ANTILITHIATIC ACTIVITY OF SACCHARUM SPONTANEUM LINN. ON ETHYLENE GLYCOL – INDUCED LITHIASIS IN RATS

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Abstract

The ethanolic extract of roots of Saccharum spontaneum Linn. was evaluated for its antilithiatic activity in rats. Lithiasis was induced by oral administration of ethylene glycolated water (0.75%) in adult male wistar albino rats for 28 days. The ionic chemistry of urine was altered by ethylene glycol (EG), which elevated the urinary concentration of calcium, oxalate, urea, uric acid and creatinine. However, treatment with ethanolic root extract of S. Spontaneum (200 and 300 mg/kg body weight) in group III and IV significantly (p<0.05) reduced the elevated level of these ions in urine. Also, it elevated concentration of urinary magnesium, which is considered as one of the inhibitors of crystallization. The levels of serum calcium, oxalate, phosphorus, magnesium and protein were significantly increased (p < 0.05) in urolithiatic rats. Treatment with plant extract restored the levels and it brought back the values to near normal range in urolithiatic rats. All these observations revealed that ethanolic root extract of S. Spontaneum has curative effect on stone formation induced by ethylene glycol.
Urolithiasis (renal stone formation) is a recurrent disorder predominant in males. The present day medical management of urolithiasis is either costly or not without side effects. Hence, the search for antilithiatic drugs from natural sources has assumed greater importance. Many Indian plants have been quoted to be useful as antilithiatic agents. They are effective with fewer side effects and are also inexpensive. Hence; the Indian plants are constantly being evaluated for possible antilithiatic effects in systematic manner\textsuperscript{1}. One such plant is *Saccharum spontaneum* L. known as Kasa (Family: Poaceae) is a traditional herb, it has excellence medicinal value; has been advocated in the treatment gynaecological troubles, respiratory disease. Roots are used as galactagogue and diuretic and in ayurveda system roots are also used as astringent, emollient, refrigerant, diuretic, purgative, tonic, and aphrodisiac and useful in treatment of dyspepsia, burning sensation, piles and sexual weakness\textsuperscript{2}. The stems (culm) are useful in vitiated conditions of pitta and vata burning sensation strongly, renal and vesicol calculi dyspepsia, haemorrhoids, menorrhagia dysentery, agalactia phthisis and general debility\textsuperscript{3}.

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**MATERIALS & METHODS**

Collection of the plant material

*Saccharum spontaneum* Linn. Was collected from Koorappalayam, Erode district, Tamil Nadu, India during the month of September to November, 2011. The plant was...
identified and authenticated by taxonomist Dr. K. Arumugasamy, Assistant Professor, Department of Botany, Kongunadu Arts and Science College, Coimbatore, Tamilnadu, India. Voucher specimen was deposited in herbarium centre, Department of Botany, Kongunadu Arts and Science College, Coimbatore.

**Preparation of the ethanolic root extract for in vivo studies**

Roots of the plants were washed, shade dried, powdered and stored in tight containers under refrigeration. 100g of *S. spontaneum* powder was taken in a conical flask. To this 500ml of 99% ethanol was added. The content of the flask was kept in the shaker for 48 hr. and the suspension was filtered and residue was re suspended in an equal volume of 99% ethanol for 48hr. and filtered again. The two filtrates were pooled and the solvents were dried in an oven at 37°C and a crude residue was obtained. The yield was 21.8 g, and the residue was suspended in water and administered orally to the experimental rats.

**Selection of animals for In vivo studies**

For the purpose of antilithiatic studies, adult male wistar albino rats weighing about 150 to 200 g were collected from animal breeding centre, Kerala Agricultural University, Mannuthy, Thrissur, Kerala, India. The rats were kept in properly numbered large polypropylene cages with stainless steel top grill having facilities for pelleted food. The animals were maintained in 12 hr. light and dark cycle at 28°C ± 2°C in a well ventilated animal house under natural conditions in large polypropylene cages and they were acclimatized to laboratory conditions for 10 days prior to the commencement of the experiment. The animals were fed with standard pelleted diet supplied by AVM foods, Coimbatore, Tamilnadu, India. All animal experiments were performed according to the ethical guidelines suggested by the Institutional Animal Ethics Committee (IAEC). Paddy husk was used as bedding material and changed twice a week.

**Experimental design of animals for in vivo studies**

The method of Selvam et al. (2001) was followed to evaluate the antilithiatic effect. The acclimatized animals were divided into five groups of six each designated as Group...
I, II, III, IV, and V. The animals of Group I served as the normal control. Group II animals received 0.75% ethylene glycol in drinking water _ad libitum_ for 28 days and served as the lithiatic control.

The Group III and Group IV group animals received 0.75% ethylene glycol in drinking water _ad libitum_; along with ethanolic root extract of (200 and 300mg/ kg body weight and Group-V group animals received 0.75% ethylene glycol in drinking water _ad libitum_; along with thiazide (150μg/ kg body wt) by oral route for 28 days.

Biochemical parameters assayed for pharmacological screening studies

The 24-h urine samples were collected in metabolic cages, on the 7th, 14th, 21st, and 28th days and the volume noted. Urinary calcium, oxalate, magnesium, urea, uric acid, creatinine and the serological parameters were estimated on 28th day of lithiasis.

RESULTS AND DISCUSSION

In the present study, chronic administration of 0.75% (v/v) ethylene glycol aqueous solution to male Wistar albino rats resulted in hyperoxaluria. As mentioned in table 1 and 2. The urinary excretion of calcium and oxalate are increased (p < 0.05) significantly on in calculi-induced (group II) animals when compared with normal control rats. Maximum levels of excretion were observed with group II on the 28th day.

However the calcium and phosphorus excretion was normalized in the extract treated rats (group III and IV), When S. _Spontaneum_ extract treated rats (Group III and IV) were compared with thiazide treated rats (Group V), there was no significant difference between these groups.

Urinary magnesium levels were significantly decreased (p < 0.05) in ethylene glycol induced lithiatic rats (group II). The above alterations were reverted to near normal in rats treated with plant extract in group III and IV rats. Treatments with plant extract treated rats (Group III and IV) were compared with thiazide treated rats (Group V) there was no significant difference between these groups of rats (Table 3).

Experimental design

Group I: Control rats – received normal pelleted diet
Group II: Received 0.75% ethylene glycol in water for 28 days

Group III and IV: Received 0.75% ethylene glycol in drinking water ad libitum; along with ethanolic root extract of 200mg/ kg body weight by oral administration at a rate of 1.0 ml / rat / day

Group IV: Received 0.75% ethylene glycol in drinking water ad libitum; along with ethanolic root extract of 300mg/ kg body weight by oral administration at a rate of 1.0 ml / rat / day

Group V: Received 0.75% ethylene glycol in drinking water ad libitum; along with thiazide (150μg/ kg body wt) by oral route for 28 days

Comparison between the groups

‘a’ represents comparison between II and I
‘b’ represents comparison between III and II
‘c’ represents comparison between IV and II
‘d’ represents comparison between V and II
‘e’ represents comparison between III and V
‘f’ represents comparison between IV and V

From the tables 4, 5 and 6 it is evident that the levels of biochemical parameters i.e. urea, uric acid and creatinine increased (p < 0.05) significantly in urolithiatic rats (Group II), when compared to control rats (Group I). Maximum levels of excretion were observed with group II on the 28th day. Treatment with plant extract, these values was reduced to near normal range in group III and IV rats. Treatment with thiazide significantly decreased (p < 0.05) the levels and brought back the values to near normal range in group V rats. When S.spontaneum root extract treated rats (Group III and IV) were compared with thiazide treated rats (Group V), there was no significant difference between these groups of rats.

In urolithiasis, the glomerular filtration rate (GFR) decreases due to the obstruction to the outflow of urine by stones in the urinary system. Due to this, the waste products, particularly nitrogenous substances such as urea, creatinine, and uric acid get accumulated in blood. Also, increased lipids per oxidation and decreased levels of antioxidant potential have been reported in the kidneys of rats supplemented with a calculi producing diet. In this context, oxalate has been reported to induce lipid
peroxidation and to cause renal tissue
damage by reacting with polyunsaturated
fatty acids in the cell membrane$^4$.

In the present study, higher concentration
of nitrogenous substances was observed in
ethylene glycol induced urolithic rats.
*S.spontaneum* ethanolic root extract
restored the uric acid level to normal thus
reducing the risk of stone formation.

From the table$^7$ it is evident that the levels
of serum calcium, oxalate, magnesium,
phosphorus and protein were significantly
increased ($p < 0.05$) in urolithiatic rats
(Group II). Whereas, the levels of
magnesium and protein were significantly
decreased ($p < 0.05$) in group II rats when
compared to control rats (Group I).

Treatment with plant extract restored the
levels and it brought back the values to near
normal range in group III and IV rats. When
*S.spontaneum* extract treated rats (Group
III) were compared with thiazide treated
rats (Group V), there was no significant
difference between these groups of rats.
This result gives a supportive evidence for
the antiurolithiatic activity of ethanolic
extract of *S.spontaneum* which is similar to
standard drug thiazide.

**Discussion**

Changes in ionic pattern of urine are the
major determinant of stone formation. In
this study, the ionic pattern was found
disturbed by treatment with ethylene
glycol. It has been reported that daily oral
administration of ethylene glycol for more
than 4 weeks resulted in a significant
increase in oxalate excretion and that
kidneys are the targets for ethylene glycol
toxicity $^5$. Ethylene glycol gets oxidized to
oxalic acid leading to hyper oxaluria $^6$. Hyper
oxaluria is reported to be a more significant
risk factor in the pathogenesis of stone
formation$^7$. Likewise, ethylene glycol
administration increased the urinary
calcium level. It has been stated that hyper
calciuria favors precipitation of calcium
oxalate from urine. $^8$ Thus the high oxalate
and calcium ion concentration in urine
tends to form calcium oxalate crystals.

Calcium and oxalate excretion are
progressively increased in calculi induced
animals (Group II). Oxalate plays an
important role in stone formation and has
about 15 fold greater effect than urinary
calcium $^{10}$. Calcium oxalate crystals and high
oxalate levels in nephrons can produce
damages in the epithelial cells, and consequently, the cells may produce some products, as well as free radicals, inducing heterogeneous crystal nucleation and causing aggregation of crystals\textsuperscript{9}.

Soundararajan \textit{et al.} (2006) showed that calcium oxalate excretion was significantly increased in urine of ethylene glycol induced urolithic rats. Additionally, they stated that ethylene glycol disturbs oxalate metabolism by way of increasing the substrate availability that increase the activity of oxalate synthesizing enzymes in rats. Moreover, several investigations demonstrated that ethylene glycol treatment increased urinary calcium excretion significantly in lithiatic rats\textsuperscript{10, 11, 12}.

Magnesium one of the inhibitor for stone formation, reduces the super saturation of calcium oxalate by reducing the saturation of calcium oxalate and the growth of calcium oxalate crystals\textsuperscript{13}. Increased excretion of proteins has been noted in hyperoxaluric rats and stone formers\textsuperscript{14}. A high urinary colloidal concentration favors crystal growth\textsuperscript{15}. Such a condition was observed with ethylene glycol treated rats, in this study.

Uric acid is known to promote calcium oxalate crystal growth. The predominance of uric acid crystals in calcium oxalate stones and the observation that uric acid binding proteins are capable of binding to calcium oxalate and modulate its crystallization also suggest its primary role in stone formation\textsuperscript{16}. In the present study, higher concentration of urinary uric acid was observed in ethylene glycol induced urolithiatic rats.

Our results coincides with that of Karadi \textit{et al.} (2006) who showed that root woods of \textit{Moringa oleifera} Lam. reduced the oxalate level in serum of ethylene glycol induced urolithiatic rats.

Christiana \textit{et al.} (2006) showed that aqueous extract of \textit{Melia azedarach} Linn. reduced calcium and oxalate and elevated magnesium levels in serum of urolithiatic rats.

Christiana \textit{et al.} (2002) showed that \textit{Cyclea peltata} root powder increased serum magnesium and phosphorous levels in urolithiatic rats.

Karadi \textit{et al.} (2008) reported that the root bark of \textit{Moringa oleifera} Lam. normalized
the serum levels of urea, uric acid and creatinine in experimental animals.

Anand et al. (1993) showed that alcoholic extract of *Crataeva nurvula* has reversed the levels of biochemical parameters in blood and serum to normal levels in urolithiatic rats.

From the above results it was evident that the levels of the serum mineral constituents were restored to its near normal range on treatment with the plant extract.

**ACKNOWLEDGEMENT**

The authors are thankful to the college management for their guidance, valuable suggestion, encouragement and constant supporting during this investigation.

**Table 1**

Effect of ethanolic root extract of *Saccharum spontaneum* on calcium excretion in experimental nephrolithiasis (urine analysis)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Calcium before EG treatment</th>
<th>Calcium after EG treatment (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Day 7</td>
</tr>
<tr>
<td>I</td>
<td>1.53 ±0.07</td>
<td>1.51 ±0.07</td>
</tr>
<tr>
<td>II</td>
<td>1.67 ±0.26</td>
<td>2.96 ±0.04 a*</td>
</tr>
<tr>
<td>III</td>
<td>1.48 ±0.56</td>
<td>2.01 ±0.54 b* e ns</td>
</tr>
<tr>
<td>IV</td>
<td>1.58 ±0.19</td>
<td>2.08 ±0.19 c*f ns</td>
</tr>
<tr>
<td>V</td>
<td>1.61 ±0.25</td>
<td>2.16 ±0.84 d*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of six animals
### Table 2

**Effect of ethanolic root extract of S. spontaneum on oxalate excretion in experimental nephrolithiasis**

<table>
<thead>
<tr>
<th>Group</th>
<th>Oxalate before EG treatment (Days)</th>
<th>Oxalate after EG treatment (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 7</td>
<td>Day 14</td>
</tr>
<tr>
<td>I</td>
<td>0.82±0.05</td>
<td>0.76±0.01</td>
</tr>
<tr>
<td>II</td>
<td>0.80±0.06</td>
<td>2.01±0.03 a*</td>
</tr>
<tr>
<td>III</td>
<td>0.79±0.05</td>
<td>1.29±0.06 b<em>e</em>ns</td>
</tr>
<tr>
<td>IV</td>
<td>0.73±0.02</td>
<td>1.31±0.04 c<em>f</em>ns</td>
</tr>
<tr>
<td>V</td>
<td>0.77±0.04</td>
<td>1.39±0.06 d*e</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of six animals

Experimental design and comparison between the groups are as in table 1

The symbols represent statistical significance p* < 0.05, ns – not significant

**Units** ψψ mg/ 24 hr. urine sample

### Table 3

**Effect of ethanolic root extract of S. spontaneum on magnesium excretion in experimental nephrolithiasis**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Magnesium before EG treatment</th>
<th>Magnesium after EG treatment (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 7</td>
<td>Day 14</td>
</tr>
<tr>
<td>I</td>
<td>4.35±0.17</td>
<td>4.78±0.16</td>
</tr>
<tr>
<td>II</td>
<td>4.30±0.14</td>
<td>2.03±0.23 a*</td>
</tr>
<tr>
<td>III</td>
<td>4.40±0.03</td>
<td>2.52±0.02 b<em>e</em>ns</td>
</tr>
<tr>
<td>IV</td>
<td>4.46±0.02</td>
<td>2.50±0.08 c<em>f</em>ns</td>
</tr>
<tr>
<td>V</td>
<td>4.57±0.88</td>
<td>2.47±0.09 d*</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of six animals

Experimental design and comparison between the groups are as in table 1

The symbols represent statistical significance p* < 0.05, ns – not significant

**Units** ψψ mg/ 24 hr. urine sample
Table 4

**Effect of ethanolic root extract of *S.*spontaneum** on urea excretion in experimental nephrolithiasis

<table>
<thead>
<tr>
<th>Groups</th>
<th>Urea ( \psi \psi ) before EG treatment</th>
<th>Urea ( \psi \psi ) after EG treatment (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>26.81 \pm 0.24</td>
<td>26.58 \pm 0.05</td>
</tr>
<tr>
<td>II</td>
<td>26.87 \pm 0.14</td>
<td>31.59 \pm 0.46 a*</td>
</tr>
<tr>
<td>III</td>
<td>27.12 \pm 0.51</td>
<td>27.86 \pm 0.13 b*e( ns )</td>
</tr>
<tr>
<td>IV</td>
<td>26.67 \pm 0.24</td>
<td>27.66 \pm 0.36 c*f( ns )</td>
</tr>
<tr>
<td>V</td>
<td>27.06 \pm 0.29</td>
<td>27.87 \pm 0.56 d*</td>
</tr>
</tbody>
</table>

Values are expressed as mean \( \pm \) SD of six animals

Experimental design and comparison between the groups are as in table 10

The symbols represent statistical significance \( p^* < 0.05 \), \( ns \) – not significant

**Units**

\( \psi g/24\text{hour urine} \)

Table 5

**Effect of ethanolic root extract of *S.*spontaneum** on uric acid excretion in experimental nephrolithiasis

<table>
<thead>
<tr>
<th>Groups</th>
<th>Uricacid ( \psi \psi ) before EG treatment</th>
<th>Uricacid ( \psi \psi ) after EG treatment (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>47.68 \pm 0.56</td>
<td>47.25 \pm 0.30</td>
</tr>
<tr>
<td>II</td>
<td>46.68 \pm 0.35</td>
<td>60.22 \pm 0.08 a*</td>
</tr>
<tr>
<td>III</td>
<td>47.02 \pm 0.63</td>
<td>48.35 \pm 0.05 b*e( ns )</td>
</tr>
<tr>
<td>IV</td>
<td>47.69 \pm 0.21</td>
<td>48.18 \pm 0.11 c*f( ns )</td>
</tr>
<tr>
<td>V</td>
<td>47.53 \pm 0.41</td>
<td>48.17 \pm 0.12 d*</td>
</tr>
</tbody>
</table>
Values are expressed as mean ± SD of six animals

Experimental design and comparison between the groups are as in table 10

The symbols represent statistical significance p* < 0.05, ns – not significant

Units

ψψ mg/ 24 hr. urine sample

Table 6

Effect of ethanolic root extract of *S. spontaneum* on creatinine excretion in experimental nephrolithiasis

<table>
<thead>
<tr>
<th>Group</th>
<th>Creatinine before EG treatment</th>
<th>Creatinine after EG treatment (Days)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Day 7</td>
<td>Day 14</td>
</tr>
<tr>
<td>I</td>
<td>140.63±0.19</td>
<td>140.32±0.16</td>
</tr>
<tr>
<td>II</td>
<td>141.35±0.13</td>
<td>160.66±1.11 a*</td>
</tr>
<tr>
<td>III</td>
<td>140.96±0.28</td>
<td>142.63±0.14 b*e&lt;sub&gt;ns&lt;/sub&gt;</td>
</tr>
<tr>
<td>IV</td>
<td>140.86±0.23</td>
<td>142.79±0.12 c*f&lt;sub&gt;ns&lt;/sub&gt;</td>
</tr>
<tr>
<td>V</td>
<td>141.58±0.24</td>
<td>142.63±0.03 d*s</td>
</tr>
</tbody>
</table>

Values are expressed as mean ± SD of six animals

Experimental design and comparison between the groups are as in table 10

The symbols represent statistical significance p* < 0.05, ns – not significant

Units ψψ mg/ 24 hr. urine sample

Table 7

Effect of ethanolic root extract of *Saccharum spontaneum* on serological parameters on 28<sup>th</sup> day of lithiasis

<table>
<thead>
<tr>
<th>Serological Parameters</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
<th>Group V</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calcium **</td>
<td>8.57±0.16</td>
<td>12.23±0.11 a*</td>
<td>8.90±0.11 b*e&lt;sub&gt;ns&lt;/sub&gt;</td>
<td>8.89±0.13 c*f&lt;sub&gt;ns&lt;/sub&gt;</td>
<td>8.93±0.09 d*</td>
</tr>
<tr>
<td>Oxalate **</td>
<td>1.50±0.14</td>
<td>4.73±0.17 a*</td>
<td>1.67±0.19 b*e&lt;sub&gt;ns&lt;/sub&gt;</td>
<td>1.65±0.18 c*f&lt;sub&gt;ns&lt;/sub&gt;</td>
<td>1.74±0.17 d*</td>
</tr>
<tr>
<td>Magnesium **</td>
<td>2.85±0.15</td>
<td>1.53±0.05 a*</td>
<td>2.42±0.10 b*e&lt;sub&gt;ns&lt;/sub&gt;</td>
<td>2.41±0.02 c*f&lt;sub&gt;ns&lt;/sub&gt;</td>
<td>2.33±0.04 d*</td>
</tr>
<tr>
<td>Phosphorus **</td>
<td>6.39±0.20</td>
<td>8.81±0.12 a*</td>
<td>6.75±0.15 b*e&lt;sub&gt;ns&lt;/sub&gt;</td>
<td>6.39±0.20 c*f&lt;sub&gt;ns&lt;/sub&gt;</td>
<td>6.78±0.18 d*</td>
</tr>
</tbody>
</table>
Values are expressed as mean ± SD of six animals

Experimental design and comparison between the groups are as in table 1
The symbols represent statistical significance p* < 0.05, ns – not significant

**Units**

mg/dl *g/dl


