Bioevaluation of Sirupeelai samoolam herbal formulation for Antiurolithiatic activity

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Abstract
Ayurveda offers vast scope for the successful treatment of urolithiasis. Although most remedies were herbal and proved useful, a systematic scientific evaluation has been reported for only few remedies. Sirupeelai samoolam a herbal formulation was claimed to be useful in the treatment of urinary stones in Ayurvedic literature. Toxicity study confirms that the therapeutic dose of Sirupeelai samoolam was 360mg/kg. The antilithiatic effect of Sirupeelai samoolam was determined on lactose diet + ethylene glycol induced and ammonium chloride + ethylene glycol induced lithiasis in male albino wistar rats. The results obtained after 4 weeks of treatment for the urine estimation of calcium, oxalate, magnesium and protein were significant (p<0.01) in reducing the calculus when compared with the standard drug cystone (750mg/kg) treated animals. Urine calcium oxalate crystals were also reduced in test drug treated and standard treated groups. The histopathological examination of kidney shows normal pattern of the cells, tissues, less damage and no microcrystalline deposition. These observations and results conclude that Sirupeelai samoolam herbal formulation possesses significant antiurolithiatic action and can be used safely for treating urinary calculus.

Keywords: Antirolithiatic activity, Sirupellai samoolam, Urinary calculus.

Introduction
Urolithiasis in its different forms is frequently common causes are inadequate urinary drainage, foreign bodies in the urinary tract, microbial infections, diet with excess oxalates and calcium, vitamin abnormalities, viz. Vitamin A deficiencies, Vitamin D excess, metabolic diseases like hyperparathyroidism, cystinuria, gout and intestinal dysfunction generally stones of two types i.e., non-calcium and calcium stones are formed. Calcium, albumin, creatinine, urate and oxalate are some necessary analytical markers in serum and urine for clinical diagnosis of this type of urological disorders. Urolithiasis is an extremely painful disease that afflicts the human population since ancient times. The mechanism of calcium oxalate renal calculi formation has attracted the attention of medical scientists because of its widespread clinical occurrence and the difficulty of treatment. Hyperoxaluria is one of the main risk factors of human idiopathic calcium oxalate disease. Oxalate, the major stone-forming constituent, is known to induce lipid per oxidation which causes disruption of the cellular membrane integrity. Lipid per oxidation is a free radical induced process leading to oxidative deterioration of polyunsaturated lipids. This alters the membrane fluidity, permeability and thereby affects the ion

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transport across the cellular organelle\(^3\). In herbal medicine plant based formulations are used to alleviate the diseases. But the most important challenges faced by these formulations arise because of their lack of complete evaluation\(^4\). So evaluation is necessary to ensure quality and purity of the herbal product. It is very important to establish a system of evaluation for every plant medicine in the market, since the scope for variation in different batches of medicine is enormous. The present study is to investigate antiurolithiatic activity of Sirupeelai samoolam in preclinical animal models.

**Materials and Methods**

**Herbal formulation**

The herbal formulation Sirupeelai samoolam was purchased from SKM Siddha and Ayurvedha Company (India) Limited, Erode, Tamil Nadu, India. The purchased formulation was evaluated for toxicity assessment and preclinically for antiurolithiatic action in the suitable animal models.

**Experimental animals**

Male albino rats of wistar strain weighing between 150-200gm were used, the animals were fed with commercial rat feed pellets (Amrut laboratory animal feed Ltd, Bangalore) and were given water *ad libitum*. Animals were housed in plastic cages with filter tops under controlled conditions of 12:12 light dark cycle, 50% humidity and 28°C. All animal experiments and maintenance were carried out according to the ethical guidelines suggested by the IAEC of Teegala Ram Reddy College of Pharmacy, Meerpet, Hyderabad. (Approval no. 890/ac/05/CPCSEA).

**Toxicity study**\(^5\)

Toxicity study was performed to find out the toxic dose and therapeutic dose confirmation. The human therapeutic dose (5gm) was converted into animal therapeutic dose (360mg/kg) and acute toxicity test was performed for that particular dose. Subacute toxicity study was done for the sub minimal therapeutic dose (260mg/kg), therapeutic dose (360mg/kg) and the sub maximal therapeutic dose (460mg/kg).

**Pharmacological screening**

**Lactose (30%) and Ethylene glycol (1%) induced urolithiasis\(^6\)**

Adult healthy male albino wistar rats were divided into 4 groups of 6 animals each. Group I served as the control received 1ml/kg of distilled water and rat Chow diet *ad libitum* for 4 weeks. Group II received Ethylene glycol intoxicated rats with diet for inducing urolithiasis were fed with a lactose rich Lab diet (which contains 3.68% sucrose, 30% lactose, 23.4% protein, 10% fat, 5.3% crude fiber, 6.9% ash minerals – calcium (0.95%), phosphorus (0.67%), magnesium (0.21%), Vit A 22IU/g, Vit D3 4.5IU/g, Vit E 49 IU/g, with 1% ethylene glycol in drinking water for 4 weeks. Group III received lactose rich lab diet + 1% ethylene glycol + Cystone 750mg/kg and Group IV received lactose rich lab diet + 1% ethylene glycol + Sirupeelai samoolam 360mg/kg for weeks. The crystalluria and stone formation was verified by different biochemical marker analysis of urine and serum. The urine samples of the test animals in different groups were collected in their respective day of the experiment. The collected urine sample volume were measured followed by centrifugation at 3000 rpm for 10 minutes. After centrifugation of the urine samples were examined under light microscope to ensure the presence of oxalate micro crystals followed by biochemical analysis (urine oxalate, calcium and uric acid). The blood samples were collected from the animals under anesthesia before sacrificing. The collected blood samples were then centrifuged to obtain serum for the analysis of serum creatinine and serum calcium.

**Ammonium chloride (2%) and Ethylene glycol (0.75%) induced Urolithiasis\(^7\)**

Male Wistar rats (180-200gm) are acclimatized to laboratory conditions for 1 week and then placed in groups of 4 groups 6 animals each. Ammonium chloride (2%) and (0.75%) ethylene glycol were mixed to the standard chow diet and given to animals for 4 weeks. Group I served as lithiatic control and received vehicle 1%twen 80, Group II received standard antiurolithiatic drug, cystone (750mg/kg) from 15th day till 28th day\(^8,9\). Group III received aqueous extract Sirupeelai samoolam (360mg/kg) from 15th day till 28th day and served as curative regimen. Group IV received aqueous
extract Sirupeelai samoolam (360mg/kg) from 1st day till 28th day and served as preventive regimen. All drugs were given once daily by oral route using gastric tube. On day 28 animals of all the groups were kept in metabolic cages and urine samples were collected for 24h and analysed for calcium, magnesium, oxalate, inorganic phosphate, protein and creatinine using standard methods\textsuperscript{10,11,13}. The serum creatinine levels and urinary output volumes of all groups were also noted.

**Histopathological studies**

To confirm the incidence of lithiasis, the animals were sacrificed and their kidneys were isolated and subjected to histopathological studies. The kidneys were washed, weighed and fixed rapidly with 10% neutralized formalin (pH7.4), and soaked in paraffin, cut at 5μm intervals and the slices were stained with hematoxylin and eosin. Tissue slices were photographed using optical microscopy and observed the pathological changes\textsuperscript{15}.

**Statistical analysis**

The results were expressed as Mean±SEM. Statistical analysis was performed by ANOVA test for multiple comparisons followed by Dunnett’s test and P<0.05 was considered as significant.

**Results and Discussion**

The acute urolithiasis in both the conventional models was evidenced by the significant elevation in urine and serum biochemical parameters along with the reduced urine output as compared to the normal animals. The Sirupeelai samoolam (360mg/kg) employing lactose (30%) + ethylene glycol (1%) induced urolithiasis resulted in a significant reduction (P<0.001) in urine uric acid (0.79±0.05) and oxalate (1.50±0.20) level as compared to toxic group. Serum calcium (3.20±0.30) and urine calcium (2.32±0.017) level was significantly (P<0.005) lowered when compared with the toxic group along significant elevation in urine volume (13.3±0.10, P<0.05) output. Hence, the herbal formulation showed a reduction in serum creatinine level, employing both models but the reduction is not significant when compared with urolithiatic control group. The urine analysis of the animals showed that clear and no stone formation in control treated groups, lithiasis control animals showed more number of crystal formation in the urine. The formulation showed reduction in crystal formation when compared with that of the standard drug cystone treated animals. The results of urine and serum biochemistry showed significant reduction in urine calcium, uric acid, oxalate and serum calcium with significant elevation in urine volume output, the markers previously reported which affirmed potent antiurolithiatic activity\textsuperscript{16}. Urinary super saturation with respect to stone forming constituents is generally considered to be one of the causative factors in calculogenesis. Administration of ammonium chloride (2%) and ethylene glycol (0.75%V/V) to the animals for 14 days forms renal calculi composed mainly of calcium oxalate. Stone formation in ethylene glycol fed animals is caused by hyperoxaluria which causes increased renal retention and excretion of oxalate\textsuperscript{17}. The urine output was markedly decreased in lithiatic control animals on 28\textsuperscript{th} day, however in herbal formulation and standard drug treated animals the urinary volume was increased when compared to the lithiatic group. This suggested that formulation have mild diuretic effect. Following ethylene glycol administration the excretion of calcium, oxalate, phosphate and protein were found to be increased in lithiatic group while in standard, curative and preventive groups these levels were significantly decreased (P<0.01). The formulation treated curative and preventive regimen groups, the urinary outputs increased significantly (P<0.01). The chronic administration of 0.75%V/V ethylene glycol to animals resulted in hyperoxaluria. And oxalate, calcium, phosphate and protein excretion were significantly (P<0.01) lowered, in curative and preventive regimen groups. In lithiatic control group the magnesium excretion was gradually following ethylene following et.

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accumulated inside the tubules, dilation of the proximal tubules along with the interstitial inflammation, arrowed area shows calcium oxalate crystals (D) All these results concluded to the inhibitory and curative potential of herbal formulation has both prophylactics as well as treatment property in urolithiasis of rats. The phytoconstituents of the active ingredient *Aerva lanata* like alkaloids, phytosterols, mucilage and fixed oils in the plant may be responsible for antiurolithiatic action. Further more studies were required for the exact mechanism of antiurolithiatic action of the herbal formulation selected for the present study.

**Table-1 Urine and Serum biochemistry of 30% Lactose and 1% Ethylene glycol induced Urolithiasis on 28<sup>th</sup> day of experiment**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Urine volume (ml)</th>
<th>Urine calcium (mg/dl)</th>
<th>Urine oxalate (mg/dl)</th>
<th>Uric acid (mg/dl)</th>
<th>Serum creatinine (mg/dl)</th>
<th>Serum calcium (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group I Control</td>
<td>18.1±0.95</td>
<td>2.21±0.10</td>
<td>0.48±0.02</td>
<td>0.65±0.02</td>
<td>7.40±0.30</td>
<td>4.12±0.19</td>
</tr>
<tr>
<td>Group II Lithiatic</td>
<td>9.94±0.65 **</td>
<td>3.25±0.20*</td>
<td>3.9±0.12***</td>
<td>1.7±0.09</td>
<td>8.64±0.30</td>
<td>2.18±0.21***</td>
</tr>
<tr>
<td>Group III Cystone</td>
<td>14.5±0.50***</td>
<td>2.25±0.21*</td>
<td>1.19±0.17***</td>
<td>0.70±0.02</td>
<td>7.73±0.27</td>
<td>3.49±0.25**</td>
</tr>
<tr>
<td>Group IV Sirupeelai samoolam treated</td>
<td>13.3±1.10*</td>
<td>2.32±0.17*</td>
<td>1.50±0.20***</td>
<td>0.79±0.05**</td>
<td>7.94±0.70</td>
<td>3.20±0.30*</td>
</tr>
</tbody>
</table>

n=6, Values are given in Mean±SEM, *P<0.05, **P<0.01, ***P<0.001

**Figure-1** Urine analysis of 30% Lactose and 1% Ethylene glycol induced Urolithiasis on 28<sup>th</sup> day of experiment for Calcium oxalate crystals
Figure: T.S of Kidney shows the effect of Sirupeelai samoolam on Ammonium chloride (2%) and Ethylene glycol (0.75%) induced Urolithiasis in rats

Table: Effect of Sirupeelai samoolam on urinary biochemical parameters on Ammonium chloride (2%) and Ethylene glycol (0.75%) induced Urolithiasis in rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Calcium mg/dl</th>
<th>Oxalate</th>
<th>Phosphate</th>
<th>Magnesium</th>
<th>Protein</th>
<th>Serum creatinine mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group-I Control</td>
<td>4.7±0.35</td>
<td>0.42±0.02</td>
<td>5.6±0.12</td>
<td>0.92±0.00</td>
<td>3.04±0.10</td>
<td>0.58±0.03</td>
</tr>
<tr>
<td>Group-II Lithiasis control</td>
<td>10.90±0.25*</td>
<td>2.23±0.30*</td>
<td>11.20±0.20*</td>
<td>0.45±0.01*</td>
<td>6.80±0.13*</td>
<td>1.90±0.1*</td>
</tr>
<tr>
<td>Group-III Cystone treated</td>
<td>5.7±0.20**</td>
<td>0.71±0.01**</td>
<td>7.01±0.17**</td>
<td>0.8±0.01**</td>
<td>3.60±0.20**</td>
<td>0.50±0.21**</td>
</tr>
<tr>
<td>Group-IV Curative regimen</td>
<td>6.30±0.10**</td>
<td>0.70±0.07*</td>
<td>7.70±0.15**</td>
<td>0.83±0.05**</td>
<td>3.80±0.05**</td>
<td>0.60±0.12**</td>
</tr>
<tr>
<td>Group-V Preventive regimen</td>
<td>5.80±0.15**</td>
<td>0.62±0.01**</td>
<td>7.2±0.20**</td>
<td>0.81±0.05**</td>
<td>3.20±0.24**</td>
<td>0.58±0.01**</td>
</tr>
</tbody>
</table>

n=6, Values are expressed as mean±SD for in each group. One way ANOVA followed by Dunnett’s test. *P<0.001, **P<0.01
References


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