

# Evaluation of Antiurolithiatic potential of *Kigelia africana* fruits in albino rats

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### Summary

*Kigelia africana* (Lam.) Benth. (Family; Bignoniaceae) is a widely used for treatment of various diseases including renal disorders in Africa and India as traditional medicine.

The aim of this study was to evaluate the antiurolithiatic activity of alcoholic and aqueous extract of *Kigelia africana* fruits (KAFE) as treatment for renal stones.

*In vitro* calcium oxalate (CaOx) crystallization inhibitory effect of KAFE was determined by measuring crystal size microscopically and crystal dissolution quantitatively in synthetic urine. In a rat model of urolithiasis, induced by adding 0.75% ethylene glycol (EG) in drinking water and effect of simultaneous treatment of KAFE (100-200 mg/kg) was observed for 28 days.

KAFE inhibited CaOx nucleation, aggregation and crystal formation in the synthetic urine *in vitro*. The lithogenic treatment caused polyurea, weight loss, hyperoxaluria and impairment of renal function which was prevented by KAFE. Hyperoxaluria and CaOx crystal deposition in the renal tubules caused by EG intake was prevented by KAFE treatment.

This study indicates that the antiurolithiatic activity of *Kigelia africana* fruit extracts (KAFE) possibly mediated through inhibition of CaOx crystallization, hypo-oxaluria and improvement of kidney function as well as the cytoprotective effect may justify its curative and prophylactic use in urolithiasis.

**Key Words:** Calcium oxalate, Ethylene glycol, Hyperoxaluria, Urolithiasis

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## *Kigelia africana* meyvelerinin albino sıçanlarda antiürolitiyatik aktivitesinin değerlendirilmesi

### Özet

*Kigelia africana* (Lam.) Benth. (Bignoniaceae) böbrek hastalıkları gibi çeşitli hastalıkların tedavisi için geleneksel ilaç olarak Afrika ve Hindistan'da yaygın olarak kullanılan bir bitkidir.

Bu çalışmanın amacı böbrek taşlarının tedavisi için *Kigelia africana* meyvelerinin alkolü ve sulu ekstre (KAFE) lerinin antiürolitiyatik aktivitelerini değerlendirmektir.

*In vitro* kalsiyum oksalat (CaOx) KAFE kristalleştirilmesi inhibitör etkisi mikroskopik olarak kristal boyutunu ölçmek ve sentetik idrar içinde kantitatif olarak kristal erime ile tespit edilmiştir. Ürolitiazisin bir sıçan modelinde, KAFE (100-200 mg / kg) 28 gün süreyle gözlemlendi eşzamanlı tedavi içme suyu ve sonuç olarak% 0.75 etilen glikol (EG) eklenmesi ile indüklenen.

KAFE CaOx çekirdeklenme, toplama ve *in vitro* olarak sentetik idrar içinde kristal oluşumunu inhibe etmiştir. Litojenik tedavi poliüre, kilo kaybı, hiperoksalüri ve KAFE tarafından önlenmiş böbrek fonksiyon bozukluğundan kaynaklanır. EG alımı nedeniyle renal tübüllerde Hiperoksalüri ve CaOx kristal depolanması KAFE tedavisi ile önlenmiştir.

Bu çalışmada *Kigelia africana* meyve özleri (KAFE) ve antiurolithiatic etkinlik muhtemelen CaOx kristalleşme, hipo-oxaluria ve böbrek fonksiyonu gibi sito-koruyucu etki ürolitiazisi içinde tedavi edici ve profilaktik kullanım uygulanmasına izin verebilir ve iyileştirilmesi inhibe etmek yoluyla olduğunu gösterir.

**Anahtar Kelimeler:** Kalsiyum oksalat, Etilen glikol, Hiperoksalüri, Ürolitiazis

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## INTRODUCTION

Despite the technological and conceptual developments in the practice of medicine, the formation and growth of renal calculi continue to afflict the mankind. The incidence of kidney stones has increased in western societies in the last five decades, in association with the economic development; most calculi in the urinary system arise from a common component of urine; calcium oxalate (CaOx), representing up to 80% of all analyzed stones [1]. Treatment procedures for renal stones such as surgical removal, percutaneous techniques and extra corporal shock wave lithotripsy (ESWL) are prohibitively costly and with these procedures, recurrence is quite common. However, herbal remedies have been found to be effective in reducing the recurrence rate of renal stones [2]. In India, in the Ayurvedic system of medicine, Pashanabheda (Pashana-stone; bheda-break) is the Sanskrit term used for a group of plants with diuretic and antiurolithiatic activities. In Indian folk medicine, many plants have been used to treat kidney stones, including *Kigelia Africana*. *Kigelia africana* (Lam.) Benth. (Family; *Bignoniaceae*) commonly known as sausage tree in Africa and India [3]. It is used as traditional medicine for treatment of various diseases, including renal disorders. Hydro alcoholic fruit extracts of this plant is used as a trusted drug to treat kidney stones in many regions of India. However, no scientific study has been conducted to justify the use of plants in urolithiasis.

The aim of this study was to establish the scientific validity for the prophylactic and treating role of *Kigelia africana* fruit extracts in urolithiasis, using an ethylene glycol induced hyper-oxaluria model in rats.

## PATIENTS AND METHODS

### Plant material

The fruits of *Kigelia africana* (Lam.) Benth. were collected from Forest Research Institute (FRI), Dehradun (Uttarakhand), identified and authenticated by taxonomist Dr. S. K. Srivastava, Botanical Survey of India (BSI) Dehradun (Uttarakhand). A specimen sample was preserved in the herbarium section of the Botanical Survey of India (BSI) Dehradun, with The Acc. No. 113498 for future reference.

### Preparation of extracts

#### Alcoholic extract

Shade dried, and coarsely powdered fruit (200g) was extracted exhaustively with 95% ethanol by cold percolation method (3×72 h). The solvent was distilled off over heating mantle and the extract obtained was dried in vacuum desiccators until free of moisture (yield: 3.78%). It was then stored in glass bottles and labeled for further use.

#### Aqueous extract

Shade dried, and coarsely powdered fruit (100 g) was extracted exhaustively with distilled water (1 lt.) by cold percolation method (3×72 h) followed by sonication. The solvent was distilled off over boiling-water bath and the extract obtained was dried in vacuum desiccators till free from moisture (yield: 22%). It was then stored in glass bottles labeled for further use.

### Animals

Animals (Albino Wistar rats & mice) were procured from disease free animal house of Indian Veterinary Research Institute Bareilly (U.P.) and acclimatized in the animal house facility of the department under ambient condition at room temperature 25°C ±2°C) with 12h light / dark cycle. The animals were provided with pellet chow and water *ad libitum*, except during experimentation. Ethical clearance was obtained from Institutional Animal Ethical committee 1156/PO/a/07/CPCSEA (IAEC) of Dehradun Institute of Technology, Dehradun and CPCSEA India.

### *In-vitro* study: CaOx crystallization assay in synthetic urine

Test tubes were divided in 4 groups; each group has 6 test tubes, in each tube 1 ml of calcium chloride anhydrous and 1 ml sodium oxalate were added along with 2 ml of tris buffer (disodium hydrogen phosphate and potassium dihydrogen phosphate) and adjusted to 7.4 pH which is the value of the kidney's pH and incubated at 36.7°C over night. The next day, the test tubes were centrifuged for 10 min to decant to remove top liquid layer [4,5]. The calcium oxalate crystal formed in the test tube were checked using the compound microscope under 45x magnification, the crystal formed was resembling

prisms shape, to this 5 ml (5 mg/ml) equivalent to 25mg to each test tube of the different extracts of plant *Kigelia africana* were introduced into the tubes and at the same quantity the poly herbal formulation Cystone was administered to the test tube, all the above treating agents were administered as aqueous suspension using tween-80 as suspending agent and again incubated at 36.7°C for 3 days, on the fourth day all the test tubes were taken and checked for dissolution of the crystals under the microscope at the same superimposition, to this test a drop of conc. HCl was added to separate the oxalate ion, calcium and both the ions were spectroscopically analyzed [6]. Crystal's dissolution was observed under 45x microscope and prism shape CaOx crystals were sized / measured by eye piece and stage micrometer mean size of more than 50 crystals were observed. Calcium, in an alkaline medium, reacts with o-cresolphthalein to form an intense chromophore which absorbs light at 575 nm (570-580 nm) [7]. Oxalate is co-precipitated with calcium sulphate, reduced to glycolic acid by boiling with dilute sulphuric acid and a zinc pellet and estimated colorimetrically with chromotropic acid [8]. Set the Auto-analyzer instrument with the parameters given along with the kit (AGAPPE diagnostics Ltd., Kerala). Prepare the working, standard and test solutions as per the protocol. Incubate for 5 min at room temp mix and read at 575 nm and 570 nm respectively.

#### **In-vivo study: Antirolithiatic activity**

The rat models of ethylene glycol (EG) induced hyperoxaluria described by Atmny et al 2003 were used to assess this study [9]. Wistar albino adult rats weighing 180-240 g were housed in metabolic cages three days prior to the start of the experiment. Animals were divided in 5 groups comprising six animals each. All animals had free access to regular rat chow and drinking water *ad libitum* for 28 days. Renal calculi were induced in group II to V by supplementing with 0.75%v/v ethylene glycol (a renotoxic agent) in drinking water *ad libitum* for 28 days. Group IV to V were treated with plant extracts starting from 7<sup>st</sup> day to 28<sup>th</sup> day (Preventive and treating regimen).

- **Group-I.** Normal control → Vehicle treatment.

- **Group-II.** Lithiatic control → 0.75%v/v ethylene glycol → Vehicle treatment.
- **Group-III.** Standard drug treated → 0.75%v/v ethylene glycol → Cystone (100 mg/kg).
- **Group-IV.** Test-1<sup>st</sup> → 0.75%v/v ethylene glycol → Alcoholic extracts (100 mg/kg).
- **Group-V.** Test-2<sup>nd</sup> → 0.75%v/v ethylene glycol → Aqueous extracts (100 mg/kg).

During the study of 28 days various urine and kidneys biochemical parameters were assessed for Antirolithic activity.

#### **General observations**

During the study period body weight, water intake and animal health was observed regularly, so that stressed and unhealthy animals were excluded from study [10].

#### **Urine analysis**

Urine was collected on days 7, 14, 21 and 28 for 24h by keeping the animals in metabolic cages. A drop of concentrated hydrochloric acid was added to the urine before being stored at 4°C. The collected urine was analyzed for calcium (using calcium liquid kit; raichem method), magnesium (Erba magnesium Aresnazo method), oxalates (Hodkinson and William's procedure) and inorganic phosphates (using phosphorous reagent kit; raichem method) by UV spectroscopy using standard methods. The volume of urine collected from all groups also recorded [11].

#### **Kidney homogenate analysis**

The abdomen incised to remove both kidneys from each animal. Isolated kidneys were cleaned of extraneous tissue. Kidneys were dried at 80°C in a hot air oven. A sample of 100 mg of the dried kidney was boiled in 10 ml of 1N HCl for 30 min and homogenized. The homogenate was centrifuged at 2000 rpm for 10 min and supernatant was separated [12]. The calcium, phosphate and oxalate content in kidney homogenate were determined.

#### **Statistical analysis**

Data are expressed as mean ± standard error of mean (SEM). Comparisons between groups were made by



one way analysis of variance (ANOVA) with post hoc Dunnett’s t-test (Level of significance was kept at  $P < 0.05$ ). Concentration response curves were analyzed by linear regression Graphpad Prism 5.0 software [13].

RESULTS

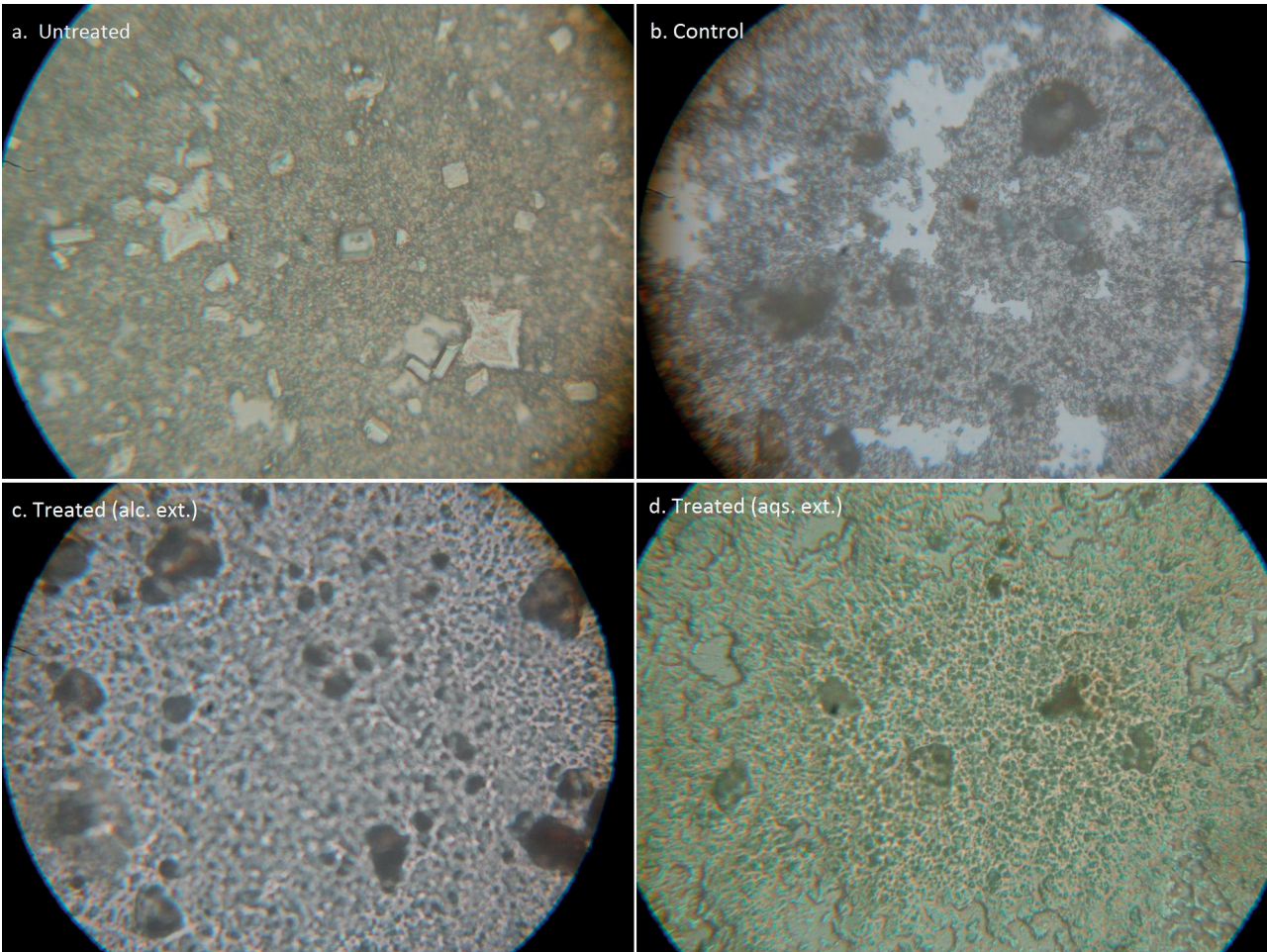
Effects on *in-vitro* CaOx crystallization

The effect of administration of aqueous and alcoholic extract (5 mg/ml-5 ml) / Cystone (5 mg/ml-5 ml) on size and dissolution of Calcium-Oxalate (CaOx) crystals was determined by microscopy and chemical analysis. The prism shape Calcium-Oxalate (CaOx) crystals were sized/measured by eye piece and stage micrometer. Mean size of more than 50 crystals were observed (Fig. 1).

In microscopic examination the crystal size reduction in aqueous extract (2.98  $\mu\text{m}$ ) is more significant ( $p < 0.001$ ) than both alcoholic extract (4.06  $\mu\text{m}$ ;  $p < 0.001$ ) and standard cystone (3.46  $\mu\text{m}$ ;  $p < 0.001$ ) compared to normal untreated crystal size (9.84  $\mu\text{m}$ ). Values are in mean  $\pm$ SEM (n=50) \*\*\* $p < 0.001$ , \* $p < 0.05$  vs. Normal control.

The aqueous extract shows more activity then alcoholic extract in examination of both the analytical parameters. In alimantal ion analysis also the aqueous extract shows maximum hike in concentration of calcium and oxalate ions (44.2; 99.47 mg/dl) than alcoholic extract (42.104; 94.73 mg/dl) and slight bit near to the standard cystone control (44.033; 98.28 mg/dl) compared to the normal control (13.185;

Figure 1. CaOx crystals of different treatment groups under 45 x magnification



Observed generated CaOx crystals of different treatment groups under 45x magnification (a) untreated showed pyramidal and prism shape crystals (b) control showed very few no. of crystals (c & d) treated groups showed significantly reduced size and number of crystals.

**Table 1.** Effect of *K. africana* fruit extracts on alimantal analysis.

Group	Calcium (mg/dl) $\pm$ SEM	Oxalate (mg/dl) $\pm$ SEM
Normal control	13.185 $\pm$ 3.240	28.78 $\pm$ 1.0
Standard control (cystone)	44.033 $\pm$ 0.3059***	98.28 $\pm$ 1.0***
Test-1 (Alcoholic extract)	42.104 $\pm$ 0.526***	94.73 $\pm$ 1.0***
Test-2 (Aqueous extract)	44.20 $\pm$ 0.526***	99.47 $\pm$ 1.0***

Values are in mean  $\pm$ SEM (n = 6) \*\*\*p <0.001, \*p <0.05 vs. Normal control.

28.78 mg/dl). The increase in calcium and oxalate concentration in both test drug extracts and standard drug cystone treated groups were found to be very significant (p <0.001) compared to normal untreated group (Table 1).

#### Effect observed in animal model

Body weight, water intake, urine volume and pH before the start of experiment were not significantly different among the study groups. The parameters recorded during the experimental period are shown in Table 2. The weight of the untreated group decreased significantly (p <0.01) compared to the normal and treated groups. However the KAFE treated groups have shown significant increase in body weight. There was no significant increase or decrease in water intake in all groups.

EG treatment reduced the urine pH in the untreated group compared to that of the control group, although not significantly. Co-treatment with KAFE increased urine volume (p <0.05) in a dose dependant manner, although these parameters remained higher than those of the untreated animals. Our results are in agreement with these studies, as shown by the significant increase in urinary calcium and oxalate levels were found to be highly significant. KAFE treatment at a dose of 100 and 250 mg/kg b. wt. revealed a dose related response. KAFE treatment at dose levels of 250 mg/kg b. wt showed a better protective effect. However, there was no significant

difference observed between 100 and 250 mg/kg b. wt of KAFE treatment. This finding revealed that 100 mg/kg b. wt of KAFE is the minimum dose required for eliciting an optimal activity. Cystone (std. drug) treatment significantly lowered the oxalate values (p <0.001) compared to lithiatic control group animals, probably by its inhibitory action on glycolate oxidase. KAFE (alcoholic and aqueous) treatment also significantly lowered the oxalate values (p <0.001) compared to lithiatic control. The reduction in the urinary oxalate level will be beneficial in preventing the urinary super-saturation with respect to oxalate. Calcium and phosphorus play vital roles in renal calculogenesis. Calcium and inorganic phosphorus levels were also elevated in the rat's urine receiving a calculi-producing diet. The increase in calcium excretion may be due to defective tubular reabsorption in the kidneys [14]. Cystone and KAFEs treatment markedly reduced the levels of calcium and phosphorus (p <0.001) in urine (Table 3). The excretion of magnesium (0.30  $\pm$ 0.64 mg/24h) decreased gradually in group-2 animals after 4<sup>th</sup> week following EG treatment which is quite significant (p <0.001) compared to normal control group. But in other animal treatment groups (3,4 & 5), it showed an enhanced excretion of magnesium and values are significant (p <0.001) compared to lithiatic control animals.

The kidneys were heavier in the untreated group, but not in the KAFE treated groups, compared with the control group. The calcium and oxalate content of the kidneys was significantly (p <0.01) increased in the untreated group, but not in the KAFE treated groups (Table 2).

#### DISCUSSION

Kidney stone formation is a physicochemical process including various events that starts with super saturation, nucleation, growth, aggregation, and retention within renal tubules [15]. Different *in-vitro* models were used to study various physicochemical events and simulate the urinary conditions by various authors [16]. We have evaluated the effect of KAFE on CaOx crystallization kinetics by the time course measurement of crystal size and alimantal ion analysis. The aqueous and alcoholic extracts of fruit

**Table 2.** Various parameters recorded for assessment of antiurolithiatic activity during 28 days of study.

Parameter		Normal control	Lithiatic control	Cystone (std.)	Alcoholic Ext.	Aqueous Ext.
General	Change in body weight (gm)	3.76 ±1.92	-7.23 ±44.43 <sup>b</sup>	2.13 ±2.13	4.57 ±1.82 <sup>b</sup>	6.14 ±2.43 <sup>c</sup>
	Water intake (ml/24hr)	11.2 ±0.23	13.73 ±0.25 <sup>b</sup>	17.33 ±0.55 <sup>b,d</sup>	15.42 ±1.08 <sup>b</sup>	16.92 ±0.62 <sup>b,d</sup>
	Urine volume (ml/24hr)	6.67 ±0.38	11.15 ±0.84 <sup>b</sup>	16.05 ±0.36 <sup>b,d</sup>	13.74 ±0.48 <sup>b</sup>	14.06 ±1.32 <sup>b,d</sup>
	Urine pH	6.8 ±0.03	6.4 ±0.06	6.8 ±0.04	6.8 ±0.03	6.8 ±0.07
Kidney homogenate analysis	Weight (gm)	0.63 ±0.03	1.44 ±0.22 <sup>b</sup>	0.74 ±0.12	0.79 ±0.02	0.67 ±0.15
	Calcium (mg)	0.20 ±0.35	1.27 ±0.34 <sup>b</sup>	0.40 ±0.56 <sup>d</sup>	0.47 ±0.64 <sup>d</sup>	0.34 ±0.42 <sup>d</sup>
	Oxalate (mg)	0.44 ±0.06	3.04 ±0.15 <sup>b</sup>	1.12 ±0.06 <sup>d</sup>	1.27 ±0.21 <sup>d</sup>	0.94 ±0.08 <sup>d</sup>
	Phosphorus (mg)	2.39 ±0.01	2.53 ±0.17	2.35 ±0.02	2.17 ±0.03	2.89 ±0.78

Values are in mean ±SEM (n = 6) a p <0.05 vs normal control group, b p <0.01 vs normal control; c p <0.05 vs lithiatic control group, d p <0.01 vs lithiatic control.

of *K. africana* strongly inhibited the precipitation of calcium and oxalate. The result of our study clearly showed the utility of *K. africana* in the treatment of renal and urinary calculi.

In microscopical examination the crystal size reduction in aqueous extract was more significant (p <0.001) than both alcoholic extract and standard cystone compared to normal untreated crystal size. Thus it can be inferred that the test drug extract contribute to heal renal calculi by crystal/stone size reduction.

In alimantal ion analysis, the aqueous extract also shows maximum hike in concentration of calcium and oxalate ions than alcoholic extract and standard cystone compared to the normal control. The increase in calcium and oxalate concentration in both test drug extracts and standard drug cystone treated groups were found to be very significant (p <0.001) compared to normal untreated group. That is under physiological condition of the reaction system inhibited calcium and oxalate ions precipitation. Our results conclude that these inhibitors of crystallization along with crystal dissolution would potentially contribute in ailment of urolithiasis.

Renal CaOx deposition induced by EG in rats is frequently used by various researchers to mimic the urinary stone formation in humans [5]. Oxalate is produced during metabolism and excreted harmlessly in normal individuals. However increased concentration of oxalate in urine can be highly toxic because it crystallizes at physiological pH to form CaOx [15]. EG metabolize to oxalate in body and increase its concentration in the untreated group, which was prevented by KAFE.

Urinary stone formation takes place due to changes in urinary chemistry, such as hyperoxaluria and hypercalciuria, leading to urinary super saturation, which later crystallizes, aggregates and ends up in stone formation [17,18,19]. Evidences in previous studies indicated that, in response to 14 days period of ethylene glycol (0.75%v/v) administration, young albino rats form renal calculi composed mainly of CaOx. The principal precursor of oxalic acid in mammals is glyoxalic acid [20]. The enzymatic oxidative conversion of glycolate to oxalate via glyoxylate is the major metabolic pathway involved in endogenous oxalate synthesis. The enzymatic disturbances are the causative factors for the idiopathic hyperoxaluria; while, the defective intestinal absorption of oxalate plays a vital role in



**Table 3.** Effect of drugs on various urinary parameters recorded for assessment of antiurolithiatic activity during 28 days of study.

	Group	Week-1	Week-2	Week-3	Week-4
	Dose→	100mg/kg	100mg/kg	100mg/kg	250mg/kg
<b>Urine Oxalate</b>	Normal control	14.48 ±0.54	14.081 ±0.27	14.28 ±0.43	13.3 ±0.1
	Lithiatic control	24.28 ±0.1***	22.24 ±0.08***	23.24 ±0.72***	24.12 ±0.08***
	Cystone (std.)	17.14 ±0.26***	16.53 ±0.01***	16.83 ±0.62***	15.79 ±0.11***
	Alcoholic Ext.	18.16 ±0.22**	17.55 ±0.06***	17.75 ±0.64***	16.02 ±0.68***
	Aqueous Ext.	14.28 ±0.43***	14.48 ±0.08***	14.08 ±0.91***	13.22 ±1.2***
<b>Urine Calcium</b>	Normal control	4.43 ±0.23	4.25 ±0.54	4.73 ±0.26	4.32 ±0.22
	Lithiatic control	13.2 ±0.14***	12.28 ±0.72***	12.84 ±0.33***	11.93 ±0.37***
	Cystone (std.)	8.68 ±0.54***	8.18 ±0.35***	8.33 ±0.38***	5.92 ±0.62***
	Alcoholic Ext.	7.832 ±0.9***	7.28 ±0.87***	7.55 ±0.15***	6.02 ±0.74***
	Aqueous Ext.	7.83 ±0.53***	6.53 ±0.86***	7.08 ±0.55***	5.87 ±0.87***
<b>Urine Inorganic phosphorus</b>	Normal control	0.579 ±0.59	0.723 ±0.47	0.661 ±0.37	0.87 ±0.38
	Lithiatic control	1.436 ±0.14***	1.336 ±0.28***	1.286 ±0.36***	1.496 ±0.54***
	Cystone (std.)	1.145 ±0.25**	1.142 ±0.56**	1.043 ±0.34***	1.121 ±0.12***
	Alcoholic Ext.	1.123 ±0.23**	1.228 ±0.32***	1.075 ±0.62***	1.136 ±0.36***
	Aqueous Ext.	1.18 ±0.87***	1.128 ±0.42**	1.004 ±1.0***	1.032 ±0.9***
<b>Urine Magnesium</b>	Normal control	1.17 ±0.73	1.2 ±0.43	1.35 ±0.84	1.22 ±0.25
	Lithiatic control	1.3 ±0.45*	0.9 ±0.57**	0.7 ±0.14***	0.3 ±0.64***
	Cystone (std.)	0.73 ±0.14**	0.81 ±0.28***	0.84 ±0.24***	0.96 ±0.34***
	Alcoholic Ext.	0.78 ±0.42**	0.88 ±0.32***	0.89 ±0.49***	0.92 ±0.39***
	Aqueous Ext.	0.63 ±0.24**	0.78 ±0.29**	0.84 ±0.28***	0.97 ±0.25***

Values are in mg/24 hours (n = 6)

\*\*\*p <0.001, \*\*p <0.01, \*p <0.05 vs. lithiatic control group. Whereas lithiatic control group was compared with normal control group.

enteric hyperoxaluria and lead to an increase in the urinary oxalate concentration [21].

In the present study, chronic administration of 0.75% ethylene glycol aqueous solution to male Wistar rats resulted in hyperoxaluria. Oxalate and calcium excretion in urine were grossly increased in calculi-induced animals (Table 3, Group II). Since it is accepted that hyperoxaluria is far more significant risk factor in the pathogenesis of renal stones than

hypercalciuria, the changes in urinary oxalate levels are relatively much more important than those of calcium [22]. Increased urinary calcium is a factor favouring the nucleation and precipitation of CaOx or apatite (CaPh) from urine and subsequent crystal growth [23]. The increase in calcium deposition in kidney and its urinary excretion may be due to defective renal tubular reabsorption or an increase in absorption from the intestine as the patients with renal calcium stones are reported to have hyperabsorption

of calcium [24]. Aq.E. and Alc.E. of *K. africana* fruits lower the levels of oxalate and calcium in urine and even their retention in kidney.

Remarkable increase in urinary phosphate was observed in calculi-induced rats. Increased urinary phosphate excretion along with oxalate stress seems to provide an environment appropriate for stone formation by forming CaPh crystals, which epitaxially induces CaOx deposition [25]. Treatment with KAFE restores urinary phosphate level, thereby reducing the risk of stone formation.

In urolithiasis, the glomerular filtration rate (GFR) decreases, due to the obstruction to the outflow of urine by stones in urinary system. Due to this, the waste products, particularly nitrogenous substances such as urea, creatinine and uric acid get accumulated in blood [26]. In addition, increased lipid peroxidation and decreased levels of antioxidant potential have been reported in the kidneys of rats supplemented with a calculi-producing diet [27,28]. In this context, oxalate has been reported to induce lipid peroxidation and to cause renal tissue damage by reacting with polyunsaturated fatty acids in cell membrane [29]. Hence hastens the process of dissolving the preformed stones and in prevention of new stone formation in urinary system on prophylactic treatment.

## CONCLUSIONS

In conclusion, the drug *Kigelia africana* fruit extracts demonstrated anti-lithogenic properties in both *in-vivo* and *in-vitro* antilithiatic models. In *in-vitro* model, the crystal dissolution was observed microscopically and confirmed by detecting increase in calcium and oxalate concentration in elemental ion analysis. In *in-vivo* model there was reduced urine concentration of calcium, oxalate, inorganic phosphorus and increased concentration of magnesium ions. An overall result from both the lithiatic models concludes that is *Kigelia Africana* has a potent anti-urolithogenic activity and would help in renal stone dissolution and elimination.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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