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PREVENTIVE EFFECT OF *SACCHARUM SPONTANEUM* LINN.AGAINST GLYCOLIC ACID – INDUCED UROLITHIASIS IN MALE WISTAR ALBINO RATS

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ABSTRACT

The aim of the present study was to evaluate the antilithiatic activity of *Saccharum spontaneum* Linn. Healthy male Wistar rats were used in the present study and were divided randomly into 5 groups. Group I was served as normal control. Lithiasis was induced in rats by fed with a calculi-producing diet (CPD: commercial diet mixed with 3% glycolic acid) for 28 days (group II). The levels of protein, sodium, potassium, and chloride and lipid peroxidation were altered in urolithiatic rats. Supplementation with ethanolic extract of *S.spontaneum* (200 and 300 mg / kg body weight) by oral administration for subsequent 28 days at a rate of 1.0 ml / rat / day restored the levels and it brought back the values to near normal range in urolithiatic rats. The results indicate that the ethanolic extract of *S.spontaneum* is endowed with significant antiurolithiatic activity. Accordingly, it can be concluded that the supplementation of *S.spontaneum* root has a beneficial effect on urolithiasis induced by glycolic acid.

KEY WORDS: ***Saccharum spontaneum*, urolithiasis, glycolic acid, protein, sodium potassium chloride**

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INTRODUCTION

Medicinal plants are part and parcel of human since the dawn of civilization. In India, medicinal plants form the backbone of several indigenous traditional systems of medicine. Pharmacological studies have acknowledged the value of medicinal plants as potential source of bioactive compounds[1]. Medicinal plants are believed to be an important source of new chemical substances with potential therapeutic effects. Herbalism is a traditional medicinal or folk medicine practice based on the use of plants and plant extracts[2]. Kidney stone, are one of the most painful of the urologic disorders. Urolithiasis is the medical term used to describe stones occurring in the urinary tract[3]. Calcium containing stones, especially calcium oxalate monohydrate (whewellite), calcium oxalate dehydrate (weddellite) and basic calcium phosphate (apatite) are most commonly occurring ones to an extant of 75-90% followed by magnesium ammonium phosphate (struvite) to an extant of 10-15%, uric acid 3-10% and cystine 0.5-1[4]. The worldwide incidence of urolithiasis is quite high and in north India more than 80% of urinary calculi are calcium oxalate stones alone or calcium oxalate mixed with calcium phosphate[5]. Hyperoxaluria is the main initiating factor of human idiopathic calcium oxalate (CaOx) stone disease. Oxalate is a powerful crystallization-driving factor present in the urine. Retention of which enhances cell injury and causes early stages of lithogenesis[6]

Urinary stone disease continues to occupy an important place in everyday urological practice. The average life time risk of stone formation has been reported in the range of 5-10 %. A predominance of men over women can be observed with an incidence peak between the fourth and fifth decade of life. Recurrent stone formation is a common part of the medical care of patients with stone disease[7]. Urolithiasis is a recurrent renal disease affects 4-8% in UK, 15% in US, 20% in Gulf countries and 11% population in India. Stone formation tends to recur at very high rate; without preventative measures after a first stone. After 3 years this is about 40%, by 10 years up to 75% and by 25 years virtually every patient has formed at least one more stone[8]. There are several types, most commonly consisting of calcium phosphates and calcium oxalates; others are composed of magnesium ammonium phosphate (struvite), uric acid or cystine[9]. Epidemiological data suggests that 60-80% of stone is composed mainly of calcium oxalate (CaOx). Stones formation occurs when urinary concentrations of stone forming salts, exceed the limit of metastability for that salt in solution. This most often reflects excessive excretion of one or more stone constituents, deficient inhibitory activity in urine, or simply a low urine volume resulting in excessively concentrated urine[10].

The pathogenesis of calcium oxalate stone formation is a multi-step process, which includes-nucleation, crystal growth, crystal aggregation and crystal retention[11]. Various substances in the body have an effect on one or more of the above stone forming processes, thereby influencing a person's ability to promote or prevent stone formation. Promoters of stone formation facilitate stone formation whilst inhibitors prevent it. Low urine volume, low urine pH, calcium, sodium, oxalate, and urate are known to promote stone formation[12]. *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[13].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[14]. The recent resurgence of plant remedies results from several factors like effectiveness of plant medicines and no side effects compared to modern medicines. Plant medicine was commonly used for traditional treatment of some significant diuretic activity. Many investigators have demonstrated that studies of herbal plant used in traditional medicine as diuretic have increased recent years[15]and might be a useful tool in the treatment of urolithiasis. A large number of Indian medicinal plants have been used in the treatment of urolithiasis and they have been reported to be effective with fewer side effects[16].Even today, plants provide a cheap source of drugs for majority of world's population. Several pharmacological in vitro and in vivo investigations on the medicinal plants used in traditional antiurolithiatic therapy revealed their therapeutic potential[16]. Accordingly, we undertook the present study to assess the effectiveness of *saccharum spontaneum* Linn. a medicinal plant used to treat kidney stones, as a prophylactic agent against calcium oxalate stones in experimentally induced urolithiasis in rats

MATERIALS AND METHODS

Collection of plant material

Saccharum spontaneum Linn. was collected from Koorappalayam, Erode district, Tamil Nadu, India during the month of September to November, 2008. 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 sub acute toxicity, diuretic, pharmacological screening of anti urolithiatic and *In vivo* biological evaluation of urolithiatic studies in adult male wistar albino rats weighing about 150 to 200g 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 for in vivo biological evaluation studies

The rats were divided into 5 groups of six animals in each group and the experimental design of animals is given in table1 for *in vivo* studies

Group I : **Control rats** - received normal pelleted diet.

Group II : **Glycolic acid intoxicated rats -** Urolithiasis induced by fed with a calculi-producing diet (CPD: commercial diet mixed with 3% glycolic acid) for 28 days.

Group III : **Root extract treated rats -** Urolithiasis induced rats received ethanolic root extract of *S.spontaneum* (200 mg / kg b.w.) by oral administration for 28 days at a rate of 1.0 ml / rat / day.

Group IV : **Root extract treated rats -** Urolithiasis induced rats received ethanolic root extract of *S.spontaneum* (300 mg / kg b.w.) by oral administration for 28 days at a rate of 1.0 ml / rat / day.

Group V : **Standard drug thiazide treated rats -** Urolithiasis induced rats received thiazide (150µg/ kg b.w.) by oral administration for 28 days at the rate of 1.0 ml / rat / day.

Collection of serum sample

After the experimental regimen the animals were sacrificed by cervical decapitation under light ether anesthesia. Blood was collected and centrifuged for 10 min. at 2500 rpm. The serum supernatant was collected and then diluted with water in the ratio of 1:10. Aliquots of the diluted serum were then used for the determination of serum constituents and serum enzymic activities.

Collection of urine sample

Before the day of sacrifice the rats were placed in metabolic cages and urine was collected for 24 hours. Urine was freed from faecal contamination. Rats were provided with water but no feed. Urine collected in 50 ml beaker maintained at 0^{o}C in an ice bath. The collected urine samples were centrifuged for 10 minutes and any sediment present was discarded. The urine was used for further analysis.

Collection of liver and kidney samples

The experimental animals were sacrificed, liver and kidney were removed immediately, washed with ice cold saline10% tissue homogenate was prepared by homogenizing 1.0g of chopped liver or kidney tissue in 10ml of 0.1M tris HCl homogenizing buffer at pH 7.5. The homogenate was used for assaying the enzyme activities

Chemicals

All the chemicals used in the present study were of analytical reagent grade.

Statistical analysis

The results of the biochemical estimations were reported as mean ± SD of six animals in each group. Total variations, present in a set of data were estimated by one way Analysis Of Variance (ANOVA) followed by the analysis of level of significance between different groups based on ANOVA using SPSS statistical package (Version 15.0). Difference among means were analysed by least significant difference (LSD) at 5% level ($p<0.05$).

RESULTS

Protein levels in serum, urine, kidney and liver

Table 1 shows the protein levels in serum, urine, liver and kidney in control and experimental rats. From the table 1 it is evident that the levels of protein in serum, kidney and liver were significantly decreased in glycolic acid induced rats (group II) whereas, in urine the levels were significantly increased in group II when compared to control rats (group I). Treatment with *S.spontaneum* root extract

significantly increased the levels of protein in serum, liver and kidney and minimizing the excretion of protein and thus might have prevented the nidus formation or crystal nucleation Whereas, in urine, the levels were significantly decreased and it brought back the values to normal range in groups (Group III and IV) When *S.spontaneum* root extract treated rats (group III-IV) were compared with thiazide treated rats (Group V), there was no significant difference between the groups of rats.

Electrolytes levels in serum and urine

Table 2 represents the activities of serum electrolytes such as sodium, potassium and chloride in control and experimental rats. From the table 2 it is evident that serum electrolytes sodium and chloride were significantly increased ($p < 0.05$), whereas potassium level were significantly decreased ($p < 0.05$) in urolithiatic rats (Group II), when compared to control rats (group I). The alterations in these electrolyte levels may be due to the crystal retention in the membrane which alters the transport mechanism of electrolyte. Group III and IV rats treated with the *S.spontaneum* root extract showed a significant restoration of serum electrolyte levels when compared to urolithiatic rats (group II) which might be an indication of recovery due to antiurolithiatic property of *S.spontaneum*.

Table 1
Effect of *S.spontaneum* root extract on protein in serum, urine, kidney and liver of control and experimental rats

Group	Serum $^{\Psi}$	Urine*	Kidney * *	Liver* *
I	6.27 ± 0.08	1.16 ± 0.08	197.22 ± 0.05	220.81 ± 0.14
II	4.64 ± 0.04 a*	8.68 ± 0.15 a*	195.49 ± 0.26 a*	215.97 ± 0.57 a*
III	6.26 ± 0.01 b* e^{ns}	1.35 ± 0.13 b* e^{ns}	196.26 ± 0.15 b* e^{ns}	220.53 ± 0.14 b* e^{ns}
IV	6.23 ± 0.02 c*f^{ns}	1.32 ± 0.22 c*f^{ns}	196.22 ± 0.11 c*f^{ns}	220.51 ± 0.15 c*f^{ns}
V	6.29 ± 0.03 d*	1.37± 0.17 d*	196.31 ± 0.57 d*	220.61 ± 0.07 d*

Values are expressed as mean ± SD of six animals

Experimental design

Group I : Control rats - received normal pelleted diet

Group II : Urolithiasis induced rats - Fed with a calculi-producing diet (CPD: commercial diet mixed with 3% glycolic acid) for 28 days.

Group III : Plant drug treated rats - urolithiasis induced rats received *S.spontaneum* root extract (200 mg / kg body weight) by oral administration for subsequent 28 days at a rate of 1.0 ml / rat / day

Group IV : Plant drug treated rats - urolithiasis induced rats received *S.spontaneum* root extract (300 mg / kg body weight) by oral administration for subsequent 28 days 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 subsequent 28 days at a rate of 1.0 ml / rat / day.

Group comparison

'a' represents comparison between group II and I
'b' represents comparison between group III and II
'c' represents comparison between group IV and II
'd' represents comparison between group V and II
'e' represents comparison between group III and V
'f' represents comparison between group IV and V

The symbols represent statistical significance p* < 0.05; ns - not significant

Units
$^{\Psi}$g/dl
★ g/24hour urine
★ ★ mg/g tissue

Table 2
Effect of oral administration of *S.spontaneum* root on electrolytes in normal and glycolic acid induced urolithiatic rats

Group	Sodium★	Potassium★	Chloride★
I	140.66 ± 0.18	4.33 ± 0.20	96.62 ± 0.28
II	190.53 ± 0.19 a*	3.01 ± 0.044 a*	120.81 ± 0.16 a*
III	141.08 ± 0.01 b* e^{ns}	4.07 ± 0.11 b* e^{ns}	97.03 ± 0.09 b* e^{ns}
IV	141.03 ± 0.21 c*f^{ns}	4.02 ± 0.12 c*f^{ns}	97.01 ± 0.12 c*f^{ns}
V	141.11 ± 0.12 d*	4.08 ± 0.21 d*	97.97 ± 0.012 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
★mEq/L

Table 3
Effect of S.spontaneum root extract of on urinary electrolytes parameters of urolithiatic rats

Group	Sodium★★	Potassium★★	Chloride★★
I	69.17 ± 0.08	81.39 ± 0.61	170.72 ± 0.27
II	17.16±0.09 a*	190.41± 0.11 a*	82.15 ± 0.21 a*
III	67.75.±0.15b*e^{ns}	80.18 ±0.23 b*e^{ns}	168.69 ± 0.06b* e^{ns}
IV	68.52±0.11c*f^{ns}	80.58±0.12 c*f^{ns}	168.46±0.20c*f^{ns}
V	67.83 ± 0.12 d*	80.34± 0.13 d*	167.91 ± 0.03 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
★★mEq/24hour urine

Thiazide also showed the similar curative effect like that of plant extract 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. Table 3 represents the activities of urinary electrolytes such as sodium, potassium and chloride of control and experimental rats. From the above table it is evident that these urinary electrolytes, sodium and chloride were significantly decreased and potassium was significantly ($p < 0.05$) increased in calculi induced animals (Group II) when compared to control rats (Group I). Treatment with plant extract restored the levels to near normal range in group III and IV rats. Thiazide also showed the similar curative effect like that of plant extract in group V rats. When *S.spontaneum* root extract treated rats (Group III and IV) were compared with thiazide treated rats (Group III), there was no significant difference between these groups of rats.

Lipid peroxidation in liver and kidney

Table 4 shows the activity of lipid peroxidation in liver and kidney of control and experimental rat

Table 4
Effect of *S.spontaneum* root extract of on LPO in liver and kidney of control and experimental rats

Group	Liver¥¥	Kidney ¥¥
I	2.51 ± 0.09	2.61 ± 0.17
II	8.09 ± 0.05 a*	4.64 ± 0.21 a*
III	2.95 ± 0.01 b* e^{ns}	2.48 ± 0.01 b* e^{ns}
IV	2.92 ± 0.01 c*f^{ns}	2.41 ± 0.11 c*f^{ns}
V	2.31 ± 0.08 d*	2.52 ± 0.21 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

n moles of MDa formed/mg protein

From the table 4, it is evident that the levels of lipid peroxidation were significantly increased ($p < 0.05$) in liver and kidney homogenate on glycolic acid intoxication (Group II) when compared to control rats (Group I). Group III and IV rats treated with the *S.spontaneum* root extract showed a significant decrease in the levels of lipid peroxidation in homogenates of liver and kidney when compared to urolithiatic rats (group II), which might be an indication of recovery due to the administration of ethanolic extract of *S.spontaneum* which possess free radical scavenging activity and antiurolithiatic property. 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. This result gives a supportive evidence for the antiurolithiatic activity of ethanolic extract similar to standard drug thiazide**.**

DISCUSSION

Kidney stone disease has afflicted humankind since antiquity and can persist, with serious medical consequences, throughout a patient's lifetime. In addition, the incidence of kidney stones has been increased in most societies in the last five decades, especially in association with economic development. In spite of tremendous advances in the field of medicine, there is no truly satisfactory drug for the treatment of nephrolithiasis. Recently, there is increasing evidence that many healthy natural food and medicinal herbal and supplements have the potential to become valuable complementary therapy in the treatment of various renal disorders and in the protection against iatrogenic nephrotoxicity. Proteinuria reflects proximal tubular dysfunction. Supersaturation of urinary colloids results in precipitation crystal initiation particle which when trapped acts as a source leading to subsequent crystalgrowth[17]. Protein excretion was increased in hyperoxaluric rats[18].Supersaturation of urinary colloids results in precipitation as a crystal initiation particle, which when trapped, act as a nidus and leading to subsequent crystal growth[16] .Protein urea reflects proximal tubular dysfunction [19].

Renal injury whether a result of renal calculi decreases nephron population. The effect of this reduction in renal mass is to place an increased workload on surviving nephron. Through a series of microcirculary adaptations, glomerular blood flow and capillary hydrostatic pressure is increased, and the filtration rate in each glomerulus is augmented. Gomeruli undergoing this hyperfiltration process are subjected to hemodynamic stress; which damages capillary integrity and predisposes to leakage of protein into the urine[20]. Sodium and chloride ions excretion from the body is a function of arterial blood pressure[21]. Sodium depletion stimulates rennin release and subsequent production of Angiotensin II, a potent vasoconstrictor[22]. Increased blood sodium levels inhibit rennin release from the juxtaglomerular cells and consequent withdrawal of angiotensin II[23] .When modulation of the rennin angiotensin system is pharmacologically prevented, changes in salt intake markedly affect long term levels of arterial blood pressure [24]. There is therefore a need to strike a balance in the levels of blood sodium and chloride to avoid either of the extreme of hypotension or hypertension. [25]reported that the hypernatremia is rare but does occur when there is loss of body fluids containing less sodium than blood along with water intake restriction or if there is excessive sodium intake with limited liquid intake. [26]reported that the hypernatremia almost always indicates water depletion. The present increase of serum sodium level is suspected to be due to the inability of the kidneys to excrete adequate sodium from the tubular fluid.

LPO is a degenerative pathway of membrane components mediated through free radicals produced in the cell [27].Membrane injury facilitated the fixation of calcium oxalate crystals and subsequent growth into kidney stones [28].Oxalate the major stone forming constituent has been reported to induce free radical generation, which results in peroxidative injury to renal epithelial cells [29].Oxalate-induced peroxidative injury is one of the major mechanisms in promoting crystal attachment to renal epithelial cells[30].

CONCLUSION

The present study indicates that the administration of ethanolic root extracts of *Saccharum spontaneum* to rats with glycolic acid induced nephrolithiasis reduced and prevented the growth of kidney stones, renal and hepatic impairment. Administration of root extract of the plant against glycolic acid induced urolithiasis, mediated possibly

through a combination of CaOx crystal inhibitory, diuretic, antioxidant and hyper magneseuric effects, rationalize its medicinal use for urinary pathologies in the Indian folk medicine. Accordingly, it can be concluded that the supplementation of *S.spontaneum* root has a beneficial effect on nephrolithiasis induced by glycolic acid and may be also by other chemical factors.

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