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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.

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INTRODUCTION

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 asystematic manner[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[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[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 37ºC 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 $28^{o}C \pm 2^{o}$ 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 beding 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 1and 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 28thday. 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 28 th 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 table7 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[9].

Soundararajan *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[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[13].Increased excretion of proteins has been noted in hyperoxaluric rats and stone formers[14].A high urinary colloidal concentration favors crystal growth[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[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 *et al.* (2006) who showed that root woods of *Moringa oleifera* Lam. reduced the oxalate level in serum of ethylene glycol induced urolithiatic rats.

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

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

Karadi *et al*. (2008) reported that the root bark of *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.

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Table1

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

Groups	Calcium $^{\psi\psi}$ before EG treatment	Calcium $^{\psi\psi}$ after EG treatment (Days)			
		Day 7	**Day 14**	**Day 21**	**Day 28**
I	1.53±0.07	1.51 ±0.07	1.59 ±0.10	1.66 ±0.10	1.73 ±0.07
II	1.67 ±0.26	2.96 ±0.04 a*	3.08 ±0.70 a*	4.05 ±0.10 a*	5.24 ±0.19 a*
III	1.48 ±0.56	2.01 ±0.54 b* e^{ns}	2.04 ±0.02 b* e^{ns}	1.97 ±0.10 b* e^{ns}	1.89 ±0.12 b* e^{ns}
IV	1.58 ±0.19	2.08 ±0.19 c*f^{ns}	2.03 ±0.25 c*f^{ns}	2.06 ±0.13 c*f^{ns}	1.87 ±0.11 c*f^{ns}
V	1.61 ±0.25	2.16 ±0.84 d*	2.09 ±0.11 d*	2.13±0.10 d*	1.94 ±0.07 d*

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

Group	Oxalate $^{\Psi\Psi}$ before EG treatment	Oxalate $^{\Psi\Psi}$ after EG treatment (Days)			
		Day 7	**Day 14**	**Day 21**	**Day 28**
I	0.82±0.05	0.76 ±0.01	0.79 ±0.01	0.83 ±0.03	0.91 ±0.03
II	0.80±0.06	2.01 ±0.03 a*	2.37 ± 0.17 a*	3.35 ±0.17 a*	4.30 ±0.14 a*
III	0.79±0.05	1.29 ±0.06 b*e^{ns}	1.19 ±0.12b*e^{ns}	0.93 ±0.05 b*e^{ns}	0.85 ±0.03 b*e^{ns}
IV	0.73±0.02	1.31 ±0.04 c*f^{ns}	1.26 ±0.06 c*f^{ns}	0.96 ± 0.03 c*f^{ns}	0.84 ± 0.03 c*f^{ns}
V	0.77±0.04	1.39 ±0.06 d*	1.10± 0.07 d*	0.91 ± 0.03 d*	0.98 ± 0.06d*

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 $^{\Psi\Psi}$ mg/ 24 hr. urine sample

Table3

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

Groups	Magnesium $^{\Psi\Psi}$ before EG treatment	Magnesium $^{\Psi\Psi}$ after EG treatment (Days)			
		Day 7	**Day 14**	**Day 21**	**Day 28**
I	4.35±0.17	4.78 ±0.16	4.66 ±0.11	4.75 ±0.13	4.79 ±0.16
II	4.30±0.14	2.03 ±0.23 a*	1.89 ±0.12 a*	1.61 ±0.26 a*	0.85 ±0.08 a*
III	4.40±0.03	2.52±0.02 b*e^{ns}	2.63 ±0.02 b*e^{ns}	3.96 ±0.04 b*e^{ns}	4.04 ±0.13 b*e^{ns}
IV	4.46±0.02	2.50 ±0.08 c*f^{ns}	2.67 ±0.03 c*f^{ns}	3.99 ±0.03 c*f^{ns}	4.05 ±0.13 c*f^{ns}
V	4.57±0.88	2.47±0.09 d*	2.62 ±0.17 d*	3.94 ±0.01 d*	3.99 ±0.12 d*

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 $^{\Psi\Psi}$ mg/ 24 hr. urine sample

Table 4

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

Groups	Urea $^{\Psi\Psi}$ before EG treatment	Urea $^{\Psi\Psi}$ after EG treatment (Days)			
		Day 7	**Day 14**	**Day 21**	**Day 28**
I	26.81.81±0.24	26.58 ±0.05	26.60±0.04	26.75 ±0.07	26.72±0.19
II	26.87±0.14	31.59 ±0.46 a*	36.72 ±0.17 a*	39.46 ±0.24 a*	43.66±0.10 a*
III	27.12±0.51	27.86 ±0.13 b*e^{n}	27.34 ±0.12b*e^{ns}	27.63 ± 0.12 b*e^{ns}	27.20 ±0.21b*e^{ns}
IV	26.67±0.24	27.86 ±0.36 c*f^{ns}	27.56 ±021 c*f^{ns}	27.78 ±0.14 c*f^{ns}	27.23 ±0.36 c*f^{ns}
V	27.06±0.29	27.87 ±0.56 d*	27.79 ±0.31 d*	27.76 ±0.40 d*	27.45 ±0.42 d*

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

$^{\Psi}$ g/24hour urine

Table 5

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

Groups	Uricacid $^{\Psi\Psi}$ before EG treatment	Uricacid $^{\Psi\Psi}$ after EG treatment (Days)			
		Day 7	**Day 14**	**Day 21**	**Day 28**
I	47.68±0.56	47.25 ±0.30	47.40 ±0.19	47.69 ±0.09	47.91±0.24
II	46.68±0.35	60.22 ±0.08a*	70.99 ±0.74 a*	80.30 ±0.33 a*	90.27 ±0.39 a*
III	47.02±0.63	48.35 ±0.05b*e^{ns}	47.66 ±0.13b*e^{ns}	47.99 ±0.12 b*e^{ns}	47.38 ±0.14 b*e^{ns}
IV	47.69±0.21	48.18 ±0.11 c*f^{ns}	47.93 ±0.14 c*f^{ns}	48.08 ±0.17 c*f^{ns}	47.37±0.11 c*f^{ns}
V	47.53±0.41	48.17 ±0.12 d*	48.36 ±0.09d*	47.94 ±0.03 d*	47.53±0.415 d*

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

Groups	Creatinine$^{\psi\psi}$ beforeEG treatment	Creatinine$^{\psi\psi}$ after EG treatment (Days)			
		Day 7	**Day 14**	**Day 21**	**Day 28**
I	140.63±0.19	140.32±0.16	140.42±0.08	140.70 ±0.07	140.78±0.16
II	141.35±0.13	160.66 ±0.11 a*	175.69 ±0.24 a*	187.90 ±0.18 a*	197.92 ±0.19 a*
III	140.96±0.28	142.63±0.14 b*e^{ns}	142.11±0.41 b*e^{ns}	141.55 ±0.13 b*e^{ns}	141.30 ±0.04 b*e^{ns}
IV	140.86±0.23	142.79 ±0.12 c*f^{ns}	141.65 ±0.13 c*f^{ns}	142.35 ±0.16 c*f^{ns}	141.35 ±0.10 c*f^{ns}
V	141.58±0.24	142.63 ±0.03 d*s	141.61±0.17 d*	142.39±0.18 d*	141.37±0.02 d*

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$^{\psi\psi}$ mg/ 24 hr. urine sample

Table 7

Effect of ethanolic root extract of *Saccharum spontaneum* on serological parameters on 28th day of lithiasis

Serological Parameters	Group I	Group II	Group III	Group IV	Group V
Calcium**	8.57 ±0.16	12.23 ±0.11 a*	8.90 ±0.11 b*e^{ns}	8.89±0.13 c*f^{ns}	8.93±0.09 d*
Oxalate**	1.50±0.14	4.73±0.17 a*	1.67±0.19 b*e^{ns}	1.65±0.18 c*f^{ns}	1.74±0.17 d*
Magnesium**	2.85±0.15	1.53±0.05 a*	2.42±0.10 b*e^{ns}	2.41±0.02 c*f^{ns}	2.33±0.04 d*
Phosphorus**	6.39±0.20	8.81±0.12 a*	6.75±0.15 b*e^{ns}	6.39±0.20 c*f^{ns}	6.78±0.18 d*

Values are expressed as mean ± SD of six animals

Experimental design and comparison between the groups are as in table1

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

Units

**mg/dl *g/dl

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