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Research Article

Exploring The Antiurolithiatic Activity of Hydroalcoholic Extract of Aquatic Weed *Eichhornia Crassipes,* In Rats

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ABSTRACT

Urolithiasis or simply kidney stone is defined as the formation of stones in the urinary tract. It is a common problem troubling humankind since centuries with frequent relapse. Numerous plants have been used as reliable source of antiurolithiatic agents and been widely used in the treatment and management of kidney stones in several folklore therapy. Presently there are several plants based marketed formulations with antiurolithiatic effect including Cystone (Himalaya), Calcuri (Charak Pharma Pvt Ltd) and Chandraprabha bati (Dabur). These formulations have been enormously used in clinical practice to disintegrate and dissolute renal stones in situ as to remove urinary calculi from the kidney and/or urinary bladder along with the urine.

INTRODUCTION

The kidney stone disease also known as "Urolithiasis" or simply urinary stone formation is a crystal concretion accumulated usually in the kidneys (1). It is a growing kidney ailment in the present human health context and is increasingly affecting population across the globe (2). In India, kidney stone disease is predominant, with an anticipation of 12% of the population to be at higher risk to develop urinary stones (3). In addition to that statistics revealed that 12%, and 50% of the population are brutally affected by kidney damage (3). Besides it has been also established that an abrupt 15% of the population in north India suffers from urolithiasis as compared to southern parts of the country (4). The stone belt of India predominantly involves areas of Maharashtra, Gujarat, Rajasthan, Punjab, Haryana, Delhi, Madhya Pradesh, Bihar, and West Bengal (Fig. 1).

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Many a times, a minute stone may pass out in urine without generating any symptoms, however a stone that grows more than 5 mm in size can cause some obstruction of the ureter, which results in severe ache in lower side of abdomen. A kidney stone may cause bloody urine, nausea, or severe pain during urination (1,2). However, the symptoms usually are linked with the location of the stone either in the kidney, ureter, or urinary bladder (1,2). It is usually found that urolithiasis is a condition that is asymptomatic initially. But intense renal colic cramping pain, flank pain, hematuria, hydronephrosis, obstructive uropathy and urinary tract infections are found to occur in the later stages of the disease (1,2). The pathogenesis of renal calculi (kidney stone or biomineralization) has been depicted in Fig. 4. This complex pathway primarily involves physicochemical changes and supersaturation of urine. The supersaturation condition of the urine causes solutes present in the urine to precipitate in urine leading to nucleation in turn formation of crystal concretions. The alteration of a liquid state into a solid one is highly determined by pH and excessive amounts of specific stone forming ingredients including calcium, phosphorus, uric acid, oxalate and cystine. A low urine volume is also a menace for calculi development as it facilitates crystallization. (5) Reports suggest that, crystallization is the most rate limiting step of kidney stone formation and predominantly hinge on the thermodynamics specifically regulating nucleation process of a supersaturated solution. Therefore, avoiding supersaturation and nucleation could be the important approach of treating and managing lithiasis (6). However, it must be distinguished that the process of renal stone formation is typically reliant on on the side by side of inequity amid inhibitors and promoters of the crystallization process as almost all forms of stones show analogous proceedings with reverence to the mineral-phase of stone creation (7). Substances decreasing the initiation of supersaturation, nucleation, or other procedures obligatory to stone formation are called inhibitors such citrate (small organic anions), pyrophosphates (small inorganic anions), magnesium (multivalent metallic cations) or macromolecules including osteopontin, glycosaminoglycans, glycoproteins, urinary prothrombin fragment-1, and Tamm–Horsfall proteins (8). Whereas substances which ease stone formation processes are called promoters such as cell-membrane lipids (phospholipids, cholesterol, glycolipids), calcitriol hormone, oxalate, calcium, sodium, cystine, and low urine volume (5,8). Over-all, an inequity among these inhibitors and promoters' components has been proposed to be the major reason for stone formation (5,8). Table 1 enlist the different important A list of inhibitors or promoters of stone formation. Urolithiasis could possibly lead to sever kidney damage through numerous ways. Obstructive uropathy, a major consequence of urolithiasis may lead to renal vasoconstriction and inflammation induced acute injury because of increased intratubular pressure (9). This causes renal perfusion reduced ischemia, that if continues long leads to conditions including glomerulosclerosis, tubular atrophy, and interstitial fibrosis (10). Therefore, if kidney stones caused recurrent episodes of obstructive uropathy, then there could be substantial loss of functional nephrons. Further, the Renin Angiotensin Aldosterone System in the kidney triggers the NADPH oxidase producing Reactive Oxygen Species and they in turn activates phospholipase A2 through nuclear transcription factor NF-κB and upsurge cell death and crystal formation (11).

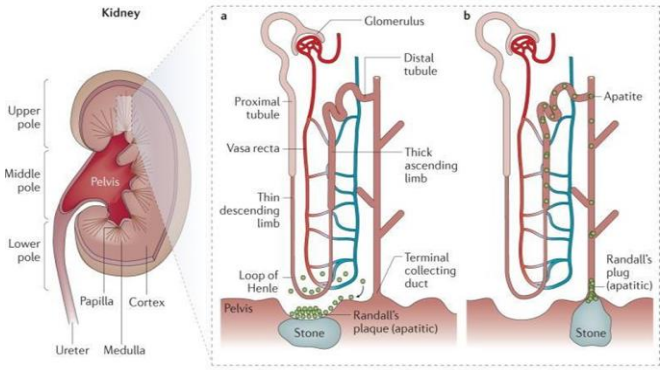


Fig. 1: Locations of Kidney stones in the human urinary system.

Source: Khan, S. R., Pearle, M. S., Robertson, W. G., Gambaro, G., Canales, B. K., Doizi, S., Traxer, O., & Tiselius, H. G. (2016). Kidney stones. *Nature reviews. Disease primers*, *2*, 16008.

At present, there is no pleasing medication to cure and/or prevent kidney stone reappearances. In the present scenario therapeutic managing of nephrolithiasis is either expensive or associated with side-effects. Invasive measures for the treatment and management of nephrolithiasis possibly might lead to grave complications and correspondingly execute a countless freight of costs on the healthcare system. Further, numerous mechanisms are found to be involved in the pathogenesis of urolithiasis and this is the one of the major hindrances in the development of antiurolithiatic drugs. As discussed earlier reactive oxygen species are recognized to agitate the cellular damage in kidney cells).

Table 1: Inhibitors and promotors of urolithiasis

Serial Number	Name of protein	Role in crystallization			
		Nucleation	Growth	Aggregation	Cell adherence
1	Nephrocalcin (NC)	I	I	I	—
2	Tamm–Horsfall protein (THP)	P	—	I/P	—
3	Osteopontin/uropontin (OPN)	I	I	I	I/P
4	Albumin	P	—	I	—
5	Urinary prothrombin fragment-1 (UPTF1)	I	I	I	—
6	Alpha-1-microglobulin	—	—	I	—
7	S100A	—	I	I	—
8	Inter-alpha-inhibitor	I	I	I	I
9	Bikunin	I	I	I	I
10	Renal lithostathine	—	I	—	—
11	Alpha defensin	—	P	P	—

12	Human phosphatecytidyl yl transferase 1	—	I	—	—
13	Myeloperoxidas e	—	P	P	—
14	Nucleolin	—	—	—	P
15	Histone-lysine N methyltransferas e	—	I	I	—
16	Inward rectifier K channel	—	I	I	—
17	Protein Wnt-2	—	I	I	—
18	Alpha-2HS glycoprotein	P	I	—	—
19	Crystal adhesion inhibitor (CAI)	—	—	—	I
20	Hyaluronic acid (HA)	—	—	—	P

I: inhibitor; P: promoter; "—": no effect.
Source: Alelign, T., & Petros, B. (2018). Kidney Stone Disease: An Update on Current Concepts. *Advances in urology*, *2018*, 3068365. https://doi.org/10.1155/2018/3068365

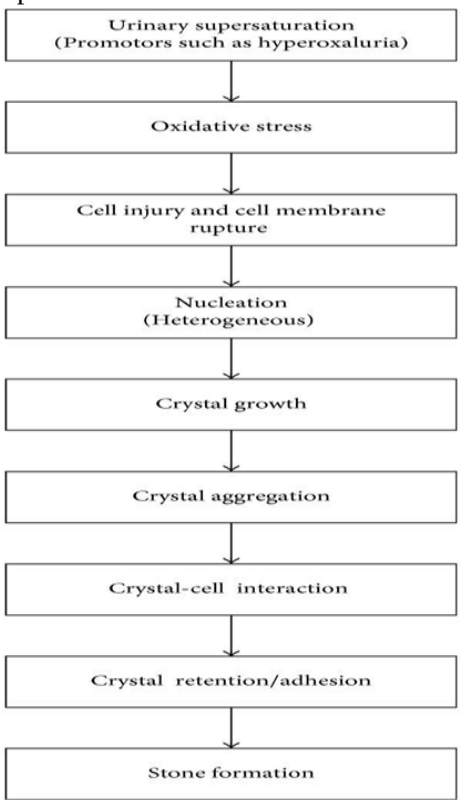


Fig.2: The schematic representation of the kidney stone formation or urolithiasis.

Source: Alelign, Tilahun, and Beyene Petros. "Kidney Stone Disease: An Update on Current Concepts." *Advances in urology* vol. 2018 3068365. 4 Feb. 2018

Eichhornia crassipes (Family -Pontederiaceae), commonly known as "Water hyacinth" is one of the free floating macrophytes, seen in the water milieu such a channels, ponds, and lakes. It is

extremely supplemented in phytochemicals. It is enumerated as the greatest prolific plants on earth and measured the top 10 biosphere's nastiest aquatic plants (17). The whole aquatic plant *Eichhornia crassipes* is carefully selected for the study as different plant parts are reported to contain phenolics, flavonoids and tannins compounds (18) and there is no scientific validation till date for its use in urolithiasis to the best of our knowledge. A study also revealed that, this plant species contains nine flavonoids comprised of four groups isolated by TLC (19). The entire plant will be imperilled to both in-vitro and in-vivo screening for antiurolithiatic activity to investigate and justify its use in the management of urolithiasis.

1. Plant Profile

1.1. *Eichhornia crassipes* (Mart.) Solms



Fig. 3: *Eichhornia crassipes* (Mart.) Solms in its natural habitat. (Whole plant)

Source:

https://www.google.com/url?sa=i&url=http%3A%2F%2Feichh ornia

1.2. Preferred Scientific Name (1)

a. *Eichhornia crassipes* (Mart.) Solms

1.3. Pharmacological importance

Numerous investigators have assessed various extracts and isolated compounds of the plant for possible therapeutic indications.

1. *Anti-microbial activity*

The methanolic extract of *E. crassipes* showed significant reduction in the growth of *Aspergillus niger* (16).

2. *Antioxidant activity*

In a study, *Eichhornia crassipes* unprotected to various concentrations of Ag, Cd, Cr, Cu, Hg, Ni, Pb and Zn hydroponically for 21 days presented upsurges in the action of catalase, peroxidase and superoxide dismutase (19).

3. *Wound healing activity:*

The methanol leaf extract of this plant was examined for its wound healing potential in an excision experimental model of wounds in rats (21).

4. *Antitumor activity:*

A methanolic leaf extract of water hyacinth presented virtuous response contrary to B16F10 in-vivo melanoma tumor bearing hybrid mice models (from Swiss albino female and C57BL male) proving its anticancer effect (22).

5. *Larvicidal activity*:

Chironomus ramosus Chaudhuri eggs and larvae were exposed to variable concentrations of crude root extracts of *E. crassipes* (final concentrations 0.25–2.5%) and it showed 100% efficiency (23).

2. MATERIALS AND METHODS

2.1. Plant Extract

Hydroalcoholic extract (50% v/v) of *Eichhornia Crassipes* (100 g) was obtained from Vital Herbs (Z-26/27 Commercial Enclave Mohan Garden Uttam Nagar Delhi- 110059).

3.2.1. Preliminary phytochemical screening

Preliminary phytoconstituents present in the hydroalcoholic extract of *Eichhornia Crassipes* were identified based on the following qualitative phytochemical tests. (1,2,3)

3.2.2. Test for alkaloids

(a) Dragendorff's test: 1 ml of the extract was taken in a test tube and 1 ml of dragendorff's reagent (Potassium Bismuth iodide solution) was added in it. Formation of an orange-red precipitate confirmed the presence of alkaloids.

(b) Mayer's test: 1 ml of the extract was taken in a test tube and 1 ml of mayer's reagent (Potassium mercuric iodide solution) was added in it.

Formation of whitish yellow/cream colour precipitate confirmed the presence of alkaloids.

(c) Hager's test: 1 ml of the extract was taken in a test tube and 3ml of Hager's reagent (Saturated aqueous solution of picric acid) was added in it. Formation of yellow colour precipitate confirmed the presence of alkaloids.

3.2.3. Test for Protein

(a) Biuret test: 1ml of 40% sodium hydroxide solution and 2 drops of 1% CuSO4 solution was mixed in a test tube till a blue colour showed. Then 1ml of the extract was added in it. Formation of pink or purple violet colour confirmed the presence of proteins.

Million's test: 3ml of extract was taken in a test tube and 5ml of Million's reagent was added in it. Formation of white precipitate occurred, then warm it and precipitate turns brick red or precipitate get dissolved given red colour solution confirmed the presence of protiens.

(b) Xanthoprotein test: 3ml of extract was taken in a test tube and 1ml of concentrated H2SO4 was added in it. Formation of white colour confirmed the presence of proteins.

3.2.4. Test for Glycosides

(a) Legal test: 1 ml of extract was taken in a test tube and pyridine was added in it, then 1-2 ml of sodium nitroprusside solution was in the solution to make it alkaline. Formation of pinkish red to red colour confirmed the presence of glycosides.

(b) Baljet test: 1 ml of extract was taken in a test tube and 1ml of sodium picrate solution was added in it. Formation of yellow to orange colour confirmed the presence of glycosides.

(c) Keller-Killiani test: 1gm of extract was taken and heated with 10ml of 70% alcohol for 2 minutes, filter it. 10ml of water and 0.5ml of strong solution of lead acetate was added in precipitate, mixed the solution and again filtered. 5ml of chloroform was added on the obtain precipitate and shaken continuously for 3 minutes. The chloroform layer was separated in a porcelein dish and the solvent was removed by gentle evaporation. The cool residue in 3ml of glacial acetic acid was dissolved which was containing 2 drops of 5% ferric chloride solution. Carefully this solution was transferred to the surface of 2ml of concentrated sulphuric acid. A reddish-brown layer indicated at the junction of the two liquids and the upper layer slowly indicated bluish green, that got darkened with standing. It confirmed the presence of glycosides.

3.2.5. Test for carbohydrates and sugars

(a) Molisch's test: 2ml of the extract was taken 1ml of alpha-napthol solution was added in it, then concentrated sulphuric acid was added through the side of the test tube. Formation of reddish violet color at the junction of the two liquids was not showed which confirmed the absence of carbohydrates.

(b) Fehling's test: 1ml of the extract was taken and equal quantity of Fehling solution A and B was added in it. It was heated and during heating no formation of a brick red colour precipitate was found.

(c) Benedict's test: 1 ml of extract was taken in a test tube and 5ml of Benedict's reagent was added in it. Boiled for 2 minutes and cooled. No formation of red precipitate confirmed the absence of sugars.

3.2.6. Test for tannins and phenolic compounds

a) 1 ml of extract was taken, and basic lead acetate solution was added in it. Formation of white precipitates confirmed the presence of tannins.

b) 1 ml of extract was taken, and ferric chloride solution was added in it. Formation of a dark blue or greenish black colour product confirmed the presence of tannins.

c) 1 ml of extract was taken and treated with potassium ferric cyanide and ammonia solution. Formation of dark red colour confirmed the presence of tannins.

3.2.7. Test for flavonoids

Shinoda's test: 1 ml of extract was taken and treated with magnesium foil and concentrated HCl. Formation of dark cherry red colour confirmed presence of flavanones or orange red colour confirmed the presence of flavanols.

3.2.8. Test for steroids

(a) Libermann-Burchard test: 1gm of the extract was taken and dissolved in a few drops of chloroform, add 3ml of acetic anhydride and 3ml of glacial acetic acid in it, heated it and cool under the tap. Two drops of concentrated sulphuric acid were added along the sides of the test tube. Formation of bluish green color confirmed the presence of sterols.

(b) Salkowski test: Small quantity of extract was dissolved in chloroform and equal amount of conc. H2SO4 was added in it. Formation of blue, red to cherry colour in chloroform layer and green fluorescence in the acid layer confirmed the presence the steroid.

3.2.9. Test for saponins

(a) Foam test: 1ml of extract was taken in a measuring cylinder and 9ml of distilled water was added and shaken for 15seconds and was allowed to stand for 10min. Formation of stable foam confirmed the presence of saponins.

3.2.10. Test for Phenols

(a) Ferric chloride test: Small amount of extract was treated with 5% ferric chloride. Formation of deep blue or black colour confirmed the presence of phenols.

(b) Liebermann's test: 1 ml of extract was taken and sodium nitrite and h2so4 was added and heat it. Formation of deep red or green or blue colour confirmed the presence of phenols.

3.2.11. Test for Quinones

Extract was taken and treated with concentrated HCl and observed for the formation of yellow colour precipitate confirmed the presence of quinones.

3.2.12. Test for Anthraquinones

(a) Borntrager's test: 50 mg of extract was taken with 10% ferric chloride solution and 1ml concentrated HCl and heat it. Then cool the solution, filter it and the filtrate was shaken with diethyl ether and then ether extract was further extracted with strong ammonia. Formation of pink or deep red colour of aqueous layer confirmed the presence of anthraquinone.

3.3. Proximal analysis

The parameters determined for proximate analysis include ash value, moisture content, total solid content and acid value of the extract.

3.3.1. Ash value

2 gm of the Eichhornia Crassipes extract powder was weighed and taken in a tarred silica dish and it was incinerated in muffle furnace at a temperature 450°C for 10-12 hours until free from carbon. The sample was cooled at desiccator and weighed. Carbon free extract was silver, white in colour (4).

3.3.2. Acid value

1 gm of extract was taken and weighed accurately and place it in a 250-mL conical flask, then 2.5-2.5 ml of ethanol-ether(v/v) solution was added. It was shaken well. The it was titrated with potassium hydroxide titrant until pink colour persist for at least for 30 second. The titration was done for 3 times. The volume of potassium hydroxide titrant used was measured and the acid value was calculated (5).

3.3.3. Moisture content

1 gm of extract was taken and weighed accurately and kept in clean and dry petri dish. It was allowed to dry in a hot air oven at 60˚c and checked after every 5 minutes until its constant weight occurred. (6)

3.4. In-vitro assessment of antiurolithiatic activity of hydroalcoholic extract of *E. crassipes*

3.4.1. Preparation of semipermeable membrane

A complete egg was taken. A hole was made on the outer side of the eggshell with the help of sharp pointer carefully and the yolk (inner content) of the shell was removed carefully and then washed with distilled water three to four times. The eggshell was decalcified by putting it into 30 % v/v HCL for 10 hours. Membrane was removed out from decalcification process and then it was washed with distilled water. Membrane was placed in an ammonia solution to neutralize the traces of acid present on the egg. After that, the egg membranes were washed with distilled water. (7)

3.4.2. Preparation of calcium oxalate crystals

1.4 g of calcium chloride dihydrate was taken and dissolved in 100 ml of distilled water and

1.34 g of sodium oxalate was dissolved in 100ml of 2N H2SO4. Solution was mixed equally with continuously stirring for 15 minutes and kept aside for 1hour. Calcium oxalate precipitate was obtained and freed from the traces of sulphuric acid using ammonia solution. Then washed with distilled water and dried at 60˚C in hot air oven for 2 hours. Thus, calcium oxalate crystal was formed. (8)

3.4.3. Group division

Group 1 (Blank): Blank, 10mg of calcium oxalate only

Group 2 (Standard): 10mg of calcium oxalate + 100 mg of cystone

Group 3 (Test, 50 mg): 10mg of calcium oxalate + 50 mg of extract of *Eichhornia crassipes* Group 4 (Test, 100 mg): 10mg of calcium oxalate + 100 mg of extract of *Eichhornia crassipes* Group 5 (Test, 200 mg): 10mg of calcium oxalate +200 mg of extract of *Eichhornia crassipes* Group 6 (Test, 400 mg): 10mg of calcium oxalate +400 mg of extract of *Eichhornia crassipes*

3.4.4. Estimation of calcium oxalate by titrimetric method

10 mg of CaOx, 100 mg of Cystone, 50 mg, 100 mg, 200 mg and 400 mg of extract/compound was packed together in the semi permeable membrane by suturing it, which was suspended in a 250 ml conical flask containing 100ml of 0.1 M Tris HCL buffer. First group served as negative control which contains only 10 mg of CaOx. Second group served as standard and third group served as test. Prepared a conical flask of all groups was placed in an incubator for 7 to 8 hours, which is already preheated to 37˚C. The component of semi permeable membrane from each group was squeezed out into the flask or in test tube, 2ml of 1N H2SO4 was added and finally titrated with 0.9494 N KMnO4 till light pink color occurred and end point was obtained. 1ml of 0.9494 N KMnO4is equal to 0.1898 mg of calcium. (9)

3.5. In-vivo assessment of antiurolithiatic activity of hydroalcoholic extract of *E. crassipes*

3.5.1. Experimental Animals

Wistar rats of either sex, weighing 150-200g were maintained in animal house of Amity Institute of Pharmacy, Amity University Uttar Pradesh, Lucknow campus. The selected animals were grouped by randomization process and housed in polypropylene cages in standard environmental conditions at 23 ± 20˚ with 12:12 hour dark and light cycle. The animal had free access to food and water. All animals were housed standard hygienic laboratory condition one week prior to testing.

3.5.2. Induction of urolithiasis

0.75% v/v ethylene glycol was administered orally for 14 days (10, 11).

The curative doses of aquatic plant extract as decided based on the preexisting literature (12) was administered from 15th to 28th day. On the 28th day, urine and blood sample was collected followed by estimating the following:

a. various biochemical parameters were estimated in urine and serum

b. various biochemical parameters were estimated kidney homogenate

c. histology of kidney was estimated

3.5.3. Grouping

The experiments on animals were conducted in accordance with the experimental protocols duly approved by Institutional Animal Ethical Committee. Wistar rats of either sex, weighing 150-200g were used and grouped and treated as depicted in Table

Table 2: Grouping of animals for evaluating the in-vivo antiurolithiatic activity of hydroalcoholic extract of E. crassipes

No. Of Groups	Treatment	Animals	Total no. of animals
1. Healthy control	Vehicle	Wistar rats	5
2. Disease control	0.75% v/v ethylene glycol in drinking water from day 1- day 28	Wistar rats	5
3. Standard group	0.75% v/v ethylene glycol in drinking water from day 1-day 28 + Cystone (750 mg/kg b.w) from 14th day to 28th day.	Wistar rats	5
4. Test group (200 mg/kg body wt.)	0.75% v/v ethylene glycol in drinking water from day 1-day 28 + low dose aquatic plant extract from 14th day to 28th day.	Wistar rats	5
5. Test group (400 mg/kg body wt.)	0.75% v/v ethylene glycol in drinking water from day 1-day 28 + high dose aquatic plant extract from 14th day to 28th day.	Wistar rats	5
Total animal required			**25**

3.3. Parameters evaluated

3.3.1. Urine collection and analysis

On 29 th day, the animals were kept separately in metabolic cages for 24 hours for urine collection. Animals had free access to food and drinking water during the urine collection period. The collected urine samples were measured for the following parameters: Urine volume, Creatinine, Calcium and uric acid. (10,11)

3.5.4. Biochemical estimations in plasma

0n 29th day, the animals were sacrificed, and blood samples were collected by cardiac puncture. Plasma was separated by centrifugation at 1500 rpm for 15 minutes and used for the estimation of creatinine, total protein and calcium using Auto zyme diagnostic kits according to the manufacturer's instructions. Fully automated autoanalyzer (Erba EM-200, Transasia Biomedicals Ltd, Mumbai) was used for the estimations. (10,11)

The collected blood samples were measured for the following parameters using commercially available biochemical kits ():

Calcium: determined by autoanalyzer **Creatinine**: determined by autoanalyzer **Uric acid**: determined by autoanalyzer

3.5.5. Biochemical estimations in kidney tissue homogenate

On 29th day, the animals were sacrificed (cervical disc location) and dissected. Then both kidneys were removed and washed 3 to 4 times by saline and dried with tissue paper. The weight of the kidneys was recorded, and the right kidney was stored in formalin (10 %) for histopathological evaluation whereas the left was kept in buffer solution for each animal. After that, the kidney tissue was cut into small pieces and homogenized with the help of a tissue rotor. After homogenization the sample was centrifuged at

6000 rpm for 10minutes. After centrifugation the supernatant was transferred to different test tubes by using micropipette (1000 µl) and then stored in refrigerator by covering with silver foil. Supernatant was used for the analysis of GSH, MDA at 432 nm and 512 nm. (13,14) The collected supernatant samples were measured for the following parameters using commercially available biochemical kits :

a. **Calcium**: determined by autoanalyzer
b. **Creatinine**: determined by autoanalyzer
c. **Uric acid**: determined by autoanalyzer

3. RESULTS AND DISCUSSIONS

7.1. Results of preliminary phytochemical screening

The result of preliminary phytochemical screening has been depicted in table 6. The results revealed that hydroalcoholic extract of *E. crassipes* (HAEEC) contains several phytoconstituents including alkaloids, proteins, tannins, phenols, flavonoids, steroids and anthraquinones. Hence, it can be predicted that HAEEC could be a promising antiurolithiatic agent as studies have reported that plants containing phenols, flavonoids, carotenoids show promising antioxidant and antiurolithiatic effects (1, 2).

Table 6: Preliminary phytochemical screening

S.NO.	Phytochemicals	HAEEC
1	**Alkaloids**	
a.	Dragendorff's test	+
b.	Mayer's test	+
c.	Hager's test	+
d.	Wagner's test	+
2.	**Proteins**	
a.	Biuret test	+
b.	Million's test	+
c.	Xanthoprotein test	+
3.	**Glycosides**	
a.	Legal test	-
b.	Baljet test	-
c.	Keller-Killiani test	-
d.	Borntrager;s test	-
4.	**Carbohydrate and Sugar**	
a.	Molisch's test	-
b.	Fehling's test	-
c.	Benedict's test	-
5.	**Tannins and Phenolic Compound test**	+
6.	**Flavonoids**	
a.	Shinoda's test	+
7.	**Steroid**	
a.	Liebermann-Burchard test	+
a.	Foam test	+
9.	**Phenols**	

a.	Ferric chloride test	+
b.	Liebermann's test	+
10.	**Quinones test**	-
11.	**Anthroquinones**	
a.	Borntrager's test	+

+: indicates presence; -: indicates absence; HAEEC: hydroalcoholic extract of *E. crassipes*

7.2. Results of proximal analysis

a. Ash value of hydroalcoholic extract of *E. crassipes* (HAEEC) was found to be 0.069gm.

b. **Result for acid value was calculated as per the formula given below:**

Mean = 3.13

Acid value = 5.61 × n / W

5.61 × 3.13 / 1 = 17.55

where n= Volume of potassium hydroxide titrant used (mL) W = Weight of the Eichhornia extract sample (g)

Acid value of hydroalcoholic extract of *E. crassipes* (HAEEC) was found to be 17.55

d. Result for moisture content was calculated as per the formula given below:

Weight of empty Petri dish (W1) = 72.147 g

Weight of sample (W2) = 1g

Weight of empty petridish + sample + water (Ws) = 73.147 g

Weight of empty petridish + sample (After drying at constant value) (W2) = 73.113 g

Formula: 1-(Ws-W1) / W2 × 100 1-(73.113-72.147) / 1 × 100

0.034 /1 × 100 = 3.4 %

Moisture content of hydroalcoholic extract of *E. crassipes* (HAEEC) was found to be 3.4%.

7.3 In-vitro assessment of antiurolithiatic activity of hydroalcoholic extract of *E. crassipes* (HAEEC)

The result of in-vitro assessment of antiurolithiatic activity of hydroalcoholic extract of *E. crassipes* (HAEEC) has been depicted in Fig. 7 and Fig. 8. The result depicted that the HAEEC significantly($p<0.001$) reduced the content of CaOx crystals at 50 mg, 100 mg, 200 mg and 400 mg when compared against the control group. However, when compared against the standard group (Cystone 100 mg) at higher dose of the extract (400 mg) there was no significant difference found to be exiting in between and the results of dissolution of the stone thus produced were found to be in close association.

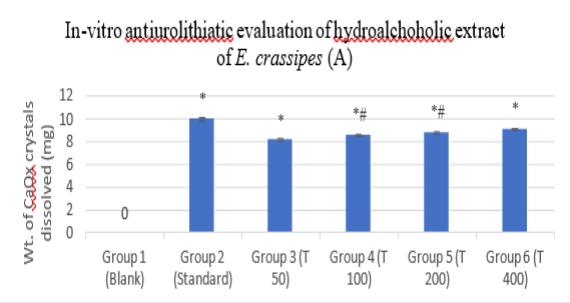


Fig. 17: CaOx crystal dissolving effect of hydroalcoholic extract of *E. crassipes* (HAEEC)

(All values represent mean ± S.E.M, *: $p<0.01$ w.r.t Group 1 (Blank); #: $p<0.01$ w.r.t Group 2

(standard drug Cystone), N=3. One-way analysis of variance (ANOVA) followed by "Dunnett's test.")

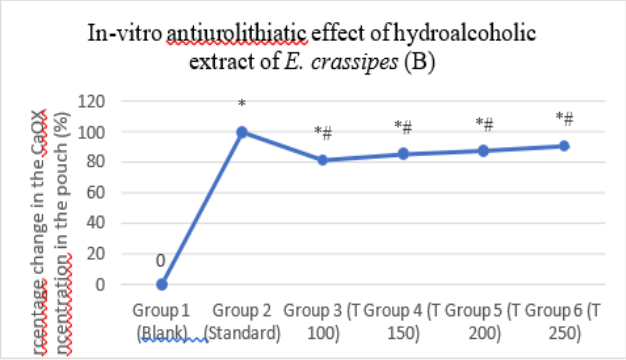


Fig. 18: Percentage dissolution of CaOx crystal by hydroalcoholic extract of *E. crassipes* (HAEEC)

(All values represent mean ± S.E.M, *: p<0.01 w.r.t Group 1 (Blank); #: p<0.01 w.r.t Group 2 (standard drug Cystone), N=3. One-way analysis of variance (ANOVA) followed by "Dunnett's test.")

DISCUSSION

The result of in-vitro assessment of antiurolithiatic activity of hydroalcoholic extract of *E. crassipes* (HAEEC) has been depicted in Fig. 11 and Fig. 12. The results depicted that the HAEEC significantly($p<0.001$) reduced the content of CaOx crystals at 50 mg, 100 mg, 200 mg and 400 mg when compared against the control group. However, when compared against the standard group (Cystone 100 mg) at higher dose of the extract (400 mg) there was no significant difference found to be exiting in between and the standard group and results of dissolution of the CaOx crystals were found to be in close association (9.92 mg was dissolved by Cystone and 9.01 mg was dissolved by HAEEC). This suggest that HAEEC is potent at higher dose.

- (99.24%) Cystone treated group > (90%) 400 mg HAEEC treated group > (90%) 400 mg HAEEC treated group > (87%) 200 mg HAEEC treated group > (85%) 100 mg HAEEC treated group > (81%) 50 mg HAEEC treated group.
- The results of the in-vitro antiurolithiatic assessment of HAEEC has specified primary sign for *E. crassipes* as the plant possessing lithotriptic assets. Studies have shown that, phenolic compounds, flavonoids, tannins are usually responsible for the dissolution of calcium oxalate stones (41,42). Hence, referring the results as provided in Table 6, the presence of all these phytoconstituents in hydroalcoholic extract of *E. crassipes* (HAEEC) might be responsible for degrading the calcium oxalate crystals and antiurolithiatic effect of the extract in the in-vitro assay.

In-vivo assessment of antiurolithiatic activity of hydroalcoholic extract of *E. crassipes* (HAEEC)

7.4.1.Body weight

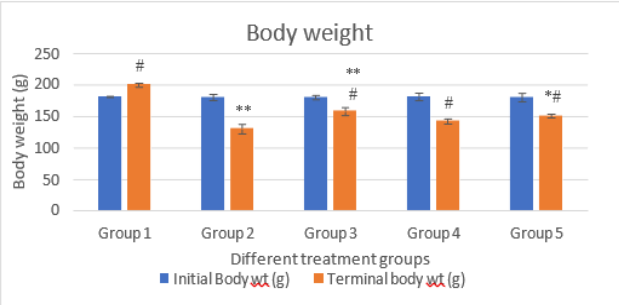


Fig. 19: Change in body weight of the rats by hydroalcoholic extract of *E. crassipes* (HAEEC)

(All values represent mean ± S.E.M, **: p<0.01 w.r.t Group 1; *: p<0.05 w.r.t Group 1; #: p<0.01 w.r.t Group 2,N=5. One-way analysis of variance (ANOVA) followed by "Dunnett's test." Group 1: Normal control, Group 2: Disease control,
Group 3: Cystone (750 mg/kg b.w), Group 4: HAEEC 200 mg/kg, Group 5: HAEEC 400 mg/kg)

DISCUSSION

The variation in the body weights of the treatment of standard (Cystone 100 mg/kg) and HAEEC at 200 and 400 mg/kg has been depicted in the Figure 13. The terminal body weight recordings disclosed that, the mean body weight of the diseased animals (Group 2) was significantly reduced (p<0.01) as compared to healthy animals (Group 1). The loss in the mean body weight of the animals in Group 2 might be because of the anorexia caused by ethylene glycol administration (5). However, the body weight was significantly improved (p<0.01) in the Cystone treated group (Group 3) and HAEEC 400 mg/kg treated group (Group 5) (p<0.05) as compared to Group 2. The lower dose (200 mg/kg) treated groups though presented with improvements, but it was not significant, thus suggesting the higher dose to be more potential (42).

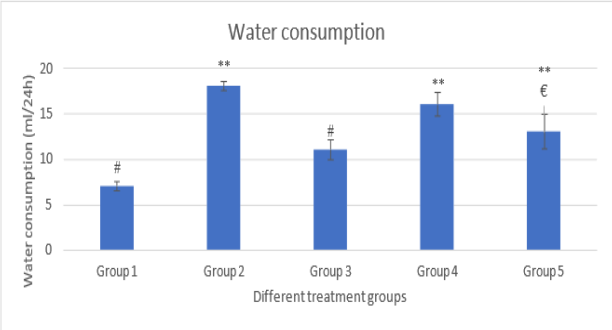


Fig. 20: Change in water consumption of the rats by hydroalcoholic extract of *E. crassipes* (HAEEC)

(All values represent mean ± S.E.M, **: p<0.01 w.r.t Group 1; *: p<0.05 w.r.t Group 1; #: p<0.01 w.r.t Group 2, €: p<0.05
w.r.t Group 2. N=5. One-way analysis of variance (ANOVA) followed by "Dunnett's test." Group 1: Normal control, Group 2: Disease control, Group 3: Cystone (750 mg/kg b.w), Group 4: HAEEC 200 mg/kg, Group 5: HAEEC 400 mg/kg)

DISCUSSION

The water consumption of the disease control group (Group 2) was significantly (p<0.01) high as compared to the healthy control animals (Group 1). The groups treated with Cystone (p<0.01) and HAEEC 400 mg/kg (p<0.05) significantly lowered down the water intakes of the animal (Fig. 14).

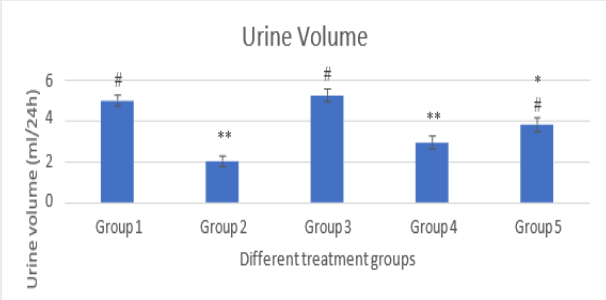


Fig. 21: Change in urine output of the rats by hydroalcoholic extract of *E. crassipes* (HAEEC)

(All values represent mean ± S.E.M, **: p<0.01 w.r.t Group 1; *: p<0.05 w.r.t Group 1; #: p<0.01 w.r.t Group 2, α: p<0.05 w.r.t Group 2. N=5. One-way analysis of variance (ANOVA) followed by "Dunnett's test." Group 1: Normal control, Group 2: Disease control, Group 3: Cystone (750 mg/kg b.w), Group 4: HAEEC 200 mg/kg, Group 5: HAEEC 400 mg/kg).

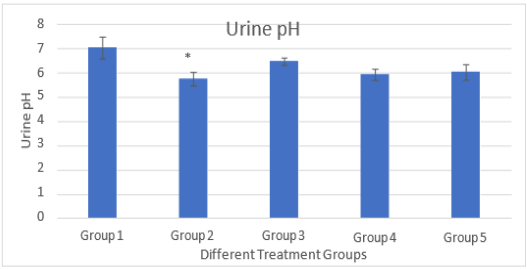


Fig. 22: Change in urine pH of the rats by hydroalcoholic extract of *E. crassipes* (HAEEC)

(All values represent mean ± S.E.M, *: p<0.05 w.r.t Group 1; N=5. One-way analysis of variance (ANOVA) followed by "Dunnett's test." Group 1: Normal control, Group 2: Disease control, Group 3: Cystone (100 mg/kg b.w), Group 4: HAEEC 200 mg/kg, Group 5:
HAEEC 400 mg/kg)

DISCUSSION

The effect of HAEEC showed a significant increase in urinary volume (p< 0.01) when compared with disease control indicating that it might have potent diuretic effect at 400 mg/kg (Fig.15). Though there was an increase in the urine output in HAEEC 200 mg/kg treated animals as well, but the change was not significant. Hence, these findings revealed that diuretic property of *E. crassipes* could be one of the major causes of its antiurolithiatic potential.

Ethylene glycol metabolites causes metabolic acidosis with compensatory hyperventilation and low urine pH usually, < **5.0** is perceived in metabolic acidosis (43,44). The similar effect was obtained in Group 2 animals. The urine pH of disease control animals (Group 2) was found to be 5.72±0.28 that closely resembles the foresaid notion. As depicted in Fig. 16 the mean urine pH was significantly (p<0.05) as compared to Group 1 (healthy animals). Though the treated groups revealed a raise in the mean urine pH, but the changes were not significant to that of Group 2 animals.

Table 7: Effect of hydroalcoholic extract of *E. crassipes* (HAEEC) on urine parameters

sl.no.	name of the	creatinine (mg/dl)	uric acid (mg/dl)	calcium (mg/dl)

	treatment groups			
1	Group 1	0.68±0.14 #	2.16±0.2#	8.55±0.15#
2	Group 2	1.84±0.13**	8.752±0.4**	14.84±0.6**
3	Group 3	0.94±0.3 €	5.7±0.3** #	11.2±1.2€
4	Group 4	1.3±0.3	7.34±0.8**	13.98±0.5**
5	Group 5	1.04±0.3	6.44±0.4** #	10.34±1.3#

(All values represent mean ± S.E.M, **: p<0.01 w.r.t Group 1; *: p<0.05 w.r.t Group 1; #: p<0.01 w.r.t Group 2, €: p<0.05 w.r.t Group 2. N=5. One-way analysis of variance (ANOVA) followed by "Dunnett's test." Group 1: Normal control, Group 2: Disease control, Group 3: Cystone (750 mg/kg b.w), Group 4: HAEEC 200 mg/kg, Group 5: HAEEC 400 mg/kg)

DISCUSSION

- The administrations of ethylene glycol chronically led to metabolic acidosis and in turn hyperoxaluria (45). The reason could be the leakage of renal calcium into the gut due to the acidosis caused due to ethylene glycol thus leading to hyperoxaluria and finally to CaOx crystal formation (45).
- Several studies have also reported that urolithiasis induced by ethylene glycol are mainly due to deposition of CaOx stone and hence there is always an increased level of calcium and phosphorous in the urine (46, 47). Similar responses have been found in our study.
- The creatinine, uric acid and calcium levels were significantly enhanced in the disease-controlled animals (Group 2). However, as depicted in the Table 11, HAEEC at 400 mg/kg treated animals showed a significant decrease (p<0.01) in the urine uric acid and calcium levels.

Table 8: Effect of hydroalcoholic extract of E. crassipes (HAEEC) on blood parameters

sl.no.	name of the treatment groups	creatinine (mg/dl)	Uric acid (mg/d)	calcium (mg/dl)
1	Group 1	0.82±0.1#	2.04±0.2#	8.55±0.2#
2	Group 2	2.4±0.3**	8.462±0.4**	19.84±1.3**
3	Group 3	1.06±0.2 #	5.54±0.3** #	9.96±10.1#
4	Group 4	1.6±0.2	7.44±0.8**	14.74±0.2**#
5	Group 5	1.14±0.2#	6.44±0.4** #	10.6±0.8#

(All values represent mean ± S.E.M, **: p<0.01 w.r.t Group 1; *: p<0.05 w.r.t Group 1; #: p<0.01 w.r.t Group 2, €: p<0.05 w.r.t Group 2. N=5. One-way analysis of variance (ANOVA) followed by "Dunnett's test." Group 1: Normal control, Group 2: Disease control, Group 3: Cystone (750 mg/kg b.w), Group 4: HAEEC 200 mg/kg, Group 5: HAEEC 400 mg/kg)

Discussion (Serum Analysis)

The results of the serum analysis have been tabulated in Table 12. The findings showed that there was significant (p<0.01) increase in the serum creatinine, uric acid and calcium levels of Group 2 animals as compared to the healthy

control Group 1 animals (46,47). The HAEEC 400 mg/kg treated animals significantly ($p<0.01$) lowered serum creatinine, uric acid and calcium levels. However, HAEEC 200 mg/kg treated animals could significantly ($p<0.01$) lower the serum calcium level only as compared against the disease control animals (Group 2).

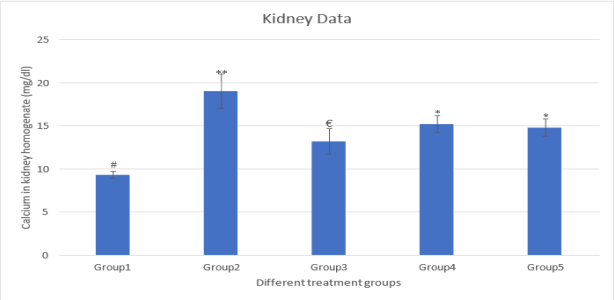


Fig. 23: Change in calcium level in kidney homogenate of the rats by hydroalcoholic extract of *E. crassipes* (HAEEC)

(All values represent mean ± S.E.M, **: $p<0.01$ w.r.t Group 1; *: $p<0.05$ w.r.t Group 1; #: $p<0.01$ w.r.t Group 2, €: $p<0.05$ w.r.t Group 2. N=5. One-way analysis of variance (ANOVA) followed by "Dunnett's test." Group 1: Normal control, Group 2: Disease control, Group 3: Cystone (750 mg/kg b.w), Group 4: HAEEC 200 mg/kg, Group 5: HAEEC 400 mg/kg)

DISCUSSION

Fig. 17 represents the change in calcium level in kidney homogenate of the rats by hydroalcoholic extract of *E. crassipes* (HAEEC). It was found that there was significant ($p<0.01$) increase in calcium levels in the kidney tissue as compared to the healthy control (Group 1) animals (48), but significant reduction ($p<0.05$) was only obtained in the cystone treated animals. The HAEEC treated animals at both doses though showed decrease in the kidney tissue calcium level but that was not a significant figure.

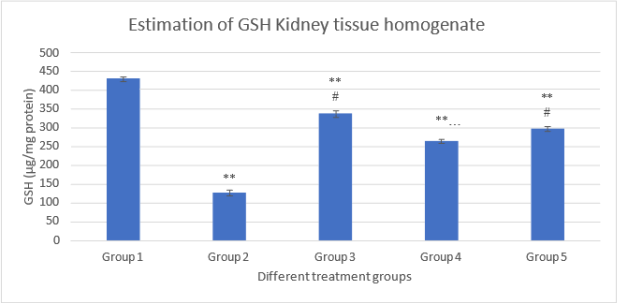


Fig. 24: Change in GSH level in kidney homogenate of the rats by hydroalcoholic extract of *E. crassipes* (HAEEC)

(All values represent mean ± S.E.M, **: $p<0.01$ w.r.t Group 1; #: $p<0.01$ w.r.t Group 2; N=5. One-way analysis of variance (ANOVA) followed by "Dunnett's test." Group 1: Normal control, Group 2: Disease control, Group 3: Cystone (750 mg/kg b.w), Group 4: HAEEC 200 mg/kg, Group 5: HAEEC 400 mg/kg)

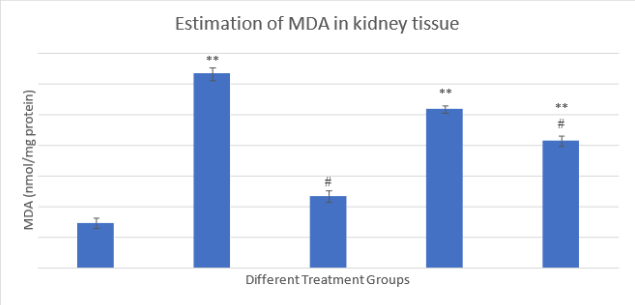


Fig. 25: Change in MDA level in kidney homogenate of the rats by hydroalcoholic extract of *E. crassipes* (HAEEC)

(All values represent mean ± S.E.M, **: p<0.01 w.r.t Group 1; #: p<0.01 w.r.t Group 2; N=5. One-way analysis of variance (ANOVA) followed by "Dunnett's test." Group 1: Normal control, Group 2: Disease control, Group 3: Cystone (100 mg/kg b.w), Group 4: HAEEC 200 mg/kg, Group 5: HAEEC 400 mg/kg)

- The in- vivo antioxidant profile of HAEC was evaluated based on evaluating the GSH and MDA level in the kidney tissue homogenate (Fig.18 and Fig.19). This study discovered the effect in terms of a significant increase in lipid peroxidation (MDA levels) and decreased levels of antioxidant potential (GSH levels) in the kidneys of rats of disease control (Group 2).
- This could be due to the accumulation of oxalate crystals in the kidney tissues that induces lipid peroxidation and further reacts with the polyunsaturated fatty acids in cell membranes of kidney tissue leading to kidney damage.
- As depicted in Fig. 18 there was significant (p<0.01) decrease in GSH level in Group 2 animals as compared against Group 1, whereas HAEEC at both doses significantly (p<0.01) enhanced the GSH levels as compared to Group 2.

Similarly, Fig. 19 represents that the MDA level was significant (p<0.01) higher in diseased animals (Group 2) as compared against Group 1, whereas HAEEC 400 mg/kg significantly (p<0.01) reduced the MDA levels as compared to Group 2 (49-50).

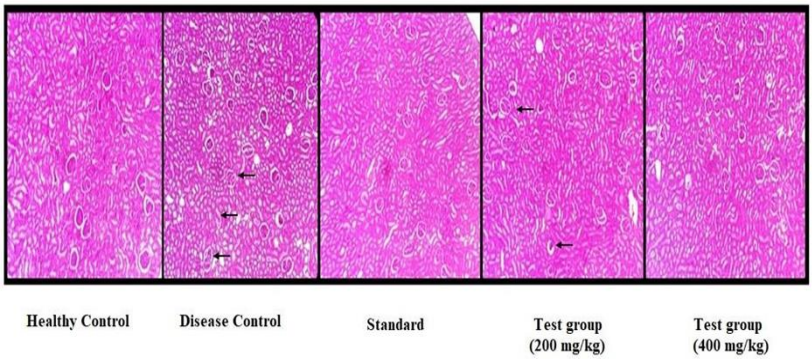


Fig. 26: Effect of hydroalcoholic extract of *E. crassipes* (HAEEC) on the histopathological parameters of the kidney of rats.

(Group 1: Normal control, Group 2: Disease control, Group 3: Cystone (750 mg/kg b.w), Group 4: HAEEC 200 mg/kg,
Group 5: HAEEC 400 mg/kg)

Histopathology of kidney tissue of healthy control group shows normal artitechture. Disease control group shows diffuse thickening of bowman's capsule with fibrous connective tissue and increase

in the subcapsular space. Moreover, it shows tubular degeneration and nodular structure formation. However, test and standard drug treated groups reversed the pathological changes in the kidney tissue.

CONCLUSION

The results of the present investigation "Exploring the antiurolithiatic activity of hydroalcoholic extract of aquatic weed *Eichhornia crassipes* in rats" have directed to the following conclusions.

- The hydroalcoholic extract of *E. crassipes* showed significant reduction in calcium, uric acid, and creatinine levels in urine & led to a rebuilding of normal urine output volume. In addition to that the levels of creatinine, uric acid and calcium in the serum were normalized by the extracts. Histopathological observations also confirmed that the extract treated groups normalized the structure of the bowmen's capsule to a greater extent.
- The promising antioxidant effect of the extracts was also revealed by in-vivo lipid peroxidation study in which the elevated MDA levels were significantly reduced by the treatment of the plant extract and improved GSH content in the kidney tissue, both at higher (400 mg/kg) and lower dose (200 mg/kg) treated groups.
- The presence of phytoconstituents such as flavonoids, polyphenols, terpenoids might be responsible for the antioxidant and antiurolithiatic activity of the extract of ***Eichhornia crassipes.***
- These verdicts designate the potential of the hydroalcoholic extract of aquatic weed ***Eichhornia crassipes*** in inhibiting urolithiasis as well as the lithotripsic action of the same on the formed renal stones has been confirmed in the in-vitro study.
- However, the hydroalcoholic extract of aquatic weed ***Eichhornia crassipes*** is more potent at higher dose i.e at 400 mg/kg than the lower dose 200 mg/kg in the rat model.

Hence, it can be concluded that the plant *Eichhornia crassipes* has antiurolithiatic property and it can be employed as an alternative medicine for the management of urolithiatic conditions.

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