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Original Research Article

In-vitro Antiurolithic activity of Kigelia africana fruit extracts

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ABSTRACT

Objectives: The plant Kigelia africana (Lam.) Benth. Family: Bignoniaceae is used in traditional medical practices of Africa and India to treat various diseases including renal disorders. The present study is designed to evaluate the effect of K. africana fruit extract (KAFE) for in-vitro anti-urolithic activity on generated calcium-oxalate crystals. Method: The aqueous and alcoholic (ethanolic) extracts of fruits were tested for anti-urolithiatic potential on generated calcium-oxalate crystals by homogenous precipitation method and simultaneously a supporting two step vice-versa reactions were assessed (New method). The activity was assessed by studying the crystal dissolution by microscopy and quantitative alimental ions analysis for calcium and oxalates. Result: They exhibited significant activity when compared to standard drug Cystone- a poly herbal formulation. The aqueous and alcoholic extracts significantly decreased (p < 0.001) crystal size and increased calcium and oxalate concentration in reaction setup of all tested groups as compared to normal control. Simultaneously a supporting two step vice-versa reaction was assessed that have shown significant inhibition of crystal formation. Conclusion: All the interpretations of various result outcomes direct the use of this drug for urolithiasis prophylaxis and treatments.

Introduction

Urolithiasis is the third most common disorder of the urinary tract, the others being frequently occurring urinary tract infections and benign prostatic hyperplasia [1]. The worldwide incidence of urolithiasis is quite high and in spite of tremendous advances in the field of medicine, there is no truly satisfactory drug for the treatment of renal calculi [2]. Most patients still have to undergo surgery to be rid of this painful disease. Hyperoxaluria is the main initiating factor for urolithiasis, most calculi in the urinary system arise from a common component in urine e.g. calcium-oxalate (CaOx), representing up to 80% of analyzed stones [3]. Ayurveda, an indigenous system of Indian medicine, offers vast scope for the successful treatment of urolithiasis.

A number of plant drugs have been used in India and elsewhere which claim efficient cure of urinary stones. Kigelia Africana (Lam.) Benth. is a tropical African plant widely grown and distributed in South, Central and West Africa. It belongs to the family of Bignoniaceae and commonly called the Sausage tree because of its huge fruits. The sausage tree has a long history of use by rural African communities especially for its medicinal properties. The fruits are believed to be a cure for a wide range of ailments including rheumatism, snakebites, evil spirits and venereal diseases like syphilis. The fruits are a popular source of traditional medicine throughout Africa and beyond. Kigelia africana fruit pulp and extracts have been exploited in a variety of ways; traditional/folklore, dietary/herbal supplement, cosmeceutical, nutraceutical and pharmaceutical purposes. It has strong anti-oxidative effects against hepatotoxicity induced by paracetamol toxicity. It is speculated that the antioxidant activity is attributed to the caffeic acid derivative and compounds unique to Kigelia. Other notable bioactivities include its antimicrobial action against sexually transmitted diseases, Antiprotozoal activity against Plasmodium falciparium, Trypanosoma cruzi, Trypanosoma brucei and Leishmania major, anti-amoebic activity against E. histolytica anti-diarrheal activity, anti-inflammatory/ analgesic activity and anticancer activity. The Bignoniaceae family is noted for the occurrence of iridoids, naphthoquinones, flavonoids, terpenes,
tannins, steroids, saponins and caffeic acid in the fruits, stem, leaves and roots. The anti-oxidant actions of *Kigelia africana* have been attributed to the abundance of flavonoids and saponins in the fruits. *Kigelia africana* is reported to have low acute systemic toxicity [4].

Based on these findings and others, in the present study, an effort has been made to establish the scientific validity for the anti-urolithiatic property/effect of *Kigelia africana* fruit extract on generated calcium-oxalate crystals.

**Materials 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 of the same 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 so obtained was dried in a vacuum desiccator till free from 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 so obtained was dried in a vacuum desiccator till free from moisture (yield: 22%). It was then stored in glass bottles labeled for further use.

**Experimental design**

Test tubes were divided in 4 groups, each group has 6 test tubes, in each tube 1ml of calcium chloride anhydrous and 1ml sodium oxalate were added along with 2 ml of tris buffer (disodium hydrogen phosphate and potassium dihydrogen phosphate) and adjusted at 7.4 pH which to the kidney pH and incubated at 36.7°C over night. The next day the test tubes were centrifuged for 10min to decant to remove top liquid layer [5, 6].

The calcium oxalate crystal formed in the test tube were checked using the compound microscope under 45x magnification, the crystal formed was resembling the prisms shape, to this 5ml (5mg/ml) equivalent to 25mg to each test tube of the different extracts of plant *Kigelia africana* were introduced to 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 (Merck specialties Pvt. Ltd., Mumbai) 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 con. HCl was added to separate the oxalate ion, calcium and both the ions were spectroscopically analyzed [7].

**Method- 2 (New method)**

Simultaneously a supporting two step vice-versa reactions were assessed as per the following reaction setup.

a) Calcium chloride anhydrous (2ml) + KAFE (5ml)\\nIncubated for 24 hours → Sodium oxalate (2ml) → Mixed thoroughly → Incubated for 24 hours → Observed under microscope (45X magnification) for shape and size of calcium oxalate crystals.

b) Sodium oxalate (2ml) + KAFE (5ml) → Incubated for 24 hours → Calcium chloride anhydrous (2ml) → Mixed thoroughly → Incubated for 24 hours → Observed under microscope (45X magnifications) for shape and size of calcium oxalate crystals.

**Microscopical Examination**

Crystal 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.

**Elemental ions analysis**

**Calcium**

Calcium, in an alkaline medium, reacts with o-cresolphthalein to form an intense chromophore which absorbs light at 575 nm (570-580 nm). 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 [8].

**Oxalate**

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 chromatopic acid. 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 570 nm [9].

**Statistical analysis**

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Results

Calcium-Oxalate crystal size analysis

Effect of administration of aqueous and alcoholic extract (5mg/ml – 5ml) / Cystone (5mg/ml – 5ml) 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 (Table-1).

Table 1: Effect of *K. africana* fruit extracts on generated calcium-oxalate mean crystal size

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Group</th>
<th>Mean crystal size (µm) ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Normal control</td>
<td>9.84 ± 2.62</td>
</tr>
<tr>
<td>2.</td>
<td>Standard control (cystone)</td>
<td>3.64 ± 1.346***</td>
</tr>
<tr>
<td>3.</td>
<td>Test-1 (Alcoholic extract)</td>
<td>4.06 ± 1.846***</td>
</tr>
<tr>
<td>4.</td>
<td>Test-2 (Aqueous extract)</td>
<td>2.98 ± 1.047***</td>
</tr>
</tbody>
</table>

Values are in mean ± SEM (n=50) ***p<0.001, *p<0.05 vs. Normal control.

The aqueous extract is showing more activity then alcoholic extract in examination of both the analytical parameters. In microscopical examination the crystal size reduction in aqueous extract (2.98µm) is more significant (p<0.001) than both alcoholic extract (4.06µm; p<0.001) and standard cystone (3.46µm; p<0.001) compare to normal untreated crystal size (9.84µm).

![Microscopical calcium-oxalate crystal observation](image)

Figure 1: Effect of *K. africana* fruit extracts on generated calcium-oxalate mean crystal size.
Fig. 2: 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.

Elemental Ions Analysis

Calcium and oxalate ions were colorimetrically and spectroscopically analyzed using calcium and oxalate detection kits (AGAPPE diagnostics Ltd., Kerala).

Table 2: Effect of K. africana fruit extracts treated on generated calcium oxalate analysis

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Group</th>
<th>Calcium (mg/dl) ± SEM</th>
<th>Oxalate (mg/dl) ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Normal control</td>
<td>13.185 ± 3.240</td>
<td>28.78 ± 1.0</td>
</tr>
<tr>
<td>2.</td>
<td>Standard control (cystone)</td>
<td>44.033 ± 0.3059***</td>
<td>98.28 ± 1.0***</td>
</tr>
<tr>
<td>3.</td>
<td>Test-1 (Alcoholic extract)</td>
<td>42.104 ± 0.526***</td>
<td>94.73 ± 1.0***</td>
</tr>
<tr>
<td>4.</td>
<td>Test-2 (Aqueous extract)</td>
<td>44.20 ± 0.526***</td>
<td>99.47 ± 1.0***</td>
</tr>
</tbody>
</table>

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

Figure 3: Effect of K. africana fruit extracts treated on generated calcium oxalate analysis.

The aqueous extract is showing more activity than alcoholic extract in examination of both the analytical parameters.

In microscopical examination the crystal size reduction in aqueous extract (2.98µm) is more than both alcoholic extract (4.06µm) and standard cystone (3.46µm) compare to normal untreated crystal size (9.84µm).

In alimental ion analysis also the aqueous extract shows maximum hike in concentration of calcium and oxalate ions (44.2; 99.47mg/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) compare to the normal control (13.185; 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-2).
Calcium-Oxalate crystal size analysis in method-2

There was no crystal formation in both the reactions for both the extracts. That is this drug may help in prophylactic regimen of calculogenesis.

Discussion

The aqueous and alcoholic extracts of fruit 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 cystine compared to normal untreated crystal size. Thus it can be inferred that the test drug extracts contribute to heal renal calculi by crystal/stone size reduction.

In alimental ion analysis also, the aqueous extract shows maximum hike in concentration of calcium and oxalate ions than alcoholic extract and standard cystine compared to the normal control. The increase in calcium and oxalate concentration in both test drug extracts and standard drug cystine 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. Simultaneously a supporting two step vice-versa reaction was assessed that have shown significant inhibition of crystal formation.

Conclusion

Both the ions which were analyzed by visible spectroscopy as well as by microscopical examination, on detail in-vitro study, it was found that the aqueous extract of the fruits of plants *Kigelia africana* has shown more significant anti-liathiatic activity in dissolution of generated calcium oxalate crystals compared to alcoholic extract. And this is a drug substance of quite interest would contribute potentially for the ailment of urolithiasis for curative as well as prophylactic regimens.

References


Conflict of interest statement

We declare that we have no conflict of interest.