Anti Urolithiatic Activity of *Rhus Mysorensis* against Experimentally Induced Urolithiasis in Male Albino Rats

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Abstract

To evaluate the anti urolithiatic activity of *Rhus mysorensis* in experimentally induced urolithiasis in male wistar albino rats. The plant was shade dried at room temperature and coarsely powdered, extracted with 80% v/v alcohol. Male wistar albino rats were used for assessment of anti urolithiatic activity. Urolithiasis was induced in the animals using ethylene glycol and ammonium chloride. Standard drug cystone was used to treat standard group animals whereas ethanolic extract of *Rhus mysorensis* 200mg/kg b.w and 400mg/kg b.w were given to the test groups. Development of urolithiasis was confirmed by serum and urine biochemical estimations at the end of the experiment. Ethylene glycol and ammonium chloride developed calculi in the animals. Treatment with *Rhus mysorensis* reduced the formation of calculi. This was confirmed by biochemical estimations of urine and serum creatinine, calcium, oxalate, urea and uric acid. The mechanism underlying this effect is unknown but is apparently related to diuresis and lowering of urinary concentrations of stone forming agents. The protective effect against oxalate induced lipid peroxidation may be contributory to the recovery of renal damage. The present study revealed the anti urolithiatic activity of *Rhus mysorensis* against experimentally induced urolithiasis, reduced the formation of stones.

Keywords: Ammonium chloride, Calculi, Ethylene glycol, Rhus mysorensis, Urolithiasis,

Introduction

Man’s existence on this earth has been made possible only because of the vital role played by the plant kingdom.\(^1\) Traditional medicine using herbal drugs exists in every part of the world. The major areas are Chinese, Indian and European traditions. The philosophies of these traditional medicines have some resemblance to each other but differ widely from modern Western medicine. Herbal drugs have the advantage of being available for patients in the geographical area of the special traditional medicine.\(^2\) The raw
materials for ayurvedic medicines were mostly obtained from plant sources in the form of crude drugs such as dried herbal powders or their extracts or mixture of products. India unquestionably occupies the top position in the use of herbal drugs. It is one of the foremost countries exporting plant drugs or their derivatives and excels in home consumption too. Chemists have so far been unable to reproduce the complex structure of many plant compounds. Further coordinated research into folk traditions, plant species, growing conditions and local medical needs is urged.

Urolithiasis refers to calcifications that form in the urinary system, primarily in the kidney (nephrolithiasis) or ureter (ureterolithiasis), and may also form in or migrate into the lower urinary system. Urolithiasis is a complex process that occurs from series of several physic chemical events including super saturation, nucleation, growth, aggregation and retention within the kidneys. Urolithiasis is the third most disorder of the urinary tract. Stones can be composed of calcium (80%), oxalate, urea, uric acid, cystine, phosphate or all of these. Though the cause of stone formation is hard to determine some factors include a genetic predisposition, metabolic disorders such as diabetes, myeloproliferative diseases like leukemia or hypocalcaemia, diet imbalance a poor intake of water, parasites such as bladder thread worm and bacterial infections such as E.coli, Klebsiella, Staphylococcus, and Mycoplasma. Rhus mysorensis is a small aromatic, often gregarious shrub with a thin brown bark and spiny branches. It contains alkaloids, flavonoids, glycosides, phenols, and triterpenoids. Its flowering occurs in the month of February-June. It is commonly called Mysore sumac, belongs to the family Anacardiaceae. The plant is traditionally claimed to treat diabetes. The ethanolic extraction of the whole plant of mysore sumac protects the liver against toxic effects of paracetamol by reducing the levels of serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), serum bilirubin, serum alkaline phosphatase (SALP).

Materials and methods

Animals: Male wistar albino rats weighing between 150 to 200 g were selected for acute toxicity studies and for the anti urolithiatic activity. The animals were acclimatized to standard laboratory conditions of temperature (22±3°C) and maintained on 12:12 h light: dark cycle. They were provided with regular rat chow diet and distilled water ad libitum. The animal and experimental protocols were in accordance with CPCSEA guidelines. The animals were divided into five groups normal (I), control (II), standard (III), lower test T1 (IV) and higher test T2 (V) groups.

Plant collection: Plant sample was collected in March 2014 from S.V University. Preparation of extract: About 100g of the plant powder was separately extracted for 24h with 90% ethanol with soxhlet apparatus. The extracts were filtered concentrated and dried over a water bath at 45°C and the material after dying was used for experimental analysis.

Preparation of suspension: Ethanolic extract was suspended in distilled water using 2% tween 80 as a suspending agent. The extracts were subjected to phytochemical screening and acute oral toxicity study and depending upon LD50 the calculated quantity of each extract was given to each animal in corresponding group once daily through oral route.

Acute toxicity study: Acute toxicity study was carried out according to OECD guidelines. The animals were observed for 24 hours for any change in their behavior, colour of skin, salivation, convulsions etc. The table is given under results. No mortality of the animals was observed and the dose was calculated as 200mg/kg and 400mg/kg bodyweight.

Induction of urolithiasis: Ethylene glycol (0.75%) and ammonium chloride (2%) in drinking
water was given to groups II-V for induction of renal calculi for 10 days. All extracts were given once daily by oral route. The animals were fed with normal lab diet. After the experimental period, urine and serum were collected for biochemical estimations as well as the physiological parameters like urine volume and pH. Animals were decapitated, sacrificed for weighing kidneys at the end of the experiment. Standard drug cystone 5ml/kg b.w was induced to the III group animals.

Ethanolic suspension of the drug was induced to the IV and V group animals at 200mg/kg and 400mg/kg b.w respectively.

**Results**

**Acute toxicity studies**

Title: Evaluation of LD50 of *Rhus mysorensis*  
Drug: *Rhus mysorensis*  
Dose: 2000 mg/kg BW  
Species: Albino rats  
Male & Female  
Duration: 24 hours

<table>
<thead>
<tr>
<th>S.no.</th>
<th>Code</th>
<th>Toxicity</th>
<th>Time</th>
<th>Skin color</th>
<th>Eyes</th>
<th>Resp</th>
<th>CNS</th>
<th>Tre</th>
<th>Sal</th>
<th>Diah</th>
<th>Sleep</th>
<th>Leth</th>
<th>Con</th>
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<tbody>
<tr>
<td>1.</td>
<td>ERM</td>
<td>X X X</td>
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<td>X</td>
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</tr>
</tbody>
</table>

(*TRE-Tremor, CON-Convulsions, SALI-Salivation, Diah-Diarrhea, LET-Lethargy)  
x = Negative, □ = Positive

**ERM:** Ethanolic extract of *Rhus mysorensis*

The results of serum and urine biochemistry are reported in table 1 and table 2. The physiological reports like urine volume, kidney weight and urine pH are reported in table 3. The ethanol extract resulted in significant reduction in urine and serum, uric acid urea and oxalate compared to toxic group. Also the calcium, creatinine levels were also lowered in both serum and urine. Significant elevation in the volume of urine was found in the treated groups compared to the toxic. The results of urine and serum biochemistry showed significant reduction in urine calcium, uric acid and oxalate level, serum calcium with significant elevation in urine volume output, the markers previously reported which affirmed potent antiurolithiatic activity.
### Table no 1: Estimation of serum parameters

<table>
<thead>
<tr>
<th>Treated groups</th>
<th>SERUM PARAMETERS</th>
<th></th>
<th></th>
<th></th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Creatinine</td>
<td>Calcium</td>
<td>Uric acid</td>
<td>Urea</td>
<td>Oxalate</td>
</tr>
<tr>
<td>Normal (0.9% saline)</td>
<td>7.2±0.114</td>
<td>2.35±0.09</td>
<td>4.43±0.057</td>
<td>30.43±0.07</td>
<td>2.58±0.012</td>
</tr>
<tr>
<td>Control (EG 0.75%+2%AC)</td>
<td>8.9±0.061</td>
<td>4.1±0.021</td>
<td>7.45±0.07</td>
<td>50.38±0.068</td>
<td>5.17±0.009</td>
</tr>
<tr>
<td>Standard (cyst 5ml/kg)</td>
<td>6.2±0.04</td>
<td>3.54±0.018</td>
<td>5.41±0.08</td>
<td>41.17±0.107</td>
<td>2.68±0.017</td>
</tr>
<tr>
<td>ETT 200mg/kg</td>
<td>6.8±0.009</td>
<td>3.15±0.01</td>
<td>3.58±0.094</td>
<td>45.44±0.173</td>
<td>2.76±0.018</td>
</tr>
<tr>
<td>ETT 400mg/kg</td>
<td>6.42±0.014</td>
<td>2.82±0.018</td>
<td>3.48±0.107</td>
<td>46.7±0.029</td>
<td>2.72±0.008</td>
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### Table no 2: Estimation of urine parameters

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<tr>
<th>Treated groups</th>
<th>URINE PARAMETERS</th>
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<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Creatinine</td>
<td>Calcium</td>
<td>Uric acid</td>
<td>Urea</td>
<td>Oxalate</td>
</tr>
<tr>
<td>Normal (0.9% saline)</td>
<td>0.66±0.011</td>
<td>2.23±0.007</td>
<td>0.65±0.011</td>
<td>49.08±0.206</td>
<td>1.8±0.033</td>
</tr>
<tr>
<td>Control (EG 0.75%+ 2%AC)</td>
<td>4.87±0.05</td>
<td>3.46±0.01</td>
<td>1.83±0.007</td>
<td>62.14±0.122</td>
<td>4.04±0.01</td>
</tr>
<tr>
<td>Standard (cyst 5ml/kg)</td>
<td>1.36±0.038</td>
<td>2.54±0.01</td>
<td>0.74±0.013</td>
<td>53.93±0.155</td>
<td>2.25±0.006</td>
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<tr>
<td>ETT 200mg/kg</td>
<td>1.89±0.07</td>
<td>3.05±0.01</td>
<td>1.45±0.008</td>
<td>58.21±0.204</td>
<td>3.55±0.007</td>
</tr>
<tr>
<td>ETT 400mg/kg</td>
<td>1.6±0.02</td>
<td>2.85±0.007</td>
<td>0.94±0.007</td>
<td>56.46±0.155</td>
<td>2.74±0.007</td>
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</tbody>
</table>

### Table no 3: Estimation of physiological parameters:

<table>
<thead>
<tr>
<th>Treated groups</th>
<th>PHYSIOLOGICAL PARAMETERS</th>
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<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Urine volume</td>
<td>Urine PH</td>
<td>Kidney weight</td>
<td></td>
</tr>
<tr>
<td>Normal (0.9% saline)</td>
<td>18.01±0.18</td>
<td>7.2±0.08</td>
<td>0.87±0.014</td>
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</tr>
<tr>
<td>Control (EG 0.75%+2%AC)</td>
<td>9.51±0.17</td>
<td>4.03±0.12</td>
<td>0.5±0.013</td>
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<tr>
<td>Standard (cyst 5ml/kg)</td>
<td>16.5±0.08</td>
<td>6.9±0.088</td>
<td>0.81±0.015</td>
<td></td>
</tr>
<tr>
<td>ETT 200mg/kg</td>
<td>12.5±0.07</td>
<td>6.2±0.122</td>
<td>0.6±0.01</td>
<td></td>
</tr>
<tr>
<td>ETT 400mg/kg</td>
<td>15.5±0.06</td>
<td>6.6±0.11</td>
<td>0.71±0.012</td>
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</tr>
</tbody>
</table>
Discussion
Urinary stone formation takes place due to changes in urinary chemistry, such as hyperoxaluria and hypercalciuria, leading to urinary super saturation, which later crystallizes, aggregates and ends up in stone formation. Evidences in previous studies indicated that, in response to 10 days period of ethylene glycol (0.75% v/v), and ammonium chloride (2% w/v) administration, young albino rats form renal calculi composed mainly of CaOx. The principal precursor of oxalic acid in mammals is glycoxalic acid. The enzymatic oxidative conversion of glycolate to oxalate via glycoxylate is the major metabolic pathway involved in endogenous oxalate synthesis. The enzymatic disturbances are the causative factors for the idiopathic hyperoxaluria; while, the defective intestinal absorption of oxalate plays a vital role in enteric hyperoxaluria and lead to an increase in the urinary oxalate concentration. Oxalate, the major stone-forming constituent, has also been reported to induce lipid peroxidation and to cause tissue damage.

In the present study, chronic administration of 0.75% ethylene glycol aqueous solution to male Wistar rats resulted in hyperoxaluria. Oxalate and calcium excretion in urine were grossly increased in calculi-induced animals.

Increased urinary calcium is a factor favoring the nucleation and precipitation of CaOx from urine and subsequent crystal growth. Calcium binds to the free fatty acids that cannot be absorbed. This reduces the normal calcium oxalate precipitation in the feces, thereby allowing the absorption of soluble oxalate from the gut. The increase in calcium deposition in kidney and its urinary excretion may be due to defective renal tubular reabsorption or an increase in absorption from the intestine as the patients with renal calcium stones are reported to have hyper absorption of calcium process starts either in the loop of Henle or in the distal part of the distal tubule. At these nephron levels, the urine might be supersaturated with calcium phosphate. When the calcium phosphate crystals are transported down the nephron and meet the acid environment in the collecting ducts, crystal dissolution might occur. At that level, the urine commonly is supersaturated with calcium oxalate, and nucleation of calcium oxalate crystals becomes possible. The ethanolic extract of R. mysorensis lowered the levels of oxalate and calcium in urine and even their retention in kidney. The plant extraction showed significant antiurolithiatic activity.

Conclusion
The result exhibited by the ethanol extract of Rhus mysorensis showed significant antiurolithiatic activity when compared with the standard drug cystone.

Acknowledgement
I firstly would like to thank my beloved parents who have been the support for the fulfillment of my dreams in pursuing the higher education. I then would acknowledge Dr. A. Srinivasa Rao, Principal, Bhaskar Pharmacy College, and Mr. M. Sri Rama Chandra, Head, Department of Pharmacology, who guided me throughout the project. I take pleasure thanking the staff and managers of SICRA LABS, Hyderabad for providing me the required infrastructure to carry out the work. I would also express my gratitude towards Sukesh Krishna Chaitanya Loka, Intern, Department of Doctor of Pharmacy, Malla Reddy Narayana Multi Specialty Hospital, who had been the pillar of support in article making and processing.

References


