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 crucial ions, viz. calcium, phosphorus and protein thereby contributing to renal stone formation. 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. The levels of serum urea, uric acid and creatinine 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.

KEYWORDS: Antilithiatic, *Saccharum spontaneum*, Ethylene glycol, Calcium, Phosphate and Protein.

INTRODUCTION

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 a systematic manner[1].Several *in vivo* model have been developed for the study of nephrolithiasis and to investigate the mechanisms involved in the formation of urinary stones and to ascertain the effects of various therapeutics agents on development and progression of the disease. Rats have been a suitable species for study of calcium oxalate deposition in the kidneys, a process that mimics the etiology of kidney stone formation in humans[2].

Several methods were used for induction of urolithiasis, which cause predominantly two types of hyperoxaluria, one acute, when the rat is challenged by a large single dose of lithogen and secondly chronic, when the rat is continuously challenged by small doses of lithogen for a period of time. In the present study, ethylene glycol-induced hyperoxaluria model was used to assess the antilithiatic activity in albino rats. Chronic administration of 0.75% ethylene glycol aqueous solution to male Wistar rats resulted in hyperoxaluria. The study of the urinary chemistry with respect to the stone forming minerals will provide a good indication of the risk of stone formation. In general, the crystallization of stone forming salts is due to an abnormal urinary composition that is either higher in crystallization promoters (e.g. calcium, oxalate and uric acid) or lower in inhibitors (e.g. citrate, glycosaminoglycans, kidney proteins such as nephrocalcin, Tamm- Horsfall mucoprotein uropontin), or both [3].As traditional medicines are usually taken by the oral route, same route of administration was used for evaluation of antilithiatic effect of the *S.spontaneum* against ethylene glycol induced urolithiasis in rats.

MATERIALS AND 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 resuspended 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 ethical committee permission license number is 659/02/a/CPCSEA.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 7^{th}, 14^{th}, 21^{st}, and 28^{th} days and the volume noted. Urinary calcium, phosphorus and protein and the serological parameters were estimated on 28^{th} day of lithiasis. To confirm the incidence of lithiasis, the animals were sacrificed and their kidneys were subjected to histopathological studies.

RESULTS

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, 2 and 3.The urinary excretion of calcium, oxalate and phosphorus are increased ($p < 0.05$) significantly on the 14^{th} day in calculi- induced (group II) animals when compared with normal control rats. Maximum levels of excretion were observed with group II on the 28^{th}day. However the calcium and phosphorus excretion was reduced significantly in the extract treated group (group III and IV), though normal values were reached. 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 of rats.

The present observation showed proteinuria in ethylene glycol induced urolithic rats on the 14^{th} and 28^{th} day (Table3). Administration of the extract had profound effects on minimizing the excretion of protein and thus has prevented the nidus formation for crystal nucleation (Group III and IV).

Table 1. Effect of ethanolic root extract of *Saccharum spontaneum* on calcium excretion in experimental nephrolithiasis (urine analysis)

Group	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 ± 0.25	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 ± 0.19 c*f^{ns}	1.87 ±0.11 c*f^{ns}
V	1.61±0.25	2.16 ±0.84 d*	2.09 ± 0.50 d*	2.13± 0.84 d*	1.94 ±0.07 d*

Values are expressed as mean ± SD of six animals

Experimental design

Group I : Control rats – received normal pelleted diet

Group II : Received 0.75% ethylene glycol in water for 28 days

Group III : Treated rats –Urolithiasis induced rats received *S.Spontaneum* extract (200 mg / kg body weight) by oral administration for 28 days at a rate of 1.0 ml / rat / day

. Group IV : Treated rats – Urolithiasis induced rats received ethanolic root extract of *S.Spontaneum* (300 mg / kg body weight) by oral administration for 4 weeks at a rate of 1.0 ml / rat / day

Group V : Standard drug thiazide treated rats – Urolithiasis induced rats received thiazide (150 µg / kg body weight) by oral administration for 30 days at a rate of 1.0 ml / rat / day.

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

Table 2. Effect of ethanolic root extract of S.spontaneum on phosphorus excretion in experimental nephrolithiasis

Group	Phosphorus ΨΨ before EG treatment	Phosphorus ΨΨ after EG treatment (Days)			
		Day 7	Day 14	Day 21	Day 28
I	6.54± 0.20	6.80 ± 0.15	6.82 ± 0.11	6.82 ± 0.11	6.76 ± 0.15
II	6.71± 0.15	8.18 ± 0.04 a*	9.77 ± 0.32 a*	10.41 ± 0.18 a*	12.01 ± 0.69 a*
III	6.58± 0.14	7.01 ±0.06 b*e^{ns}	6.95 ± 0.06 b*e^{ns}	6.98 ±0.16 b*e^{ns}	6.94 ±0.08 b*e^{ns}
IV	6.78± 0.08	6.96 ±0.12 c*f^{ns}	6.93 ±0.14 c*f^{ns}	6.88 ± 0.16 c*f^{ns}	6.86 ±0.13 c*f^{ns}
V	6.79± 0.02	7.03 ±0.12 d*	6.97 ±0.16 d*	6.96 ±0.08 d*	6.91 ± 0.12 d*

Values are expressed as mg/ 24 hr. urine sample

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

ΨΨ mg/ 24 hr. urine sample

Table 3.Effect of ethanolic root extract of *S.spontaneum* on protein excretion in experimental nephrolithiasis

Group	Before EG treatment	After EG treatment (Days)			
		Day 7	Day 14	Day 21	Day 28
I	1.02± 0.04	1.04 ± 0.10	1.08 ± 0.09	1.19 ± 0.07	1.14 ± 0.08
II	1.05± 0.02	4.12 ± 0.24 a*	5.95 ± 0.23 a*	7.68 ± 0.18 a*	9.29 ± 0.06 a*
III	1.13± 0.01	1.74 ±0.99 b*e^{ns}	1.48± 0.05 b*e^{ns}	1.60 ± 0.04 b*e^{ns}	1.27 ±0.04 b*e^{ns}
IV	1.16± 0.03	1.79 ±0.12 c*f^{ns}	1.46 ± 0.03 c*f^{ns}	1.58 ± 0.03 c*f^{ns}	1.26 ±0.04 c*f^{ns}
V	1.18± 0.03	1.69 ±0.09 d*	1.56 ±0.03 d*	1.49 ±0.03 d*	1.28 ±0.02 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

ΨΨ mg/ 24 hr. urine sample.

SERUM BIOCHEMICAL PARAMETERS

From the table 4 it is evident that the levels of serum urea, uric acid and creatinine were significantly increased (p < 0.05) in urolithiatic rats (Group II).

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* similar to standard drug thiazide.

Table 4.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
Urea (mg/ dl)	10.37±0.18	25.06±1.33 a*	10.75±0.16 b*e^{ns}	10.74±0.15 c*f^{ns}	10.76±0.12 d*
Uric acid (mg/ dl)	4.29±0.18	10.85±0.15 a*	4.87±0.09 b*e^{ns}	4.85±0.11 c*f^{ns}	4.91±0.23 d*
Creatinine (mg/ dl)	0.83±0.13	2.73±0.19 a*	0.96±0.01 b*e^{ns}	0.95±0.03 c*f^{ns}	0.98±0.07 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

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 [4].Ethylene glycol gets oxidized to oxalic acid leading to hyper oxaluria[5].

Hyper oxaluria is reported to be a more significant risk factor in the pathogenesis of stone formation [6].Likewise, ethylene glycol administration increased the urinary calcium level. It has been stated that hyper calciuria favors precipitation of calcium oxalate from urine[7].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[8].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].

A gradual increase in urinary phosphorus excretion was observed in ethylene glycol induced urolithic rats. Increased urinary phosphorus excretion along with oxalate stress seems to provide an environment appropriate for stone formation by forming calcium phosphate crystals, which epitaxially induces CaOx deposition.[10] Increased excretion of phosphorus has been reported in stone formers[11]and hyper oxaluric rats[12].Increased phosphorus excretion along with the oxalate stress seems to provide an environment appropriate for stone formation by forming calcium phosphate crystals, which epitaxially induces calcium oxalate deposition[13].

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 increase 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 (Christina *et al*., 2002; Karadi *et al*., 2006; Verma *et al*., 2009).

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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