Anti-Urolithiatic Activity of An Aqueous Extract of *Leucas Aspera* Leaves in Ethylene Glycol (Eg)-Induced Urolithiasis Activity

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Abstract- Kidney stones, a major urology problem, are formed due to decreased urine volume or increased excretion of stone-forming components. Men are more likely to develop stones due to increased testosterone and estrogen levels, tissue breakdown, and metabolic waste. Pharmacological therapy for urolithiasis utilizing medicinal plants has been increasingly used to prevent recurrence. The evaluation of an aqueous extract of *Leucas Aspera* leaves in ethylene glycol induced (EG) urolithiasis activity on Albino Rat. Group I-Control were as received 5 % CMC (10ml/kg), Group II- received Ethylene glycol (1ml/kg), Group III- Received Ethylene glycol (0.75%) and cystone (Std) 750 mg/kg, Group IV- Received Ethylene glycol(075%) + AELA 250 mg/kg and Group V- Received Ethylene glycol(0.75%) + AELA 500mg/kg. Urolithiasis was induced by supplying drinking water mixed with 2% Ammonium chloride and 0.75% Ethylene glycol for 10 days. On 11th day three rats from each Group were kept in one metabolic cage and urine (pooled) collected for 24h was subjected for estimation of biochemical parameters like Urine volume, Urine PH, Creatinine, Calcium, Magnesium, Oxalate, Phosphate, Serum Constituents (Urea, Creatinine & Blood Urea Nitrogen). The result was showed that the ethanolic extracts of the plant produced significant anti-urolithiatic activity like that of standard.

Key Words: Ethylene glycol, Cystone, *Leucas Aspera,* Calcium oxalate.

INTRODUCTION

Urolithiasis, commonly known as kidney stones, is a prevalent urological condition affecting a significant portion of the global population. In Western societies, urolithiasis affects around 10% of individuals by the age of 70, and the incidence is increasing worldwide, particularly in developing countries (Monico and Milliner, 2011; Razi et al., 2024). The condition exhibits significant variations in prevalence depending on geographic and demographic factors. For instance, a study in Iran indicated that 5.7% of the country's adult population had experienced urinary stones (1).

Urolithiasis can manifest in various forms and is categorized based on the stone's composition. The most common type is calcium oxalate stones, which result from metabolic tendencies like hypercalciuria (2). In children, these stones often occur in the context of metabolic abnormalities such as hypercalciuria and uric acid hyperexcretion, leading to significant symptoms like abdominal pain and hematuria (Perrone et al., 1992; Gearhart et al., 1991). In adults, additional factors including dietary influences, genetic predispositions, and lifestyle components like obesity and hypertension appear to contribute to urolithiasis risk (1).

A broad range of symptoms is associated with urolithiasis, though these can often be nonspecific in nature. Commonly reported symptoms include flank or abdominal pain, restlessness, vomiting, and haematuria (Baştuğ et al., 2012; Perrone et al., 1992). The presence of urinary stones often correlates with urinary tract infections, which may exacerbate symptoms and risks associated with stone formation (3).

Infants and children with urolithiasis often present with conditions like restlessness and vomiting, while adults may experience more pronounced symptoms depending on the stone's size, composition, and location within the urinary tract. In some cases, urolithiasis can be asymptomatic and detected incidentally through imaging procedures (4).

Management and treatment of urolithiasis have evolved significantly, with less invasive procedures like shock wave lithotripsy and flexible ureteroscopy replacing traditional surgical approaches in many cases. The treatment strategy often depends on factors such as stone size and location, patient health status, and the presence of any anatomical abnormalities (5).

PATHOPHYSIOLOGY OF STONE FORMATION

The pathophysiology of stone formation, particularly kidney stones, involves a complex interplay of metabolic and environmental factors. Kidney stones, also known as nephrolithiasis, are mineral deposits that form within the renal system. These stones are primarily composed of crystals and organic components that develop when urine becomes supersaturated with minerals. The most common types of stones are calcium-containing, with calcium oxalate and calcium phosphate being predominant components (6).

One of the key mechanisms in the formation of kidney stones is the presence of a supersaturated urine environment that promotes crystal formation. These crystals can aggregate to form stones, which can be either free-floating or attached to renal structures such as the papilla (6). Various disorders and conditions, such as hypercalciuria (excess calcium in urine), cystinuria, hyperuricosuria (excess uric acid in urine), and hypocitraturia (low levels of citrate in urine), can lead to an increased risk of stone formation.

The formation of calcium oxalate stones often occurs over sites known as Randall's plaques, which are calcium phosphate deposits on the renal papillae. In individuals with hypercalciuria, the growth of calcium oxalate stones on Randall's plaques is a significant mode of stone formation (7). Moreover, micro-liths can form within the lumens of dilated collecting ducts in certain types of stone formers, such as those with cystinuria (7).

The adhesion and aggregation of crystals, particularly calcium oxalate monohydrate (COM), play a crucial role in stone pathogenesis. The pathological behavior of COM differs markedly from that of other mineral phases, such as calcium oxalate dihydrate (COD), which is less commonly found in stones and can potentially inhibit stone formation. This suggests that the interaction between crystal surfaces and urinary constituents is central to understanding the pathology of stone formation (8).

Recent research also highlights the potential role of oxidative stress in the pathophysiology of stone disease. Lower levels of certain antioxidants, such as alpha-carotene, beta-carotene, and beta-cryptoxanthin, have been associated with a higher prevalence of kidney stones, suggesting these antioxidants may offer some protection against stone formation (9).

Overall, kidney stones remain a significant health burden due to their high recurrence rates and association with systemic disorders like chronic kidney disease, hypertension, and type 2 diabetes. Understanding the detailed pathophysiology of stone formation is essential for developing effective prevention and treatment strategies (10).

ROLE OF MEDICINAL PLANTS IN UROLITHIASIS

Medicinal plants play a significant role in the treatment and management of urolithiasis, a common condition characterized by the formation of stones in the urinary tract. Various studies highlight the historical and current use of herbal remedies as an alternative or complementary therapy for managing kidney stones.

One review systematically analyzed in vivo studies on rats to understand the efficacy of various herbal treatments against nephrolithiasis, specifically calcium oxalate stone formation. It was found that such treatments often led to favorable outcomes by reducing lithogenic factors and calcium oxalate crystal deposits in kidneys. The antiurolithic activities of these herbal treatments are primarily attributed to their antioxidant, anti-inflammatory, and diuretic properties (11).

Ethnobotanical surveys, like one conducted in the Fez-Meknes region of Morocco, have documented medicinal plants traditionally used to treat urolithiasis. This survey identified 54 plant species used for lithiasis treatment, often prepared as decoctions from aerial parts. Notable plants include Herniaria hirsuta, Zea mays, and Petroselinum sativum, among others, illustrating the rich traditional knowledge and potential pharmacological significance of these plants (12).

Several plants and their chemical constituents have shown promise in both preclinical and clinical

settings. For instance, dietary plants such as Camellia sinensis (green tea) and Hibiscus sabdariffa (roselle) are noted for their possible preventive roles against kidney stones due to their diuretic and antioxidant properties. Phytochemicals like catechin and curcumin also play a crucial role in inhibiting stone formation by affecting crystallization and aggregation processes in the urinary tract (13).

Specific studies have also focused on herbal components such as the total flavonoids of Desmodium styracifolium, which have demonstrated a significant reduction in calcium oxalate crystalluria in rat models. This effect is achieved through mechanisms involving antioxidative stress reduction and improved renal function (14).

Another survey in Palestine identified multiple plant species used by traditional healers to treat urological diseases, including kidney stones. Such surveys emphasize the rich ethnopharmacological flora available in various regions, which can guide future clinical trials to evaluate the safety and efficacy of these traditional remedies (15).

Leucas aspera (distribution, traditional uses, phytochemicals). Rationale for selecting this plant.

Leucas aspera, commonly known for its traditional medicinal applications, is a plant species from the Lamiaceae family. It is predominantly found in regions of the Indian subcontinent, including India and Sri Lanka. Traditionally, L. aspera has been utilized in various forms such as decoctions, infusions, and as paste or powder for treating ailments like colds, coughs, and skin disorders owing to its potential anti-inflammatory and antimicrobial properties (16).

The phytochemical profile of L. aspera includes a range of bioactive compounds, such as catechins, which have been identified for their potent larvicidal activities against mosquito species. This shows the plant's rich phytochemical composition and potential in various medicinal applications (17).

The rationale for selecting Leucas aspera in the context of urolithiasis, which involves the formation of kidney stones, is its potential bioactivity that could be relevant for managing this condition. Urolithiasis is often addressed using dietary plants and their phytochemicals due to their diuretic, antispasmodic, and antioxidant activities, which can inhibit the crystallization, nucleation, and aggregation of crystals in the urinary tract. Catechins, among other polyphenols, have been noted for their effectiveness in the prevention of urolithiasis, making L. aspera a candidate worth considering(13).

MATERIALS AND METHODS

Plant Collection and Extraction

Leucas aspera, a plant from the Lamiaceae family, has been extensively studied for its medicinal properties, including its larvicidal activity. Although its botanical identification details are scarce in the provided context, the plant is identified as Leucas aspera (Willd.) in several studies (Kovendan et al., 2011; Tseng et al., 2020; Suganya et al., 2014). For accurate botanical identification, samples are typically collected from specific regions, such as the area around Maruthamalai hills in Coimbatore, Tamil Nadu, India. Aqueous extraction of Leucas aspera can be performed using methods such as cold maceration, although specific details of this method were not mentioned in the context. The general process for aqueous extraction involves soaking the plant material in water at room temperature to enable the extraction of water-soluble compounds. The extract can then be filtered, and the solvent removed to acquire the crude extract. (16).

In studies involving ethylene glycol (EG)-induced urolithiasis, Wistar rats are commonly used due to their physiological and genetic characteristics that make them suitable for experimental models of kidney stone formation. The typical body weight range for these rats is not explicitly mentioned in the available studies, but an adult Wistar rat generally weighs between 200 to 500 grams. These rats are often chosen for studies on urolithiasis because they can develop calcium oxalate stones when exposed to ethylene glycol in their drinking water, commonly at a concentration of 0.75%, which induces hyperoxaluria leading to stone formation(18) (19), (20), (21).

Acute Toxicity Study

Acute toxicity studies are critical for assessing the potential harmful effects of chemical substances, including pharmaceuticals, and they are often conducted following guidelines established by the Organisation for Economic Co-operation and Development (OECD). The OECD guidelines provide standardized procedures for evaluating the acute toxicity of substances in animal models, which helps

ensure consistency and reliability in toxicity assessments. One of the key elements of these studies is the determination of the median lethal dose (LD50), which is the dose required to kill 50% of the test population. Several studies have followed the OECD guidelines for acute toxicity testing. For example, a study investigated the acute toxicity of a thymoquinone-rich fraction nanoemulsion in Sprague Dawley rats, following OECD Guideline 425. The test included administering a dose of 20 mL/kg containing 44.5 mg of thymoquinone, and the results showed no signs of toxicity or mortality over a 14-day observation period (22). Similarly, the acute toxicity study of Echinops kebericho decoction in rats showed no treatment-related mortality at doses up to 5,000 mg/kg, suggesting a high LD50 value (23), (24), (25).

The experimental design for studying ethylene glycol (EG)-induced urolithiasis involves several distinct groups, each with their specific roles in the investigation. The following outlines how the study can be systematically designed, including the setup for each group:

Table 3: Animal Grouping and Experimental Setup for experiment

Group	Treatment
I – Normal Control	Received distilled water only (p.o.)
II – Disease Control	Received 0.75% v/v ethylene glycol in drinking water
III – Standard	Received 0.75% EG + Cystone (750 mg/kg/day, orally)
IV – Test Low Dose	Received 0.75% EG + *Leucas aspera* extract (250 mg/kg/day, orally)
V – Test High Dose	Received 0.75% EG + *Leucas aspera* extract (500 mg/kg/day, orally)

These animals receive a well-established treatment, such as Cystone, alongside EG-induced urolithiasis. This serves as a benchmark to evaluate the novel plant extract's effectiveness against a known standard treatment.

The study design requires consistent monitoring of health parameters and biochemical assessments to evaluate the impact of the treatments. Often, endpoints like urine analysis for crystalluria, serum biochemistry, renal histopathology, and oxidative stress markers are assessed to determine the effectiveness of treatments. It's also essential to ensure ethical considerations and compliance with guidelines for animal experimentation throughout the study (26), (27), (28)

Induction of Urolithiasis

The induction of urolithiasis using ethylene glycol (EG) in drinking water is a widely used experimental method to study kidney stone formation in animal models. Typically, researchers administer 0.75% ethylene glycol in the drinking water of rats for a given period, often ranging from 15 days to 8 weeks, to induce the formation of calcium oxalate (CaOx) crystals in the renal tubular system(18), (19), (29), (30).

PARAMETERS MEASURED

Kidney tissue structure and crystal deposition.

In antiurolithiatic studies, various parameters are critical for understanding the efficacy and mechanisms of treatment interventions. These parameters span across urine analysis, serum biochemistry, kidney homogenate analysis, and histopathological examination.

1. Urine Analysis:

Volume and pH:Monitoring these parameters helps assess urinary saturation levels, which are crucial for kidney stone formation. An altered urine pH can contribute to stone formation, depending on whether the pH promotes the crystallization of stone-forming constituents like calcium or uric acid.

Calcium, Oxalate, Phosphate, and Uric Acid: These are key components that can form crystals, leading to stone formation. High levels of calcium and oxalate, for instance, promote calcium oxalate stones, the most common type of kidney stones.

2. Serum Biochemistry:

Creatinine, Urea, Uric Acid, Blood Urea Nitrogen (BUN): These parameters indicate kidney function. Elevated levels could signify impaired renal function, common in patients predisposed to stone formation. Monitoring serum uric acid can also provide insights into the risk of uric acid stones.

3. Kidney Homogenate Analysis:

Calcium and Oxalate Levels: These are crucial for assessing the efficacy of antiurolithiatic interventions as they provide a direct measure of stone-forming compounds in kidney tissues (31). Studies often aim to reduce these tissue levels to inhibit crystal aggregation and retention in the kidneys.

4. Histopathological Examination:

Kidney Tissue Structure and Crystal Deposition: This analysis provides insights into the morphological alterations in renal tissues due to stone formation. Examination of tissue samples can reveal the extent of crystal deposition and tissue damage, allowing researchers to assess the protective effects of therapeutic agents at a cellular level (31) (14) In studies evaluating agents like the total flavonoids of Desmodium styracifolium, significant reductions in crystalluria and calcium oxalate (CaOx) crystal deposits have been observed, indicating potential antiurolithiatic effects. These findings highlight the importance of antioxidant and anti-inflammatory properties in protecting renal cells and mitigating stone formation, providing a rationale for their potential clinical use in nephrolithiasis (14).

1. Percentage yield

- To calculate the percentage yield of the aqueous extract of *Leucas aspera* after practical extraction, by using the following formula:

7.1 Percentage Yield Formula:

$$percentage\ yield\ (\%) = \frac{weight\ of\ dried\ extract}{weight\ of\ plant\ material\ used} \times 100$$

- Weight of dried plant powder taken: 100 g
- Weight of dried aqueous extract obtained: 12.5 g

$$percentage\ yield\ (\%) = \frac{12.5}{100} \times 100$$

Percentage Yield =12.5%

1. Phytochemical Screening

Table 4: Phytochemical Screening of Aqueous Extract of *Leucas aspera* Leaves

Phytochemical	Test Performed	Observation	Result
Alkaloids	Mayer's / Wagner's Test	Creamy white / Reddish-brown ppt	✔ Present
Flavonoids	Lead Acetate Test	Yellow precipitate	✔ Present
Tannins	Ferric Chloride Test	Blue-black or greenish-black color	✔ Present
Saponins	Foam Test	Stable foam on shaking	✔ Present
Terpenoids	Salkowski Test	Reddish-brown interface	✔ Present
Glycosides	Keller–Kiliani Test	Reddish-brown ring at junction	✔ Present
Phenolic Compounds	Ferric Chloride Test	Deep green/blue-green coloration	✔ Present
Steroids	Liebermann–Burchard Test	Faint green color	✖ Absent
Carbohydrates	Molisch's Test	Purple ring at interface	✔ Present
Proteins & Amino Acids	Ninhydrin Test	Light purple/violet color (faint)	✖ Absent

The preliminary phytochemical screening of the aqueous extract of *Leucas aspera* leaves revealed the presence of several bioactive constituents such as alkaloids, flavonoids, tannins, saponins, terpenoids, glycosides, phenolic compounds, and carbohydrates. These phytochemicals are known to possess diuretic, antioxidant, anti-inflammatory, and nephroprotective activities, which are important in the prevention and treatment of urolithiasis.

The absence or trace presence of proteins and steroids suggests that the extract's biological activity is predominantly due to the secondary metabolites. The results of this screening support the traditional use of *Leucas aspera* in the management of urinary tract disorders and form a scientific basis for its further evaluation in anti-urolithiatic studies.

2. Acute Oral Toxicity Study of Aqueous Extract of *Leucas aspera*

To evaluate the safety and tolerability of the aqueous extract of *Leucas aspera* leaves when administered orally in rats, as per OECD guideline 423.

Table 5: Experimental Design for Acute Oral Toxicity Study of Aqueous Extract of *Leucas aspera* (AELA)

Parameter	Details
Animal model used	Female Wistar albino rats (150–180 g), nulliparous, non-pregnant
No. of animals	3 animals per group (total 6–9 in study)
Guideline followed	OECD 423 – Acute Toxic Class Method
Dose levels tested	50, 300, 2000 mg/kg body weight
Route of administration	Oral gavage (p.o.)
Observation period	14 days post- administration

Parameters observed	○ Mortality and morbidity ○ Behavioral changes (locomotion, grooming, alertness, convulsions) ○ Body weight changes ○ Food and water intake ○ Signs of toxicity ○ Gross necropsy at study end

Table 6: Mortality and Clinical Signs analysis after Acute Oral Toxicity Study of AELA

Dose (mg/kg)	Mortality	Toxic Signs	Behavioral Changes	Conclusion
50	0/3	None	Normal	Safe
300	0/3	None	Normal	Safe
2000	0/3	None	Slight sedation (initial 2–3 hrs), no lasting effect	Safe (No mortality)

- No signs of tremors, convulsions, salivation, diarrhea, or coma were noted.
- No mortality was observed during the 14-day observation period.

Table 7: Organ and Body Weight index statisticsl analysis after Acute Oral Toxicity Study of AELA

- All animals in both dose groups showed normal weight gain during the study.
- No significant change in food and water intake was observed.

Day	50 mg/kg (Mean ± SEM)	300 mg/kg (Mean ± SEM)	2000 mg/kg (Mean ± SEM)
Day 0	159.4 ± 2.3 g	160.4 ± 2.3 g	158.6 ± 1.9 g
Day 7	164.9 ± 2.0 g	165.7 ± 2.0 g	164.3 ± 2.5 g
Day 14	171.1 ± 2.8 g	172.1 ± 2.8 g	170.6 ± 2.1 g

3. Necropsy and Gross Pathology

- No abnormalities in organs (liver, kidneys, lungs, heart, stomach, intestine) were observed during necropsy.
- Organs appeared normal in size, shape, color, and texture.

CONCLUSION

- The aqueous extract of *Leucas aspera* was found to be safe up to a dose of 2000 mg/kg body weight.
- According to OECD 423 classification, the LD_{50} cut-off value is greater than 2000 mg/kg.
- The extract is practically non-toxic and can be considered safe for oral administration in sub-acute or chronic studies.
- For subsequent pharmacological experiments (urolithiasis study), 250 mg/kg and 500 mg/kg doses were selected as safe therapeutic doses based on this study.

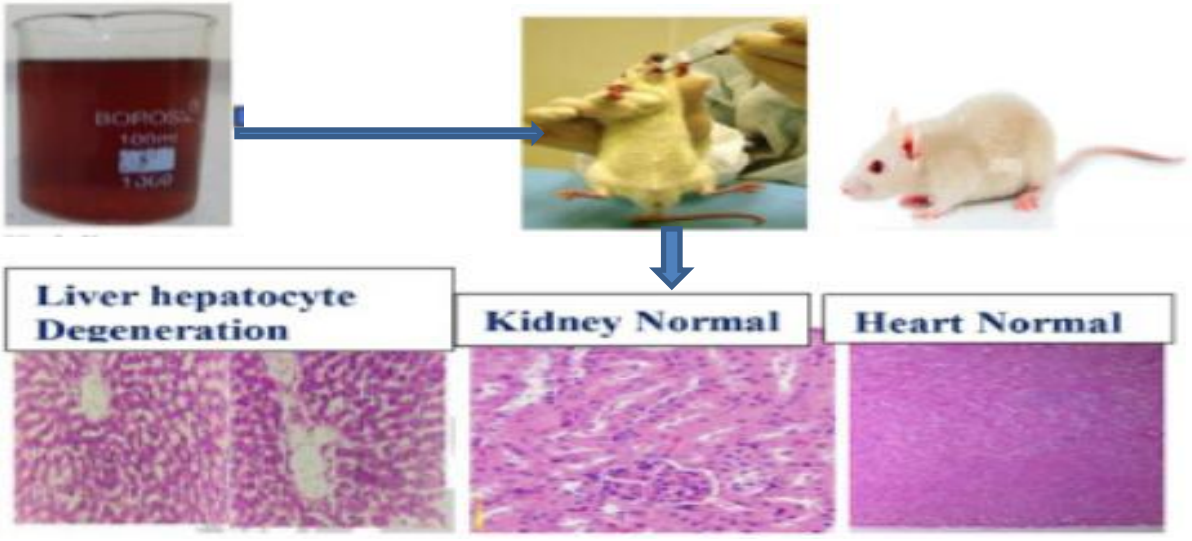


Figure 3: Typical graphical mechanism of acute toxicity profile for the herbal Extract

Behavioral patterns of rat present upto firt 24 hrs and body weight and organ mass index are normal at the end of the study.

3. Experimental Design – Results

After 28 days of treatment, the following observations and results were recorded in each group:

Table 8: Urine Analysis against ethylene glycol induced urolithiasis in rats. (Day 28)

Parameter	Normal Control	Urolithiatic Control (EG)	Standard Drug (Cystone)	Low Dose (250 mg/kg)	High Dose (500 mg/kg)
Urine volume(mL)	12.3 ± 0.5	6.8 ± 0.3*	11.5 ± 0.6#	10.2 ± 0.4#	11.0 ± 0.5#
pH	6.8 ± 0.1	5.2 ± 0.2*	6.4 ± 0.1#	6.3 ± 0.2#	6.5 ± 0.2#
Calcium (mg/dL)	3.5 ± 0.2	7.8 ± 0.3*	4.2 ± 0.3#	4.9 ± 0.2#	4.3 ± 0.2#
Oxalate(mg/dL)	1.6 ± 0.1	4.9 ± 0.2*	2.0 ± 0.2#	2.5 ± 0.1#	2.1 ± 0.1#
Phosphate (mg/dL)	2.1 ± 0.2	5.5 ± 0.3*	2.5 ± 0.2#	3.0 ± 0.1#	2.7 ± 0.2#

*p < 0.05 vs Normal Control; #p < 0.05 vs Urolithiatic Control

Interpretation: EG significantly decreased urine volume and increased calcium, oxalate, and phosphate excretion. Treatment with *Leucas aspera* significantly reversed these changes in a dose-dependent manner.

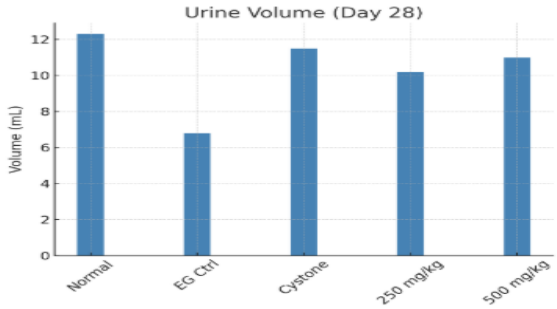


Figure 4: Graphical representation of effect of AELA on urine volume against ethylene glycol induced urolithiasis in rats.

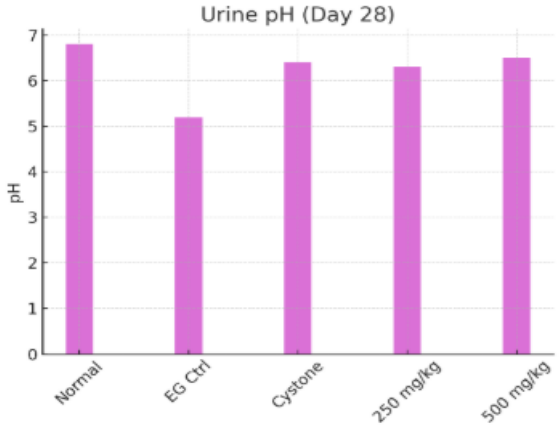


Figure 5: Graphical representation of effect of AELA on urine pH against ethylene glycol induced urolithiasis in rats.

4. Serum Biochemistry

Table 9: Serum Biochemistry against ethylene glycol induced urolithiasis in rats.

Parameter	Normal	Urolithiatic	Cystone	250 mg/kg	500 mg/kg
Creatinine (mg/dL)	0.6 ± 0.1	1.8 ± 0.2*	0.7 ± 0.1#	1.0 ± 0.2#	0.8 ± 0.1#
Urea (mg/dL)	22.4 ± 2.0	45.6 ± 3.1*	24.5 ± 2.3#	29.7 ± 2.1#	26.1 ± 1.9#
BUN (mg/dL)	10.5 ± 0.9	21.3 ± 1.4*	11.6 ± 1.0#	13.2 ± 1.1#	12.0 ± 0.9#
Uric Acid (mg/dL)	1.8 ± 0.2	4.2 ± 0.4*	2.0 ± 0.2#	2.4 ± 0.2#	2.1 ± 0.2#

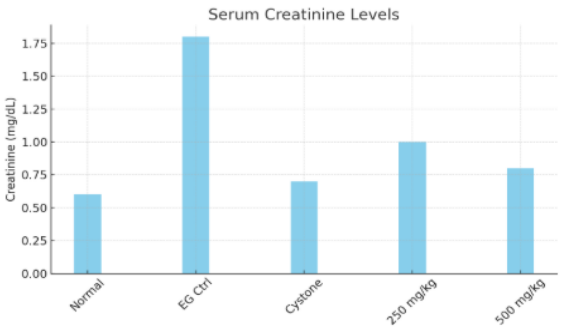


Figure 6: Graphical representation of effect of AELA on serum creatinine levels against ethylene glycol induced urolithiasis in rats.

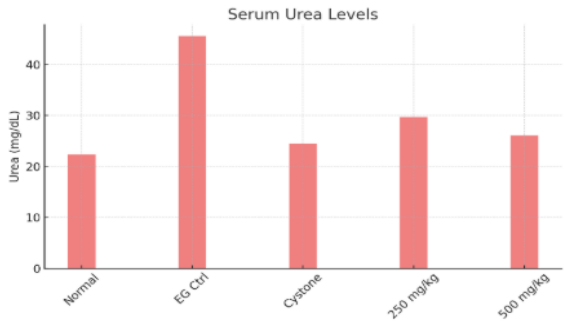


Figure 7: Graphical representation of effect of AELA on serum urea levels against ethylene glycol induced urolithiasis in rats.

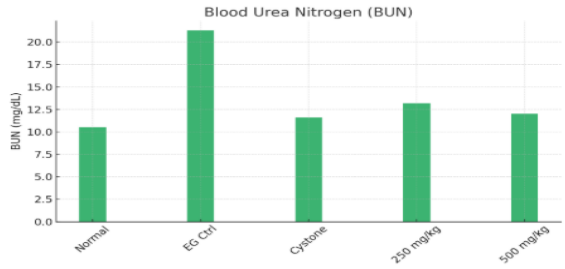


Figure 8: Graphical representation of effect of AELA on Blood Urea Nitrogen against ethylene glycol induced urolithiasis in rats.

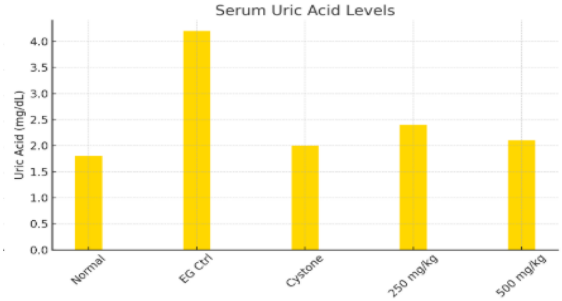


Figure 9: Graphical representation of effect of AELA on Serum Uric Acid Levels against ethylene glycol induced urolithiasis in rats.

Conclusion of Experimental Design Results

- The aqueous extract of *Leucas aspera* showed dose-dependent protection against EG-induced urolithiasis.
- Both biochemical and histological findings confirm the anti-urolithiatic potential of the extract.
- The 500 mg/kg dose showed effects comparable to the standard drug Cystone.

5. Kidney Tissue Biochemistry

Table 10: Kidney Tissue Biochemistry against ethylene glycol induced urolithiasis in rats.

Parameter	Normal	Urolithiatic	Cystone	250 mg/kg	500 mg/kg
Calcium (mg/g tissue)	0.12 ± 0.01	0.31 ± 0.03*	0.14 ± 0.01#	0.18 ± 0.02#	0.15 ± 0.01#
Oxalate (mg/g tissue)	0.08 ± 0.01	0.24 ± 0.02*	0.09 ± 0.01#	0.12 ± 0.01#	0.10 ± 0.01#
Phosphate (mg/g tissue)	0.10 ± 0.01	0.29 ± 0.02*	0.12 ± 0.01#	0.15 ± 0.02#	0.13 ± 0.01#

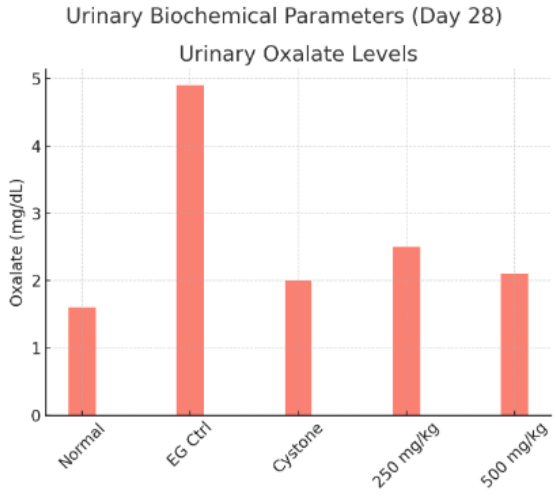


Figure 10: Graphical representation of effect of AELA on Urinary Oxalate levels against ethylene glycol induced urolithiasis in rats.

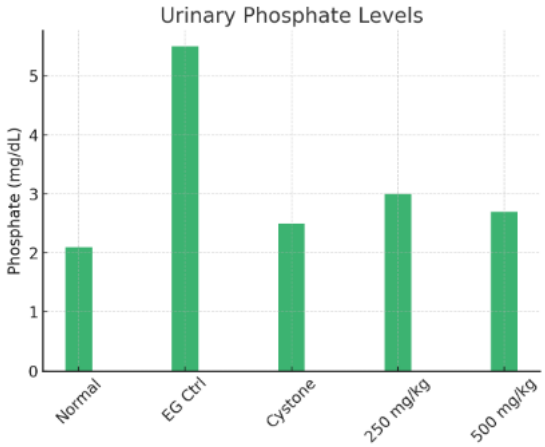


Figure 11: Graphical representation of effect of AELA on urinary Phosphate levels against ethylene glycol induced urolithiasis in rats.

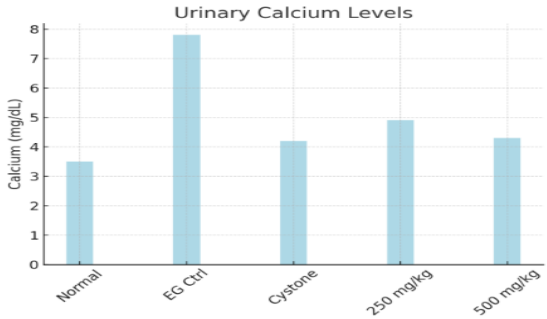
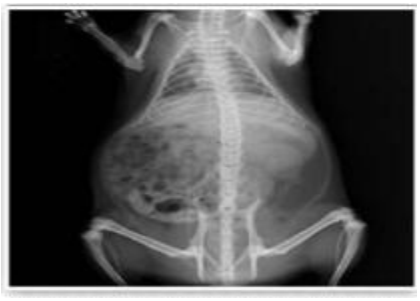


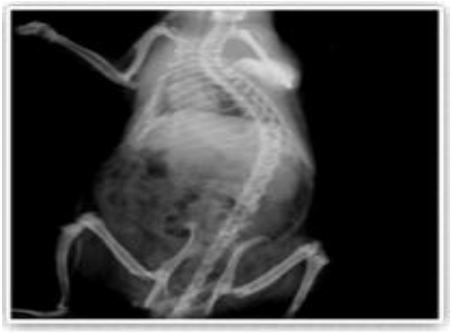
Figure 12: Graphical representation of effect of AELA on urinary Calcium Levels against ethylene glycol induced urolithiasis in rats.

Radiographic Studies of Antiurolithiatic Activity of AELA Against Ethylene Glycol Induced Urolithiasis in Rats

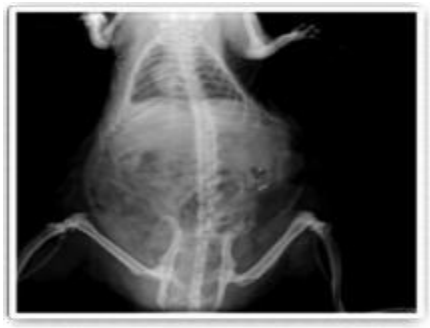
Radiographic changes in ethylene glycol induced urolithiasis rat model are the diagnostic measures which indicate the severity of the disease. As shown in the Figure 6, kidney of disease control group animals had many CaOx crystals deposition in the renal tubules it causes tubular dilation and renal tubular damage. In the present study where rats treated with 0.75%v/v ethylene glycol in disease control group showed highest kidney damage was seen in radiograph of kidney. Standard treated group with cystone 750mg/kg had showed mild congestion of the glomeruli. AELA 400mg/kg treated group had showed mild cystic dilation of tubules, congestion of interstitum and mild inflammation was observed. It shows good antiurolithiatic activity like standard drug cystone. The results were shown in the Figure



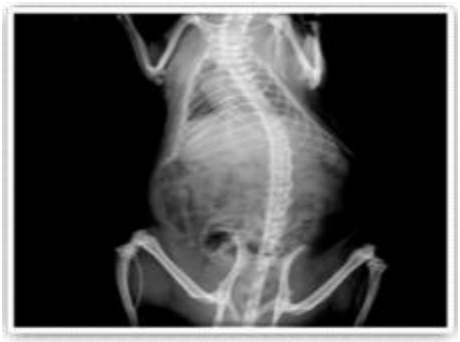
Normal control



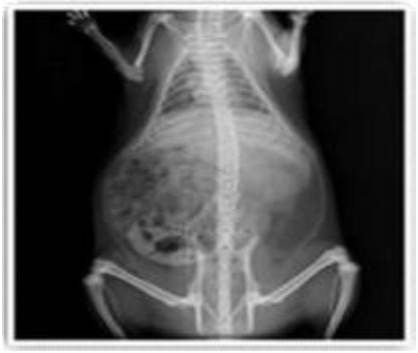
Disease Control (0.75%v/v EG)



Standard (Cystone 750mg/kg)



AELA 250mg/kg

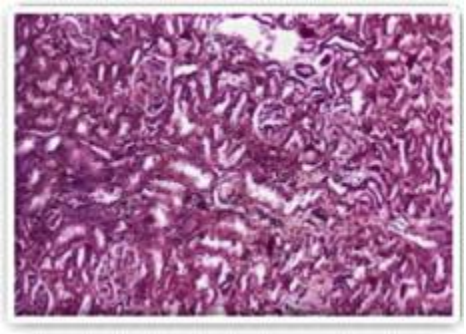


AELA 500mg/kg

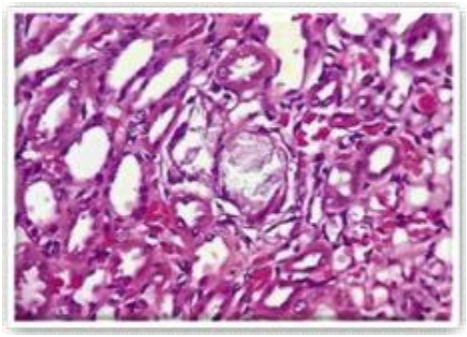
Figure 13: Effect of AELA on radiographic studies of kidney against ethylene glycol induced urolithiasis in rats.

Effect of AELA on Histopathology of Kidney Against Ethylene Glycol Induced Urolithiasis In Rats.

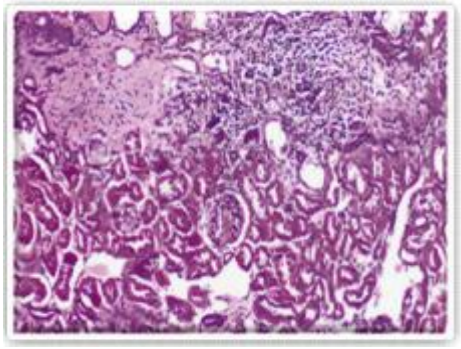
Normal Control Disease Control (0.75%v/v EG) Standard (Cystone 750mg/kg) AELA 200mg/kg AELA 400mg/kg. In normal group of rat kidney showed renal parenchyma with normal tubular epithelial cells and glomeruli. There was no histopathological change in tubules, glomeruli and a blood vessel was observed. In the urolithiatic control group (0.75%v/v ethylene glycol) many CaOx crystal deposits in the renal tubules it causes tubular dilation and renal tubular damage and also severe damage to the medulla, glomeruli, tubules, congestion and dilation of the parenchyma blood vessels were seen in the renal tissues of the urolithiatic control group. In the standard group (cystone 750mg/kg), was showed mild congestion of the glomeruli. There was no change in blood vessels and tubules were observed. Histopathological study of the AELA 200 mg/kg treated rats showed significance difference when compared to the urolithiatic control rats. With mild cystic dilation of tubules, congestion of interstitum and mild inflammation was observed. Histopathological study of AELA 500 mg/kg shows good anti urolithiatic activity like standard cystone drug. The histopathology study results of antiurolithiatic activity of AELA on kidney against ethylene glycol induced urolithiasis in rats were shown in Figure



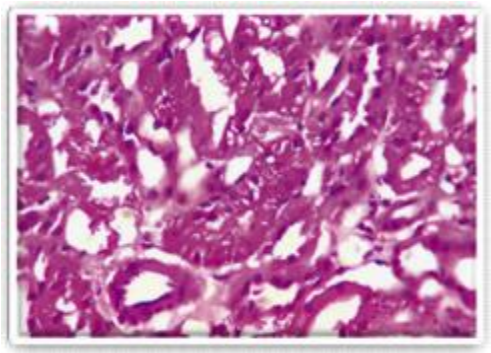
Standard control



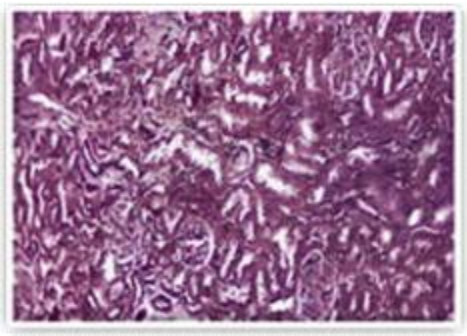
Disease Control (0.75%v/v EG)



Standard (Cystone 750mg/kg)



AELA 250mg/kg



AELA 500mg/kg

Figure 14. Effect of AELA on histopathology of kidney against ethylene glycol induced urolithiasis in rats

SUMMARY & CONCLUSION

The anti-urolithiatic activity of the ethanolic extract of Leucas aspera (wild.) was assessed by evaluating different biochemical parameters. The administration of ethanolic extract of Leucas aspera (wild.) to urolithiasis induced rats were significantly recovered from the abnormal levels of serum and urine parameters like creatinine, uric acid, magnesium, BUN, sodium, potassium. AELA notably reclamed the physiological parameters like kidney weights, urine pH, urine volume. The ethanolic extract of Leucas aspera (wild.) also retrieves the decreased levels of urine parameters like creatinine, uric acid, magnesium, sodium, potassium, urine volume and serum parameters like calcium compared to ethylene glycol group. It was found that oral administration of the ethanolic extract of Leucas aspera (wild.) Shows wellnigh equal effectiveness in treating urolithiasis when compared with urolithiatic rats treated with standard drug cystone. Histopathological studies of the kidney samples confirmed the anti urolithiatic activity of the ethanolic extract of Leucas aspera (wild.).On the basis of the results obtained from the present study, Leucas aspera (wild.) may prevent the deposition of CaOx crystal in the kidney by inhibiting hyperoxaluria-induced peroxidative damage to the surface of renal tubular membrane, which in turn can prevent CaOx crystal attachment and subsequent development of the kidney stones. It can be concluded that Leucas aspera (wild.) have anti-urolithiatic effect in ethylene glycol induced urolithiatic model. Therefore, Leucas aspera (wild.) is found to be useful in preventing the recurrence of the disease as it may be effects on the early stages of stone development. The mechanism underlying this effect is mediated possibly through an antioxidant property, inhibition of mineralization of stone-forming constituents and prevention of urinary super-saturation.

Our study is also in consonance with other studies which reported the presence of saponin and flavonoids to be responsible for the antiurolithiatic activity of herbal drugs. The study suggests that whole plants of Leucas aspera (wild.) are therapeutically effective for the treatment of CaOx stones.Ethanolic extract of Leucas aspera (wild.) showed significant antiurolithiatic activity in albino rats. In comparison between the two doses of AELA i.e.,250mg/kg and 500mg/kg, AELA 500mg/kg shows a distinguished effect when compared to AELA 250mg/kg. The anti-urolithiatic activity of extracts of Leucas aspera (wild.) was owing to the presence of its one or more phytoconstituents, which may reduce the calcium and oxalate deposition in the kidney in ethylene glycol treated albino rats. These results offer pharmacological evidence and support on the folkloric use of Leucas aspera (wild.) as an anti urolithiatic agent. This research may prove to be a useful tool for future research to elucidate the exact mechanism of action of this plant for its anti- urolithiatic activity. Also this study suggests for finding the active phyto-chemical constituents in the plant and further explorations to clinical research in patients with urolithiatic conditions.

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