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### IN VIVO AND IN VITRO MODELS FOR EVALUATING ANTI-UROLITHIASIS ACTIVITY OF HERBAL DRUGS

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**Abstract:** Today large number of population suffers from kidney stone disease has increasing due to change in living conditions. Kidney stone formation or urolithiasis is a complex process that results from a succession of several physicochemical events including supersaturation, nucleation, growth, aggregation, and retention within the kidney. The treatments used are extracorporeal shock wave lithotripsy (ESWL). The exposure to shock waves in therapeutic doses may cause acute renal injury, in addition increases in stone recurrence, possibility of infection after exposing to shock. Further, progress in the study of the biological and physical manifestations of kidney stones, there is no satisfactory drugs to use in clinical therapy. The data from *in vivo* and *in vitro* reveal that phytotherapeutic agents may be useful as alternative or an adjunctive therapy in the management of urolithiasis. The medicinal plants are used from centuries for the treatments of kidney stone, due to its safety, efficacy, cultural acceptability and lesser side effects compared to synthetic drugs. This systemic review reveals are used of single herbs, in treatments of urolithiasis animals models. We conducted an electronic and by hand search treatment studies focusing on animals model and antiurolithiasis activity of plants in urolithiasis. The proven antilithiasis activity of plants in animals models and also traditional, ethno veterinary claimed medicinal plants. Plants extracts and its effective in reducing kidney stone and oxalate, calcium, Phosphate creatinine, uric acid and BUN. The present review article provides basic idea about the different animal's model and parameter to be considered for evaluation of antiurolithiasis animal's models.

**Keywords:** Animals models, urolithiasis, Calcium oxalate, Oxalate, Plants extracts



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## INTRODUCTION

The formation of urinary tract stone is worldwide, sparing no geographical, cultural or racial group, most common urolithiasis accounting for more than 80% of the stone<sup>[1]</sup>. The urinary stone disease is a common disorder estimated to occur in approximately 12% of the population with a recurrence rate of 70-80% in male, and 47-60% in female<sup>[2]</sup>. The mechanism involved in the formation of calcific stone are not fully understood, it is agreed that involving events leading to crystal nucleation, aggregation and growth of insoluble particles<sup>[3]</sup>. Urine is supersaturated with common stone forming minerals, however, the crystallization inhibiting capacity of urine does not allow urolithiasis in most of individual, whereas, the natural inhibition capacity is on deficient in stone former<sup>[4]</sup>. The term supersaturation means beyond the metastable limit that could form crystals spontaneously. Although most kidney stone are found in the renal pelvis, the stone-forming process actually starts in the nephrons. Intranephronic crystals of calcium oxalate (CaOx) were found intracellular in renal biopsies of a patient with type I primary hyperoxaluria, crystals are frequently precipitated in the tubular fluid of both stone-forming and non-stone forming individual, such crystals are harmless as long as they are excreted into the urine. A single microcrystal is usually too small to occlude the tubular lumen due to its sluggish growth rate. However, high levels of urine of stone-forming<sup>[5]</sup>. It has multifactorial etiopathogenesis, involving genetic, nutritional, socio-economic, environmental, metabolic, and anatomic and infections factors<sup>[6]</sup>.

### Pathphysiology of Nephrolithiasis

The kidney stones are classified according to their chemical composition. For the crystal to form, urine must be supersaturated with respect to the stone material, meaning the concentrations are higher than the thermodynamic solubility for that substance. The levels of urinary supersaturation correlate with the type of stone formed, and lowering supersaturation is effective for preventing stone recurrence<sup>[7]</sup>. The CaOx is the predominant component of most stones accounting for more than 80% of stone<sup>[8]</sup>. The remaining 20% are composed of struvite, cystine, uric acid, and other stones<sup>[9]</sup>. The metabolic abnormalities such as hypercalciuria (Urinary calcium excretion >200 mg/d) hypocitraturia (Urinary citrate excretion >320 mg/d), hyperoxaluria (Urinary oxalate excretion >40 mg/d) hyperuricosuria (Urinary acid excretion >600 mg/d), hypomagnesuria (Urinary magnesium excretion <50 mg/d) and gouty diathesis<sup>[10]</sup>. Any cellular dysfunction that can affect various urinary ions and other substance can also influence CaOx supersaturation and crystallization in the kidney. The crystal formation, particularly of calcium phosphate (CaP) and CaOx, within the urinary tract is widespread. Since humans excrete millions of urinary crystal daily without developing kidney stones, at least a transient development of supersaturation is likely<sup>[10,11]</sup>. For stone formation crystal need to be retained within the kidney and they should also be located at sites from where crystal can ulcerate to the renal papillary surface to form a stone nidus<sup>[12]</sup>. Renal injury promotes crystal

retention and the development of a stone nidus on the renal papillary surface and further supports crystal nucleation at lower super saturation. Thus one approach to prevent the stone formation would be stop crystal retention. Reactive oxygen species (ROS) seem to be responsible for cellular injury therefore a reduction of renal oxidative stress could be an effective therapeutic approach<sup>[13]</sup>. The medical plants have played a significant role in various ancient traditional systems of medication. Even today, plants provide a cheap source of drugs for majority of world population<sup>[1]</sup>. Which are regarded as quite, safe, with minimal or no side effects, cost effective, readily available and easily affordable. The accepted management of stone disease ranges from observation to surgical removal of the stone. The Various factors such as size of calculi, severity of symptoms, and degree of obstruction, kidney function, location of the stone and the presence or absence of associated infection influence the choice of one type of intervention over the other<sup>[10]</sup>. Today surgical endoscopic stone removal and extracorporeal shock wave lithotripsy (ESWL) have revolutionized the treatment of urolithiasis, but not prevent the like hood new stone formation<sup>[14]</sup>. However, in addition to the traumatic effect of shock waves, persistent residual stone fragments, and the possible of infection and may decrease renal function and increase stone recurrence<sup>[15]</sup>. The recurrence rats is also high (50-80%) and no suitable medical therapy is available for such stone disorders<sup>[16]</sup>. The various therapies including thiazide diuretic and alkali-citrate are being used in attempt to prevent recurrence of hypercalciuria and hyperoxaluria-induced calculi but evidence for their efficacy is less<sup>[17]</sup>. It remain unclear which treatment modality is better than the other and the final decision should be based on the patient's preference, on the size and the location of the stone, expertise of the physician and the costs of the procedure<sup>[10]</sup>. In current we reviewed the effect of plants extracts as single herb in preventing or curing kidney stone in rats model to determined, if the evidence from the animals studies of plants extracts supports it use in clinical practice. The aim of this work was to review the available *in vivo*, *in vitro* and surgical model of urolithiasis in order to provide adequate tolls to investigate the mechanism of action of drugs with potential antiurolithiatic properties.

In view of urolithiasis the various medicinal plants was studies to evaluate its antiurolithiasis by using *in vivo*, *in vitro* and surgical. Due to insufficient reporting of data, the safety of plants was not assessed in this our review. Many *in vivo* and *in vitro* models have been developed to investigate the mechanisms involved in the formation of urinary stones, and to ascertain the effect of various therapeutic agent on the development and progression of the disease. Rats are the most frequently used animals in model of CaOx deposition in the kidneys, a process that mimics the etiology of kidney stone formation in humans<sup>[18]</sup>.

#### LITERATURE SEARCH AND CRITERIA

We made an attempt to search various screening animals model for urolithiasis and potential antiurolithiasis plants extracts. This study will help scientist for selection of suitable animal's

model in search of antiurolithiasis compounds. We included studies that adopted at least one measurement relevant to renal urolithiasis, such oxalate, calcium, phosphate creatinine, uric acid and BUN etc.

### ***IN VIVO* ANIMAL MODEL**

#### **Ethylene glycol induced urolithiasis in rats.**

The toxicity of ethylene glycol has been related to the metabolic breakdown product of oxalic acid, which, when present in sufficient concentration, crystallizes as calcium oxalate within body tissue, when given in the drinking water at concentrations of 25-10% produce oxalate stone in the urinary tract of the animals. The stone formation in ethylene glycol is caused by hyperoxaluria, which causes increased renal retention and excretion of oxalate. Changes of urinary oxalate levels are relatively much more important than of calcium. Increased urinary calcium is a factor favoring the nucleation and precipitation of calcium oxalate or apatite (calcium phosphate) from urine and subsequently crystal growth. Increased urinary phosphate excretion along with oxalate stress seems to provide an environment appropriate for stone formation by forming calcium phosphate crystals, which epitaxially induce calcium oxalate deposition. In urolithiasis, the glomerular filtration rate (GFR) decreased due to obstruction to the outflow of urine by stone in urinary systems. Due to this, the waste products, particularly nitrogenous substance such as urea, creatinine and uric acid get accumulated in blood. Magnesium as well-known inhibitor of crystallization in urine. This is substantiated by the low levels of urinary magnesium observed in stone former. It also reported that magnesium decreased the growth rate of calcium oxalate crystals<sup>[19,20,21,22,23,24,25,26]</sup>.

#### **Ethylene glycol and ammonium chloride induced urolithiasis in rats.**

The lithogenic effect caused by 0.75% ethylene glycol (EG) must be mainly attributed to the oxidative damage caused by the high level of oxalate generated by EG. Thus, although EG rat model can be questioned as a general model to study renal stone formation, it must be considered as an interesting model to evaluate renal papillary stone development, at least for those stones which genesis is linked to oxidative cell damage. The 2% of ammonium chloride (w/v) was in order to promote hyperoxaluria and CaOx deposition in the kidneys<sup>[18]</sup>.

#### **Sodium oxalate (NaOx) induced urolithiasis in rats.**

Oxalocalcic lithiasis was induced by an intraperitoneal injection of sodium oxalate (NaOx). The loss of weight observed is due to anorexia due to disturbance in carbohydrates, proteins or fat metabolism which is affected by the injection of sodium oxalate. Its injurious effects are considered salts CaOx which are insoluble at physiological pH inducing CaOx nephrolithiasis. The intensity of necrosis, the size of CaOx particles, their number and their distribution within

the inner medulla appeared to be dependent on the time interval following NaOx injection [27,28]

### **Calculi-Producing diet induced urolithiasis**

The elevation of the oxalate synthesizing liver enzymes, glycolate oxidase, produced by feeding glycolic acid. CaOx stone were induced by feeding 3% glycolic acid with normal diet. The commercial diet mixed with 3% glycolic acid for 40 d, results in renal tissue deposition of calcium and oxalate. The increased deposition of calcium and oxalate in the renal tissue is known to lead papillary calcification and eventual calculi formation. The hyperoxaluria and CaOx deposition in the kidney was induced using gentamycin (40 mg/kg/day s.c) and calculi producing diet (CPD). The latter was made from powdered standard rat pellet feed mixed with ammonium oxalate (5%), then made pellets and dried [29,30].

### **Glyoxylate induced acute lithiasis**

Glyoxylic lithiasis by acute glyoxylate intoxication significantly raised the deposition of stone forming components- calcium, oxalate and phosphorus in the renal issue. A four-fold increase in the urinary oxalate level reported [31]. The glycollate and glyoxylate are the effective oxalate precursors in isolated rat hepatocytes. The only compound demonstrated to be an immediate precursor of oxalate in man is glyoxylate. The glycoxylate has two advantages 1). The quantity of toxic material absorbed can be perfectly controlled 2) the agent used is an immediate precursor of oxalic acid and permits the study of true antilithiatic compounds. The animals received sodium glyoxylate 120 mg/kg per rat intraperitoneally for one 1 d, given in two divided doses in the morning and evening, to induce oxalate stones within 24 h [31].

### **2. Zinc disc induced urolithiasis in rats**

Rats were anesthetized with sodium pentobarbitone (40 mg/kg, ip). A suprabic incision was made and the urinary bladder was exposed. A small cut was made at the top of the bladder. The urine was then aspirated aseptically into a sterile vial for bacteriological examination and pH determination (using narrow range pH paper BDH). Previously weighted sterile zinc discs were inserted into bladder, and the incision was closed with a single suture using absorbable 4-0 chronic catgut (Ethicon) and the rats were allowed to recover for one week. The implantation of zinc foreign bodies into the urinary bladder induce growth of urinary stones and hypertrophy of the organ smooth musculature that were greater in males than in female from 4 & 8 w surgery, respectively [32,33,34]

### 3. *IN VITRO* STUDIES ON UROLITHIASIS.

#### Determination of effect on $\text{CaC}_2\text{O}_4$ crystallization

The  $\text{CaC}_2\text{O}_4$  Crystallization was determined by the time course measurement of turbidity change due to the crystal formation and aggregation in the metastable situations of  $\text{Ca}^{2+}$  and oxalate, stock solutions of  $\text{CaCl}_2$  (8.5 Mm) and  $\text{Na}_2\text{C}_2\text{O}_4$  (1.5 Mm). Containing 200 mM NaCl and 10 mM sodium acetate were adjusted to pH 5.7. An aggregometer devised for platelet aggregation studies based on the measurement of optical density at 620 nm was used to investigate the event of  $\text{CaC}_2\text{O}_4$  crystallization. The  $\text{CaCl}_2$  solution (0.5 ml) was stirred constantly both in the absence and presence of different concentrations of the test material or reference drug: potassium citrate at 37 °C. After obtaining a stable base line, crystallization was induced by the addition of  $\text{Na}_2\text{C}_2\text{O}_4$  solution (0.5) to obtain the final concentration of  $\text{Ca}^{2+}$  as 4.25 and oxalate as 0.75 Mm. The time course measurement of turbidity was simultaneously started on a chart, moving at the speed of 30 mm/h and continued for 15 min with constant stirring of the solutions. All experiments were run in triplicate, Slope of nucleation ( $S_N$ ) and aggregation ( $S_A$ ) phases were calculated using linear regression analysis. Using the slopes, the percentage inhibition was calculated as  $[(1-S_m/S_c) \times 100]$ , where  $S_m$  is slope in the presence of modified and  $S_c$  is slope of the control experiment.

To determine the effect of incubation with the test material on  $\text{CaC}_2\text{O}_4$  crystal formation, stock solutions of  $\text{CaCl}_2$  and  $\text{Na}_2\text{C}_2\text{O}_4$  having composition similar to those in the kinetic study were used.  $\text{CaCl}_2$  solutions, containing different concentrations of the test material or potassium citrate, were aliquoted (0.5) to the flat bottomed tubes in a 24 well plate. To each of these tubes  $\text{Na}_2\text{C}_2\text{O}_4$  solution (0.5) was added to obtain the final concentration of  $\text{Ca}^{2+}$  as 4.25 and oxalate as 0.75 mM. Each concentration of the test material was prepared in triplicate. The plates were then incubated in a shaking water bath at 90 oscillations/min at a temperature of 37 °C for 45 min. Each tube then observed under an inverted microscope for crystal morphology and count in five randomly selected fields (200x)<sup>[1]</sup>.

#### Nucleation Assay

The method used similar to that described<sup>35</sup>. The solutions of calcium chloride and sodium oxalate were prepared at the final concentration of 3 mmol/l and 0.5 mol/l, respectively, in a buffer containing Tris 0.05 mol/l and Nacl 0.15 mol/l at pH 6.5. Both solutions were filtered through a 0.22  $\mu\text{m}$  filter; 33 ml of calcium chloride solution was mixed with 3.3 ml of the test at different concentration. Crystallization was started by adding 33 ml of sodium oxalate solution. The final solution was magnetically stirred at 800 rpm using a PTFE-coated stirring bar. The temperature was maintained at 37 °C. The absorbance of the solution was monitored at 620 nm after every 1 min. The percentage inhibition produced by test was calculated as  $[1-(T_{si}/T_{sc})] \times 100$ , Where  $T_{sc}$  was the turbidity slope in the presence of the inhibitor<sup>[36]</sup>.

### Growth assay

Inhibitory activity against CaOx crystal growth was measured using the seeded, solution-depletion assay. A TEST solution of 10 mM Tris-HCl containing 90 mM NaCl was adjusted to pH 7.2 with 4 N HCl. Stone slurry (1.5 mg/ml) was prepared in 50 mM sodium acetate buffer (pH 5.7). CaOx monohydrate crystal seed was added to a solution containing 1 mM CaCl<sub>2</sub> and 1 mM sodium oxalate (Na<sub>2</sub>C<sub>2</sub>O<sub>4</sub>). The reaction of CaCl<sub>2</sub> and Na<sub>2</sub>C<sub>2</sub>O<sub>4</sub> with crystal seed led to deposition of CaOx (CaC<sub>2</sub>O<sub>4</sub>) on the crystal surfaces, thereby decreasing free oxalate that is detectable by Spectrophotometer at λ214 nm. When test is added into this solution, Depletion of free oxalate ions will decrease if the test sample inhibits CaOx crystal growth. Rate of reduction of free oxalate was calculated using the baseline value and the value after 30- second incubation with or without test sample. The relative inhibitory activity was calculated as follows: % Relative inhibitory activity = [(C-S)/C]x100, where C is the rate of reduction of free oxalate without any test sample and S is the rate of reduction of free oxalate with a test sample [36,37].

### Calcium phosphate assay

Calcium phosphate (CaP) assay was studied on *in vitro* homogeneous systems of initial mineral phase formation for CaP, its subsequent growth and demineralization by employing 5.0 ml systems which was prepared by adding 0.5 ml of KH<sub>2</sub> PO<sub>4</sub> (50 mM), 0.5 of CaCl<sub>2</sub> (50 mM), 2.5 ml of Tris buffer (210 mM NaCl + 0.1 mM tris HCl) and increasing volume of test ranging from 0.2 ml to 1.5 ml by subsequently decreasing the volume of water ranging from 1.5 ml to 0.0 ml. This system was centrifuged at 4500 rpm and precipitates so obtained were dissolved in 5 ml of 0.1 N HCl. This 5 ml system for mineralization [38,39]. For the growth, firstly 5 ml systems were prepared using standard protocols then again 5 ml were re-grown on the same tubes with the additions of increasing volumes of the test. Calcium and phosphate were then estimated on the precipitates obtained and dissolved in 0.1 N HCl. In case of control no test was added. To check the demineralization, again 5 ml system was prepared having no test added to that and precipitates were obtained. To these precipitates, 2.5 ml of Tris buffer (210 mM NaCl+0.1 mM Tris HCl) and increased volumes of test ranging from 0.2 ml to 1.5 ml with subsequently reduced volume of water was added and then centrifuged at 4500 rpm for 15 min. Calcium and phosphate were then estimated in supernatant obtained after centrifuged. The Ca<sup>2+</sup> and HPO<sub>4</sub><sup>4-</sup> ions were estimated by the methods<sup>40,41</sup> respectively. Percentage inhibition of mineral phase in the presence of test was calculated as % inhibition= ((C-T)/C)x100, where T is the concentration of Ca<sup>2+</sup> or HPO<sub>4</sub><sup>2-</sup> ion of the precipitates formed in test having test ranging from 0.2 ml to 1.5 ml in the assay systems and C is the concentration of Ca<sup>2+</sup> or HPO<sub>4</sub><sup>2-</sup> ion of the precipitate formed in control systems which had distilled water (Millipore) and no test [42].

### Calcium oxalate crystal assay:

Inhibitory activity of test was also checked on calcium oxalate crystal growth. A 4 ml system was prepared to check the effect of the test in inhibiting growth of calcium oxalate crystals. In this systems, 1 ml each of 4 mM calcium chloride and 4 mM sodium oxalate were added to a 1.5 ml solution, containing NaCl (90 Mm) buffered with Tris HCl (10 mM) pH 7.2. To this 30 µl of calcium oxalate monohydrate (COM) crystal slurry (1.5 mg/ml acetate buffer) was added consumption of oxalate begins immediately after COM slurry addition and was monitored for 600 sec by disappearance of absorbance at 214 nm. When test is added into this solution, depletion of free oxalate ions will decrease if test inhibits calcium oxalate crystal growth. Rate of reduction of free oxalate was calculated using the baseline value and the value after 30 sec incubation with or without the test. The relative inhibitory activity was calculated  $=((C-S)/C) \times 100$ , where C is the rate of reduction of free oxalate without any test sample and S is the rate of reduction of free oxalate with a test sample <sup>[42]</sup>

### Lactate dehydrogenase leakage assay

LDH leakage assay was performed by the methods described<sup>43</sup> The 6.6 mM NADH and 30 mM sodium pyruvate were prepared in Tris (0.2M, pH 7.3) reaction was initiated with the addition of 50 µl of the test sample and the disappearance of NADH was monitored at 340 nm, for 5 min at an interval of 1 min. The percentage of LDH release was calculated by dividing the activity of LDH in the supernatant by the LDH activity measured after complete cell lysis achieved by sonication <sup>[36]</sup>.

## 1. Traditional claimed plant for Urinary disorders (UD) and Polyurea<sup>[44,45,46,47,48,49]</sup>

### Urinary disorders

Plants	Part	Plants	Part
<i>Artabotry hexapetalus</i>	Seeds	<i>Cassia auriculata</i> L	Seeds
<i>Cissampelos pareira</i> L	Root	<i>Tinospora cordifolia</i> (Willd)	Root
<i>Sida cordifolia</i> L	Root	<i>Grewia villosa</i> Willd	Root
<i>Boswellia serrata</i> Roxb	Bark	<i>Mangifera indica</i>	Root
<i>Pueraria tuberosa</i>	Root	<i>Tephrosia purpurea</i> L	Root
<i>Bauhinia variegata</i> L	Flower	<i>Psidium guajava</i> L	Leaf
<i>Indigofera tinctoria</i> L	Root	<i>Citrullus colocynthis</i> (L)	Root bark
<i>Saccharum officinarum</i> L	Root and stem	<i>Diospyros melanoxylon</i>	Flower



<i>Solanum erianthum</i> D.	Root	<i>Hemidesmus indicus</i> L	Root
<i>Ehretia canarensis</i>	Bark	<i>Merremia tridentate</i> L	Whole plant
<i>Scoparia dulcis</i> L	Leaf	<i>Utricularia bifida</i> L	Whole plant
<i>Tecomella undulata</i>	Bark	<i>Plectranthus amboinicus</i>	Whole Plant
<i>Phyllanthus urinaria</i> L	Whole plant	<i>Barleria longiflora</i> L.F	Leaf
<i>Phyllanthus virgatus</i> Forst	Whole plant	<i>Phoenix sylvestris</i> (L)	Root

## 2. Traditional claimed plant for Urinary disorders (UD) and Polyurea<sup>[44,45,46,47,48,49]</sup>

### Urinary/troubles/complaint/disease

Plants	Part	Plants	Part
<i>Mimosa pudica</i> L	Leaves	<i>Scoparia dulcis</i> L	Whole plant
<i>Ananas comosus</i> L	Fruit	<i>Glycyrrhiza glabra</i> L	Root
<i>Pavetta Crassicaulis</i> Bremek.	Root	<i>Acorus calamus</i> L.	Rhizome
<i>Mucuna pruriens</i> L DC	Seeds	<i>Citrullus colocynthis</i> shcrad	Root
<i>Eleusine indica</i> L	Root	<i>Abutilon theophrastii</i>	Seeds
<i>Cucurbita Maxima</i> Duch	Seeds	<i>Imperata cylindrica</i> L	Whole plant
<i>Michelia champaca</i> L	Flowers	<i>Momordica dioica</i> Roxb	Root
<i>Kalanchoe laciniata</i> L	Leaves	<i>Cissampelos pareira</i> L.	Leaves
<i>Alismataceae</i>	Fruit	<i>Coleus amboinicus</i> Lour. syn	Leaves
<i>Papaver somniferum</i> L	Seeds	<i>Areca catechu</i> L	Nut
<i>Rubia cordifolia</i> L	Root	<i>Crataeva nurvala</i>	Leaves
<i>Caryota urens</i> L	Spadices	<i>Ageratum conyzoides</i>	Root
<i>Ocimum gratissimum</i> L	Whole plant	<i>Acorus calamus</i> L	Leaves
<i>Prunus cerasoides</i> D.	Seed	<i>Sesamum orientale</i> L.	Seeds

**3. Traditional claimed plant for Urinary disorders (UD) Polyurea and Kidney stone<sup>[44,45,46,47,48,49]</sup>**

Plants	Part	Plants	Part
<i>Cocculus hirsutus</i> L	Root	<i>Crataeva magna</i> Lour	Root, bark
<i>Ananas comosus</i> L	Fruit	<i>Hybanthus enneaspermus</i> L	Whole plant
<i>Polycarphaea corymbosa</i> L	Whole plant	<i>Acorus calamus</i> L.	Rhizome
<i>Abelmoschus moschatus</i>	Seed	<i>Cansjera rheedii</i> Gmel	Leaf
<i>Eleusine indica</i> L	Root	<i>Buchanania axillaris</i>	Seed
<i>Caesalpinia bonduc</i> L	Leaf	<i>Imperata cylindrica</i> L	Whole plant
<i>Mimosa pudica</i> L	Whole plants	<i>Boerhavia erecta</i> L.	Whole plant
<i>Kalanchoe laciniata</i> L	Leaves	<i>Pongamia pinnata</i> L	Seeds
<i>Commicarpus chinensis</i> L	Whole plant	<i>Coleus amboinicus</i> Lour. syn	Leaves
<i>Dalbergia sissoo</i> Roxb.	Root barks	<i>Polygonum plebeium</i> R.Br.	Root
<i>Rubia cordifolia</i> L	Root	<i>Vigna radiate</i> L	Seeds
<i>Euphorbia thymifolia</i> L	Whole plant	<i>Ageratum cogyzoides</i>	Root
<i>Cassia occidentalis</i> L	Leaf	<i>Phyllanthus acidus</i> L	Root
<i>Prunus cerasoides</i> D.	Seeds	<i>Saraca asoca</i> (Roxb)	Stem bark
<i>Dioscorea bulbifera</i> L	Tuber	<i>Spinacia oleracea</i> L.	Whole plant
<i>Acacia nilotica</i> L Willd	Fruit	<i>Dioscorea hispida</i> Dennst.	Tuber
<i>Anogeissus latifolia</i>	Stem bark	<i>Dioscorea oppositifolia</i> L	Tuber
<i>Terminalia arjuna</i>	Bark	<i>Dioscorea pentaphylla</i> L	Tuber

4. Antiurolithiasis proven plants

Plants	Model	Duration	Parameter
<i>Moringa oleifera</i> Lam	Ethylene glycol urolithiasis induced	28 d	↓oxalate, ↓calcium in urine and ↓Phosphate ↓oxalate, ↓calcium and ↓Phosphate ↓creatinine, ↓uric acid and ↓UN in renal content of those constituents stone forming <sup>[15]</sup> .
<i>Moringa oleifera</i>	Zinc disc foreign body insertion in the bladder supplemented with 1% ethylene glycol in drinking water	4 w	↓significant in the ↓wt. of stone ↓in the bladder stone formation with ↑ in the dose <sup>[50]</sup> .
<i>Paronychia argentea</i>	Sodium oxalate induced urolithiasis rats	28 d	↑Body weight of rats, ↑RBC, Packed cell In the lymphocyte levels ↓renal urea, ↓creatinin levels Proteinimia restoration to normal levels <sup>[28]</sup> .
<i>Crataeva nurvala</i>	calcium-producing diet, diet mixed with 3% glycolic acid) for 40 days	10 d	Kidney LDH is stimulated PPase activity no significant change ACP showed a slight ↓oxalate, ↓calcium and phosphorus did not change ↑ magnesium <sup>[30]</sup> .
<i>Costus Spiralis</i>	Implants of 0.5mg/kg calcium oxalate crystals or zinc disc in the urinary	-----	Not change of either EC <sub>50</sub> bladder of rats Values of bethanecol or PA <sub>2</sub> values of atropine in both control and extract treated with zinc . disc in plants <sup>[53]</sup> .

<b>Hibiscus Sabdariffa Linn.</b>	Ethylene glycol induced urolithiasis rats	28 d	↑urine volume ↓calcium and ↓oxalate in urine ↑Magnesium low serum levels and improved creatinine clearance and improve kidney function <sup>[14]</sup> .
<b>Raphanus Sativus</b>	Insertion of zinc disc	-----	↓Size of stone dose dependent urinary In the 140 mg/kg extract treated group There was almost normalization 140 dose produced maximum urine volume of urine <sup>[34]</sup> .
<b>Salix Taxifolia</b>	Insertion of zinc disc into bladders in rats	8 w	Dose dependent reduction in the size of stone 60 mg/kg showed 90.63% reduction in the stone deposition.

Plants	Model	Duration	Parameter
<b>Betulin and lupeol</b>	Pyridoxime deficient diet containing 3%glycolic acid	21 d	↓calcium extraction to normal levels L ↓oxalate excretion ↑phosphorus excretion no significant decrease in the urinc acid levels ↑Magnesium excretion to near normal Proteinuria was effectively normalized ↑Moderate in the treated groups <sup>[52]</sup> .
<b>Aerva Lanata</b>	Ethylene glycol induced urolithiasis rats	28 d	The lactate dehydrogenase and oxalate levels to near normal control, calcium and oxalate excretion citrate and magnesium the <i>aervalanata</i> therapy reverted, uric acid and proteins to near normal <sup>[20]</sup> .
<b>Ammi their</b>	<b>Visnaga</b> diet given for the period of 4 weeks.	-----	The prophylactic effects of acts on Ammi Visnaga may be attributed to its diuretic activity to maintain the oxalate, below the supersaturation to precipitate as Calcium oxalate <sup>[53]</sup> .

<b><i>Spirulina</i></b>	Ethylene glycol induced urolithiasis rats	-----	the ↑ in the levels of sodium chloride, BUN Blood urea nitrogen ALT and a decrease in the levels Calcium were noted in rats treated with ethylene glycol. Supplementation spirulina for the last three weeks mostly recovered the rats from nephrolithiasis <sup>[54]</sup> .
<b><i>Trigonella foenum L. graceumand ammi majus</i></b>	Ethylene glycol induced urolithiasis rats and 2% of ammonium chloride	-----	the calcification in the kidneys and the total renal tissue calcium amount of the in rats treated with trigonella foenum raceum were significantly ↓with the untreated group <i>ammi majus</i> found to statistically insignificant <sup>[55]</sup> .
<b><i>Herniaria hirsuta</i></b>	Ethylene glycol 0.75% induced	3 w	Calcium remain constant on treated rats Urolithiasis treated animals excreted mostly small CaOx dihydrate crystals <sup>[56]</sup> .
<b>Berberine</b>	Ethylene glycol induced urolithiasis rats and 1% of ammonium chloride	3 w	↓urinary crystal count↓ visible reduced crystal size prevent the ↑in urine volume and water intake, Urinary pH↑ ↑oxalate excretion not significant ↓calcium raised BUN and serum creatinine and ↓creatinine clearance which is prevented treated with berberine <sup>[57]</sup>

Plants	Model	Duration	Parameter
<b><i>Tribulus terrestris</i></b>	Inhibition of calcium oxalate crystallization in vitro	-----	The demonstrated the % inhibition By <i>tribulus terrestris</i> on the calcium oxalate crystal growth. The <i>tribulus terrestris</i> extract inhibition in a concentration dependent manner <sup>[36]</sup> .
<b><i>Tribulus terrestris</i></b>	artificially induced urolithiasis	-----	It exhibited dose dependent antiurolithiatic activity and almost completely inhibited stone formation <sup>[58]</sup> .

<i>Phyla nodiflora</i> (Linn)	Gentamycin and calculi producing diet (5% ammonium oxalate in standard rat Pellet feed)	15 d	↓Urinary excretion of calcium and oxalate ↑Urinary pH (7.0-8.0) ↓Kidney weight ↓Significant in calcium and oxalate deposition <sup>[59]</sup> .
<i>Asparagus racemosus</i> Willd and <i>Tamarindus indica</i>	Zinc disc foreign body insertion technique supplemented with 1% ethylene glycol.	4 w	↓in the Wt of stone ↓was not statistically Significant <sup>[60]</sup> .
<i>Sesbania grandiflora</i>	Gentamicin (Subcutaneously) and 5% ammonium oxalate in rats feed to induce calcium oxalate-type stones.	-----	showed significant antiurolithiatic activity against calcium oxalate-type stones <sup>[61]</sup> .
<i>Pinus eldarica medw</i>	ethylene glycol 1% for 30days.	30 d	↑urinary calcium excretion Prevented stone formation in the Kidney significantly. prophylactic treatment did not ↑urine volume <sup>[62]</sup> .
<i>Homonnia riparia</i> Lour	3% glycolic acid along with normal& implantation of zinc discs in the urinary bladder	-----	effective in reducing deposition of calcium in the kidney and dissolving the pre-formed ammonium magnesium phosphate <sup>[63]</sup> .
<i>Mimusops elengi</i>	Ethylene glycol (0.75) in dirking	28 d	Significantly ↓the elevated levels of oxalate, calcium and phosphate in the urine and kidney, significantly ↓the renal content of stone.

Plants	Model	Duration	Parameter
		-----	forming constituents. Significant ↓levels of the elevated serum creatinine, uric acid, and BUN <sup>[64]</sup> .
<i>Rubia cordifolia</i>	Ethylene glycol induced urolithiasis	-----	Significantly prevented change in urinary Calcium, oxalate crystals deposits in the Kidney tissue of calculogenic rats were significantly reverted by <i>Rubia cordifolia</i> treatment also prevents the impairment of renal functions <sup>[65]</sup> .
<i>Rotula aquatica</i> flour	Feeding glycolic mixed feed for 45 days	3% acid for 45 d	↓calcium, ↓oxalate ion concentration in urine, confirming the stone inhibitory effect <sup>[66]</sup> .
<i>Phyllanthus niruri</i>	calcium oxalate (CaOx) seed into the bladder	42 d	The creatinine clearance or urinary and plasma concentration of Na <sup>+</sup> , K <sup>+</sup> , Ca <sup>2+</sup> , oxalate phosphate and uric acid were unaffected. Strongly inhibited the growth of the matrix calculus and ↓he number of stone  The urinary excretion of citrate and magnesium was unaffected <sup>[67]</sup> .
<i>Phyllanthus niruri</i>	calcium oxalate (CaOX) seed into the bladder	30 d	↓the number (75%) and the weight (65%) of calculi that frequently exhibited a matrix-like material on its surface. X-Ray analysis revealed the precipitation of struvite crystals over the CaOX seed. And did not change the crystalline

composition of the of the calculi <sup>[68]</sup> .			
<i>Cyclea peltata</i>	Ethylene glycolated water (1%) induced urolithiasis	35 d	↓urinary oxalate and ↓calcium, ↓serum potassium was elevated <sup>[69]</sup> .
<i>Melia azedarach</i>	Ethylene glycol- induced nephrolithiasis	28 d	↓reduced urinary calcium, ↓oxalate, ↓phosphate, And, ↑urinary magnesium level. ↑the urine volume, thereby reducing the Tendency Crystallization <sup>[70]</sup> .

Plants	Model	Duration	Parameter
<i>Terminalia arjuna</i>	<i>In vitro</i> evaluation of on calcium Phosphate and calcium oxalate crystallization	-----	% inhibition of 20% was found to be 30 sec and 48% at 120 sec time interval <sup>[42]</sup> .
<i>Jasminum auriculatum</i> Vahl	Ethylene glycol urolithiasis induced	28 d	↓oxalate, ↓calcium in urine an ↓Phosphate <sup>[71]</sup> .

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