ANTIROLITHIATIC EFFECT OF VARIOUS WHOLE PLANT EXTRACT OF AGERATUM CONZOIDES LINN. ON ETHYLENE GLYCOL INDUCED UROLITHIASIS IN MALE WISTAR ALBINO RATS

Soundararajan Muthukrishnan

Department of Pharmacology, Sankaralingam Bhuvaneshwari College of Pharmacy, Sivakasi, Tamil Nadu, India

Keywords:
Urolithiasis, Ageratum conzoides, Calcuri, Ethylene glycol, Calcium, Oxalate

ABSTRACT: In present study antiurolithic activity of various extract of whole plant of Ageratum conzoides Linn was investigated on experimentally urolithiasis induced male albino wistar rats. Urolithiasis was induced in animals by using ethylene glycol (5% v/v, 2ml/rat/7days). Experimental induction of hyperoxaluria results in rapid formation of calcium oxalate crystals in the renal tubules of experimental animals. Investigation was done on the basis of estimation of stone forming constituents oxalate, calcium, and phosphate, in kidney and urine. Treatment with ethyl acetate, ethanol and aqueous extract (500mg/kg, p.o) of Ageratum conzoides, standard group treated with calcuri (500mg/kg, p.o) and positive control group treated with only saline. The results are compared with calcuri, ethanolic extracts and ethyl acetate extract are significantly lowered the increased levels of oxalate, calcium and phosphate in urine and also significantly reduced their retention in kidney. The presented data indicate that administration of Ageratum conzoides extracts decrease urolithiasis and also prevented the formation of urinary stones; it proves the antirolithiatic activity of the plant.

INTRODUCTION: Urolithiasis is a condition in which urinary calculi are formed anywhere in the urinary system. The term urolithiasis comes from the Greek word, ouron means urine; lithos means stone. Almost 3 million of peoples were affected this disease. It is called nephrolithiasis, kidney stones or renal calculi. It can be classically explained as the imbalance between promoters and inhibitors of crystallization. Deficiency of any one of inhibitors or excess of any one of promoters plays an important role a stone formation. It is a succession of several physicochemical events including supersaturation, nucleation, growth, aggregation and retention within the kidneys. The calculi may sometimes stay in a position from where it is originated or migrate down the urinary tract. The recurrence rate of this disease is very high. The other factors which aggravate kidney stone are patient with metabolic syndrome, gout and person with high body mass index. Majority of the kidney stones contain calcium salt (calcium oxalate and calcium phosphate) as main crystalline compound. This is because commonly human urine is supersaturated with calcium or uric acid and...
crystalluria is very common. Daily excretion of solute and urine pH is the important factor which produces kidney stone. The irritation produced by the stone leads to secondary infection like pyelonephritis, cystitis and urethritis. It is more common in males than females because of longer urethra. Other stone types include the metabolites of certain drugs like Indinavir, Topiramite, Vitamin D analogues.

Drug treatments are available for elimination of kidney stones. The purpose of medical management is breaking the stone or dissolving the stone and prevent recurrence of stone. Citrate prevents the recurrences rate of stone. So patients are advised to increase the consumption of citric acid contain juices. Alpha blockers inhibit ureteral muscle spasm and decrease the basal tone, reduce the peristaltic frequency and colic pain and there by improve the stone expulsion. Nifedepine is increases spontaneous passage of stone.

The overuse of synthetic drug results in higher incidence of adverse drug reaction. So nowadays humans are return to natural herbs for safe remedies. It does not produce any type of complication like synthetic drugs to patients. Ageratum conyzoides used as folkloric medicine for urolithiatic condition.

In the present study, the main objective to evaluate the preventive antiurolithiatic activity of various extract in whole plant of Ageratum conyzoides in ethylene glycol induced urolithiasis in rats.

MATERIALS AND METHODS:

Plant: The plant Ageratum conyzoides Linn was collected from the nearest area of kallannai in Thiruchy, Tamilnadu, India. It was authentically verified by Ms.M.Shanthi,M.Sc., M.phil., Ph.D., Botanist, Department of Botany, S.F.R.college of arts and science, sivakasi. A voucher specimen has been kept in the herbarium of this institute for future reference.

Preparation of extracts: The whole plant 4 kg of Ageratum conyzoides after collection was washed in running tap water to remove the soil and adhering materials, shade dried. Dried materials were coarsely powdered before extraction and placed in a 2 liter RBF. The powdered crude drug was allowed for cold maceration process for 72 hours for each solvent like petroleum ether, ethyl acetate, ethanol and water. Then the extracted compounds were concentrated by distillation and solvent was evaporated to dryness. Then the final products were dried in a vacuum desiccator containing anhydrous calcium chloride. The dried products were weighed and the percentage yield was calculated. The color consistencies of the extracts were noted.

Drugs: The urolithiasis inducing agent, Ethylene glycol was also purchased from ponmani chemical agencies, Madurai. The standard drug for screening anti-urolithiatic activity is calcure which was purchased from local marked in sivakasi.

Animals: Healthy male albino wistar rats weighing between 150-180 g were used throughout the present study. They were housed, grouply in polypropylene cages, maintained under standard conditions (12 hrs light and 12 hrs dark cycle; 21±3°C; 35-60% humidity). These animals were fed with pelleted diet manufactured by amrut laboratory animal feed company, sangli, maharashtra and drinking water ad libitum.

Pharmacological studies:

Ethylene glycol induced urolithiasis in rats: The method of Mitra et al was employed for the assessment of anti-urolithiatic activity. Twenty four albino rats of sex (150-250gm) were taken. They are divided into six groups of four rats each.

Ethylene glycol induced urolithiasis model: This study is designed to find out the effect of Ageratum conyzoides (AC) on therapeutic usage against ethylene glycol induced urolithiasis.

Group and Treatment:

Group I: Positive control group treated with only vehicle

Group II: Negative control group treated with only 5% ethylene glycol 2ml\rat

Group III: Standard group treated with calcure (500mg/kg.P.O)
Group IV: Treated with aqueous extract of AC (500mg/kg, P.O)

Group V: Treated with Ethyl acetate extract of AC (500mg/kg, P.O)

Group VI: Treated with Ethnolic extract of AC (500mg/kg, P.O)

The all animals except Group-I urolithiasis were induced by the administration of 5% Ethylene glycol of a dose of 2ml/rat for 7 days.

Group-I served as positive control and received regular rat food and drinking water ad libitum.

Group-II received ethylene glycol (5% v/v) for seven days and it’s served as urolithiatic control. Group-III received ethylene glycol for 7 days and standard antiurolithiatic drug calcuri (500mg/kg. P.O), Group-IV, V and VI received ethylene glycol for 7 days and aqueous, ethyl acetate and ethanolic extract of AC (500mg/kg. P.O) 23.

Assessment of antiurolithiatic activity:

Collection and analysis of urine: One day after 7 day treatment 24 hours urine sample was collected and calcium, phosphate and oxalate were determined. All animals were kept in individual metabolic cages and urine samples of 24h were collected on 8 day. Animals will be having free access to drinking water during the urine collection period. The total volume of urine collected was measured for both control and drugs treated groups.

Urine was stored at 4°C and analyzed for calcium, phosphate and oxalate content.

Kidney homogenate analysis: After the experiment period the animals were sacrificed. The abdomen was cut open to remove both kidneys form each animal. Isolated kidneys were carefully removed and cleaned off extraneous tissue, washed in ice cold 0.15m kcl. Kidney of each animal was homogenized in normal saline. The homogenate was centrifuged at 3000rpm for 10 minutes and the supernatant was separated. The calcium, phosphate and oxalate content in kidney homogenate were determined 24.

Statistical analysis: Results expressed as mean ± S.E.M. Difference among data was determined using one-way ANOVA followed by Dunnet test.

RESULTS: The percentage yield of various extract of Ageratum conyzoides was 0.72%, 2.856%, 5.018%, and 12.08%w/w were the percentage yield obtained for petroleum ether, ethyl acetate, ethanol and aqueous extract of Ageratum conyzoides respectively. The colour and consistency of various extracts of Ageratum conyzoides was greenish black, brownish black, dark-brown color and black were the colors obtained for petroleum ether, ethyl acetate, ethanol and aqueous extract of Ageratum conyzoides respectively. All the extracts were obtained in sticky consistency.

The detailed result of the phytochemical tests carried out on the whole plant of Ageratum conyzoides was presented in Table 1.

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Phytoconstituents</th>
<th>Petroleum ether</th>
<th>Ethyl acetate</th>
<th>Ethanol</th>
<th>Aqueous</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Alkaloids</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Carbohydrate</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Glycoside</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Fixed oil &amp; Fat</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Saponins</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>6</td>
<td>Tannins &amp; Phenol</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>7</td>
<td>Proteins &amp; Amino acid</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>8</td>
<td>Gums mucilage</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>9</td>
<td>Flavonoids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>10</td>
<td>Lignin</td>
<td>-</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>11</td>
<td>Steroids</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>Total number of constituents</td>
<td>4</td>
<td>5</td>
<td>8</td>
<td>8</td>
</tr>
</tbody>
</table>
In this present investigation, the phytochemical tests revealed the presence of fixed oil, fats, proteins, amino acid, flavonoids and steroids in petroleum ether extract. Carbohydrate, fixed oils, fats, saponins, flavonoids and steroids present in ethyl acetate extract.

The ethanolic extract contains lignin, carbohydrate, glycosides, saponins, tannins, phenolic compounds, protein, flavonoids, mucilage and lignin. The aqueous extract contains carbohydrate, glycoside, saponins, tannins, proteins, flavonoids, mucilage and gums.

Urine volume: As shown in Table 2, administration of 5% v/v ethylene glycol alone (2ml/rats) shows significant change in urine volume. Administration of standard drug calcuri (500mg/kg, P.O), various extracts shows significantly increase in urine volume compared to ethylene glycol alone treated group. Administration of ethanolic extract shows significant high increase in urine volume than others (Fig. 1).

Urinary Calcium, oxalate, phosphate: As shown in Table 2, administration of aqueous, ethyl acetate and ethanolic extracts of Ageratum conyzoides linn (500mg/kg, P.O) and calcuri (500mg/kg, P.O) to ethylene glycol treated albino rats produced increasing urine output. Administration of 5% v/v ethylene glycol alone (2ml/rats) for 7 days shows significantly increase urinary calcium, oxalate and phosphate level in group-II when compared to group-I. The treatments with ethanolic extract (500mg/kg, P.O) and calcuri (500mg/kg, P.O) for 7 days significantly reduce the excretion of calcium when compared to group-II. The treatment with aqueous, ethyl acetate (500mg/kg, P.O) for 7 days also reduce the excretion of calcium but less significant when compared to group-III.

钙、草酸盐含量在肾组织中: 表明5% v/v 乙二醇（2ml/rats）处理7天显著增加尿钙和草酸盐水平在组II型对照组相比。治疗用乙醇提取物（500mg/kg, P.O）和calcuri（500mg/kg, P.O）7天显著减少钙的排泄量相比至组II。治疗用乙醇性，乙酸盐（500mg/kg, P.O）7天也减少钙的排泄量但不显著当比较于组-III (Table 3).

### Table 2: Anti Urolithic Activity of Various Extract of Whole Plant of Ageratum Conyzoides Linn Against Ethylene Glycol Induced Lithiatic in Albino Rats (in Urine)

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Dose (mg/Kg)</th>
<th>Urine Volume (ml)</th>
<th>Calcium (mg/dl)</th>
<th>Oxalate (mg/dl)</th>
<th>Phosphate (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Positive control (NS)</td>
<td>2ml/rat</td>
<td>4.6 ± 0.34</td>
<td>0.343 ± 0.03</td>
<td>2.13 ± 0.02</td>
<td>2.08 ± 0.13</td>
</tr>
<tr>
<td>II</td>
<td>Control (5% Ethylene glycol)</td>
<td>2ml/rat</td>
<td>3.7 ± 0.04</td>
<td>0.878 ± 0.05</td>
<td>7.8 ± 0.14</td>
<td>5.15 ± 0.17</td>
</tr>
<tr>
<td>III</td>
<td>Standard (Calcuri)</td>
<td>500mg/kg P.O.</td>
<td>7.9 ± 0.05</td>
<td>0.308 ± 0.13**</td>
<td>2.38 ± 0.19***</td>
<td>2.815 ± 0.17**</td>
</tr>
<tr>
<td>IV</td>
<td>Aqueous extract</td>
<td>500mg/kg P.O.</td>
<td>6.8 ± 0.36</td>
<td>0.802 ± 0.31***</td>
<td>7.28 ± 1.46****</td>
<td>4.7 ± 0.33****</td>
</tr>
<tr>
<td>V</td>
<td>Ethyl acetate extract</td>
<td>500mg/kg P.O.</td>
<td>5.2 ± 0.03</td>
<td>0.305 ± 0.02**</td>
<td>2.175 ± 0.52***</td>
<td>2.71 ± 0.02**</td>
</tr>
<tr>
<td>VI</td>
<td>Ethanolic extract</td>
<td>500mg/kg P.O.</td>
<td>7.7 ± 0.34</td>
<td>0.123 ± 0.02**</td>
<td>1.52 ± 0.04**</td>
<td>1.21 ± 0.02**</td>
</tr>
</tbody>
</table>

Values are given as mean ± S.E. n=4 (4 animals are used in each group), ****p<0.001, ***p<0.01, **p<0.02, *p<0.05, NS-Non significant as compared to control, Oneway ANOVA followed by Dunnet’s t-test

### Table 3: Anti Urolithic Activity of Various Extract of Whole Plant of Ageratum Conyzoides Linn Against Ethylene Glycol Induced Lithiatic in Albino Rats (in Kidney Homogenate)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Calcium (mg/dl)</th>
<th>Oxalate (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Positive control (NS)</td>
<td>2ml/rat</td>
<td>0.345 ± 0.03</td>
<td>2.13 ± 0.02</td>
</tr>
<tr>
<td>II</td>
<td>Negative Control (5% Ethylene glycol)</td>
<td>2ml/rat</td>
<td>0.807 ± 0.01</td>
<td>3.19 ± 0.02</td>
</tr>
<tr>
<td>III</td>
<td>Standard (Calcuri)</td>
<td>500mg/kg P.O.</td>
<td>0.4 ± 0.02**</td>
<td>2.01 ± 0.05**</td>
</tr>
<tr>
<td>IV</td>
<td>Aqueous extract</td>
<td>500mg/kg P.O.</td>
<td>0.757 ± 0.04*</td>
<td>3.07 ± 0.10**</td>
</tr>
<tr>
<td>V</td>
<td>Ethyl acetate extract</td>
<td>500mg/kg P.O.</td>
<td>0.705 ± 0.02***</td>
<td>2.29 ± 0.06**</td>
</tr>
<tr>
<td>VI</td>
<td>Ethanolic extract</td>
<td>500mg/kg P.O.</td>
<td>0.415 ± 0.03**</td>
<td>1.98 ± 0.02**</td>
</tr>
</tbody>
</table>

Values are given as mean ± S.E. n=4 (4 animals are used in each group), ****p<0.001, ***p<0.01, **p<0.02, *p<0.05, NS-Non significant as compared to control, Oneway ANOVA followed by Dunnet’s t-test
The results obtained in this study indicate that the model selected for inducing urolithiasis, i.e. ethylene glycol is suitable and reproducible. Urinary stone formation takes place due to change in urinary chemistry such as hypercalcuria and hyperoxaluria, leading to urinary super saturation which later crystalizes, aggregates and ends up in stone formation.

In the present study, an increase in deposition of calcium (134%) and oxalate (50%) in the kidney were observed in 5% ethylene glycol administered albino rats when compared to the normal. The increase in calcium deposition in the kidney and its urinary excretion may be due to an effective renal tubular reabsorption. Administration of aqueous, ethyl acetate and ethanolic extracts of *Ageratum conyzoides* linn (500mg/kg, P.O) statistically reduced. Calcium (50%) and oxalate (37%) deposition respectively in the kidney in 5% ethylene glycol administered albino rats. The plant extracts such as aqueous, ethyl acetate and ethanolic attenuated the urinary excretion of calcium oxalate without affecting the phosphate concentration in 5% ethylene glycol administered albino rats (Table 2).

**DISCUSSION:** The preliminary phytochemical studies such as determination of percentage yield colour and consistency of various extracts of *Ageratum conyzoides* helps to fix up the standards for the plant. Identification of phytoconstituents present in various extracts help to find out the nature of phytoconstituents. Phytochemical test can be used to identify and differentiate *Ageratum conyzoides* from other related species. Also these preliminary phytochemical parameters help in the detection of adulteration in commercial samples.

In spite of advances in the understanding of urolithiogenesis, there is lack of satisfactory drug treatment of the “idiopathic” oxaloacic stone formers (hyper calciuria or hyper oxaluria). This might be due to many causes that provoke the disease in non-uniform group of patients. Thus, the genesis of calculus is attributed to deficit of crystallization inhibitors (nucleation inhibitors) and/or increase of promoters (heterogenous nucleation)\(^{25}\). Rat is the suitable animal model for the present study because the urinary system of rat resemble to that of humans\(^{26}\). In this experiment Urolithiasis induced by the rats were continuously administered by 5% ethylene glycol (2ml/rats/p.o/7days) for rapid screen the anti-urolithiatic activity.

Biochemical assay for determination of calcium oxalate levels in kidney and urine were carried out during the study. These are evident from the results (Table-2 & 3) of ethylene glycol treated groups were significant elevation in calcium oxalate excretion and calcium oxalate levels in kidney were observed (Fig. 2 & 3). These results are in line with the clinical reports of calcium oxalate Urolithiasis patients\(^ {25}\). The kidney ATPase and phosphohydrolases are responsible in the process of calcification. The modulatory roles of *Ageratum conyzoides* ATPase, phosphohydrolases has been observed in earlier studies.
The appearance of calcium oxalate in renal tubules following ethylene glycol injected is associated with necrosis of tubular cells, which results in exposure of tubular, basal lamina and formation of luminal cellular debris.

The calcium oxalate crystals do causes cytolysis of polymorphonuclear leukocytes following phagocytosis and may be destructive to renal epithelium. Ethylene glycol challenge brings about radib increase in urinary excretion of calcium oxalate and formation of crystals takes place. These crystals deposit progressively in the cortex, medulla, and renal tubules.

In the present study, the increased severity of kidney crystal deposition after seven days treatment with ethylene glycol correlated well with increased calcium oxalate concentration in kidney (Table 3).

The treatment of calcuri, aqueous, ethyl acetate and ethanolic extracts of *Ageratum conyzoides* linn caused significant reduction of calcium oxalate excretion and calcium oxalate in kidney (Table 2 & 3). Suggested their beneficial effects against calcium oxalate deposition in urolithiasis.

Tannins, saponins and flavonoids present in the extract these may be responsible for the reduction in super saturation of oxalate in tissue by diuretic or protection of cells. Determination of exact mode of action is subject of further research interest.

**SUMMARY:** The three test extract of *Ageratum conyzoides* linn showed significant anti-urolithiatic activity in albino rats. Among that ethanolic extract 500mg/kg showed superior effect and ethyl acetate extract 500mg/kg showed almost equipotent effects as that of calcuri. The anti urolithiatic activity of various extracts of *Ageratum conyzoides* linn was owing to the presence of its one or more phytoconstituents, which may reduce the calcium and oxalate deposition in the kidney in ethylene glycol treated albino rats. These results offer pharmacological evidence and support on the folkloric use of *Ageratum conyzoides* linn as an anti urolithiatic agent.

**ACKNOWLEDGEMENT:** The author is thankful to Dr. A.Thanga Thirupathi, Professor, for his guidance, A.Alageswaran, K.Sheejadevi, V.sreedevi and S.B.C.P management for providing the necessary facilities and help to carry out this research work.

**REFERENCES:**


How to cite this article:

This article can be downloaded to ANDROID OS based mobile. Scan QR Code using Code/Bar Scanner from your mobile. (Scanners are available on Google Playstore)