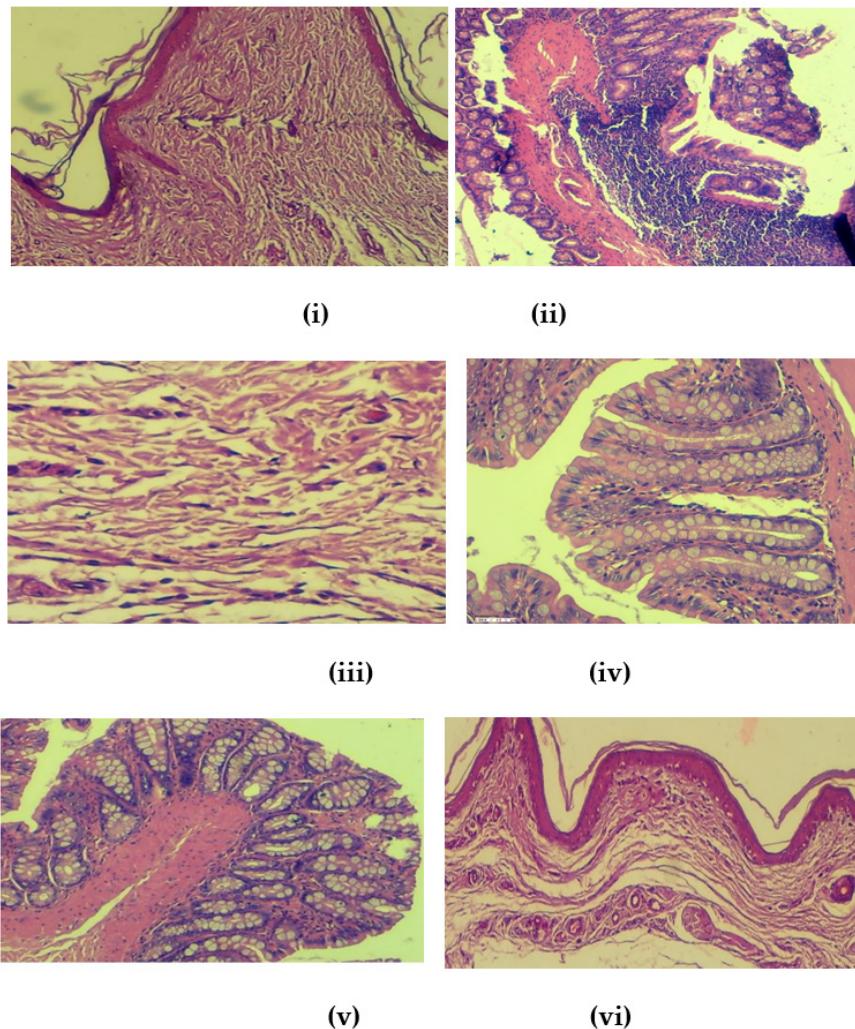


Figure 1: Effects of Polyherbal Formulation on Urinary Metrics and Urine Output.



**Figure 2:** Comparative Renal Function Markers: Standard vs. Polyherbal Treatment. All values are depicted as mean  $\pm$ SEM ( $n=6$ ): \*\*\*\* $p<0.0001$  inducing group vs standard. \* $p<0.05$  inducing group vs plant A, \*\* $p<0.01$  inducing group vs plant B, \*\*\* $p<0.001$  inducing group vs polyherbal.



**Figure 3:** Histology of kidney tissue.

**Table 1: Identification tests for phytoconstituents of *Aegle marmelos*.**

Phytochemical test	Finding	Result
Alkaloids	Reddish-brown appearance in alkaloids	+++
Flavonoids	Yellow to colourless solution	++
Tannin	Dark blue to Black	++
Polyphenol	Blue Green/red color	+
Saponins	Appearance of creamy mass	+
Terpenoids	Reddish brown interface	+
Cardiac glycosides	Brown ring formation	+
Steroids	Violet to blue to green color	+
Coumarin	Yellow color	+
Phlo-battins	No red precipitate	-
Carbonyl	No yellow crystal formation	-

(+) indicates presence and (-) indicates absence of phytoconstituents.

**Table 2: Identification tests for phytoconstituents of *Saraca asoca***

Phytochemical test	Observation	Result
Flavonoids	Yellow to colourless	+++
Tannin,	Dark blue to Black	++
Cardiac glycosides	Brown ring formation	+
Steroids	Violet to blue to green color	+
Polyphenol	Blue Green/red color	+
Saponins	Appearance of creamy mass	+
Triterpenoids	Reddish brown interface	+
Alkaloids	Reddish-brown coloration	+

(+) indicates presence and (-) indicates absence of phytoconstituents.

**Table 3: Impact of Polyherbal preparation on Urinary markers in Ethylene Glycol-triggered Urolithiasis.**

Groups	Urine volume	Calcium	Urea	Uric acid	Oxalate	Phosphate
Negative Control	9.3±0.14	0.45±0.10	44±1.41	2.50±0.20	0.36±0.03	3.43±0.41
Positive control	3.04±0.69	12.6±0.47	106.5±0.79	6.45±0.18	3.62±0.24	6.90±0.68
Standard	11.6±1.27****	2.71±0.06****	63.6±0.43****	2.98±0.3****	0.95±0.17****	3.51±0.37****
Plant A	5±0.32***	10.5±1.37*	96±6.22*	5.85±0.48*	3.03±0.54**	5.9±0.56*
Plant B	4.86±0.53***	10.1±1.47**	93.1±12.0**	5.68±0.62**	2.78±0.22***	5.5±0.94**
Polyherbal	7.11±0.47***	9.83±1.47***	87.5±10.09***	5±0.30***	1.32±0.31***	4.1±0.48***

All values are recorded as Mean ±SEM (n=6): \*\*\*\*p<0.0001 inducing group vs standard. \*p<0.05 inducing group vs plant a, \*\*p<0.01 inducing group vs plant b, \*\*\*p<0.001 inducing group vs polyherbal. Table 4 Effect of polyherbal formulation on Serum parameter (BUN, Creatinine, Uric acid) in Ethylene Glycol-Induced Urolithiasis Model.

which exhibit potent antioxidant and diuretic properties. These compounds help reduce oxidative stress-induced kidney damage and enhance urine output, aiding in the dissolution and elimination of kidney stones. *Saraca asoca*, on the other hand, is rich in tannins, glycosides, and triterpenoids, which provide nephroprotective effects by preventing calcium oxalate crystal formation and mitigating inflammatory damage in renal tissues. Additionally, their nephroprotective and anti-inflammatory effects work in conjunction to protect renal tissues. *Aegle marmelos* inhibits pro-inflammatory cytokines and oxidative stress markers, preventing ethylene glycol-induced urolithiasis, while *Saraca asoca* enhances renal blood flow and reduces inflammation, further lowering the risk of kidney stone formation. The regulation of urinary biomarkers is another crucial aspect of their synergy. Both plants help reduce urinary calcium, oxalate, phosphate, and uric acid levels, with flavonoids in *Aegle marmelos* preventing oxalate deposition and tannins in *Saraca asoca* stabilizing renal function by maintaining electrolyte balance. Moreover, their combination enhances diuretic activity,

increasing urine volume and promoting the expulsion of crystallized materials, thereby preventing stone aggregation. This synergistic interaction between *Aegle marmelos* and *Saraca asoca* results in a superior anti-urolithiatic effect compared to the use of individual plant extracts, making their combination highly effective in managing kidney stone formation and related complications.

The bar graph illustrates the serum biochemical parameters Blood Urea Nitrogen (BUN), Uric Acid, and Creatinine across six experimental groups. Group II exhibits the highest BUN levels, indicating renal dysfunction, while treatment groups (Groups III to VI) show a significant reduction in BUN levels compared to Group II, with varying degrees of statistical significance (\*p<0.05 to \*\*\*\*p<0.0001). Similarly, uric acid and creatinine levels are elevated in Group II but significantly decrease in treatment groups, suggesting nephroprotective effects. The results indicate that the polyherbal formulation effectively mitigates renal impairment, restoring serum biomarkers towards normal levels.

**Table 4: Effect of polyherbal formulation on Serum parameter (BUN, Creatinine, Uric acid) in Ethylene Glycol-Induced Urolithiasis Model.**

Experimental groups	BUN parameter	Creatinine parameter	Uric acid parameter
I Normal Control	35.5±0.82	0.77±0.06	1.6±0.23
II Positive control	50.8±0.34	0.95±0.03	3.58±0.25
III Standard	39.1±0.36****	0.76±0.23****	1.61±0.23****
IV Plant A	45.2±4.03*	0.86±0.05*	2.83±0.55*
V Plant B	45.1±3.98**	0.85±0.07**	2.75±0.74**
VI Polyherbal	43.4±5.31***	0.79±0.07***	1.73±0.16***

All values are denoted as Mean±SEM (n=6): \*\*\*\*p<0.0001 inducing group vs standard. \*p<0.03 inducing group vs plant A, \*\*p<0.01 inducing group vs plant B, \*\*\*p<0.001 inducing group vs polyherbal.

## Histopathology

Histopathological investigations revealed varying degrees of renal abnormalities, inflammation, and stone formation among treatment groups. The control group exhibited normal renal histology. Ethylene glycol-treated rats showed severe abnormalities, inflammation, and extensive stone formation. Cystone-treated rats demonstrated minimal abnormalities, inflammation, and stone formation, indicating effective recovery. Herbal treatments Plants A (*Aegle marmelos*) and B (*Saraca asoca*) showed moderate improvements, reducing abnormalities and inflammation to some extent (Figure 3). Polyherbal treatment showed significant improvement, although slightly less effective than the standard treatment.

The images presented as (i), (ii), (iii), (iv), (v), and (vi) which showed the normal control, ethylene glycol-treated, Cystone-treated (standard), Herbal Plant A, Herbal Plant B, and Polyherbal treatment groups, respectively.

## CONCLUSION

The study showed that a polyherbal blend containing *Aegle marmelos* and *Saraca asoca* significantly improved ethylene glycol-induced urolithiasis in albino Wistar rats. This formulation effectively reversed the biochemical disturbances associated with urolithiasis, reduced calcium oxalate deposits in the kidneys, and enhanced renal function indicators such as urine volume and serum parameters. Histopathological evaluations confirmed these results, revealing less tissue damage and inflammation in the treated rats. These outcomes suggest that the polyherbal blend has a strong anti-urolithiatic effect, potentially offering a natural therapeutic approach for both preventing and managing kidney stones.

This study highlights the promising therapeutic potential of the polyherbal formulation comprising *Aegle marmelos* (MEAM) and *Saraca asoca* (MESA) in managing urolithiasis. The formulation demonstrated significant anti-urolithiatic effects by reducing urinary excretion of key biomarkers such as calcium, oxalate, and uric acid, while improving kidney function markers like serum creatinine and urea. Histopathological analysis further

confirmed its nephroprotective effects. These findings suggest that the polyherbal combination could serve as an adjunct to current urolithiasis treatments, potentially providing a novel alternative therapy for individuals with recurrent kidney stones. The formulation's antioxidant and anti-inflammatory properties, as indicated by the modulation of markers like NF-kB and TNF- $\alpha$ , offer broader potential in mitigating oxidative stress and inflammation, which are crucial in the progression of renal disorders. The safety profile observed in acute toxicity studies, coupled with its efficacy, makes it a promising candidate for long-term use, particularly for individuals at high risk of developing kidney stones. Furthermore, the identification of molecular markers such as KIM-1 highlights the mechanistic pathways of the formulation, providing opportunities for personalized medicine in the management of kidney disease. Moving forward, larger clinical trials and studies exploring the pharmacokinetics, bioavailability, and long-term safety of this polyherbal remedy are essential to confirm its therapeutic efficacy in human populations. Future research could also explore synergistic formulations with other nephroprotective agents to enhance its potency and effectiveness in treating urolithiasis and other kidney-related conditions.

## ACKNOWLEDGEMENT

Authors acknowledge expressing our sincere gratitude to the management of the Noida Institute of Engineering and Technology, (Pharmacy Institute) for continuous support, motivation, enthusiasm, and immense knowledge.

## ABBREVIATIONS

**MEAM:** Methanolic extract of *Aegle marmelos*; **MESA:** Methanolic extract of *Saraca asoca*; **BUN:** Blood urea nitrogen; **OECD:** Organisation for Economic Co-operation and Development; **CCSEA:** The Committee for Control and Supervision of Experiment on Animals; **EG:** Ethylene glycol; **HCL:** Hydrochloric acid; **IAEC:** Institutional Animal Ethical Committee.

## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Study protocol was sanctioned from the Institutional Animal Ethics Committee and the regulation was approved by CCSEA (Protocol No- IAEC/NIET/2023/01/04).

## SUMMARY

A study was conducted to evaluate the anti-urolithiatic effects of a polyherbal formulation containing *Aegle marmelos* and *Saraca asoca* in albino Wistar rats, using ethylene glycol to induce urolithiasis. The results showed that the polyherbal formulation significantly reversed biochemical alterations, including reducing urinary oxalate and calcium levels, and alleviated kidney damage by reducing calcium oxalate deposits. Histopathological analysis revealed less tissue damage and inflammation in treated rats. The formulation demonstrated promising anti-urolithiatic effects, suggesting its potential for managing urolithiasis. Further studies are recommended to explore its mechanisms and clinical applications

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**Cite this article:** Pooja, Mazumder A, Das S. Evaluation of Polyherbal Preparation Containing *Aegle marmelos* and *Saraca asoca* Plants for Screening of Urolithiasis Disease. *Indian J of Pharmaceutical Education and Research*. 2026;60(1):183-90.