EVALUATING ANTI-UROLITHIATIC ACTIVITY OF ETHANOLIC EXTRACT OF *LEUCAS ASPERA* ON ETHYLENE GLYCOL INDUCED RAT MODEL

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ABSTRACT

This study aimed to determine the antiurolithiatic effect of an ethanolic extract of *Leucas aspera* (wild) on rat models of urolithiasis caused by ethylene glycol. Rats were given ethylene glycolated water (0.75% v/v) orally for 28 days to produce urolithiasis. From 15 to 28 days, a *Leucas aspera* (wild) ethanolic extract (200 and 400 mg/kg) was given orally. Due to the preventative and therapeutic routine of plant extract, treatment with ethanolic extract of *Leucas aspera* (wild) considerably reduced the high levels of ions in urine as well as blood urea nitrogen, serum creatinine, and serum uric acid levels. Because the plant extract contains one or more phytoconstituents like saponin and flavonoids to be responsible for the antiurolithiatic activity, which could reduce the calcium and oxalate deposition in the kidney, the histopathological and radiological findings also showed improvement in kidney architecture after treatment with the plant extract. These findings provide pharmacological support and proof for the traditional usage of *Leucas aspera* (wild) as an anti-urolithiatic agent.

KEYWORDS

Urolithiasis, Ethylene glycol, *Leucas aspera* (wild), Calcium, Oxalate

1. INTRODUCTION

One of the most prevalent conditions affecting the urinary tract is urolithiasis. Urolithiasis is the term used to describe the development of stones in the urinary system, including the kidney, ureter, bladder, and urethra. Populations all around the world struggle with urolithiasis. It is the third-most common urinary system condition [1]. It occurs more frequently in men than women but rare in children.

Kidney stone formation and recurrence remain a major cause of morbidity in humans and one of the foremost difficulties urologists confront today. An elevated risk of end-stage renal failure has been linked to this growing urological health problem, which affects roughly 12% of the global population. This expanding pattern is said to be caused by changes in lifestyle, such as inactivity and poor eating habits, as well as global warming [2]. The most significant problem facing patients receiving post-operative therapy for renal and ureteric calculi is likely recurrent stone development. The multifactorial process of urolithiasis production includes dietary factors, urinary tract infections, changed urine solutes and colloids, decreased urinary drainage and urinary stasis, prolonged immobility, Randall's plaque, and microliths, among other factors [3]. One of the most common and ancient diseases known to humans is kidney stone production. Urinary saturation, urinary supersaturation, nucleation, crystal development, crystal aggregation, and urinary stone formation are the steps in the production of urinary stones. Metabolic abnormalities such as hypercalciuria, hyperoxaluria, cystinuria, etc. trigger the formation of urinary stones [4].Urinary stones can occasionally develop as a result of recurrent UTIs. Calcium, cystine, uric acid, or struvite stones can all occur in the urinary system. They often develop inside the urinary bladder, ureter, or kidney (nephrolithiasis). The ureter might get obstructed by these calculi when they reach a maximum size and shape of 2.3 mm. As a result, the upper ureter and renal pelvis may enlarge or stretch, causing blockage. This may also cause a spasm that causes acute, episodic stomach pain that may be accompanied by nausea and vomiting. There is currently no medical treatment for renal stone displacement or stone disintegration [5].

Because of its complex etiology and high rate of recurrence, the issue of stone formation is regarded as a medical challenge. Additionally, an imbalance between promoters and inhibitors might result in the production of stones. Due to testosterone's ability to increase and estrogen's ability to inhibit stone formation, the incidence rate is three times higher in men than in women. In terms of a metabolic condition, changes in urinary volume or pH, decreased levels of ingredients like citric acid or magnesium, and increased excretion of chemical substances like calcium, oxalate, uric acid, or cystine can all contribute to the formation of stones [6].

When urea-splitting organisms infect the urinary system, bacteria break down the urea discharged in urine in the presence of urease enzymes. This causes the creation of ammonia, which causes the urine to become alkaline. Urine that is highly precipitated in calcium oxalate, magnesium phosphate, and calcium carbonate in an alkaline condition has a considerable propensity to create calculi. By causing crystal adhesion, bacterial infection can cause the production of stones. The majority of urea-splitting organisms are Proteus species;

however, urease production has also been documented in *Pseudomonas, Staphylococcus, Escherichia coli,* and even Mycoplasma. *Escheria coli, Proteus species, Streptococcus, Staphylococcus, Pseudomonas,* and Urea plasma *urealyticum* were all linked to infected stones[7]. There is increasing evidence that the end products of urealysis harm the renal urolithial cells' glycosaminoglycon layer, causing bacterial adhesion, biofilm development, and mineral encrustation. Therefore, in order to identify and treat the infection that is the cause of the stone formation, extensive microbiological studies are required.

Since ancient Egyptian mummies, urinary tract stone illness has been documented historically [8]. Rats are frequently used to create kidney stones using ethylene glycol-induced experimental intoxication. The body breaks down ethylene glycol to produce harmful byproducts such as glycoaldehyde, glycolate, and glyoxylate. Specifically, large anion-gap metabolic acidosis, lactic acidosis, and hypocalcemia are the metabolic abnormalities caused by these metabolites that primarily induce tissue death. Crystals of calcium oxalate are created when oxalic acid and calcium interact, and they accumulate in the kidneys. Hematuria, proteinuria, a rise in creatinine, and renal failure occurs[9].Urolithiasis occurs approximately about 12% and it is estimated at 1-5% Asia, 5-9% Europe, 13% North America. In India, 12% of the populations are expected to have urolithiasis and also nearly 15% of the population of north India suffers from kidney stones. It occurs both in sex but the risk is generally high in men and is becoming more common in young women. It is male is 70-81% and 47-60% in females with a span of 20 years [10]. The prevalence of renal stone formation is roughly 2–3% in the general population and is influenced by a number of epidemiological, biochemical, and genetic factors, including age, sex, hereditary, occupation, body size, social class, affluence, geographic location, climate, diet, fluid intake, and other medical conditions like hypertension, gout, cystinuria, hyperparathyroidism, etc. Various regions of India have reported high rates of urolithiasis with calculi that have various chemical compositions [11]. Management of stone disease depends on the size and location of the stones. Stones larger than 5mm or stones that fail to pass through should be treated by some invention procedures such as extracorporeal shock, wave lithotripsy (ESWL), lasertripsy, electrohydmulic lithotripsy, ureteroscopy (URS), or percutaneous nephrolithotomy (PNL) [12].

Medical management of urolithiasis today, includes lithotripsy and surgical procedures. Disappointingly, the underlying risk factors are not corrected by these techniques, hence there are a need to continue the medical supervision and therapy to prevent stone recurrence. Many remedies have been employed since ages to treat renal stones and most of them were from plants and proved to be useful. In ayurvedha and folk medicine many herbs are used in management of urolithiasis.

Leucas aspera (wild), a perennial, branching plant with a range of distribution across India, is a member of the Lamiaceae family. It is commonly known as “Thumbai”. *Leucas aspera* (wild.) is found in all parts of India mainly in the Himalayas [13].The major phytoconstituents present in the plants are terpenoids, tannins, alkaloids, carbohydrates, triterpenoids, steroids , flavonoids, fatty acids, nicotine, ursolic acid, glucoside, beta-sitosterol, sterols, diterpene, and phenolic compounds[14].All the parts of *Leucas aspera* (wild.) showed various pharmacological properties like antimicrobial property, anti-Inflammatory property, antioxidant property, anti-diabetic property. Anti-cancer property, hepato-protective property, antianxiety effect, anti-nociceptive property. It is also used for treating jaundice, anorexia, dyspepsia, fever, and helminthic manifestation, respiratory and skin diseases [15].The plant parts are used traditionally as an anti-pyretic and insecticidal agent, and the flowers are valued as a stimulant, expectorant, and diaphoretic. The leaves of the plants are also useful in externally in snakebites [16].

The toxicity levels of *Leucas aspera* (wild.) was evaluated by using swiss albino mice. Female mice were employed in the study on acute toxicity. These experimental protocols were approved by the committee for control and supervision of experiments on animal ethics. Rats were kept in cages. The animals were observed for any changes up to the first 4 hours and then for 24 hours for their mortality test. The results showed that no behavioral, as well as, no mortality changes happened during the study period. The administration of *Leucas aspera* (wild.) extracts was safe up to 2,000 mg/kg body weight. So, this dose can be considered as the cut-off dose for the animals in the study [17]. However, till the date there have been no investigations supporting the anti-urolithiatic properties of *Leucas aspera* (wild.) Hence, this present study has been taken with an aim to evaluate the Pre-clinical evaluation of anti-urolithiatic activity of ethanolic extract of *Leucas aspera* (wild.)(EELA) on ethylene glycol induced urolithiasis rat model.

2. MATERIALS AND METHODS

2.1. PLANT COLLECTION AND AUTHENTICATION

The whole plant of *Leucas aspera* (wild.) was collected from surrounding areas of Thingalur, Erode District, and Tamil Nadu. It was authenticated by Dr. P. Radha, Research officer - Botany, scientist grade-II, Incharge, Central Council for Research in Siddha, Ministry of AYUSH, and Government of India**. (Authentication. No: L061222052A)**

2.2. EXTRACTION OF THE PLANT MATERIAL

2.2.1. Preparation of ethanolic extract of *Leucas aspera* (wild.) by cold maceration method

The whole plant of *Leucas aspera* (wild.) was washed thoroughly in tap water to remove dust particles. The whole plants were then shade dried for 7 days at room temperature and coarsely powdered by a mechanical grinder. The dried powdered sample (100g) was subjected to extraction by cold maceration method in 100% ethanol in the ratio of (80:20) for 3 days. After 3 days, the extract was filtered using No.1 Whatmann filter paper the filtrate was then further concentrated using rotary evaporator and the final residue (92.517%w/w) is stored in air tight container for further analysis is shown in Figure 1[18].

Figure 1. Steps involved in ethanolic extract of *Leucas aspera* (wild.) by cold maceration Method

2.3. PHYTOCHEMICAL ANALYSIS

The extract was screened for various constituents (alkaloids, saponins, tannins, sterols, flavonoids, terpenoids, glycosides, phenols, carbohydrates) using standard protocol.

2.4. SELECTION OF ANIMALS FOR VIVO STUDIES

The colony inbreed Albino Wistar Rats, weighing 200- 250 gm were obtained from central animal house of Swamy Vivekanandha College of Pharmacy, Elayampalayam, Namakkal – 637 205. The animals were kept under standard environmental conditions of 12/12 light/dark rhythm, maintained under controlled room temperature (23±2ºC) and a relative humanity of 60%±10% in polypropylene cages. They were fed with standard pellet diet and water ad libitum. Rats were provided with standard pellet diet and water ad libitum freely throughout the study. The cages were cleaned daily by changing the husk bedding. The experimental protocol was approved by the Institutional Animal Ethical Committee (IAEC) of Swamy Vivekanandha College of Pharmacy, Elayampalayam, Namakkal – 637 205. Care and use of laboratory animals were confirmed to CCSEA guidelines. **IAEC Proposal Number: SVCP/IAEC/PG/6/07/2022**

2.5. DRUGS AND CHEMICALS

All the chemicals and drugs used in this study were of analytical grade. The following chemicals were used for the experimental study.

- Cystone (Himalaya drug company, Bangalore)
- Ethylene glycol 0.75%v/v (Loba Chemie Pvt. Ltd., Mumbai)
- CMC (Loba Chemie Pvt. Ltd., Mumbai).

2.6. EXPERIMENTAL DESIGN FOR IN VIVO BIOLOGICAL EVALUATION STUDIES

The rats were divided into 5 groups of six animals in each group and the experimental design of animals is given:-

Group I : Normal Control (drinking water ad libitum)

Group II : Disease control (0.75% v/v ethylene glycol in drinking water ad libitum)

Group III : Standard drug treated (0.75% v/v ethylene glycol in drinking water+ Cystone 750 mg/kg *p.o.)*

Group IV : 0.75% v/v ethylene glyol in drinking water + Ethanolic extract of Leucas aspera(wild) 200mg/kg *p.o*.

Group V : 0.75% v/v ethylene glycol in drinking water + Ethanolic extract of Leucas aspera(wild) 400mg/kg *p.o.*

2.7. Physical Evaluation

2.7.1. Measurement of Body weight

Body weight of each rats in all groups were measured weekly till end of the treatment using a weighing balance and the changes were recorded.

2.7.2. Measurement of Feed Intake

Daily Feed consumption was measured in individual treatment groups by using standard weighing balance.

2.7.3. Measurement of Water Intake

Daily water consumption was measured in individual treatment groups by using measuring cylinder.

2.7.4. Measurement of Body weight

Body weight of each rats in all groups were measured weekly till end of the treatment using a weighing balance and the changes were recorded.

2.8. ASSESSMENT OF ANTIUROLITHIATIC ACTIVITY:

2.8.1. COLLECTION AND ANALYSIS OF URINE SAMPLE

All animals were kept in individual metabolic cages and urine samples of 24-h were collected on the 28th day. Animals had free access to drinking water during the urine collection period. Urine was analyzed for calcium, phosphate and oxalate content using an automated system.

2.8.2. SERUM ANALYSIS

After the experiment period, blood was collected from the retro-orbital under 10min. serum parameters like calcium, creatinine, BUN (blood urea nitrogen), magnesium, phosphate, sodium, potassium and uric acid was analyzed using Automated Clinical Chemistry Analysis System.

2.8.3. HISTOPATHOLOGICAL EVALUATION

On 28th day, Kidneys were excised quickly and fixed in 10% neutral formalin solution and stained with hematoxylin-eosin and then observed under microscope for the presence of calcium oxalate crystals and also study the kidney architecture**.**

On 28^{th} day animals were anesthetized with chloroform and place in X-ray machine for the radiographic analysis of the kidney. X-ray taken at the kidney for the conformation of the severity of urolithiasis (CaOx deposits) in the kidney.

2.9. STATISTICAL ANALYSIS

All the values were expressed as mean ± SEM (n=6 in each group). The statistical analysis was carried out by one way analysis of variance (ANOVA) followed by Tukey's multiple comparison Test.

3. RESULTS

3.1. EXTRACTION OF PLANT MATERIAL AND PRELIMINARY PHYTOCHEMICAL ANALYSIS

The percentage yield of ethanolic extract of *Leucas aspera* (wild.) was found to be 92.51%w/w. The extracts of *Leucas aspera* (wild.) were used for preliminary phytochemicals screening such as alkaloids, flavonoids, phytosterols, glycosides, saponins, terpenoids and tannins. The results are tabulated in **Table 1.** The extract shows the presence of alkaloids, flavonoids, glycosides, phenols, terpenoids, tannins and carbohydrates.

Table 1. Phytochemicals constituents present in ethanolic extract of *Leucas aspera* (wild.)

S.NO	PHYTOCHEMICALS	ETHANOLIC EXTRACT OF *LEUCAS ASPERA* (WILD.)
1	Alkaloids	Present
2	Flavanoids	Present
3	Glycosides	Present
4	Phenolic compounds	Present
5	Tannins	Present
6	Saponins	Present
7	Phystosterols	Present
8	Proteins and aminoacids	Absent
9	Terpenoids	Present
10	Carbohydrates	Present

3.2. MEASUREMENT OF BODY WEIGHT

Body weight of each rats in all groups were measured weekly till end of the treatment using a weighing balance and changes are recorded. The body weights of animals treated with ethylene glycol groups was significantly decreased where as cystone 750mg/kg, EELA 200mg/kg, EELA 400mg/kg were significantly increased in body weights compared to the urolithiatic control group. The results are shown in Figure 2. From the study, ethylene glycol induced urolithiasis rats showed significant loss of body weight was observed as compared to other groups of rats and this may be due to stone formation is more and this leads to obstructions in urine passage and results in pain during urination. Due to pain, food consumption may be decreased and result in decreased in body weight of animal.

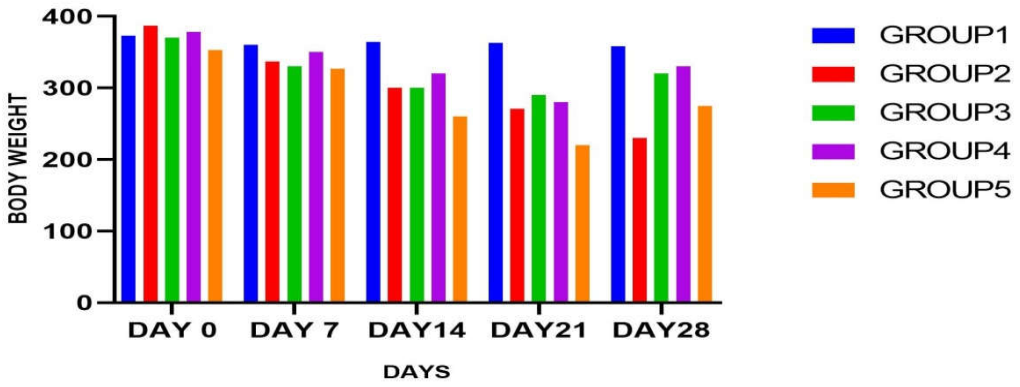


Figure 2. Graphical representation of effect of EELA on body weight against ethylene glycol induced urolithiasis in rats.

3.3. EFFECT OF EELA ON KIDNEY WEIGHT, URINE VOLUME, URINE pH AND URINE TOTAL PROTEIN LEVEL AGAINST ETHYLENE GLYCOL INDUCED UROLITHIASIS IN RATS.

In ethylene glycol group, the kidney weight, urine pH, urine total protein level of rats were significantly increased where as cystone 750 mg/kg , EELA 200 mg/kg, EELA 400 mg/kg were significantly decreased the kidney weights, urine pH, urine total protein level compared to the normal control group. whereas the urine volume of rats treated with ethylene glycol group were significantly decreased(4.32±0.12) where as cystone 750 mg/kg(8.36±0.05), EELA 200 mg/kg(6.75±0.07), EELA 400 mg/kg(7.58±0.06) were significantly increased compared to the normal control group. The results were shown in Table 2**.**

Table 2. The effect of EELA on kidney weights, urine pH, urine volume and urine total protein levels against ethylene glycol induced urolithiasis in rats

S.NO	Groups	Kidney weight(gm)	Urine pH	Urine volume(ml)	Urine total protein(mg/dl)
1	Normal	0.52±0.005	3.92±0.02	9.45±0.21	5.45±0.13
2	Ethylene glycol	1.12±0.02*	10.44±0.18*	4.32±0.12*	9.82±0.41*
3	Cystone	0.54±0.007$^{**\#}$	5.73±0.02$^{*\#}$	8.36±0.05$^{*\#}$	5.62±0.03$^{*\#}$
4	EELA 200mg/kg	0.63±0.008$^{*\#}$	6.34±0.07$^{*\#}$	6.75±0.07$^{*\#}$	7.70±0.08$^{*\#}$
5	EELA 400mg/kg	0.58±0.003$^{**\#}$	5.88±0.06$^{**\#}$	7.58±0.06$^{*\#}$	6.27±0.11$^{*\#}$

n=6; values were expressed as mean ±SEM;*P<0.001, **P<0.01, vs control group, $^{\#}$P<0.001 vs ethylene glycol group. Data were analysed by one way ANOVA followed by Tukey's multiple comparison test. Values of P<0.05 considered significant.

3.4. EFFECT OF EELA ON URINE BIOLOGICAL PARAMETERS AGAINST ETHYLENE GLYCOL INDUCED UROLITHIASIS IN RATS.

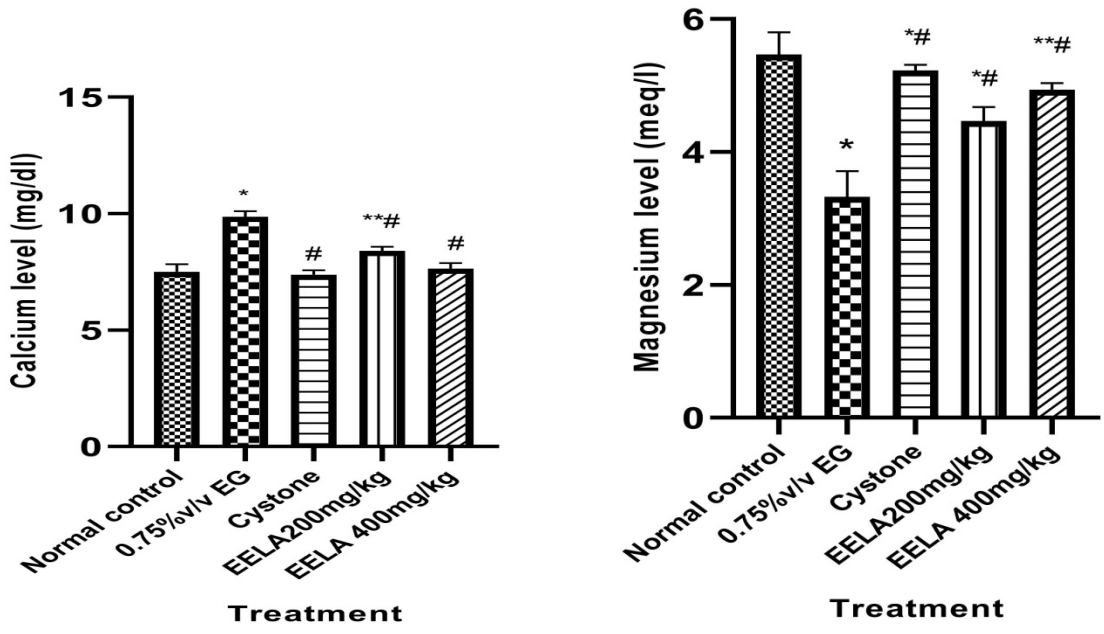
The urine calcium, phosphorous levels of rats treated with ethylene glycol group were significantly increased where as cystone 750 mg/kg, EELA 200 mg/kg and EELA 400 mg/kg

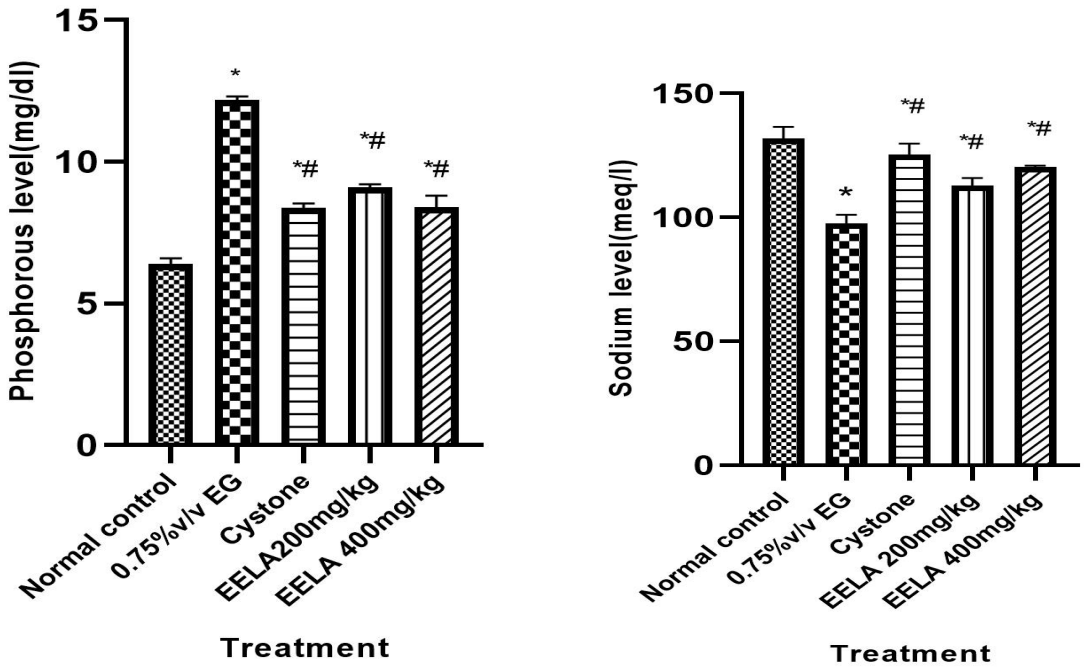
were significantly decreased compared to the normal controls group. Whereas the urine magnesium, sodium levels of rats treated with ethylene glycol group were significantly decreased where as cystone 750 mg/kg, EELA 200 mg/kg and EELA 400 mg/kg were significantly increased compared to the normal control group. The results were shown in Table 3, Figure 3.

Table 3. Effect of EELA on urine biological parameters against ethylene glycol induced urolithiasis in rats

S.No	Groups	Calcium (mg/dl)	Magnesium (meq/l)	Phosphorous (mg/dl)	Sodium (meq/l)
1	Normal	7.86±0.04	5.81±0.057	6.62±0.05	137.23±0.14
2	Ethylene glycol	10.01±0.09*	3.75±0.08*	12.72±0.04*	99.49±0.53*
3	Cystone	7.83±0.08$^{\#}$	5.32±0.04$^{*\#}$	8.54±0.076$^{*\#}$	129.67±0.67$^{*\#}$
4	EELA 200mg/kg	8.63±0.07$^{**\#}$	4.66±0.11$^{*\#}$	9.21±0.08$^{*\#}$	115.21±0.87$^{*\#}$
5	EELA 400mg/kg	7.62±0.04$^{\#}$	5.02±0.08$^{**\#}$	8.82±0.039$^{*\#}$	120.38±0.42$^{*\#}$

n=6; Values are expressed as mean±SEM;*P<0.001, **P<0.01, vs control group, $^{\#}$P<0.001 vs ethylene glycol group. Data are analysed by one way ANOVA followed by Tukey's multiple comparision Test. Values of P<0.05 considered significant.





n=6; Values are expressed as mean±SEM;*P<0.001, **P<0.01, vs control group, #P<0.001 vs ethylene glycol group. Data are analysed by one way ANOVA followed by Tukey's multiple comparision Test. Values of P<0.05 considered significant.

Figure 3. Graphical representation of effect of EELA on urine sodium level against ethylene glycol induced urolithiasis in rats

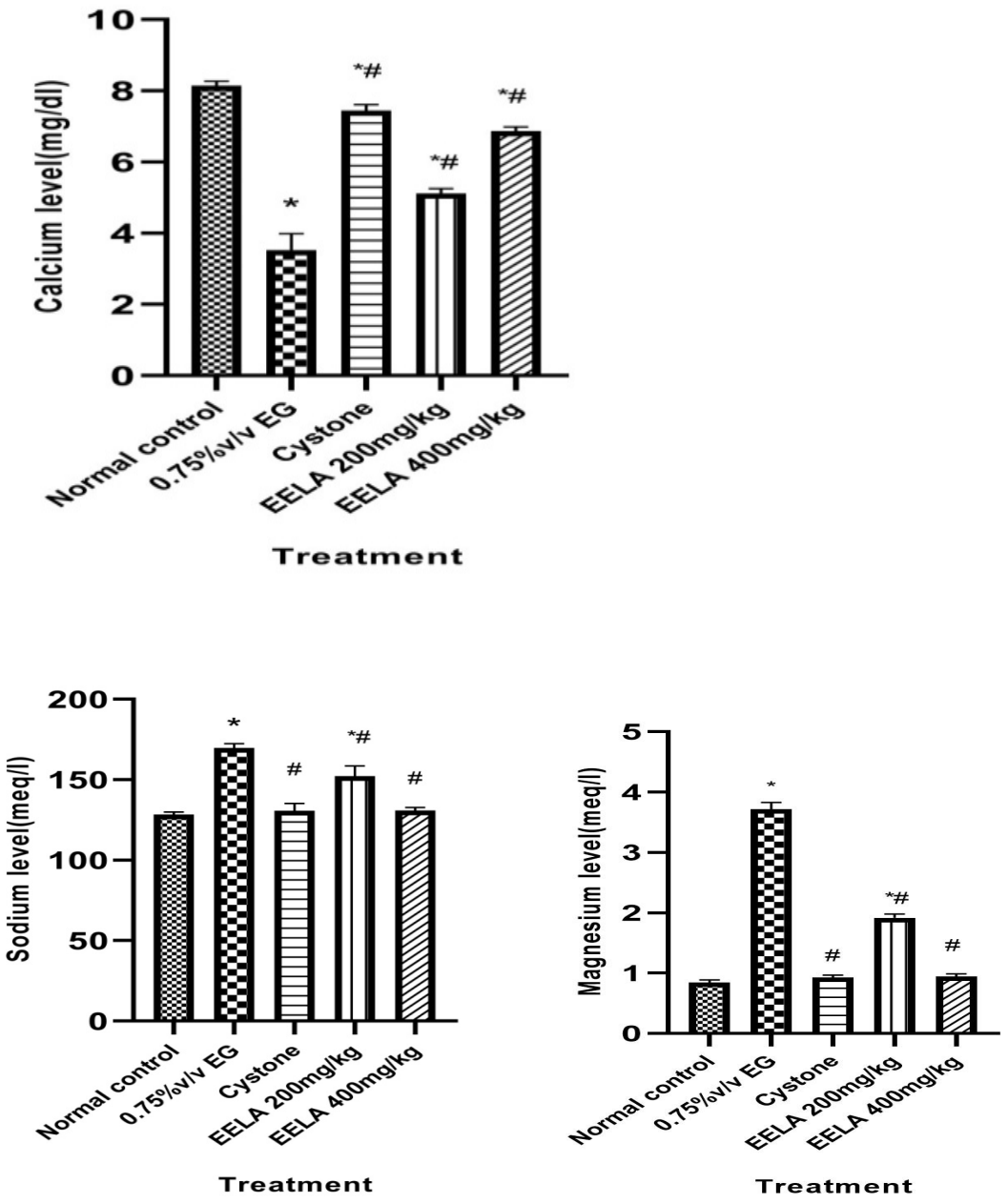
3.5. EFFECT OF EELA ON SERUM BIOLOGIAL PARAMETERS AGAINST ETHYLENE GLYCOL INDUCED UROLITHIASIS IN RATS.

The serum calcium levels of rats treated with ethylene glycol group were significantly decreased (3.82±0.02) where as cystone 750 mg/kg (7.58±0.08), EELA 200 mg/kg (5.32±0.05) and EELA 400 mg/kg (6.76±0.03) were significantly increased the serum calcium levels compared to the normal control group. Whereas the serum sodium, magnesium levels of rats treated with ethylene glycol control group were significantly increased where as cystone 750 mg/kg, EELA 200 mg/kg and EELA 400 mg/kg were significantly decreased the serum sodium, magnesium levels compared to the normal control group. The results were shown in Table 4, Figure 4.

S.NO	Treatment	Calcium (mg/dl)	Sodium (meq/l)	Magnesium (meq/l)
1	Normal	8.18±0.09	129.82±0.81	0.86±0.026
2	Ethylene glycol	3.82±0.02*	172.91±0.70*	3.72±0.049*
3	Cystone	7.58±0.08*#	135.70±0.72#	0.97±0.03#
4	EELA	5.32±0.05*#	146.88±0.90*#	1.92±0.05*#
5	EELA	6.76±0.03*#	135.96±0.53#	0.99±0.029#

Table 4. Effect of EELA on serum biological parameters against ethylene glycol induced urolithiasis in rats.

n=6; values are expressed as mean±SEM;*P<0.001, **P<0.01 vs control group, #p<0.001 vs ethylene glycol group. Data are analysed by Tukey's multiple comparison test. Values of P < 0.05 considered significant.



n=6; values are expressed as mean±SEM;*P<0.001, **P<0.01 vs control group, #p<0.001 vs ethylene glycol group. Data are analysed by Tukey's multiple comparison test. Values of P < 0.05 considered significant.

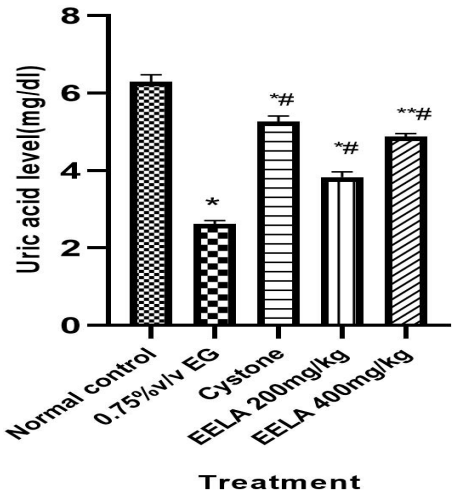
Figure 4. Graphical representation of effect of EELA on serum calcium level, potassium levels, and sodium level against ethylene glycol induced urolithiasis in rats.

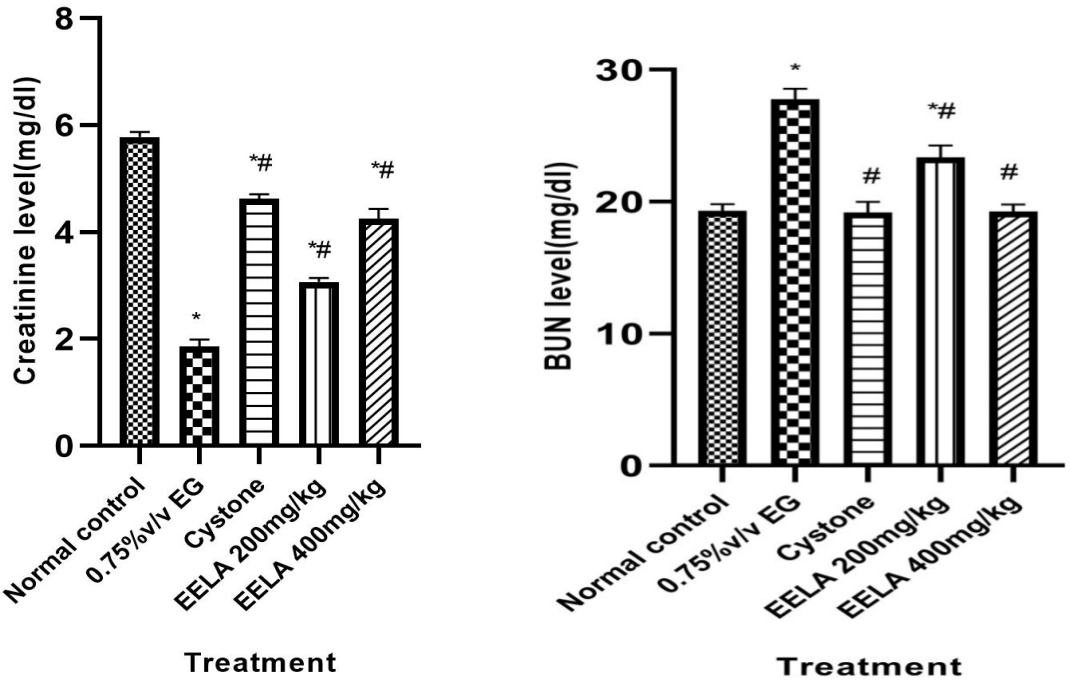
3.6. KIDNEY FUNCTION TESTS (URIC ACID, CREATININE, BUN)

The urine uric acid, creatinine levels of rats treated with ethylene glycol group were significantly decreased (2.67±0.05, 1.971±0.006) where as cystone 750 mg/kg (5.60±0.03, 4.65±0.04), EELA 200 mg/kg (3.93±0.023.07±0.08,) and EELA 400 mg/kg (4.82±0.028, 4.22±0.02) were significantly increased the urine uric acid levels compared to normal control group.whereas the serum The serum blood urea nitrogen(BUN)levels of rats treated with ethylene glycol group were significantly increased (1.971±0.006) where as cystone 750 mg/kg (4.65±0.04), EELA 200 mg/kg (24.30±0.62) and EELA 400 mg/kg (20.92±0.32) were significantly decreased the serum BUN compared to the normal control group. Blood urea nitrogen (BUN) results were shown in Table 5, Figure 5.

Table 5. Effect of EELA on kidney function tests (uric acid, creatinine, BUN) against ethylene glycol induced urolithiasis in rats.

S.NO	Group	Uric acid (mg/dl)	Creatinine(mg/dl)	BUN(mg/dl)
1	Normal	6.40±0.025	5.885±0.03	19.03±0.51
2	Ethylene glycol	2.67±0.05*	1.971±0.006*	28.91±0.50*
3	Cystone	5.60±0.03*#	4.65±0.04*#	20.75±0.83#
4	EELA	3.93±0.02*#	3.07±0.08*#	24.30±0.62*#
5	EELA	4.82±0.028**#	4.22±0.02*#	20.92±0.32#



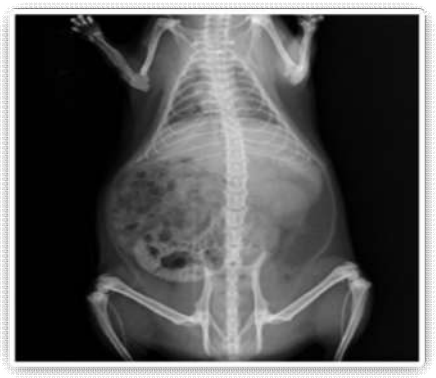


n=6; Values are expressed as mean±SEM;*P<0.001, **P<0.01 vs control group, #P<0.001 vs ethylene glycol group.Data are analysed by one way ANOVA followed by Tukey's multiple comparision Test. Values of P<0.05 considered significant.

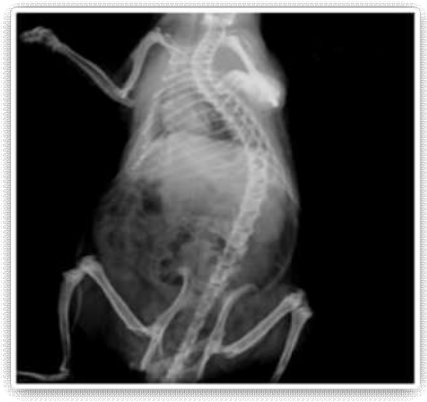
Figure 5. Graphical representation of effect of EELA on kidney function tests (uric acid, creatinine, BUN) against ethylene glycol induced urolithiasis in rats.

3.7. RADIOGRAPHIC STUDIES OF ANTIUROLITHIATIC ACTIVITY OF EELA AGAINST ETHYLENE GLYCOL INDUCED UROLITHIASIS IN RATS

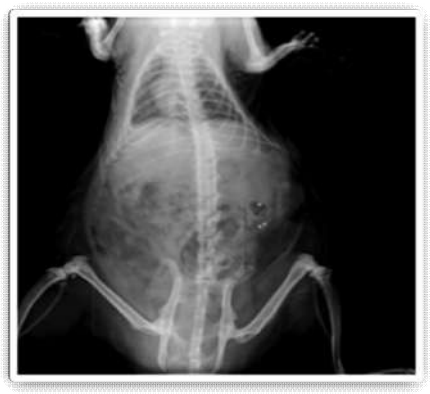
Radiographic changes in ethylene glycol induced urolithiasis rat model are the diagnostic measures which indicate the severity of the disease. As shown in the Figure 6, kidney of disease control group animals had many CaOx crystals deposition in the renal tubules it causes tubular dilation and renal 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. EELA 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 6.



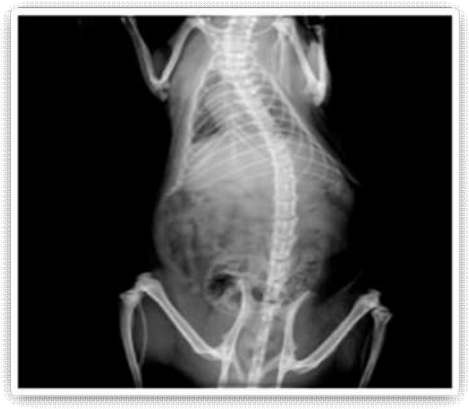
Normal Control



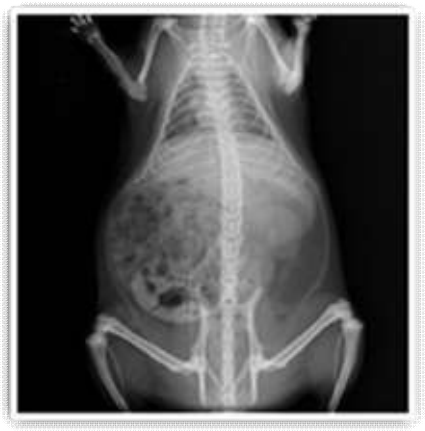
Disease Control (0.75%v/v EG)



Standard (Cystone 750mg/kg)



EELA 200mg/kg

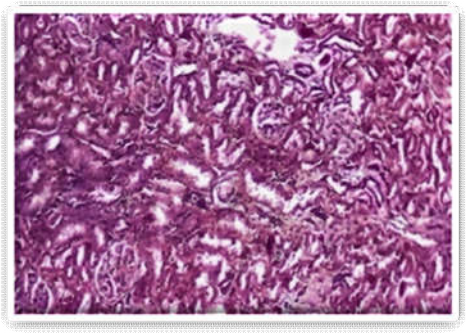


EELA 400mg/kg

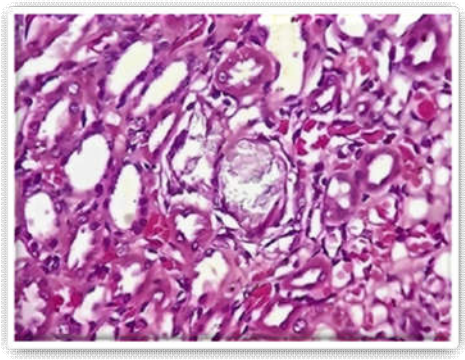
Figure 6. Effect of EELA on radiographic studies of kidney against ethylene glycol induced urolithiasis in rats.

3.8. EFFECT OF EELA ON HISTOPATHOLOGY OF KIDNEY AGAINST ETHYLENE GLYCOL INDUCED UROLITHIASIS IN RATS.

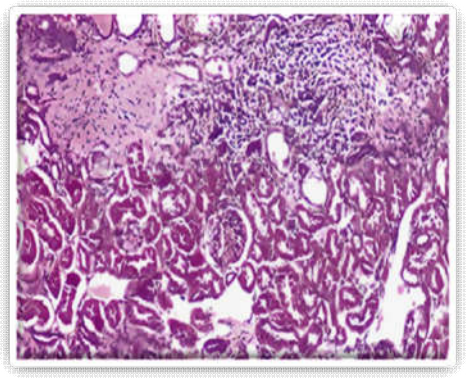
In normal group of rat kidney showed renal parenchyma with normal tubular epithelial cells and glomeruli. There was no histopathological change in tubules, glomeruli and a blood vessel was observed. In the urolithiatic control group (0.75%v/v ethylene glycol) many CaOx crystal deposits in the renal tubules 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 EELA 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 EELA 400 mg/kg shows good anti urolithiatic activity like standard cystone drug .The histopathology study results of antiurolithiatic activity of EELA on kidney against ethylene glycol induced urolithiasis in rats were shown in Figure 7**.**



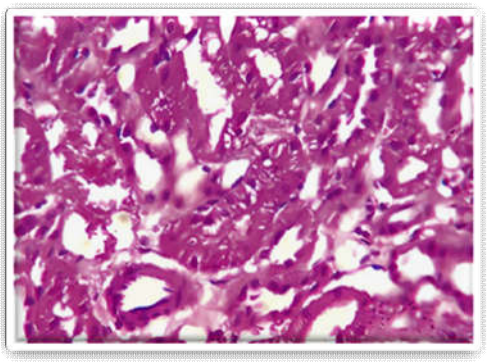
Normal Control



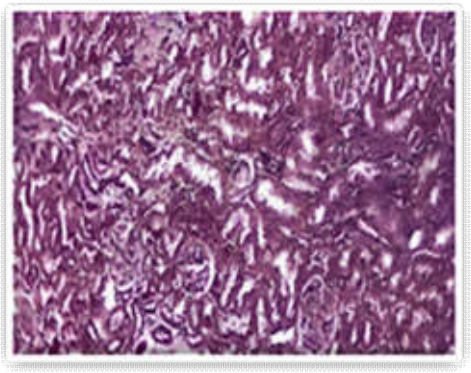
Disease Control (0.75%v/v EG)



Standard (Cystone 750mg/kg)



EELA 200mg/kg



EELA 400mg/kg

Figure 7. Effect of EELA on histopathology of kidney against ethylene glycol induced urolithiasis in rats

4. 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. EELA 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 anti-urolithiatic activity in albino rats. In comparison between the two doses of EELA i.e.,200mg/kg and 400mg/kg, EELA 400mg/kg shows a distinguished effect when compared to EELA 200mg/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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Conflict of interest

There are no financial or other conflicts of interest that the author needs to disclose.

Statement of Contribution of Researchers

Experimentation with plant extracts, data collecting, and generations of outcomes. Authors took part in the planning and writing.

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