Lithotriptic Activity of Siddha Drug Megarajanga Chooranam on Ethylene Glycol Induced Urolithiasis in Rats

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ABSTRACT
This study was aimed to evaluate the effectiveness of Megarajanga Chooranam [MRC] – A Siddha medicine on albino rats against the development of kidney stones. The activity of Megarajanga Chooranam [MRC] was studied using the ethylene glycol–induced urolithiasis model using Cystone as Standard drug. The Parameters are used including urinary volume, urine pH, urine analysis, and serum analysis to assess the activity. The results indicated that the administration of MRC to rats with ethylene-glycol-induced lithiasis significantly reduced and prevented the growth of urinary stones [p<0.01]. Also the treatment of lithiasis induced rats by MRC restored all the elevated biochemical parameters creatinine, uric acid, and blood urea nitrogen, restored the urine pH to normal and increased the urine volume significantly [p<0.01] when compared to the control drug. Treatment with cystone (750 mg/kg) and MRC reduced the biochemical changes induced by Ethylene glycol. To prove the mechanism by which MRC cures the renal damage caused by ethylene glycol, investigations on levels of various stone inhibitors like total protein, magnesium, and citrate was studied. There was significant rise on total protein, magnesium and citrate after treatment with cystone and MRC and also showed significant decrease in body weight, urine volume and Ph of urine as compared to control group. The study supports the effect of MRC in urolithiasis.

Keywords: Lithotriptic activity, diuretic activity, ethylene glycol, calcium oxalate

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INTRODUCTION
Urolithiasis is the third most common disorder of urinary tract found in humans and its incidence is quite high all over the world [1]. Calculus formation at any region in the urinary tract – kidney to urethra, leads to urolithiasis. It is clinically manifested by dysuria, burning and painful micturition, pain in the pelvis and lumbo sacral region and the presence of small stones in the urine [2]. Urinary stones affect 10-12% of the population in industrialized countries [3]. With a prevalence of > 10% and an expected recurrence rate of nearly 50%, stone disease has an important effect on the healthcare system [4]. Epidemiological studies revealed that nephrolithiasis is more common in men [12%] than in women [6%] and is more prevalent between the ages of 20 to 40 in both sexes [5]. The etiology of this disorder is multifactorial and is strongly related to dietary lifestyle habits [6]. Increased rates of hypertension and obesity, which are linked to nephrolithiasis, also contribute to an increase in stone formation [7]. The clinical features of Kalladaippu described in the classical Siddha text Yugi Vaithiya Chinthamani correlates with that of Urolithiasis [9]. Traditional herbal medicine is an important part of the healthcare system in India. Since most of the plants are claimed to be non-toxic, low cost, available in rural areas and their effectiveness in the treatment of urinary stones has been widely studied.[10]. Herbal medicines have been used to help in Urolithiasis through anti-
inflammatory, litholytic, antimicrobial and antispasmodic action [11]. Modern medication and surgical techniques like extracorporeal shock wave lithotripsy [ESWL] intracorporeal lithotripsy, urethroscopy with lithotripsy, nonsteroidal anti-inflammatory drugs are currently being used for the management of urolithiasis, which cause many adverse side effects such as haemorrhage, haematuria, tubular necrosis and subsequent fibrosis of the kidney [11]. Despite recent advances in the treatment of urolithiasis, western medical intervention continues to cause significant burden on a nation’s healthcare system. Hence, modern medical science has set its quest with traditional medicines for its cost-effective and safe medication. About 75% of renal stones are composed of either calcium oxalate or calcium oxalate crystals mixed with calcium phosphate [12]. Oxalate stones are the result of supersaturation of urine with certain urinary salts such as calcium oxalate. The supersaturation of urine with CaOX (Calcium oxalate), the most common component of kidney stones, is an important factor in crystallization, with later factors being nucleation, growth and aggregation. Megarajanga Chooranam noted in the Siddha text Athmaratchamirtham [13] has been studied initially for diuretic property. Generally, drugs having diuretic activity induced urolithiasis in rats are also known to have antiuric activity. Hence in the present study, an effort has been made to evaluate the antiuric activity of Megarajanga Chooranam using ethylene glycol-induced lithiasis in rats.

MATERIALS AND METHODS
Preparation of drug and stock solution
The suspension of Siddha drug Megarajanga Chooranam in 2% (w/v) CMC was prepared for oral administration by gastric intubation method.
Animal selection [14]
For acute toxicity studies, Wistar albino mice of either sex weighing between 28 and 30 g were selected. For the antiurolithiatic study, male Wistar weighing between 180-220 g were used. The animals were acclimatized to standard laboratory conditions (temperature: 25±2°C) and maintained on 12-h light: 12-h dark cycle. They were provided with regular rat chow and drinking water ad libitum.

Acute toxicity studies [15]
The acute oral toxicity study was carried out as per the OECD guidelines 425. One-tenth of the median lethal dose was taken as an effective dose.

Ethylene glycol induced urolithiasis model [16]
Ethylene glycol induced urolithiatic model in rat was be used to assess the effect of Megarajanga Chooranam. The study is designed to find out the effect of Megarajanga Chooranam on therapeutic usage against ethylene glycol induced urolithiasis. All rats were housed in metabolic cages for entire duration of the experiment. Animals were divided into five groups containing six animals in each. Group I served as control and received regular rat food and drinking water ad libitum. Ethylene glycol (0.75%) in drinking water was fed to Groups II-V for induction of renal calculi till 28th day. Group II received Ethylene glycol alone and served as urolithic control. Group III received standard antiurolithiatic drug, cystone (750mg/kg body weight) from 15th day till 28th day. Group IV received Megarajanga Chooranam (50mg/kg body weight) from 15th day till 28th day; Group V received Megarajanga Chooranam (100mg/kg body weight) from 15th day till 28th day.

Group and Treatment
Group 1: Treated with Normal saline
Group 2: Treated with Control (ethylene glycol) + vehicle
Group 3: Treated with Standard (ethylene glycol + Cystone)
Group 4: Treated with Megarajanga Chooranam (50 mg/kg) + ethylene glycol
Group 5: Treated with Megarajanga Chooranam (100mg/kg) + ethylene glycol

All doses were given once daily by oral route.

Assessment of antiurolithiatic activity [17]
Collection and analysis of urine
All animals were kept in individual metabolic cages and urine samples of 24h were collected on 28th day. Animals will be having free access to drinking water during the urine collection period. A drop of concentrated hydrochloric acid was added to the urine before being stored at 4°C. Urine was analyzed for calcium, phosphate and oxalate content.

Serum Analysis:
After the experimental period, blood was collected from the retro-orbital vein under anesthetic conditions and animals were sacrificed by cervical decapitation. Serum was separated by centrifugation at 10,000 x g for 10 min and analyzed for creatinine, uric acid and urea nitrogen.

Kidney homogenate analysis:
The abdomen was cut open to remove both kidneys form each animal. Isolated kidneys were cleaned off extraneous tissue and preserved in 10% neutral formalin. The kidneys were dried at 80°C in a hot air oven. A sample of 100mg of the dried kidney were boiled in 10ml of 1N hydrochloric acid for 30min and homogenized. The homogenate was centrifuged at 2000x g for 10min and the supernatant was separated. The calcium, phosphate and oxalate content in kidney homogenate were determined.

DIURETIC ACTIVITY
Standardization Of Furosemide
Seven groups of six male Wistar albino rats were employed four doses of 10, 1d, 20,25-mg/kg b.w of furosemide were administered intra peritoneal to each group of rats separately. The control animals received normal saline alone. The animals were placed in separate cages and the urine output over 24hr period was collected. This procedure was repeated. The most consistent dose (15mg/kg b.w) was adapted for dosing.

Evaluation of diuretic activity
Five groups of six male Wistar albino rats were used. First group received normal saline. Second group received Megarajanga Chooranam 50mg/kg. The third group received Megarajanga Chooranam 100
mg/kg. The fourth group was administered furosemide 20 mg/kg. Immediately after administration of the drug, the rats were placed in metabolic cages, specially designed to separate urine and fecal matter and was observed at room temperature. The animals were denied for food and water during the experiment. The urine volume (ml/day) was measured and then assayed for Na⁺ and K⁺ and Cl⁻ concentrations in mMol/l, Cl⁻ was measured using routine method.

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

**RESULTS AND DISCUSSION**
Ethylene glycol induced urolithiasis resulted in significant elevation of urine and kidney calcium, oxalate, inorganic phosphate and serum blood urea nitrogen, creatinine, uric acid compared to normal control group. Treatment with cystone (750 mg/kg) and Megarajanga Chooranam reduced the biochemical changes induced by ethylene glycol. In order to probe the possible mechanism by which Megarajanga Chooranam cures renal damage caused by ethylene glycol, investigation on levels of various stone inhibitors like total protein, magnesium and citrate was studied. There was significant rise on total protein, magnesium and citrate after treatment with cystone and Megarajanga Chooranam.

Administration of ethylene glycol significantly reduced the body weight, urine volume and pH of urine as compared to normal group. Rats treated with cystone and Megarajanga Chooranam also showed significant decreased in body weight, urine volume and pH of urine as compared to control group. The histopathological study of the kidney sections also supported the above results. In all the stone forming rats there was damage to the last part of the nephron, collecting system and peritubular interstitium as compared to the normal rat kidney architecture. The tubules appeared focally ecstatic and surrounded by inflammatory infiltration. Inflammatory infiltration was mainly composed of mature lymphocytes infiltrating tubular epithelium. Irregular crystals were present inside the tubules and in the peritubular epithelium, along the nephron and at papillary level.

The Megarajanga Chooranam treated groups showed normal histology of the kidney, and shows normal glomeruli, slight oedema of the tubular cells compared to standard drug treated animals. The kidneys excised from ethylene glycol treated group were larger and heavier than from the control animals. When observed under light microscope, many crystalline deposits in the histological preparations were seen in tubules of all regions of kidney.

In Megarajanga Chooranam along with EG treated rats, such deposits were small and less abundant. Microscopic examination of kidney sections derived from EG induced urolithic rats showed calcification inside the tubules which causes dilation of the proximal tubules. Co-treatment with Megarajanga Chooranam decreased the calcification in different parts of the renal tubules and also prevented damages to the tubules and calyces. Organ-body weight ratio is a marker of cell constriction and inflammation. The non-significant effect on the rat kidney-bodyweight ratio following the administration of various doses of the Megarajanga Chooranam suggests that the drug did not induce inflammation or constriction of the kidney cells.

**Table 1: Diuretic Activity of Megarajanga Chooranam in Rats**

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment and Dose</th>
<th>Volume of Urine (ml/4hrs)</th>
<th>Sodium (mMol/l)</th>
<th>Potassium (mMol/l)</th>
<th>Chloride (mMol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Saline (10 ml/ kg)</td>
<td>0.82±0.15</td>
<td>74.7±5.3</td>
<td>65.4±4.2</td>
<td>91.2±6.4</td>
</tr>
<tr>
<td>II</td>
<td>MRC (250 mg/ kg)</td>
<td>0.78±0.12b</td>
<td>88.0±6.9b</td>
<td>78.6±6.1c</td>
<td>110.8±10.1c</td>
</tr>
<tr>
<td>III</td>
<td>MRC (500 mg/ kg)</td>
<td>1.12±0.15b</td>
<td>100.3±6.4c*</td>
<td>89.2±5.8*</td>
<td>122.0±6.2*</td>
</tr>
<tr>
<td>IV</td>
<td>Fruosemide (20 mg/kg)</td>
<td>4.10±0.22**</td>
<td>125.1±4.8**</td>
<td>102.1±6.5**</td>
<td>142.7±8.8**</td>
</tr>
</tbody>
</table>

All values are expressed as mean±S.E.M for six rats in each group. Comparisons made between ***p<0.001; **p<0.01; *p<0.05 V, control; AP<0.001; b p<0.01; c p<0.05 V, Standard.
Table 2: Estimation of Urinary Electrolytes of Normal and Urolithiatic Rats

<table>
<thead>
<tr>
<th>S. NO.</th>
<th>Group &amp; Drug Treatment</th>
<th>Estimation of Urinary Electrolytes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Oxalate (mg/dl)</td>
</tr>
<tr>
<td>1</td>
<td>Normal control (Saline)</td>
<td>0.35±0.05</td>
</tr>
<tr>
<td>2</td>
<td>Calculi induced (0.75% EG)</td>
<td>2.28±0.05</td>
</tr>
<tr>
<td>3</td>
<td>Standard (Cystone 750 mg/kg)</td>
<td>1.22±0.05</td>
</tr>
<tr>
<td>4</td>
<td>T₁ (MRC250 mg/kg)</td>
<td>1.25±0.20</td>
</tr>
<tr>
<td>5</td>
<td>T₂ (MRC500 mg/kg)</td>
<td>0.76±0.12</td>
</tr>
</tbody>
</table>

All values are expressed as mean ±S.E.M for six rats in each group. Comparisons made between \( p<0.001,\) \( p<0.01,\) \( p<0.05; \) \( T₁, T₂ \) \( vs. \) Standard, \( **p<0.001, \) \( *p<0.01, \) \( *p<0.05; \) \( T₁, T₂ \) \( vs. \) Calculi induced, \( \alpha p<0.01, \) \( @p<0.05; \) Calculi induced \( V \) normal control, \( \#p<0.001, \) \( \#p<0.01, \) \( \#p<0.05; \) Calculi induced \( V \) Standard., One-way ANOVA followed by Tukey test.

Table 3: Estimation of Kidney Homogenate Electrolytes of Normal and Urolithiatic Rats

<table>
<thead>
<tr>
<th>S. NO.</th>
<th>Group &amp; Drug Treatment</th>
<th>Estimation of Kidney Homogenate Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Oxalate (mg/dl)</td>
</tr>
<tr>
<td>1</td>
<td>Normal (Saline)</td>
<td>0.172±0.07</td>
</tr>
<tr>
<td>2</td>
<td>Positive control (0.75% EG)</td>
<td>1.742±0.09</td>
</tr>
<tr>
<td>3</td>
<td>Standard (Cystone 750 mg/kg)</td>
<td>0.569±0.06</td>
</tr>
<tr>
<td>4</td>
<td>T₁ (MRC250 mg/kg)</td>
<td>1.156±0.09</td>
</tr>
<tr>
<td>5</td>
<td>T₂ (MRC500 mg/kg)</td>
<td>0.768±0.08</td>
</tr>
</tbody>
</table>

All values are expressed as mean ±S.E.M for six rats in each group. Comparisons made between \( p<0.001,\) \( p<0.01,\) \( p<0.05; \) \( T₁, T₂ \) \( vs. \) Standard, \( **p<0.001, \) \( *p<0.01, \) \( *p<0.05; \) \( T₁, T₂ \) \( vs. \) Calculi induced, \( \alpha p<0.01, \) \( @p<0.05; \) Calculi induced \( V \) normal control, \( \#p<0.001, \) \( \#p<0.01, \) \( \#p<0.05; \) Calculi induced \( V \) Standard., One-way ANOVA followed by Tukey test.

Table 4: Estimation of Serum Parameters of Normal and Urolithiatic Rats

<table>
<thead>
<tr>
<th>S. NO.</th>
<th>Group &amp; Drug Treatment</th>
<th>BUN (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>Uric acid (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Normal (Saline)</td>
<td>20.31±0.28</td>
<td>0.702±0.06</td>
<td>4.86±0.07</td>
</tr>
<tr>
<td>2</td>
<td>Positive control (0.75% EG)</td>
<td>28.66±0.41</td>
<td>0.924±0.07</td>
<td>6.93±0.10</td>
</tr>
<tr>
<td>3</td>
<td>Standard (Cystone 750 mg/kg)</td>
<td>23.91±0.32</td>
<td>0.846±0.09</td>
<td>5.26±0.08</td>
</tr>
<tr>
<td>4</td>
<td>T₁ (MRC250 mg/kg)</td>
<td>26.81±0.52</td>
<td>0.899±0.10</td>
<td>6.33±0.09</td>
</tr>
<tr>
<td>5</td>
<td>T₂ (MRC500 mg/kg)</td>
<td>24.35±0.40</td>
<td>0.828±0.12</td>
<td>6.00±0.11</td>
</tr>
</tbody>
</table>

All values are expressed as mean ±S.E.M for six rats in each group. Comparisons made between \( p<0.001,\) \( p<0.01,\) \( p<0.05; \) \( T₁, T₂ \) \( vs. \) Standard, \( **p<0.001, \) \( *p<0.01, \) \( *p<0.05; \) \( T₁, T₂ \) \( vs. \) Calculi induced, \( \alpha p<0.01, \) \( @p<0.05; \) Calculi induced \( V \) normal control, \( \#p<0.001, \) \( \#p<0.01, \) \( \#p<0.05; \) Calculi induced \( V \) Standard., One-way ANOVA followed by Tukey test.
Diuretic activity of Megarajanga Chooranam

Effect of Megarajanga Chooranam on Urinary Electrolytes

Effect of Megarajanga Chooranam on Kidney Homogenate Electrolytes
Pathologic studies have shown that the renal failure from EG is associated with proximal tubule cell necrosis leading to production of several metabolites (glycol aldehyde, glycolate, glyoxylate and oxalate, in that order) and accumulation of large calcium oxalate monohydrate crystals in tubular lumen. Commonly prescribed drug (Cystone) was found to contain water soluble substances, which inhibited the initial precipitation of calcium and phosphate ions in the form of a mineral phase bound to the organic matrix and the subsequent growth of the preformed mineral phase. In the present study, concurrent administration of EG with Cystone, Megarajanga Chooranam causes significant curative effect in EG induced changes. The effect is dose dependent. The effectiveness of Megarajanga Chooranam is comparable to cystone. The renal tissue of EG along with Megarajanga Chooranam shows only few stray areas of calcification in glomeruli and normal tubular structures with no congestion in blood vessels. The renal tissue of standard drug treatment still shows moderate calcification in many tubules and few glomeruli. It has been reported that the kidneys are the principle target organs for ethylene glycol toxicity and administration of ethylene glycol for 3 weeks resulted in insignificant urinary oxalate excretion and deposition of crystals in kidney, hence in our study ethylene glycol was chosen to induce lithiasis. Following the induction of lithiasis the urinary volume and composition were found to be altered. In our study also the urinary output was markedly decreased in lithiatic control rats on day 28, however in Megarajanga Chooranam and standard treated rats the urinary volumes were increased when compared to that lithiatic Group. This suggested that Megarajanga Chooranam might have moderate diuretic effect (Table1). Following ethylene glycol administration the excretion of calcium, oxalate, phosphate and protein were found to be increased in lithiatic group while in standard, test groups these levels were significantly decreased (P<0.01) (Table 2). On contrary to this the magnesium level was decreased in lithiatic group while in standard and Megarajanga Chooranam treated groups those levels were increased significantly (P<0.01) (Table 3). The serum creatinine levels of Megarajanga Chooranam treated rats restored to normal limits and the creatinine clearance was also found to be improved (Table 4). The findings of the histopathological studies suggested that no microcrystalline deposition and kidney damage in the Megarajanga Chooranam treated groups. All these observations enabled us to confirm the inhibitory potential of Megarajanga Chooranam on ethylene glycol induced urolithiasis.

CONCLUSION
The presented data indicate that administration of the Megarajanga Chooranam to rats with ethylene glycol induced urolithiasis reduced the formation of urinary stones, supporting clinical information regarding antiurolithiatic activity of the Megarajanga Chooranam. The
mechanism underlying this effect is apparently related to diuresis and lowering of urinary concentrations of stone forming constituents. These effects could conclude the antiurolithiatic property of Megarajanga Chooranam.

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