Scientific validation of *Siddha* formulation *Sirupeelai Samoola Kudineer* in the treatment of renal calculi in zinc implantation model

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*Sirupeelai samoola kudineer* prescribed for the treatment of urolithiasis was prepared as per Siddha text and validated in male Sprague Dawley rats using Zinc implantation model. Urolithiasis was induced by implantation of pre weighed and sterile zinc disc in the urinary bladder of the rats with supplementation of ethylene glycol for 3 weeks. After induction, rats were treated with SK (4.5 ml/kg) and at the end of the study, animals were scarificed and zinc disc was removed from the urinary bladder. The effect of SK on disc weight, urinary volume, pH and specific gravity besides qualitative analysis of urinary parameters was investigated at 0th, 7th, 14th and 21st day. SK treatment reduced weight of the stones significantly when compared to its vehicle control. A significant decrease in the levels of stone and renal markers was observed in the SK treated group when compared to vehicle control group. A significant increase in the levels of antioxidants such as GSH, GPx, SOD and decrease in TBARS with concomitant restoration of the membrane stabilizing enzymes Na\(^+\) K\(^+\) ATPase, Ca\(^{2+}\) ATPase and Mg\(^{2+}\) ATPase was observed. The results, concluded that SK possess significant stone dissolving property through its diuretic, antioxidant and membrane stabilizing property.

**Keywords**: Nephrolithiasis, Renal calculi, Zinc implant, Ethylene glycol, *Sirupeelai samoola kudineer*

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*Kalladdaippu* (Urolithiasis), a multifactorial urological disorder defined as the formation of calculi in any location in the kidneys, ureters, urinary tract and bladder has affected mankind since ancient times. It is the third most common disease of the urinary tract and the prevalence rate appears to be 10% of world population\(^{1,2}\) of which 3% affected in India\(^{3}\). Causative factors for the formation of renal calculi includes physiochemical changes, genetic derangements, higher intake of protein rich meat, wheat diet and climatic variations\(^{3,4}\). The pathogenesis of Urolithiasis is multifaceted including numerous physico chemical events occurring sequentially or concurrently. Inspite of its occurrence and prevalence since antiquity, the mechanism lying behind the formation of renal calculi is still complex. Hence, preclinical investigations on evaluation of anti lithiatic drugs including *in vitro* models and *in vivo* animal models have been employed\(^{5}\). Even though the *in vitro* models mimic the initial events in the formation of renal calculi; it does not fully encompass the biological mechanism lying behind. Hence, *in vivo* animal models such as chemical induction models and surgical implantation models have been done\(^{6,7}\). The implantation of Zinc disc in animals is one such urolithic model which is commonly used in the preclinical evaluation of anti lithiatic drugs.

The present study was carried out to evaluate the safety and efficacy profile of two traditional siddha formulations namely *Sirupeelai Samoola Kudineer*, a polyherbal formulation in the management of renal calculi in male Sprague Dawley rats using zinc disc implantation with supplementation of ethylene glycol induced urinary bladder calculi model.

*Sirupeelai Samoola Kudineer* (SK) is a combination of four herbs namely *Aerva lanata* (L.) Juss. ex Schult (whole plant), *Crateva nurvala* Buch. Ham. (root), *Tribulus terrestris* L. (fruit) and *Pavonia odorata* Wild. (root) and was prepared as per Siddha literature.

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Methodology

Sirupeelai samoola kudineer was prepared based on the Siddha literature. The four herbs were collected and authenticated by Prof P Jayaraman, Plant Anatomist, Plant Anatomy Research Center, Chennai in the month of July 2010 and a voucher specimen of the plants (SRU/010/2010, SRU/011/2010, SRU/012/2010, SRU/013/2010) were deposited in the Herbal Laboratory Museum, Sri Ramachandra University. Zinc disc weighing 20mg each obtained from local market were sterilized and used.

Experimental animals

Male Sprague Dawley rats of 6 weeks age (200 - 250 gm b.wt) were obtained from Centre for Toxicology and Developmental Research, Sri Ramachandra University (IAEC-XIV/SRU/54/2010). The animals were maintained on a 12 hrs dark/light cycle at a room temperature of about 22 ± 3°C and relative humidity was set to be 50-70%. The animals had free access to pelleted feed of standard composition containing all macro and micro nutrients. Purified water was provided ad libitum.

Antiurolithiatic study

Drug treatment

The dose of the test drugs were selected based on the results obtained in our earlier safety studies. Animals were divided into four groups with ten animals in each. Group I- normal control, Group II- zinc disc implanted + 0.75 % v/v ethylene glycol for 3 weeks+ water (4.5 ml/kg b.wt. p.o), Group III- zinc disc implanted + 0.75 % v/v ethylene glycol for 3 weeks + SK (4.5 ml/kg b.wt. p.o), Group IV- zinc disc implanted control.

Experimental procedure

Zinc disc implantation model was adopted from earlier method. A pre weighed sterile zinc disc was inserted into the urinary bladder of all the animals except normal control. All animals received normal pelleted food and 0.75 % v/v ethylene glycol in drinking water except Group I and IV. The stone formation in bladder was observed at regular intervals through radiological examination (Fig. 1). After 3 weeks of ethylene glycol induction, group II received water (4.5 ml/kg b.wt. p.o), group III received SK (4.5 ml/kg b.wt. p.o) for a period of 1 month, group IV was implanted with Zinc alone(Fig. 1a). At the end of the experiment, rats were sacrificed to remove the zinc implants from the bladder and weighed. Kidney and urinary bladder were dissected out for further analysis. Urine samples were collected on (0th, 7th, 14th and 21st day) for further analysis.

Effect of SK on body weight and Zinc disc weight

The effect of SK on body weight of the rats was recorded pre and post-surgery of Zinc implantation, induction period (7th, 14th and 21st day) and treatment period (7th, 14th and 21st day). SK effect was also

Fig.1— Radiographic examinations of zinc disc in rats were shown in the above. Fig.1. A: 21st day before induction, B: Zinc + EGI+ water, C: Zinc + EGI+ SK (4.5 ml/kg), D: Zinc alone
evaluated on Zinc weight (Pre- and Post–surgery) and stone weight.

**X–ray imaging of the Zinc disc**

The X-ray imaging provides a means to easily monitor the crystal growth in the urinary bladder. Hence the radiographic examinations of the implanted zinc disc before and after ethylene glycol induction as well as after the SK treatment was observed.

**Effect of SK on Urinary Volume, pH and Specific gravity**

The urinary output, pH, specific gravity and other urinary parameters (qualitative) of all experimental animals before induction, after induction and on 7th, 14th, and 21st days of treatments were recorded.

**Effect of SK on stone and renal markers**

The concentration of stone markers such as calcium, oxalate, inorganic phosphorus and magnesium were estimated in urine samples on 0th, 7th, 14th and 21st days and in renal tissue as per standard protocols. The renal markers such as urea and creatinine were analyzed in urine on 0th, 7th, 14th and 21st days and kidney homogenates using accurex kit. pH, total volume of the urine were observed (Fig. 2).

**Effect of SK on antioxidant and membrane stabilizing enzymes**

The antioxidant enzymes TBARS, SOD, GSH, GPx and membrane stabilizing enzymes Na\(^+\)K\(^+\) ATPase, Ca\(^{2+}\) ATPase and Mg\(^{2+}\) ATPase were measured in renal tissue homogenates using standard spectrophotometric methods.

**Statistical analysis**

Results are expressed as mean ± SEM (n=5). Mean difference between the groups were compared by one way analysis of variance (ANOVA) followed by LSD post hoc multiple comparison test and Wilcoxon test for non-parametric datas. P < 0.05 & 0.01 was considered to be significant. Statistical analyses were performed using SPSS 14.0.

**Results**

**Pre-Terminal deaths**

There was no abnormality in clinical signs, home cage activities, behavioral changes were observed at any stage during the study period. The fecal excretion was also recorded to be normal. 40 – 50 % of pre-terminal death was observed during the 3rd week of induction period in zinc implanted rats.

**Effect of SK on body weight**

Change in body weight during induction and treatment periods are provided in Table 1. All the experimental animals showed a decrease in body weight during induction period except zinc-implanted rats (without induction) compared to normal control. In the treatment duration, water treated rats (Group II) showed a significant (p<0.05) reduction in body weight in comparison to the normal control (Group I). On the other hand, the body weight of SK (Group III) treated rats were found to be significantly increased when compared to its vehicle control group (Group II).
Zinc alone (Group IV) was found to be coherent with the normal control.

**Effect of SK on Zinc weight**

The stone weight (difference between the pre and post implanted zinc disc) was measured at the end of the study and the data are shown in Table 1 and Fig 1. Macroscopic view of crystal deposition on implanted zinc disc of vehicle and drug treated groups are shown. Vehicle treated rats showed enormous amount of stone formation compared to zinc alone implanted group, whereas the SK treated groups showed significant decrease in the weight of stones when compared to its vehicle control.

**Effect of SK on Urinary volume, pH and Specific gravity**

The urinary output, pH, specific gravity and other urinary parameters of all experimental animals before induction, after induction and on 7th, 14th, and 21st day of treatment were noted. The data s are shown in the Table 3a. Qualitative analysis of urinary parameters was also analyzed and given in Table 3b. The urinary volume of zinc disc implanted rats was found to be increased at the end of ethylene glycol induction period in comparison with normal rats. In the present study, the urinary output was increased in SK treated group which strengthens their diuretic potential.

The pH of urine in all the experimental animals was found to be neutral (pH 7.1-7.4) before induction period. After induction, the urinary pH of rats implanted with zinc and supplemented with ethylene glycol was found to be slightly acidic than the normal rats. Administration of SK slightly neutralized the urine acidity when compared to its vehicle control rats. Specific gravity of the urine was found to be normal in all experimental groups throughout the study duration. Also there were no presence of blood, urobilinogen, ketone bodies, glucose, bilirubin and nitrite in the urine of all rats.

**Effect of SK on stone weight, stone markers and renal markers**

The levels of stone markers (calcium, oxalate, magnesium and inorganic phosphorus) and renal markers (urea and creatinine) were estimated in urine and kidney tissue. In the present study the levels of calcium and oxalate were found to be progressively increased (p<0.01) with decreased levels of magnesium and inorganic phosphorus in urine and renal tissue of zinc implanted vehicle treated rats (Group II) in comparison to the normal control. However, SK treatment significantly (p<0.05 and <0.01) lowered the levels of oxalate as well as calcium in urine and renal tissue.

The renal biomarkers such as urea and creatinine were found to be significantly elevated in urine (p<0.01) and kidney (p<0.05) tissue of zinc implanted vehicle treated rats in comparison to the normal rats. In our study, the increased urea and creatinine level in group II (Vehicle treated) rats confirm the renal damage. Post treatment with SK significantly (p<0.01) reversed the elevation of these levels in plasma, urine as well as in tissues when compared to its vehicle control.
Effect of SK on tissue lipid peroxidation and antioxidant levels

The GPx and GSH levels were found to be significantly lowered in Group II (p<0.01) whereas SK treatment significantly (p<0.01) reversed the levels of GPx and GSH. TBARS level was found to be increased significantly (p<0.01) in Group II rats in comparison to the normal rats, whereas SK treatment significantly decreased the TBARS level compared to its vehicle control groups (Fig. 3).

Effect of SK on membrane stabilizing enzymes (Fig. 4)

In renal tissues, the levels of Na\(^+\)K\(^+\)ATPase, Ca\(^{2+}\)ATPase and Mg\(^{2+}\) ATPase was found to be decreased significantly (p < 0.05) in Group II vehicle treated group in comparison with normal group. The level of Ca\(^{2+}\) ATPase and Mg\(^{2+}\) ATPase was restored to normal in SK treated groups when compared to its vehicle control.

Histopathological examinations

Histopathological examination of kidney and urinary bladder showed that, at tested dose, SK (4.5 ml/kg) has reduced the calculi number with slight congestion and inflammation with very few number of polarizing bright white oxalate crystals in the tubular region. The kidney of the vehicle control under light microscope showed presence of more number of calculi in tubules (sprinkled), congestion of blood vessels, severe inflammation near the glomeruli and vascular congestion. The vehicle control urinary...

Table 3a — Effect of SK on urinary volume, urinary \(p\)H & urinary specific gravity of zinc implanted rats

<table>
<thead>
<tr>
<th>Treatment (p.o./day)</th>
<th>Induction</th>
<th>7th day</th>
<th>14th day</th>
<th>21st day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urinary volume</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal control</td>
<td>5.40±0.24</td>
<td>6.40±0.51</td>
<td>6.00±0.32</td>
<td>6.00±0.32</td>
</tr>
<tr>
<td>ZI+ 0.75 v/v EGI+ water (4.5 ml/kg, b.wt.)</td>
<td>10.80±0.80</td>
<td>12.60±1.08</td>
<td>12.40±0.98</td>
<td>11.60±0.24</td>
</tr>
<tr>
<td>ZI+ 0.75 v/v EGI+SK (4.5 ml/kg, b.wt.)</td>
<td>9.60±0.72</td>
<td>14.40±0.60</td>
<td>16.80±0.20</td>
<td>14.20±0.66</td>
</tr>
<tr>
<td>ZI Alone</td>
<td>8.60±0.25</td>
<td>7.60±0.29</td>
<td>5.20±0.80</td>
<td>7.60±0.40</td>
</tr>
<tr>
<td>Urinary pH</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal control</td>
<td>7.4±0.10</td>
<td>7.1±0.10</td>
<td>7.4±0.10</td>
<td>7.4±0.10</td>
</tr>
<tr>
<td>ZI+ 0.75 v/v EGI+ water (4.5 ml/kg, b.wt.)</td>
<td>7.2±0.12</td>
<td>6.1±0.10</td>
<td>6.6±0.10</td>
<td>6.9±0.19</td>
</tr>
<tr>
<td>ZI+ 0.75 v/v EGI+SK (4.5 ml/kg, b.wt.)</td>
<td>7.3±0.20</td>
<td>7.3±0.12</td>
<td>7.3±0.20</td>
<td>7.8±0.12</td>
</tr>
<tr>
<td>ZI Alone</td>
<td>7.3±0.30</td>
<td>7.6±0.10</td>
<td>7.5±0.00</td>
<td>7.4±0.10</td>
</tr>
<tr>
<td>Specific gravity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal control</td>
<td>1.01</td>
<td>1.01</td>
<td>1.01</td>
<td>1.01</td>
</tr>
<tr>
<td>ZI+ 0.75 v/v EGI+ water (4.5 ml/kg, b.wt.)</td>
<td>1.01</td>
<td>1.01</td>
<td>1.01</td>
<td>1.01</td>
</tr>
<tr>
<td>ZI+ 0.75 v/v EGI+SK (4.5 ml/kg, b.wt.)</td>
<td>1.01</td>
<td>1.01</td>
<td>1.01</td>
<td>1.01</td>
</tr>
<tr>
<td>ZI alone</td>
<td>1.01</td>
<td>1.01</td>
<td>1.01</td>
<td>1.01</td>
</tr>
</tbody>
</table>

Data were expressed in mean±SEM, n= 5 in each group. Statistical analysis was done using SPSS 16 version, Non Parametric test, Wilcoxon test, p values –, * (0.05) & ** (0.01). - Normal control compared with ZI+ EGI+water, ZI and ZI Alone, # (0.05) & ## (0.01) - ZI+ EGI+water compared with ZI+ EGI+SK (4.5 ml/kg,b.wt.), † (0.05) & †† (0.01) BI- Before Induction; AI – After Induction. ZI - Zinc Implanted, EGI - Ethylene glycol Induction, SK – Sirupeelai Samoola Kudineer.

Table 3b — Effect of SK on 7th, 14th & 21st day treatment in qualitative analysis of urinary parameters of zinc implanted animals

<table>
<thead>
<tr>
<th>Parameters analyzed</th>
<th>7th day</th>
<th>14th day</th>
<th>21st day</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>I II III IV</td>
<td>I II III IV</td>
<td>I II III IV</td>
</tr>
<tr>
<td>Glucose</td>
<td>-ve -ve -ve -ve</td>
<td>-ve -ve -ve -ve</td>
<td>-ve -ve -ve -ve</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>-ve -ve -ve -ve</td>
<td>-ve -ve -ve -ve</td>
<td>-ve -ve -ve -ve</td>
</tr>
<tr>
<td>Ketones</td>
<td>-ve -ve -ve -ve</td>
<td>-ve -ve -ve -ve</td>
<td>-ve -ve -ve -ve</td>
</tr>
<tr>
<td>Nitrite</td>
<td>-ve -ve -ve -ve</td>
<td>-ve -ve -ve -ve</td>
<td>-ve -ve -ve -ve</td>
</tr>
<tr>
<td>Protein</td>
<td>-ve -ve -ve -ve</td>
<td>-ve -ve -ve -ve</td>
<td>-ve -ve -ve -ve</td>
</tr>
<tr>
<td>Urobilinogen</td>
<td>N N N N</td>
<td>T T T T</td>
<td>-ve -ve -ve -ve</td>
</tr>
</tbody>
</table>

I - Normal Control, II – ZI+ 0.75v/v EGI+ water (4.5 ml/kg, b.wt.), III -ZI+ 0.75v/v EGI+SK (4.5 ml/kg, b.wt.), IV-ZI Alone. Data were expressed in mean±SEM, n= 5 in each group ; -ve – negative; N – normal; T –trace; ZI - Zinc Implanted, EGI - Ethylene glycol Induction, SK – Sirupeelai Samoola Kudineer.
bladder showed few crystals appear in the epithelial layer with moderate inflammation under light microscope and appearance of few polarizing bright white oxalate crystals in the epithelial region when observed under polarized light (Figs. 5&6).

**Discussion**

Even though effective measures are being taken for the management of urolithiasis, it is of great importance to evaluate the role of traditional complementary practices. The anti urolithiatic
property was assessed by diuretic, stone dissolving, antioxidant and membrane stabilizing properties of SK. The foremost cause for the formation of urinary stone is the changes in the urinary chemistry such as hyper calciuria and hyper oxaluria. These changes in the urinary chemistry lead to urinary super saturation which later crystallizes aggregates and forms stone. These urinary stones are composed mainly of calcium oxalates (60–80%) and calcium phosphates.

SK was evaluated for diuretic activity at the tested dose of 4.5mg/kg b.wt. Increased urinary volume was observed in SK treated group during the treatment period of 7th, 14th and 21st day whereas groups treated with vehicle control showed decreased urinary output on the same tested days. A significant decrease in the stone weight was also observed in the SK treated group when compared to its vehicle control. Crystalluria is pH dependent factor and hence alteration in urinary pH may be a sign of stone formation. The diuretic effect increases the amount of fluid expelling out and this increased fluid output facilitates the expulsion of deposits and stones, in such a way, increased excretion in the levels of calcium, oxalate and magnesium in SK treated group indicates its stone dissolving potential. Phosphorus levels was found to be elevated in the vehicle control and Zinc implanted group whereas SK treatment showed decreased level of phosphorus.

Oxalate crystal retention in renal tubules leads to increased BUN (Urea) and creatinine levels which cause serious damage to renal functions. Decreased levels of BUN and creatinine in the SK treated group compared to its vehicle control showed the nephroprotective effect of SK. SK treatment showed a marked increase in the levels of antioxidants with subsequent reduction in the oxidative stress marker TBARS as evidenced for its antioxidant potential besides its capacity to restore the levels of membrane stabilizing enzymes such as Na⁺K⁺ATPase, Ca²⁺ATPase and Mg²⁺ ATPase. The ingredients present in SK themselves have anti urolithiatic and nephroprotective potential, the evidences of which date back since ancient time in Ayurveda.

### Conclusion

Hence from the study, it could be concluded that in built nephroprotective, diuretic, antioxidant and membrane stabilizing properties of the ingredients present in SK has proved that the polyherbal Siddha formulation used by ancient Siddhars for urolithiasis treatment is acceptable and claims scientific validity. The possible mechanism of SK in dissolution of stone and further inhibition of stone formation is elucidated as follows:

### Acknowledgement

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### References

VEERARAGHAVAN et al.: VALIDATION OF SIRUPEELAI SAMOOLA KUDINEER IN THE TREATMENT OF RENAL CALCULI


