

Assessment of Antiurolithiatic Potentials of *Crinum asiaticum* Bulbs by *in vitro* and *in vivo* Approaches

Suman Sura^{1*}, Venkata Suresh Kumar Solleti²

¹Research Scholar, Jawaharlal Nehru Technological University Anantapur, Ananthapuramu, Andhra Pradesh, INDIA.

²Department of Pharmacognosy and Phytochemistry, Creative Educational Society's College of Pharmacy, Kurnool andhra Pradesh, INDIA.

ABSTRACT

Objectives: To evaluate the antiurolithiatic potential of *Crinum asiaticum* through *in vitro* and *in vivo* methods. **Materials and Methods:** Ethanolic extract of *Crinum asiaticum* (EECA) subjected for phytochemical screening and HPTLC fingerprinting. *In vitro* antiurolithiatic activity of EECA was determined by Calcium Oxalate (CaOx) crystal nucleation and aggregation assays. Acute toxicity studies were performed as per OECD 423 guidelines. For *in vivo* antiurolithiatic activity 36 male wistar rats divided into six groups. Group I served as control, groups II to VI are administered with 0.75 % v/v ethylene glycol for 28 days to induce hyperoxaluria where in group II served as toxic control and group III as standard. Group IV to VI served as test and received respective doses of EECA from 15 to 28 days. After 28 days, creatinine, BUN, uric acid was estimated. Calcium, oxalate, phosphate were estimated in urine and kidney homogenate also subjected for histopathological studies. **Results:** Phytochemical screening revealed the presence of flavonoids, terpenoids, tannins and phenolic compounds. HPTLC fingerprinting shows the presence of 7 terpenoids and 7 flavonoids at 540nm and 366nm respectively after derivatization. *In vitro* studies

showed reduction in CaOx crystal aggregation and promoted nucleation after treatment with EECA. *In vivo* studies also showed reduction in elevated levels of serum creatinine, BUN, uric acid and levelsof calcium, oxalate and phosphate in urine and kidney homogenate as compared to control group. The results were supported by histopathological studies. **Conclusion:** The EECA have shown significant antiurolithiatic activity by reducing calculi. **Key words:** Antiurolithiatic activity, *Crinum asiaticum*, CaOx crystal aggregation, CaOx crystal nucleation, HPTLC Fingerprinting.

Correspondence

Mr. Suman Sura,

Research Scholar, Jawaharlal Nehru Technological University Anantapur, Ananthapuramu-515002, Andhra Pradesh, India.

Phone: +91 9949521100

Email: kalyan.suman1985@gmail.com

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INTRODUCTION

Crinum asiaticum (Common name: Asian poison bulb) locally known as kesaracettu, is an evergreen herb that is widely distributed throughout India along river beds and also in forest.¹ It is known as spider lily, Crinum lily and poison bulb in English, naagadamani in Ayurveda, bakong in Malaysia and morabau in Papua New Guinea. The plant has various ethnomedicinal properties and is used in traditional system of medicine. The ethnomedicinal uses of the *Crinum asiaticum* bulbs are bitter, expectorant, laxative, carminative, anthelmintic, aphrodisiac, diuretic, urinary problems, diaphoretic, nauseant,¹ analgesic and anti-inflammatory,² anti-obesity,³ emetic,⁴ antitumor.⁵

The stone formation in the urinary system, *i.e.* in the kidney, ureter, urinary bladder and in the urethra is known as urolithiasis. 'Urolithiasis' derived from, ouron also called urine and litho means stone. It is one a foremost disease of the urinary tract and is a basis of morbidity, formation of stone the kind of urologic disorders it is occur in roughly 12% of the global population and it is occurred 70-81% in males and in females 47-60%.⁶

Urolithiasis is a universal trouble, cautious no environmental, artistic or ethnic groups. Since before two decades incident of primary bladder stones has extensively reduced, but these are even now a day also reported in the developing world mostly in patients who are suffered with neurogenic bladders and benign prostatic hypertrophy. Around 80% of the stones are composed of calcium oxalate (CaOx) and calcium phosphate. Urinary stones may source for the hindrance, hydronephrosis, illness and gush of blood in the urinary tract system. Surgical operation,

lithotripsy and local calculus disruption using high-power laser are largely used to eliminate the calculi. nevertheless, these are costly and with these procedures' recurrence is quite common. The recurrence rate without preventive treatment is approximately 10% at 1 year, 33% at 5 years and 50% at 10 years. The other therapies may also be involving thiazide diuretics and alkali-citrate are used in an endeavor to prevent repetition but scientific verification for their worth is less persuasive.⁷

MATERIALS AND METHODS

Collection of plant material

The *Crinum asiaticum* plant was collected from the Tirumala hills, Chittoor district Andhra Pradesh, India and confirmed by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateshwara University, Tirupati (Voucher Number: 2011, dated 08.08.2017). The whole plant was dried in shade; bulbs were separated and pulverized to get a coarse powder.

Preparation of extract

The powered material was subjected for extraction by using ethanol as solvent by soxhlation process.

Preliminary phytochemical screening of extract

Crinum asiaticum bulb extracts were subjected to phytochemical testing for identification of alkaloids, glycosides, tannins, phenols, steroids, flavonoids and terpenoids following the standard procedures.⁸

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HPTLC analysis of ethanolic extract of *Crinum asiaticum* bulbs

The ethanolic extract of *Crinum asiaticum* bulbs were applied in a concentration of 2 µl using CAMAG Automatic TLC sampler⁴ applicator on precoated aluminium sheets with silica gel 60 F₂₅₄ TLC plates (Merck) of 0.2 mm thick, 5 cm × 20 cm, used as a stationary phase. The plates were developed in the mobile phase of n-hexane: ethyl acetate (1:1) v/v for terpenoids and ethyl acetate: formic acid: acetic acid: water (100:11:11:26) v/v/v/v for flavonoids.⁹ The plates were developed in CAMAG – Twin trough glass chamber saturated with saturated pad for 20 min, at a distance of 70 mm. After development the plates was sprayed with Anisaldehyde sulphuric acid reagent and Natural product reagent respectively for terpenoids and flavonoids using CAMAG derivatizer. The tracks were scanned using CAMAG TLC Visualizer equipped with CAMAG software© VisionCATS-Server-PH, version 2.5.18262.1 at a wavelength of 540 nm using tungsten lamp and 366 nm using mercury lamp and the finger print profiles were recorded for terpenoids and flavonoids respectively.

Evaluation of *in vitro* antiurolithiatic activity

The effect of *Crinum asiaticum* extracts on CaOx crystallization was determined by the measurement of turbidity changes due to the crystal nucleation and aggregation. The precipitation of calcium oxalate at 37°C and pH 6.8 has been examined by the measurement of turbidity at 620 nm. A spectrophotometer UV/Vis (Lab India) was used to measure the turbidity of the augmentation of calcium oxalate.

Nucleation assay

Effect of ethanolic extract of *Crinum asiaticum* bulbs on calcium oxalate (CaOx) crystal formation was determined by means of nucleation assay. Individually, calcium chloride(3 mM) and sodium oxalate(0.5 mM) solutions were filtered three times through the pore size of 0.22 µm filter, from that 950 µL of calcium chloride was took and to this added 100 µL of extract at different concentrations (50 µg - 3200 µg/mL). Then add 950 µL of sodium oxalate solution for initiation of crystals. Then finally the solution was magnetically moved at 800 rpm with a stirring bar. The temperature 37°C was maintained. At 620 nm the solution optical density has been monitored. The rate of nucleation was determined with the comparison of the induction time. (The delay ahead of the exterior of crystals that have reached a critical size then consequently become optically demonstrable) if the control with the presence of extract no need to addition of corm extracts.¹⁰

Aggregation assay

Effect of ethanolic extract of *Crinum asiaticum* bulbs on calcium oxalate (CaOx) crystal aggregation was determined by means of aggregation assay. 0.8mg/mL COM crystals were used at an ultimate concentration buffered with 0.05M Tris containing sodium chloride (0.15M) at pH 6.5. Those all were performed at the temperature 37°C in the presence and absence of the corm extract after the apprehend of stirring. The of aggregation rate was predictable as below mentioned formula, by comparing the slope of the turbidity in the sample and with that turbidity in the control.¹¹

$$Ir = \frac{\text{Turbidity of sample}}{\text{Turbidity of control}} \times 100$$

Ir = Percentage aggregation inhibition rate

Evaluation of *in vivo* pharmacological studies

Healthy wistar albino rats weighing about 150-180 g are procured from animal house, CES college of pharmacy, Kurnool. They were housed in polypropylene cages and maintained at 27±2°C, relative humidity 65 ± 10% under 12 h light/dark cycles. The animals were given standard diet manufactured by Nutrivet Life Sciences, Pune, India. The study protocol was approved by the Institutional Animal Ethics Committee (Ref. No.: IAEC/CESCOP/2017-10) constituted in accordance guidelines of the CPCSEA (Committee for the purpose of Control and Supervision of Experiments on Animals), India.

Acute toxicity studies

The acute oral toxicity study was carried out as per the OECD-423 guidelines.¹² One tenth of the non-median lethal dose (LD₅₀) was taken as effective dose.¹³

Antiurolithiatic activity

Ethylene glycol induced urolithiasis in wistar albino rats

The method described by Atmani *et al.*¹⁴ was used for the evaluation of antiurolithiatic activity of ethanolic extract of *Crinum asiaticum* bulbs in albino rats. Thirty-six rats were divided into six groups each group consisting of six rats.

Group-1 served as normal control receives regular diet and potable water for 28 days

Group-2 served as disease control receives ethylene glycol (0.75%) in drinking water for 28 days

Group-3 served as standard receives ethylene glycol (0.75%) water for 28 days and Cystone (750mg/kg) from 15th day-28th day

Group-4-6 served as test groups received ethylene glycol (0.75%) water for 28 days and ethanolic extract of *Crinum asiaticum* bulb at the doses of (100mg/kg, 200mg/kg and 400mg/kg) respectively from 15th day-28th day.

Assessment of antiurolithiatic activity

Serum analysis

After last dose of the drug treatment, blood was collected through retro-orbital plexus under slight anaesthetic conditions. Serum was separated by centrifugation (Research Centrifuge, R-22, Remi India) at 10,000×g for 10 min and analyzed for creatinine, uric acid and blood urea nitrogen (BUN). Serum parameters were estimated by semi-auto analyser (Mispa Excel Chemistry analyser) with diagnostic kits of Excel Diagnostic Pvt. Ltd, Hyderabad.

Urine analysis

On the 28th day individual animal was kept in separate metabolic cages. 24 hr of urine sample was collected. Provide the water for rats during the urine collection, urine sample was subjected for estimation of Calcium (Calcium diagnostic Kit, Agappe Diagnostics Ltd, Kerala, India), Oxalate¹⁵ and Phosphate.¹⁶

Kidney homogenate analysis and histopathology

At the end of the study period, the rats were euthanised by using CO₂ chamber and the abdomen was cut open to remove both kidneys from each animal. Isolated kidneys were washed off extraneous tissue, rinse out in ice-cold physiological saline and used for histopathology and homogenate analysis. The left kidney was finely chopped and 20% homogenate was prepared in Tris-Hcl buffer (0.02 mol/l, pH 7.4). Kidney homogenate was used for assaying tissue calcium, oxalate and Phosphate. The right kidney was fixed in 10% neutral buffered formalin, treated in a series of graded alcohol and xylene, fixed in paraffin wax, partitioned

at 5 μ m and stained with Hematoxylin and Eosin for examination under polarized light. The histopathological examination of slides was examined under polarized light microscope (40X) and photographed by an Olympus Digital Camera.

Statistical Analysis

All the values are articulated as mean \pm SEM. The data was statistically analyzed by using one-way ANOVA followed Dunnett's t test in GraphPad Prism 5.03 version software.

RESULTS

Preliminary phytochemical screening of extract

Phytochemical screening of the ethanolic extract of *Crinum asiaticum* bulbs revealed the presence of flavonoids, tannins, phenolics, steroids and terpenoids.

HPTLC analysis of ethanolic extract of *Crinum asiaticum* bulbs

To obtain the reproducible results with high resolution different combinations of solvents with various ratios were tested. Satisfactory results were obtained with n-hexane: ethyl acetate (1:1) v/v and ethyl acetate: formic acid: acetic acid: water (100:11:11:26) v/v/v/v for terpenoids and flavonoids respectively. The 2 μ l of ethanolic extract of *Crinum asiaticum* bulbs showed the presence of 7 terpenoids, 7 flavonoids with different R_f values and % of area when scanned at 540nm (Figure 1) and 366 nm (Figure 2) respectively for terpenoids and flavonoids after derivatization. (Table 1)

In vitro antiurolithiatic activity

In the present study the different graded concentrations i.e. from 50 μ g/ml to 3200 μ g/ml of ethanolic extract of *Crinum asiaticum* bulbs were used for *in vitro* evaluation of antiurolithiatic activity. The reticence of crystal formation was directly proportional to the increase in the concentration of EECA, with maximum activity was pragmatic at 3200 μ g/ml in CaOx crystal nucleation and aggregation assays (Graph 1,2).

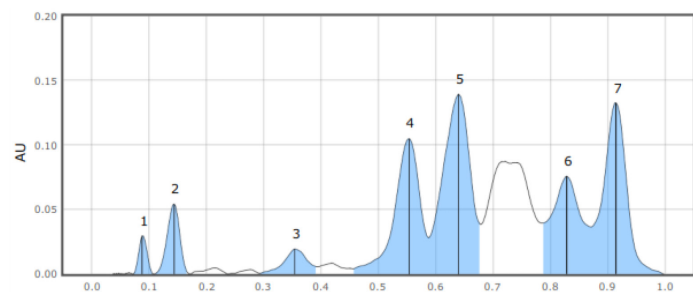


Figure 1: HPTLC chromatogram of terpenoids in 2 μ l of EECA.

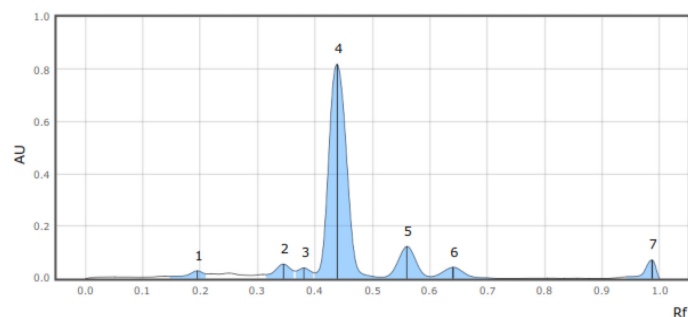
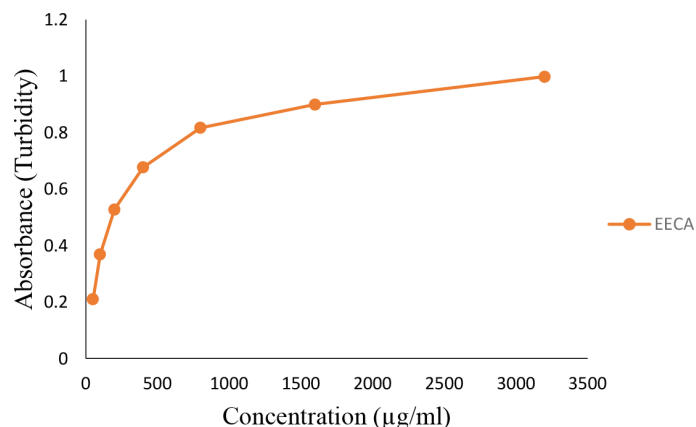


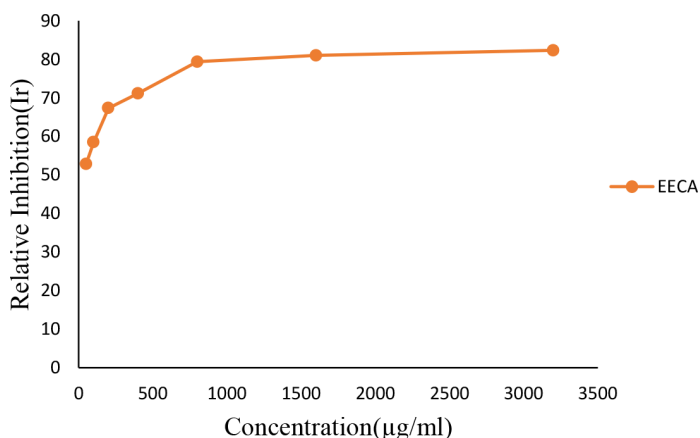
Figure 2: HPTLC chromatogram of flavonoids in 2 μ l of EECA.

Table 1: HPTLC analysis of EECA.

Compound	Terpenoids		Flavonoids	
	R_f	% Area	R_f	% Area
1	0.070	1.76	0.160	10.09
2	0.111	4.78	0.221	15.57
3	0.287	3.59	0.302	16.78
4	0.451	21.37	0.406	45.34
5	0.592	28.24	0.519	6.00
6	0.790	16.57	0.826	1.37
7	0.872	23.70	0.974	4.85



Graph 1: Effect of different concentrations of EECA on CaOx crystal nucleation.



Graph 2: Effect of different concentrations of EECA on CaOx crystal aggregation.

Evaluation of *in vivo* pharmacological studies

Acute toxicity studies

The purified and entirely dried ethanolic extract of *Crinum asiaticum* bulbs were subjected for the acute toxicity study to determine the lethal dose using wistar albino rats in forced environment. Acute toxicity studies were performed according to the OECD 423 guidelines. The limit test was performed with the dose of EECA (2000 mg/kg, b. w) was administered by oral route to a group of rats using oral feeding needle (22gauge). After treatment to rats were monitored for 14 days and it was observed that no changes in normal behaviour, hence it was confirmed that the EECA is virtually non-toxic in normal rats and fall under the sort of GHS category 5, according to 1/20th dose (100 mg/kg, b.w), 1/10th of dose

(200 mg/kg, b.w), 1/5th dose (400 mg/kg, b.w) from maximum safe dose was considered for further evaluation pharmacological studies.

Ethylene glycol induced urolithiasis in wistar albino rats Serum analysis

In the present study treatment with ethylene glycol (0.75%) results in a significant ($###p<0.001$) increase in elevated levels of (47.58 ± 3.27 , 2.503 ± 0.29 , 4.673 ± 0.54) serum BUN, creatinine and uric acid respectively when compared to normal group. These changes were restored significantly ($***p<0.001$; 30.26 ± 1.85 , 1.10 ± 0.19) BUN, Creatinine and ($*p<0.05$; 2.588 ± 0.411) uric acid in rats treated with the standard drug (cystone 750mg/kg, p.o). However, the rats treated with EECA (100mg/kg, p.o) pointedly decreases ($*p<0.05$; 36.06 ± 1.63 , 2.497 ± 0.76) in BUN, uric acid respectively and creatinine non significantly ($p>0.05$; 1.80 ± 0.41), at the dose of EECA (200mg/kg, p.o) significantly ($**p<0.01$) lowers the BUN (34.22 ± 3.11) and ($*p<0.05$; 1.366 ± 0.20 , 2.530 ± 0.49) creatinine and uric acid respectively, at the dose of EECA (400mg/kg, p.o) significantly ($***p<0.001$) lowers the BUN (31.76 ± 2.89) and ($**p<0.01$; 1.20 ± 0.33 , 1.847 ± 0.30) creatinine and uric acid respectively when compared to disease control. (Table 2).

Urine analysis

The administration of ethylene glycol (0.75%) to wistar albino rats triggers hyperoxaluria. Urinary levels of Oxalate, calcium and phosphate (8.16 ± 0.39 , 4.23 ± 0.55 , 7.25 ± 0.51) were unacceptably ($###p<0.001$) increased in the calculi-induced animals. The standard drug cystone (750mg/kg, p.o) treated group animals acceptably lowers the levels of calcium ($***p<0.001$; 3.78 ± 0.321), oxalate and phosphate ($**p<0.01$; 2.26 ± 0.20 , 4.15 ± 0.70) respectively. However, treatment with EECA (100mg/kg, p.o) lowered the elevated level of calcium ($**p<0.01$; 5.66 ± 0.74), oxalate ($*p<0.05$; 2.79 ± 0.27) and phosphate ($p>0.05$; 5.31 ± 0.55), at the dose of EECA (200mg/kg, p.o) reduces the elevated levels of calcium ($***p<0.001$; 4.26 ± 0.23), oxalate ($*p<0.05$; 2.73 ± 0.42) and phosphate ($**p<0.01$; 4.63 ± 0.47), at the dose of EECA (400mg/kg, p.o) reduces the levels of calcium and phosphate ($***p<0.001$; 4.05 ± 0.59 , 4.05 ± 0.370 and oxalate ($**p<0.01$; 2.35 ± 0.18) in urine when associated to the disease control group (Table 3).

Kidney Homogenate analysis

Supernatant from the kidney homogenate was collected and subjected for evaluation of tissue calcium, oxalate and phosphate. The levels of tissue calcium, oxalate and phosphate were significantly ($###p<0.001$) increases (6.47 ± 0.64 , 3.95 ± 0.47 , 6.48 ± 0.11) compared to normal group. The animals treated with standard drug cystone (750mg/kg, p.o) significantly reduced calcium ($***p<0.001$; 3.58 ± 0.85), oxalate ($**p<0.01$; 2.54 ± 0.70), phosphate ($**p<0.01$; 3.89 ± 0.70) and EECA (100mg/kg, p.o) significantly reduces the calcium ($**p<0.01$; 5.42 ± 0.84), oxalate ($*p<0.05$; 3.55 ± 0.75), phosphate ($*p<0.05$; 4.56 ± 0.78), EECA (200mg/kg, p.o) significantly lowers the calcium ($***p<0.001$; 4.95 ± 0.73), oxalate ($*p<0.05$; 3.38 ± 0.45) and phosphate ($**p<0.01$; 4.05 ± 0.50) phosphate respectively, EECA (400mg/kg, p.o) significantly lowers the calcium ($***p<0.001$; 4.68 ± 0.95), oxalate ($**p<0.01$; 3.68 ± 0.74) and phosphate ($***p<0.001$; 3.78 ± 0.77) correspondingly when compared to disease control group. (Table 4)

Kidney Histopathology

Histopathological examination of rat kidneys treated with ethylene glycol (0.75% v/v) revealed the presence of calcium oxalate deposits inside the renal tubules and dilation of the renal tubules along with interstitial inflammation were observed. The number of calcium oxalate deposits in the renal tubules and dilation of renal tubules of Groups III

Table 2: Effect of ethanolic extract of *Crinum asiaticum* (bulbs) on serum levels of BUN, Creatinine and uric acid in ethylene glycol (0.75%) induced urolithiasis in rats.

S.no	Groups	BUN (mg/dl)	Creatinine (mg/dl)	Uric acid (mg/dl)
1	Normal	28.28±1.74	0.354±0.09	1.78±0.36
2	Disease Control	47.58±3.27 ^{###}	2.503±0.29 ^{###}	4.673±0.54 ^{###}
3	Standard (Cystone 750mg/kg, BW, p.o)	30.26±1.85 ^{***}	1.10±0.19 ^{***}	2.588±0.411 [*]
4	EECA (100 mg/kg, BW, p.o)	36.06±1.63 [*]	1.80±0.41 ^{ns}	2.497±0.76 [*]
5	EECA (200 mg/kg BW, p.o)	34.22±3.11 ^{**}	1.366±0.20 [*]	2.530±0.49 [*]
6	EECA (400 mg/kg BW, p.o)	31.76±2.89 ^{***}	1.20±0.33 ^{**}	1.847±0.30 ^{**}

All values are expressed as mean ±S.E.M for six rats in each group.

Comparisons made between ^{###} $p<0.001$, ^{**} $p<0.01$, ^{*} $p<0.05$; Normal Vs Disease control,

^{***} $p<0.001$, ^{**} $p<0.01$, ^{*} $p<0.05$; Disease control Vs Treatment: One-way ANOVA followed by Dunnett's -t test.

Table 3: Effect of ethanolic extract of *Crinum asiaticum* (bulbs) on urinary levels of Calcium, oxalate and Phosphate in ethylene glycol (0.75%) induced urolithiasis in rats.

S.no	Groups	Calcium (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)
1	Normal	3.38±0.45	1.60±0.32	3.85±0.48
2	Disease Control	8.16±0.39 ^{###}	4.23±0.55 ^{###}	7.25±0.51 ^{###}
3	Standard (Cystone 750mg/kg, BW, p.o)	3.78±0.321 ^{***}	2.26±0.20 ^{**}	4.15±0.70 ^{**}
4	EECA (100 mg/kg, BW, p.o)	5.66±0.74 ^{**}	2.79±0.27 [*]	5.31±0.55 ^{ns}
5	EECA (200 mg/kg BW, p.o)	4.26±0.23 ^{***}	2.73±0.42 [*]	4.63±0.47 ^{**}
6	EECA (400 mg/kg BW, p.o)	4.05±0.59 ^{***}	2.35±0.18 ^{**}	4.05±0.37 ^{***}

All values are expressed as mean ±S.E.M for six rats in each group.

Comparisons made between ^{###} $p<0.001$, ^{**} $p<0.01$, ^{*} $p<0.05$; Normal Vs Disease control,

^{***} $p<0.001$, ^{**} $p<0.01$, ^{*} $p<0.05$; Disease control Vs Treatment: One-way ANOVA followed by Dunnett's -t test.

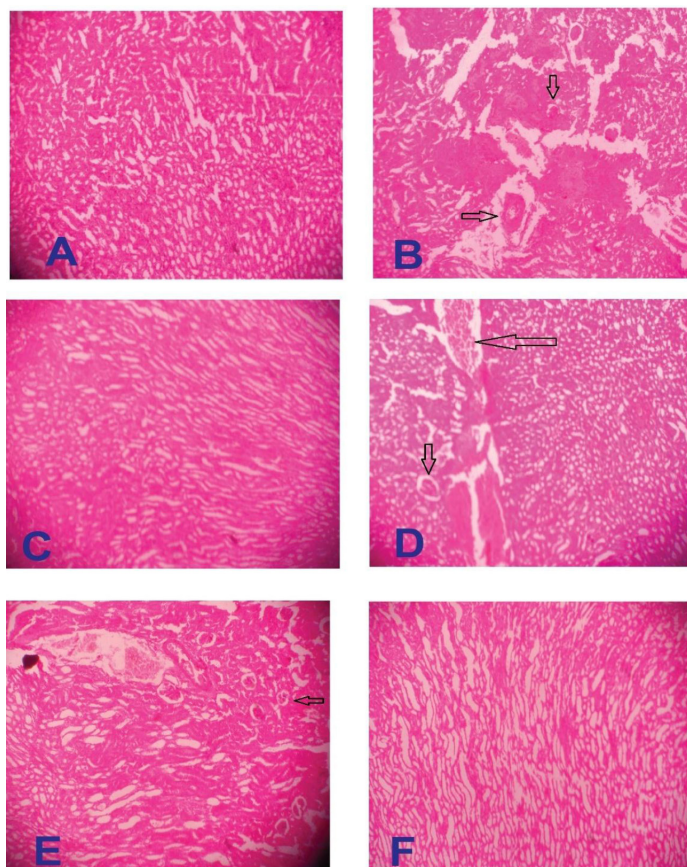
Table 4: Effect of ethanolic extract of *Crinum asiaticum* (bulbs) on Kidney homogenate levels of Calcium, oxalate and Phosphate in ethylene glycol (0.75%) induced urolithiasis in rats.

S.no	Groups	Calcium (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)
1	Normal	2.68±0.85	1.95±0.55	2.95±0.55
2	Disease Control	6.47±0.64 ^{###}	3.95±0.47 ^{###}	6.48±0.11 ^{###}
3	Standard (Cystone 750mg/kg, BW, p.o)	3.58±0.85 ^{***}	2.54±0.70 ^{**}	3.89±0.70 ^{**}
4	EECA (100 mg/kg, BW, p.o)	5.42±0.84 ^{**}	3.55±0.75 [*]	4.56±0.78 [*]
5	EECA (200 mg/kg BW, p.o)	4.95±0.73 ^{***}	3.38±0.45 [*]	4.05±0.50 ^{**}
6	EECA (400 mg/kg BW, p.o)	4.68±0.95 ^{***}	3.68±0.74 ^{**}	3.78±0.77 ^{***}

All values are expressed as mean ±S.E.M for six rats in each group.

Comparisons made between ^{###} $p<0.001$, ^{**} $p<0.01$, ^{*} $p<0.05$; Normal Vs Disease control,

^{***} $p<0.001$, ^{**} $p<0.01$, ^{*} $p<0.05$; Disease control Vs Treatment: One-way ANOVA followed by Dunnett's -t test.



- A-Normal group
 B-Disease control (Ethylene glycol 0.75% v/v)
 C-Standard (Cystone 750mg/kg, bd.wt)
 D-EECA(100mg/kg, bd. wt)
 E-EECA(200mg/kg, bd. wt)
 F-EECA(400mg/kg, bd. wt)

Figure 3: Histopathological view of the experimental groups. Sections show the hematoxylin and eosin (HE) stained kidney. Sections were reviewed using polarized light microscope (40X) and photographed by an Olympus Digital Camera.

(Figure 3-C) rats was significantly less than the Group II (Figure 3-B). Treatment with EECA with different doses (100 mg/kg, BW, p.o, 200 mg/kg, BW, p.o, 400 mg/kg, BW, p.o) i.e., Group IV-VI (Figure 3-D-F). Significantly lowers the deposition of calcium oxalate crystal, dilation of renal tubules and interstitial inflammation were observed compared to Group-II (Figure 3-B).

DISCUSSION

CaOx urolithiasis is the most predominant type of all urinary stone diseases. Key magnitudes concerned in its pathological bio mineralization include crystal nucleation, growth and aggregation. Present study was aimed to discourse these key events involved in CaOx stone formation as a resource to investigate the efficacy of *C. asiaticum* bulbs as an anti-urolithiatic agent. Nucleation is an essential in the pathogenesis of CaOx urolithiasis. Nucleation fundamentally marks a thermodynamically driven event of phase alteration wherein dissolved substances in a supersaturated solution spontaneously crystallize¹⁷⁻¹⁹. Similar phase change and formation of CaOx crystals was witnessed while carrying

out nucleation assay. The reticence of crystal formation was directly proportional to the increase in the concentration of EECA, with maximum activity was pragmatic at 3200 µg/ml in CaOx crystal nucleation.

This suggests the anticrystallization activity of EECA against CaOx crystallization. One possible mechanism of anticrystallization activity of EECA could be its proficiency to complex with free calcium and oxalate ions, thus blocking the formation of CaOx complexes, as has also been suggested for *Sarghassum wightii*.¹⁹

Aggregation is an important factor of crystal retention as large crystal agglomerates are the ones that produce renal tubular obstruction thereby promoting stone formation.¹⁹ EECA showed significant inhibitory effect on CaOx crystal aggregation.

Ethylene glycol is the most commonly used inducing agent for inducing urolithiasis in rats. The rats fed with ethylene glycol (0.75%v/v) results significant increase in serum levels of BUN, creatinine and uric acid and promote excessive excretion of urinary levels of calcium, oxalate and phosphate, indicate formation of calcium oxalate stones in kidneys. However, treatment with EECA, significantly lowers the elevated serum levels of BUN, creatinine and uric acid and urinary levels of calcium, oxalate and phosphate in a dose dependent manner.

Microscopic examination of rat kidney sections treated with ethylene glycol shows the presence of calcium oxalate deposits, dilatation of renal tubules along with interstitial inflammation. However, co treatment with EECA reduces the number of calcium oxalate deposits, dilatation of renal tubules and prevents the damage to renal tubules in dose dependent manner.

The phytoconstituent found in the extract such as flavonoids, tannins, phenolics, steroids and terpenoids may be responsible for the activity. In earlier literature it was reported that both flavonoids²⁰ and terpenoids²¹ play a significant role in antiurolithiatic activity. Attempt also made to standardise the extract by performing finger printing of flavonoids and terpenoids by HPTLC.

CONCLUSION

In conclusion, the findings of the present study provide clear evidence that the EECA inhibits the development of calcium oxalate crystals in *in vitro*. The oral administration of EECA to ethylene glycol induced urolithiasis rats' results in reduction in the elevated serum levels of BUN, Creatinine and uric acid and urinary levels of calcium, oxalate and phosphate in dose dependent manner. Therefore, EECA exhibited significant anti urolithiatic activity against ethylene glycol induced urolithiasis in rats.

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CONFLICT OF INTEREST

The author claims there is no conflict of interest.

ABBREVIATIONS

CaOx: Calcium oxalate; **COM:** Calcium oxalate monohydrate; **EECA:** Ethanolic extract of *Crinum asiaticum*.

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