

Pharmacological evaluation of ethanolic extract of *Kigelia pinnata* fruit against ethylene glycol induced urolithiasis in rats

Ravindra Kumar*, Tirath Kumar, Virender Kamboj, Harish Chander

Department of Pharmaceutical Sciences, Bhimtal Campus, Kumaun University, Nainital, Uttarakhand, India

ABSTRACT

*Urolithiasis is one of the third most common afflictions found in humans. The effect of oral administration of ethanolic extract of *Kigelia Pinnata* fruit on calcium oxalate urolithiasis has been studied in male Wistar albino rats. Ethylene glycol feeding resulted in hyperoxaluria as well as increased renal excretion of calcium, magnesium and phosphate. Supplementation with ethanolic extract of *Kigelia pinnata* fruit significantly reduced the elevated urinary oxalate, uric acid and phosphate. The increased deposition of stone forming constituents in the kidneys of calculogenic rats was also significantly lowered by ethanolic extract of *Kigelia pinnata* fruit. The results indicate that the ethanolic extract of *Kigelia pinnata* fruit is endowed with antiurolithic activity.*

Keywords: *Kigelia pinnata*; Hyperoxaluria; Urolithiasis; Ethylene glycol.

INTRODUCTION

Kigelia pinnata (Balam Kheera) belongs to the family of *Bignoniaceae* and commonly called the Sausage tree because of its huge fruits. In India, the family is distributed in 15 genera and 40 species, including *Kigelia pinnata* which mostly occur in Western and Southern India and a few species in the Himalayas. It is widely grown in the tropics and is cultivated in many parts of India but found abundantly in West Bengal as an ornamental and roadside tree [1]. It is widely grown in the tropics and is cultivated in many parts of India but found abundantly in West Bengal as an ornamental and roadside tree. The plant has maroon red flowers in long pendulous panicles and gourd like fruit. It is a tree growing up to 20 m tall. *Kigelia pinnata* is a multipurpose medicinal plant with many attributes and considerable potentials. The plant has traditional uses which include anticancer [2], antimicrobial [3], anti-aging, antioxidant [4], anti-inflammatory [5] and antimalarial properties. It is also widely applied in the treatment of genital infections [6], gynecological disorders [7], renal ailments, fainting, epilepsy [8], rheumatism, sickle-cell anemia, psoriasis, respiratory ailment, CNS depression, skin complaint, body weakness, leprosy, worm infestation etc [9].

Kidney stones are one of the most painful urologic disorders, have beset humans for centuries. Scientists have found evidence of kidney stones in a 7,000-year-old Egyptian mummy. Unfortunately, kidney stones are one of the most common disorders of the urinary tract. Each year, people make almost 3 million visits to health care providers and more than half a million people go to emergency rooms for kidney stone problems. For unknown reasons, the number of people in the United States with kidney stones has been increasing over the past 30 years [10].

Kidney stones may contain various combinations of chemicals. The most common type of stone contains calcium in combination with either oxalate or phosphate. These chemicals are part of a person's normal diet and make up important parts of the body, such as bones and muscles. Kidney stone are composed of crystal and proteins that grow until they break loose and pass into the urine collection system [11]. Stones containing calcium as oxalate, phosphate or both comprise about 80% of total. About 15% contain magnesium ammonium phosphate (struvite; these are often associated with infection), and small numbers of pure cystine or uric acid stones are found. Among

the several types of kidney stones, the most common are calcium oxalate. The formation of these stones involves several physicochemical events, beginning with crystal nucleation, aggregation, and ending with retention within the urinary tract [12].

Cystinuria and hyperoxaluria are two other rare, inherited metabolic disorders that often cause kidney stones. In cystinuria, too much of the amino acid cystine, which does not dissolve in urine, is voided, leading to the formation of stones made of cystine. In patients with hyperoxaluria, the body produces too much oxalate, a salt. When the urine contains more oxalate than can be dissolved, the crystals settle out and form stones. Hypercalciuria is inherited, and it may be the cause of stones in more than half of patients. Calcium is absorbed from food in excess and is lost into the urine. This high level of calcium in the urine causes crystals of calcium oxalate or calcium phosphate to form in the kidneys or elsewhere in the urinary tract [13].

In the present study, an effort has been made to establish the scientific validity for the Antiurolithiatic property of ethanolic extract of *Kigelia pinnata* fruit using ethylene glycol induced hyperoxaluria model in rats.

MATERIALS AND METHODS

2.1 Plant Collection:

The fresh fruits of *Kigelia pinnata* were collected from local area of Ramnagar, Nainital, Uttarakhand, India. India and Botanical authentication was carried out by National Bureau of Plant Genetic Resources Regional Station, Niglat, Bhowali, Nainital, Uttarakhand, India by Dr. K.S.Negi. A voucher specimen of plant was deposited in the RSB herbarium under the number RSB/Tech.Corrsp./2011-12/525.

2.2 Preparation of *Kigelia pinnata* Extract:

The fruit of *Kigelia pinnata* were dried under shade and then were powdered with a mechanical grinder. The powder was then passed through sieve No.40 and was stored in an airtight container for further use. Dried and coarsely powdered fruits (350 g) were extracted with petroleum ether, chloroform and ethanol respectively using soxhlet apparatus. After complete extraction the solvent was recovered with the help of recovery unit. The extract was stored at room temperature till further use in the experiment. The Percentage Yield (Ethanolic Extract) was found to be 17.2%.

2.3 Phytochemical screening of extract:

Phytochemical screening of plant extract shows the presence of carbohydrate, protein, flavanoids, alkaloids and saponin.

2.4 Drugs and Chemicals:

Ethylene glycol was obtained from Sigma Chemical Company and was used in the study to induce renal calculi in rats. Cystone was obtained from the Himalaya Drug Company Pvt. Ltd, and was used as a standard drug. All other chemicals and solvents used in this study were of analytical grade and purchased from commercial sources.

2.5 Preparation of Extract Dose:

The animals were fed with pelleted rat chow and had free access to drinking water but were starved for 12 hours prior to testing. The extracts were orally administered as 100, 200, 400, 800, 1600, 3200, 6400, 12800 mg/kg ethanolic extract of *Kigelia pinnata* fruit extract. General symptoms of toxicity and mortality were observed for 24 hours for any sign of delayed toxicity [14]. The extract was well tolerated by the animals as there were no observable signs of acute toxicity effects like restiveness, seizures or dizziness after the administration of 400 mg/kg. However, at 6400 mg/kg, the animals showed signs of toxicity like jerks and writhes with 60% death. At 12,800 mg/kg, there was 80% death of the animals. The LD50 was estimated from a log-dose curve to be 3,981.07 mg/kg [15]. The plant extract was highly soluble in 3% w/v tween 80. The test drug (*Kigelia pinnata* extract) 200mg and 400mg/kg were administered orally as a suspension in 3 % w/v tween 80.

2.6 Pharmacological screening for antiurolithic activity:

2.6.1 Animals:

Male Wistar rats weighing between 200-250g each, maintained on standard laboratory diet and tap water ad libitum were employed in the present study. They were housed in departmental animal house 12 hr light and 12 hr dark cycle. Approval was taken from the Institutional Animal Ethical Committee from Department of Pharmaceutical Sciences, Kumaun University.

2.6.2 Experimental Design:

Ethylene glycol-induced hyperoxaluria method [16] was used to assess the anti urolithiatic activity in Wistar rats. Animals were divided into seven groups containing five animals in each. Group I served as control and received regular rat food and drinking water ad libitum. Ethylene glycol (0.75% w/v) in drinking water was fed to Groups II, V, VI, and VII for induction of renal calculi for 28 days. Group III received ethanolic extract of *Kigelia Pinnata* fruit (200mg/kg body weight) once daily by oral route for 28 days. Group IV received ethanolic extract of *Kigelia Pinnata* fruit (400mg/kg body weight) once daily by oral route for 28 days. Group V received standard anti urolithiatic drug, cystone (750 mg/kg body weight), from 15th day till 28th day. Group VI received ethanolic extract of *Kigelia Pinnata* fruit (200mg/kg body weight) treated once daily by oral route from 15th day till 28th day. Group VII received ethanolic extract of *Kigelia Pinnata* fruit (400mg/kg body weight) treated once daily by oral route from 15th day till 28th day.

2.6.3 Experimental Protocol:

Group I - Control (Treated with normal diet for 28 days),

Group II- Urolithic control [Treated with ethylene glycol (0.75%) in drinking water for 28 days],

Group III- Test drug [Treated with ethanolic extract of *Kigelia Pinnata* fruit (200mg/kg) once daily by oral route for 28 days],

Group IV - Test drug [Treated with ethanolic extract of *Kigelia Pinnata* fruit(400mg/kg) once daily by oral route for 28 days],

Group V - Cystone (std.) + urolithic control (Treated once daily by oral route from 15th day till 28th day),

Group VI - Urolithic control + ethanolic extract of *Kigelia Pinnata* fruit (200mg/kg, treated once daily by oral route from 15th day till 28th day),

Group VII - Urolithic control + ethanolic extract of *Kigelia Pinnata* fruit (400mg/kg, treated once daily by oral route from 15th day till 28th day).

The test drug (*Kigelia pinnata* extract) and standard drug (cystone) were administered orally as a suspension in 3 % w/v tween 80.

2.6.4 Assessment of Antiurolithic Activity:

2.6.4.1 Collection and Analysis of Urine:

Wistar male rats were kept separately in metabolic cage and 24 h urine samples were collected on 0, 7, 14, 21 and 28th days of calculi induction treatment. Animals had free access to drinking water during the urine collection period. A drop of concentrated hydrochloric acid was added to the urine before being stored at 4°C. Urine samples were analyzed for calcium [17], uric acid [18], magnesium [19], phosphate [20], and oxalate content [21] by auto analyzer.

2.6.4.2 Serum Analysis:

At the end of the experiment, blood samples were collected from the retro-orbital plexus under anaesthetic conditions and animals were sacrificed by cervical decapitation. Serum was separated by centrifugation at 4000 rpm for 10 minutes and analyzed for creatinine [22] and uric acid [23] levels by auto analyzer.

2.6.4.3 Kidney homogenate analysis:

The abdomen was cut open to remove both kidneys from each animal. Isolated kidneys were cleaned off extraneous tissue and preserved in 10% neutral formalin. The kidneys were dried at 40°C in a hot air oven. A sample of 100 mg of the dried kidney was boiled in 10 mL of 1 N hydrochloric acid for 30 min and homogenized. The homogenate was centrifuged at 2000 rpm for 10 min. and the supernatant was separated. The calcium [17], phosphate [20], and oxalate [21] content in kidney homogenate were determined by auto analyzer.

2.6.4.4 Liver Histopathology:

The abdomen was cut open to remove liver from each animal. Isolated livers were cleaned off extraneous tissue and rinsed in ice-cold physiological saline. The livers were fixed in 10% neutral formalin and transferred to a container designed to allow reagents to freely act on the tissue inside. This cassette is immersed in multiple baths of progressively more concentrated ethanol to dehydrate the tissue followed by xylene and finally extremely hot paraffin. During this 12 to 16 hour processed paraffin was replaced the water in the tissue turning soft moist tissues into a sample miscible with paraffin. The processed of embedded then allows the sectioning of tissues into very thin (2 – 7 micrometer) sections using a microtome. The microtome slices the tissue ready for microscopic examination and stained with hematoxylin and eosin (H and E) for histopathological examination. The histological slides were examined under a microscope by a pathologist.

2.6.4.5 Statistical analysis:

The results were expressed as the mean \pm SEM and analyzed using one-way ANOVA followed by Dunnett's multiple comparison tests. Data were computed for statistical analysis using Graph Pad Prism Software and $P < 0.05$, $P < 0.01$ and $P < 0.001$ were considered to be statistically significant.

2.6.4.6 Biochemical parameters:

The biochemical parameters were estimated as per the standard procedure prescribed by the manufacturer's instruction manual provided in the standard kit using Auto analyser.

RESULTS

3.1. Urinary output determination

Control rats (Group I) did not show any significant variation in the urinary oxalate level throughout the experiment period. The urinary output was increased significantly ($P < 0.01$) in urolithic control. In the ethanolic extract of *Kigelia pinnata* fruit treated groups, the urine output was lower than that of the urolithic control but significantly ($P < 0.01$) higher than that of vehicle treated rats. Results are shown in Table 1 and figure 1.

Table 1- Determination of Urinary output in ml/24 hrs

Days	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
0	8.80 \pm 0.45	8.43 \pm 0.32	9.17 \pm 0.34	9.21 \pm 0.17	12.71 \pm 0.56	10.71 \pm 0.52	9.17 \pm 0.33
7	8.74 \pm 0.27	14.80 \pm 0.90a*	15.25 \pm 0.16	16.80 \pm 0.24	9.36 \pm 0.17	15.66 \pm 1.05a**	10.02 \pm 0.26
14	9.49 \pm 0.31	21.80 \pm 0.98a**	10.19 \pm 0.79	10.46 \pm 0.56	26.9 \pm 0.66a*b*	23.19 \pm 0.21a**	21.09 \pm 1.34a**
21	9.23 \pm 0.38	21.67 \pm 1.80a**	15.70 \pm 0.44a*	17.85 \pm 0.37a*	19.81 \pm 1.28a**	27.39 \pm 1.56a**b*	28.05 \pm 1.41a**b*
28	9.28 \pm 0.30	21.55 \pm 0.56a**	17.50 \pm 0.12a*	18.05 \pm 0.17a**	20.16 \pm 1.32a**	17.02 \pm 0.76a**b*	19.69 \pm 0.97a**b*

Values are expressed as Mean \pm SEM for five animals in each group. a values are significantly different compared to control when * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. b values are significantly different compared to the respective ethylene glycol treated group when * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

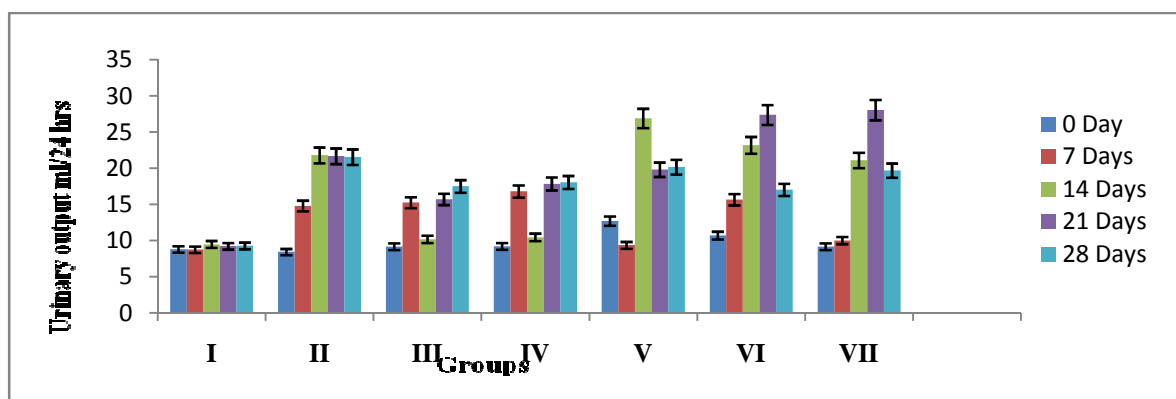


Fig.1 - Effect of ethanolic extract of *Kigelia pinnata* fruit on urinary output ml/24 hrs.

3.2. Urinary oxalate determination

In present study the male Wistar rats has shown hyperoxaluria. There was an increased oxalate in Group II. Administration of ethylene glycol (0.75% w/v) led to elevation of urinary oxalate levels in groups II, V, VI and VII as compared to control group I, which was maintained over an experimental period of 28 days. Both the doses, 200 mg and 400 mg/kg/day of ethanolic extract of *Kigelia pinnata* fruit for 15 days significantly reduced the urinary oxalate level as compared to the urolithic control.

3.3. Urinary phosphate determination

In present study the urinary phosphate control rats (Group I) did not show any significant variation in the urinary phosphate level throughout the experiment period. Both the doses 200mg and 400 mg/kg/day of ethanolic extract of *Kigelia pinnata* fruit for 15 days significantly reduced the urinary phosphate level as compared to the urolithic control. The ethanolic extract of *Kigelia pinnata* fruit at doses of 200 mg and 400 mg/kg/day have reduced the urinary phosphate level as compared to standard (cystone 750 mg/kg/day). Results are shown in Table 3 and figure 3.

Table 2 - Determination of urinary oxalate in mg/24 hrs

Days	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
0	5.07 ± 0.33	4.62 ± 0.37	4.76 ± 0.23	4.99 ± 0.27	5.13 ± 0.36	5.03 ± 0.21	4.97 ± 0.33
7	5.02 ± 0.27	6.13 ± 0.28a*	5.13 ± 0.16	6.13 ± 0.34	5.76 ± 0.17	6.32 ± 0.05a*	6.48 ± 0.26a*
14	5.24 ± 0.21	7.92 ± 0.37a**	5.13 ± 0.25	6.72 ± 0.32	8.24 ± 0.66a*b*	8.26 ± 0.21a**	7.82 ± 0.34a**
21	5.18 ± 0.38	8.83 ± 0.48a**	5.32 ± 0.44	8.44 ± 0.37	7.32 ± 0.28a**b**	8.98 ± 0.56a**	8.63 ± 0.41a**
28	5.12 ± 0.30	9.93 ± 0.42a**	6.29 ± 0.12	8.65 ± 0.17a*	7.05 ± 0.52a**b**	9.01 ± 0.26a**	8.69 ± 0.27a**b*

Values are expressed as Mean ± SEM for five animals in each group. a values are significantly different compared to control when * P < 0.05, ** P < 0.01, *** P < 0.001. b values are significantly different compared to the respective ethylene glycol treated group when * P < 0.05, ** P < 0.01, *** P < 0.001.

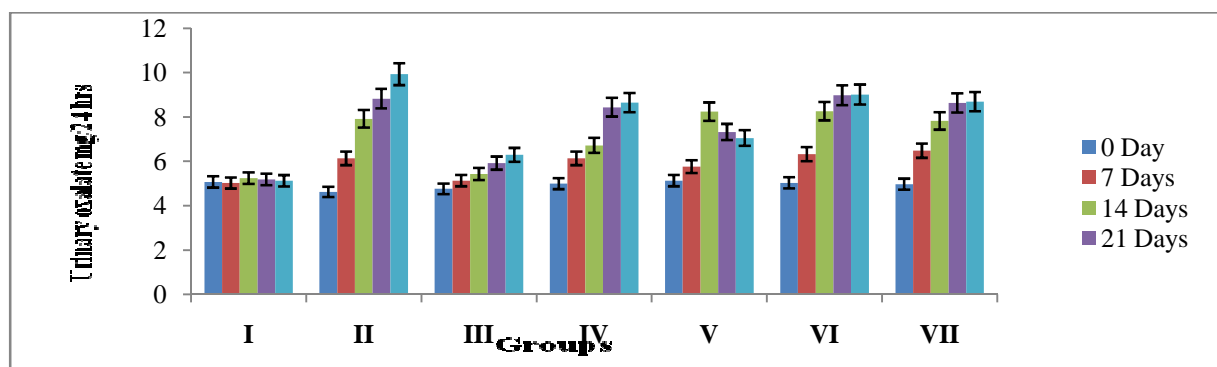
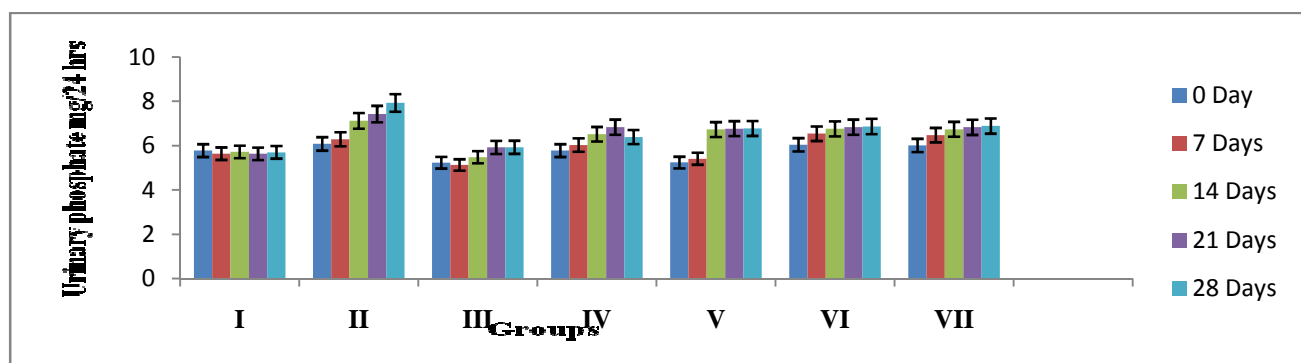
Fig.2 - Effect of ethanolic extract of *Kigelia pinnata* fruit on urinary oxalate mg/24 hrs

Table 3 - Determination of urinary phosphate in mg/24 hrs

Days	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
0	5.78 ± 0.33	6.08 ± 0.23	5.23 ± 0.23	5.78 ± 0.27	5.24 ± 0.36	6.04 ± 0.21	6.01 ± 0.33
7	5.64 ± 0.27	6.29 ± 0.28a*	5.13 ± 0.16	6.03 ± 0.34	5.41 ± 0.17a*	6.54 ± 0.05a*	6.48 ± 0.26a*
14	5.72 ± 0.21	7.12 ± 0.37a**	5.48 ± 0.25	6.92 ± 0.32	6.73 ± 0.26a**	6.76 ± 0.21a**	6.74 ± 0.34a**
21	5.63 ± 0.38	7.43 ± 0.48a**	5.92 ± 0.44	6.84 ± 0.37a*	6.77 ± 0.28b**	6.84 ± 0.56a**	6.83 ± 0.41a**
28	5.70 ± 0.30	7.93 ± 0.42a***	5.93 ± 0.12	6.39 ± 0.17a*	6.78 ± 0.52a**b**	6.87 ± 0.26a**	6.89 ± 0.27a**b**

Values are expressed as Mean ± SEM for five animals in each group. a values are significantly different compared to control when * P < 0.05, ** P < 0.01, *** P < 0.001. b values are significantly different compared to the respective ethylene glycol treated group when * P < 0.05, ** P < 0.01, *** P < 0.001.

Fig.3 - Effect of ethanolic extract of *Kigelia pinnata* fruit on urinary phosphate mg/24 hrs

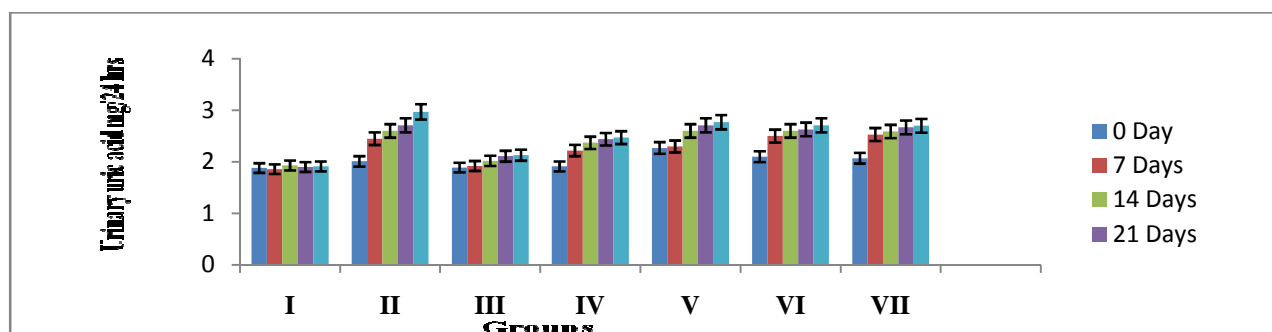
3.4. Urinary uric acid determination

The urinary uric acid control rats (Group I) did not show any significant variation in the urinary uric acid level throughout the experiment period. Both the doses 200 mg and 400 mg/kg/day of ethanolic extract of *Kigelia pinnata* fruit for 15 days significantly reduced the urinary uric acid level as compared to the urolithic control. The ethanolic extract of *Kigelia pinnata* fruit at doses of 200mg and 400 mg/kg/day reduced the urinary uric acid level as compared to the standard (cystone 750 mg/kg). Results are shown in Table 4 and figure 4.

Table 4 - Determination of urinary uric acid in mg/24 hrs

Days	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
0	1.88 ± 0.20	2.01 ± 0.13	1.89 ± 0.13	1.91 ± 0.17	2.27 ± 0.16	2.10 ± 0.12	2.07 ± 0.33
7	1.86 ± 0.24	2.45 ± 0.08a**	1.92 ± 0.16	2.22 ± 0.14	2.30 ± 0.11a*	2.50 ± 0.05a**	2.53 ± 0.26a**
14	1.93 ± 0.31	2.60 ± 0.07a**	2.02 ± 0.05	2.37 ± 0.12	2.60 ± 0.16b**	2.60 ± 0.11a**	2.59 ± 0.34a**
21	1.90 ± 0.27	2.71 ± 0.18a**	2.11 ± 0.14	2.44 ± 0.07a*	2.71 ± 0.08b**	2.63 ± 0.9a**	2.67 ± 0.41a**
28	1.91 ± 0.19	2.97 ± 0.12a**	2.13 ± 0.12a*	2.47 ± 0.10a*	2.77 ± 0.05a*b**	2.71 ± 0.06a**	2.70 ± 0.27a**b*

Values are expressed as Mean ± SEM for five animals in each group. a values are significantly different compared to control when * P < 0.05, ** P < 0.01, *** P < 0.001. b values are significantly different compared to the respective ethylene glycol treated group when * P < 0.05, ** P < 0.01, *** P < 0.001.

Fig.4 - Effect of ethanolic extract of *Kigelia pinnata* fruit on urinary uric acid mg/24 hrs

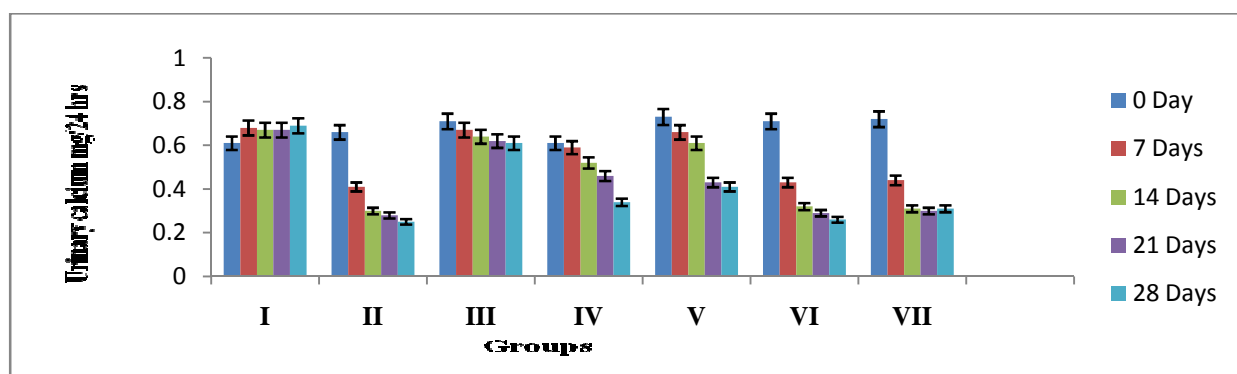
3.5. Urinary calcium determination

The present study of urinary calcium control rats (Group I) did not show any significant variation in the urinary calcium level throughout the experiment period. Both the doses 200mg and 400 mg/kg/day of ethanolic extract of *Kigelia pinnata* fruit for 15 days significantly increased the urinary calcium level as compared to the urolithic control. The ethanolic extract of *Kigelia pinnata* fruit at doses of 200 mg and 400 mg/kg/day have increased the urinary calcium level as compared to standard (cystone 750 mg/kg/day) and restores it to near-normal value. Results are shown in Table 5 and figure 5.

Table 5 - Determination of urinary calcium in mg/24 hrs

Days	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
0	0.61 ± 0.02	0.66 ± 0.03	0.71 ± 0.04	0.61 ± 0.02	0.73 ± 0.08	0.71 ± 0.08	0.72 ± 0.03
7	0.68 ± 0.03	0.41 ± 0.02a**	0.67 ± 0.06	0.59 ± 0.04	0.66 ± 0.05b**	0.43 ± 0.05a*	0.44 ± 0.06a**
14	0.67 ± 0.03	0.30 ± 0.03a**	0.64 ± 0.05	0.52 ± 0.02	0.61 ± 0.06b**	0.32 ± 0.06a	0.31 ± 0.04a**
21	0.67 ± 0.05	0.28 ± 0.06a**	0.62 ± 0.04	0.46 ± 0.07a*	0.43 ± 0.01a*b**	0.29 ± 0.01a**	0.30 ± 0.01a**
28	0.69 ± 0.01	0.25 ± 0.09a**	0.61 ± 0.02	0.34 ± 0.08a**	0.41 ± 0.05a**b**	0.26 ± 0.05a**	0.31 ± 0.07a**b*

Values are expressed as Mean ± SEM for five animals in each group. a values are significantly different compared to control when * P < 0.05, ** P < 0.01, *** P < 0.001. b values are significantly different compared to the respective ethylene glycol treated group when * P < 0.05, ** P < 0.01, *** P < 0.001.

Fig.5 - Effect of ethanolic extract of *Kigelia pinnata* fruit on urinary calcium mg/24 hrs

3.6. Urinary magnesium determination

The present study of urinary magnesium control rats (Group I) did not show any significant variation in the urinary magnesium level throughout the experiment period. Both the doses 200mg and 400 mg/kg/day of ethanolic extract of *Kigelia pinnata* fruit for 15 days significantly increased the urinary magnesium level as compared to the urolithic control. The ethanolic extract of *Kigelia pinnata* fruit at doses of 200 mg and 400 mg/kg/day have increased the urinary magnesium level as compared to standard (cystone 750 mg/kg/day) and restores it to near-normal value. Results are shown in Table 6 and figure 6.

Table 6 - Determination of urinary magnesium in mg/24 hrs

Days	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII
0	2.67 ± 0.20	2.12 ± 0.13	2.59 ± 0.18	2.23 ± 0.17	2.48 ± 0.19	2.61 ± 0.17	2.35 ± 0.12
7	2.61 ± 0.18	2.05 ± 0.08	2.48 ± 0.16	2.13 ± 0.14	2.38 ± 0.06b**	1.99 ± 0.05a**	1.92 ± 0.14a**
14	2.53 ± 0.13	1.84 ± 0.17a**	2.38 ± 0.05	2.07 ± 0.12	2.33 ± 0.09b**	1.87 ± 0.11a**	1.69 ± 0.19a**
21	2.49 ± 0.09	1.73 ± 0.18a**	2.31 ± 0.14	1.96 ± 0.07	2.28 ± 0.13a*b*	1.64 ± 0.13a**	1.62 ± 0.16a**
28	2.56 ± 0.11	1.49 ± 0.07a**	2.28 ± 0.12a*	1.91 ± 0.10a*	2.24 ± 0.05a**b**	1.78 ± 0.06a**	1.67 ± 0a**b*

Values are expressed as Mean ± SEM for five animals in each group. a values are significantly different compared to control when * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$. b values are significantly different compared to the respective ethylene glycol treated group when * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

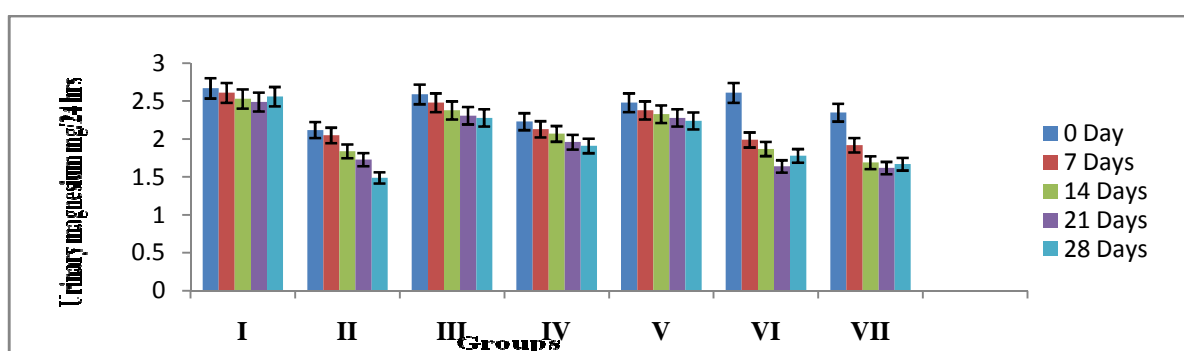


Fig.6 – Effect of ethanolic extract of *K. pinnata* fruit on urinary magnesium mg/24hrs

3.7. Kidney parameters (calcium, oxalate and phosphate) determination

The deposition of the crystalline components in the renal tissue, namely oxalate, phosphate and calcium, was increased in the stone forming rats (Group II). The ethanolic extract of *Kigelia pinnata* fruit at doses of 200mg and 400 mg/kg/day treatment significantly ($P > 0.001$) reduced the renal content. Results are shown in Table 7 and figure 7.

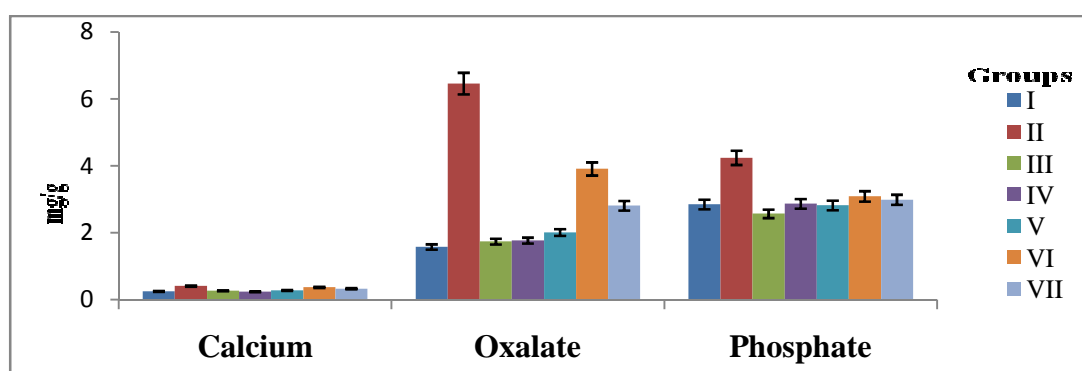


Fig.7 -Effect of ethanolic extract of *Kigelia pinnata* fruit on Kidney parameters (calcium, oxalate and phosphate) mg/g.

3.8. Serum parameters (creatinine and uric acid) determination

The serum creatinine and uric acid was remarkably increased in urolithic control (Group II). The ethanolic extract of *Kigelia pinnata* fruit at doses of 200mg and 400 mg/kg/day treatment significantly ($P < 0.001$) lowered the elevated serum levels of creatinine and uric acid. Results are shown in Table 8 and figure 8.

Table 7 - Determination of Kidney parameters (calcium, oxalate and phosphate) mg/g. Kidney mg/g

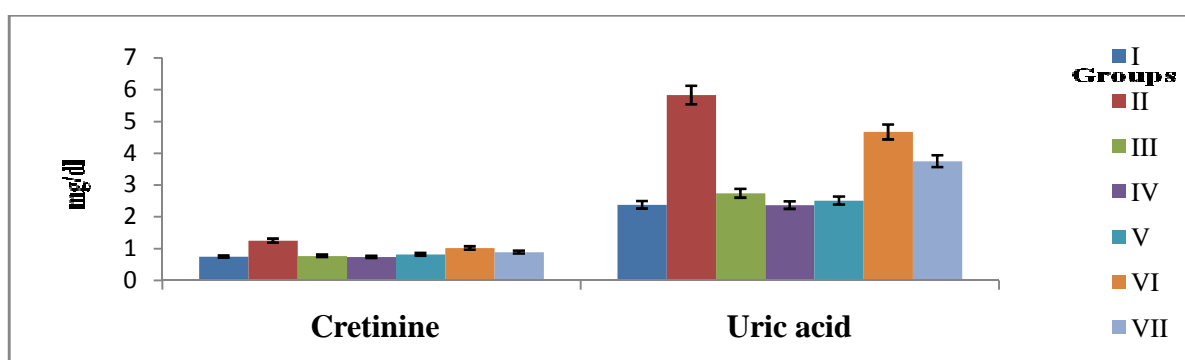
Parameter	Group I	Group II	Group III	Group VI	Group V	Group VI	Group VII
Calcium	0.25 ± 0.04	0.41 ± 0.07a**	0.27 ± 0.02	0.24 ± 0.14	0.28 ± 0.03b**	0.37 ± 0.08a**	0.33 ± 0.01a**b**
Oxalate	1.58 ± 0.14	6.46 ± 0.11a**	1.74 ± 0.12	1.77 ± 0.15a*	2.01 ± 0.17a**b**	3.91 ± 0.11a**b**	2.81 ± 0.11a**b**
Phosphate	2.85 ± 0.05	4.24 ± 0.14a**	2.57 ± 0.17a*	2.87 ± 0.09a*	2.82 ± 0.14b**	3.09 ± 0.08a**b*	2.99 ± 0.14a**b**

Values are expressed as Mean ± SEM for five animals in each group. a values are significantly different compared to control when * P < 0.05, ** P < 0.01, *** P < 0.001. b values are significantly different compared to the respective ethylene glycol treated group when * P < 0.05, ** P < 0.01, *** P < 0.001.

Table 8 - Determination of Serum parameters (creatinine and uric acid) mg/dl. Serum mg/dl

Parameter	Group I	Group II	Group III	Group VI	Group V	Group VI	Group VII
Creatinine	0.75 ± 0.04	1.25 ± 0.07a**	0.77 ± 0.02	0.74 ± 0.14	0.82 ± 0.03b**	1.02 ± 0.08a*b**	0.89 ± 0.01a**b**
Uric acid	2.38 ± 0.11	5.83 ± 0.09a*	2.74 ± 0.12a*	2.37 ± 0.15	2.51 ± 0.17a**b**	4.67 ± 0.11a**b**	3.75 ± 0.11a**b**

Values are expressed as Mean ± SEM for five animals in each group. a values are significantly different compared to control when * P < 0.05, ** P < 0.01, *** P < 0.001. b values are significantly different compared to the respective ethylene glycol treated group when * P < 0.05, ** P < 0.01, *** P < 0.001

Fig.8. Effect of ethanolic extract of *Kigelia pinnata* fruit on Serum parameters (creatinine and uric acid) mg/dl.

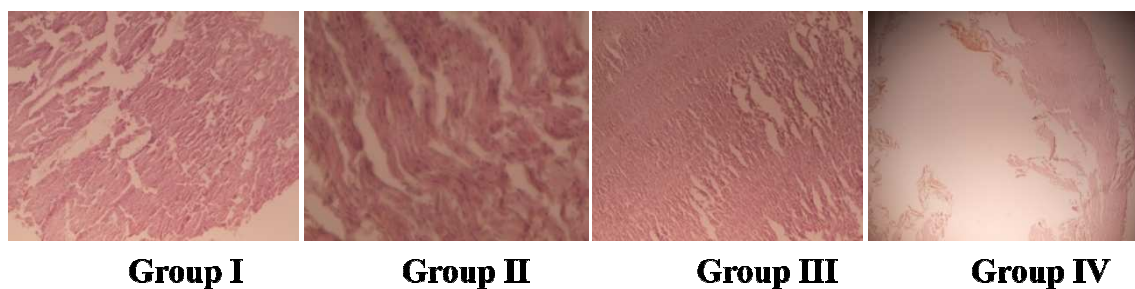
3.9 Histopathology of liver section:

Liver section of a control rat, It is composed of hexagonadal or pentagonadal lobules with central veins and peripheral hepatic triads or tetrads embedded in connective tissue. Hepatocytes are arranged in trabeculae running radiantly from the central vein and are separated by sinusoids containing Kupffer cells. They are regular and contain a large spheroidal nucleus with a distinctly marked nucleolus and peripheral chromatin distribution.

Liver section of urolithic control rat, in most hepatic lobules, the trabecular structure is lightly blurred and, in the remaining lobules, distinctly blurred. The cytoplasm of some cells shows rare empty vacuole-type spaces. A considerable number of Kupffer cells are observed in the sinusoid walls.

Liver section of group III and IV, in most hepatocytes, the structure of nuclei is normal. They are regular and contain a large spheroidal nucleus with a distinctly marked nucleolus and peripheral chromatin distribution is normal.

Liver section of group V, VI, VII, the trabecular structure of the lobules is blurred in places. The cytoplasm of some hepatocytes is enlarged, light, with vacuoles. In most hepatocytes, the structure of nuclei is normal.

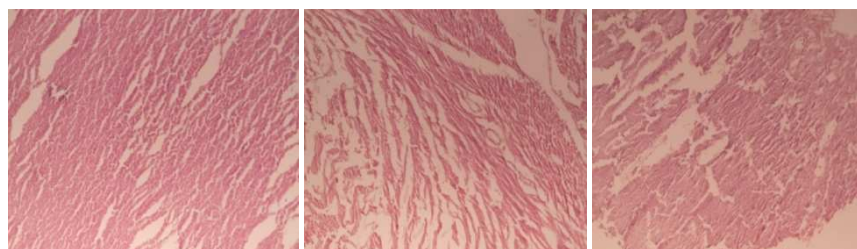


Group I

Group II

Group III

Group IV

**Group V****Group VI****Group VII**

Group I - Control, Group II - Urolithic control, Group III - Test drug (200mg/kg), Group IV - Test drug (400mg/kg), Group V - Cystone (std.) + urolithic control, Group VI - Urolithic control + Test drug (200mg/kg), Group VII - Urolithic control + Test drug (400mg/kg).

DISCUSSION

Currently available drug regimens for management of kidney stone have certain drawbacks. Therefore there is a need for safer and more effective antiurolithic drugs. The use of traditional medicine and medicinal plants in most developing countries, as a normative basis for the maintenance of good health, has been widely observed. *Kigelia pinnata* is a multipurpose medicinal plant with many attributes and considerable potentials.

Male rats were selected to induce urolithiasis because the urinary system of male rats resembles that of humans and earlier studies shown that the amount of stone deposition in female rats was significantly less [24].

Urinary supersaturation with respect to stone forming constituents is generally considered to be one of the causative factors in calculogenesis. Previous studies indicated that, upon 14 days administration of ethylene glycol to the young albino rats resulted into the formation of renal calculi composed mainly of calcium oxalate. The biochemical mechanism for this process is related to an increase in the urinary concentration of oxalate. Stone formation in ethylene glycol fed is caused by hyperoxaluria, which causes increased renal retention and excretion of oxalate. Renal calcium oxalate deposition by ethylene glycol in rats is frequently used to mimic the urinary stone formation [16], [25]. Therefore, this model was used to evaluate the protective effect of ethanolic extract of *Kigelia pinnata* fruit against urolithiasis.

Urinary chemistry is one of the important factors in determining the type of crystal formed and the nature of macromolecules included on the surface of the crystals. Hence, the study of the urinary chemistry related to the calculi forming minerals was provided a good indication of the extent of stone formation.

By studying some previous reports [26] stone induction by ethylene glycol caused an increase in oxalate and decrease in calcium urinary excretion in the Group II. The ethanolic extract of *Kigelia pinnata* fruit treatment for 15 days significantly increased the urinary calcium level as compared to the urolithic control.

Hyperoxaluria is a more significant risk factor in the pathogenesis of renal stone. It has been reported that oxalate play an important role in stone formation and has about 15-fold greater effect than urinary calcium [27]. In our studies urinary oxalate was increased in ethylene glycol induced urolithic rats. The reduction in oxalate excretion was observed on ethanolic extract of *Kigelia pinnata* fruit treatment. This decreased excretion of oxalate may be due to the inhibition of formation of oxalate by the plant extract.

Normal urine contains many inorganic and organic inhibitors of crystallization, magnesium is one such well-known inhibitors. Low levels of magnesium are also encountered in stone formers as well as in stone-forming rats. The magnesium levels return to normal on the drug treatment [28]. Magnesium complexes with oxalate and reduce the supersaturation of calcium oxalate by reducing the saturation of calcium oxalate and as a consequence reduces the growth and nucleation rate of calcium oxalate crystals [27]. Both the doses 200mg and 400 mg/kg/day of ethanolic extract of *Kigelia pinnata* fruit for 15 days significantly increased the urinary magnesium level and thus reduces the risk of stone formation.

In the present studies an increase in the urinary phosphate level was observed in ethylene glycol treated rats (Group II). Increased excretion of phosphate has been reported in stone formers [27]. Increased urinary phosphate excretion along with oxalate stress seems to provide an environment appropriate for stone formation by forming calcium phosphate crystals, which epitaxially induces calcium oxalate deposition [28], [24]. Treatment of ethanolic extract of *Kigelia pinnata* fruit lowered the excretion of phosphate and reduces the risk of stone formation.

The increase in urinary uric acid excretion was observed in urolithic rats. Increased excretion of uric acid has been reported in stone formers and hyperoxaluric. Uric acid interferes with calcium oxalate solubility [28]. Treatment of ethanolic extract of *Kigelia pinnata* fruit lowered the excretion of uric acid and reduces 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 product particularly nitrogenous substances such as creatinine and uric acid get accumulated in blood [29]. The serum creatinine and uric acid was remarkably increased in urolithic control (Group II). The ethanolic extract of *Kigelia pinnata* fruit at doses of 200mg and 400 mg/kg/day treatment significantly ($P < 0.001$) lowered the elevated serum levels of creatinine and uric acid.

CONCLUSION

The aim of the study was to assess the antiurolithic activity of ethanolic extract of *Kigelia pinnata* fruit on ethylene glycol induced hyperoxaluria model in Wistar male rats. Antiurolithic activity of *Kigelia pinnata* fruit extract was confirmed by measuring the serum marker (creatinine and uric acid), tissue homogenate marker (calcium, oxalate and phosphate), urinary parameter (calcium, oxalate, phosphate, uric acid and magnesium) and urinary output and thus it significant reduced and prevented the growth of urinary stones. These studies supported the folk information regarding antiurolithic activity of the plant.

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