Research Article

Antiurolithic activity of *Euphorbia hirta* plant extracts

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ABSTRACT

The plant *Euphorbia hirta* Family: Euphorbiaceae is used in traditional medicene of china and India to treat various diseases including female disorders, breathing disorders. The present study is designed to evaluate the effect of *Euphorbia hirta* for in-vitro anti-urolithic activity on generated calcium-oxalate crystals. The aqueous hydro alcoholic and alcoholic (ethanolic) extracts of whole plant were tested for anti-urolithiatic potential on generated calcium-oxalate crystals. The activity was assessed by studying the crystal dissolution by microscopy analysis for calcium and oxalates. They show significant activity when compared to standard drug Cystone. The aqueous hydro alcoholic and alcoholic extracts significantly decreased crystal size and increased calcium and oxalate concentration in reaction setup of all tested groups as compared to normal control. But the alcoholic extract was more significantly treated as compare to aquous and hydro alcoholic extract. Simultaneously a supporting two step vice-versa reaction was assessed that have shown significant inhibition of crystal formation. Conclusion: The various result outcomes direct the use of this drug for urolithiasis and treatment.

Key words: *anti-urolithic, anti-urolithiatic, Euphorbia hirta, Euphorbiaceae.*

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INTRODUCTION

Urolithiasis is the process to forming stones in the kidney, urethra etc. The development of the kidney stone is related to decreased urine volume and increased excretion of stone-forming component such as calcium, oxalate, and cysteine.[1] Euphorbia hirta is used traditionally for female disorder, worm infection in children, jaundice, pimple, digestive problem and tumors.[2] The largest genus of family Euphorbiaceae is Euphorbia having about 1600 species. It is identified by the presence of white milky latex.[3] India recognizes more than 2500 plant species having medicinal value, Sri Lanka around 1400, and Nepal around 700.[4]Chemical constituent of *Euphorbia hirta* plant having the shikmic acid, tinyatoxin, choline, glycoside, tennin.[5]

MATERIALS AND METHOD

The whole plant of *Euphorbia hirta* where collected from the garden of Dev Bhoomi Group of Institute, Dehradun (Uttarakhand). After collected the plant was washed, dried under shade at room tem and plant was converted in the powder form with the help of glander, pass through the sieve (coarse 10/44).

Alcoholic extract

Coarsely powdered of whole plant (50g) extracted exhaustively with 95% ethanol by cold percolation method (3×72 h). It was further sonicated for 10 min.) Left for another 48 hrs and filtered through double layered muslin cloth. The procedure was repeated for two more time

with powder residue. The filtered collected and it was then stored in a glass bottle and levelled for further use.

Hydroalcoholic extract

The powdered material (50g) was mixed with mixture of absolute alcohol and water in a ratio of 1:1 I.e. (200:200ml) and left for 24 hr. It was further sonicated for 10min, left for another 48 hrs and filtered through double layered muslin cloth. The procedure was repeated for two more times with powder residue. It was evaporated with the help of tray dried and sample was collected store in a glass bottle and labelled for further use.

Aqueous extract

The powder material (50g) was mixed with distilled water (500ml) and left for 24 hrs. It was further sonicated for 10 min,left for another 48 hrs. and filtered through double layered muslin cloth. The procedure was repeated for two more times with powder residue. The filtered was dried with the help of tray drier and moisture free sample was collected and stored in a glass bottle for further use.

Experimental Design

Method A

Test tubes of 10 ml capacity were used and marked the tubes as control and test. 5 groups, (each group of 6 tubes), in each tubes 1ml of calcium chloride (Merck specialities Pvt. Ltd., Mumbai) anhydrous and 1ml sodium oxalate (RFCL Ltd., New delhi) 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^{0}C over night. The next day the test tubes were centrifuged for 10min., decanted to remove top liquid layer.**[7,8]**

The calcium oxalate crystals formed in the test tube were checked using the compound microscope under 45X magnification. The crystal formed resembled prisms shape. 5ml (5mg/ml) (equivalent to 25mg) of extracts of plant *Euphorbia hirta* were introduced to the tubes and the same amount of Poly herbal formulation Cystone was added to the test tube. All the above treating agents were administered as aqueous suspension using tween-80 as suspending agent and incubated at 36.7^{0}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.

Group 1- Generated calcium oxalate crystals and referred as control.

Group 2- Generated calcium oxalate crystals + 5ml **Cystone**.

Group 3- Generated calcium oxalate crystals + 5ml **Aqueous extract** of *Euphorbia hirta.*

Group 4- Generated calcium oxalate crystals + 5ml **Alcoholic extract** of *Euphorbia hirta.*

Group 5- Generated calcium oxalate crystals + 5ml **Hydro-alcoholic extract** of *Euphorbia hirt.*

Crystal dissolution was observed under 45X microscope and prism shape Calcium Oxalate crystals were sized / measured by eye piece and stage micrometer. Mean size of more than 50 crystals were observed.**[7]**

Method: B

A reaction setup was established to evaluate the influence of drugs on calcium oxalate crystal generation. This is a vice versa protocol to check the drug effects in both the ways.**[9]**

Step A- Calcium chloride solution 1ml (saturated) was prepared in test tubes than drug solution 1ml (10mg/ml) was incorporated in a set of 6 tubes for all extracts an similarly standard drug Custone in one set. Incubated for 24 hours and sodium oxalate solution 1ml (saturated) was added in all the tubes including controlled group. Further incubated for 24 hours then observed microscopically the calcium oxalate crystal formation/ generation & their size.

Step B- Sodium oxalate solution 1ml (saturated) was prepared in test tubes than drug solution 1ml (10mg/ml) was incorporated in a set of 6

tubes for all extracts and similarly standard drug Custone in one set. Incubated for 24 hours and Calcium chloride solution 1ml (saturated) was added in all the tubes including controlled group. Further incubated for 24 hours then observed microscopically the calcium oxalate crystal formation/ generation & their size.**[9]**

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.

RESULT

Calcium Oxalate Crystal Size Analysis

The effect of drug extracts (5mg/ml-5ml) and cystone (5mg/ml-5ml) on size and dissolution of Calcium oxalate crystals was determined by microscopy. The prism shape calcium oxalate crystals were sized by eye piece and stage micrometer using 45X magnification. Mean size of more than 50 crystals were made as follows.

Table No. 1. Mean crystal size

Group	Mean Crystal Size (µm)± SEM
Normal Control	15.8 ± 0.141
Standard Control (cystone)	6.64 ± 0.223
Test-1 (Aqueous Extract)	7.72 ± 0.210***
Test-2 (Hydro Alcoholic)	5.84 ± 0.238***
Test-3 (Alcoholic)	7.0 ± 0.307***

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

Method- 2

In this method microscopically we observed that due to influence of drug treatment on both the reactants. It was not proper calcium oxalate crystal growth compared to normal reaction setup.

Thus there was no crystal formation observed in alcoholic and hydroalcoholic extract treated groups. The formed crystals size in aqueous extract treated was very minute measuring less than 5 µm at 45X magnification and the number of crystal size was very less. That is this drug may help in prophylactic regimen of urolithiasis.

Step: A

Table No. 2. Observed crystal size under 45X Magnification.

Group	Observation	Crystal size
Control Solution	Large size crystals were observed	=20µm
Cystone Solution	Very small and few crystals were observed	<1µm
Aqueous Extract	Small and few crystals were observed	<3 µm
Hydro alcoholic Extract	No crystals were observed	<2µm
Alcoholic/Ethanolic Extract	No crystals were observed	<3µm

Step: B

Table No. 3. Observed crystal size under 45X Magnification.

Group	Observation	Crystal size
Control Solution	Large size crystals were observed	<20µm
Cystone Solution	Very small and few crystals were observed	<1µm
Aqueous Extract Solution	Small and few crystals were observed	<3nm
Hydro alcoholic Extract	Very small and few crystals were observed	<2µm
Alcoholic/Ethanolic Extract	Very small and few crystals were observed	<3µm

DISCUSSION

The alcoholic extract and aqueous extract of *Euphorbia hirta* plant inhibit the precipitation of

calcium and oxalate. The result of our study clearly showed the utility of *Euphorbia hirta* plant for the treatment of kidney stones.

In microscopically examination the reduction of crystal size in hydroalcoholic extract was more significant then both alcoholic and aqueous extracts compared to normal untreated crystals. All three test extract decreased the crystal size with the potency order of hydroalcoholic , alcoholic and aqueous extract respectively with comparable result of standard cystone drug treatment.

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