



AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: <http://www.ajptr.com/>

Prophylaxis of Calcium Oxalate stones by *Saccharum Spontaneum* Linn. on Glycolic acid induced Urolithiasis in Male Wistar Albino rats

M.Sathya*¹, R.Kokilavani¹,

1. Kongunadu Arts and Science College, Coimbatore-29.

ABSTRACT

Urolithiasis in its different forms is a frequently encountered urological disorder. For many years it has been at the forefront of urology. In the present study ethanolic extract of whole plant of *Saccharum spontaneum* (Linn.) was studied for its antiurolithiatic activity against most common type of renal stone i.e. calcium oxalate. Lithiasis was induced in rats by fed with a calculi-producing diet (CPD: commercial diet mixed with 3% glycolic acid) for 28 days. Glycolic acid treated rats showed significant increase ($p < 0.05$) the activities of oxalate synthesizing enzymes such as glycolic acid oxidase (GAO) in liver and lactate dehydrogenase (LDH) in serum, urine kidney and liver Administration of the ethanolic extract of *S.spontaneum* (200mg and 300/kg b.wt.dose⁻¹ day⁻¹oral⁻¹) has significantly ameliorated to near normalcy in the curative group.. The results of the present study confirmed that *S.spontaneum* can be used as a curative agent for urolithiasis.

Keywords: Urolithiasis, *Saccharum spontaneum*, glycolic acid, glycolic acid oxidase, lactate dehydrogenase,

*Corresponding Author Email: sathya_biom84@yahoo.co.in

Received 21 August 2012, Accepted 28 August 2012

Please cite this article in press as: Sathya M *et al.*, Prophylaxis of calcium oxalate stones by *Saccharum spontaneum* Linn. on glycolic acid induced urolithiasis in male wistar albino rats. American Journal of PharmTech Research 2012.

INTRODUCTION

Urolithiasis is the formation of calculi, or the condition associated with urinary calculi. The incidence of renal calcium oxalate stone is increasing in most countries and crystal formation in urine has been recognized for many centuries as the first step in stone disease. It is characterized by a high recurrence rate that requires efficient preventive methods. Although various pharmacological agents are available and many are effective, such treatments can fail. The introduction of new techniques for removing stones, e.g. ESWL, has improved the management of urolithiasis, but recent studies show that, apart from the high cost that ESWL, exposure to shock waves even in therapeutic doses, is associated with several adverse effects, including renal injury, decrease in renal function, and more importantly an increase in stone recurrence¹.

Thus, more efforts are needed to better assess medical therapy and to develop new agents that can be used either alone or combined to prevent stone formation more efficiently². Our attention is particularly on phytotherapy, which is common in traditional medicine as an alternative to primary healthcare in many countries.

A good proportion of the world population particularly those living in developing countries depend mostly on herbal medicines for their health needs. Medicinal herbs are indispensable part of the traditional medicine practiced all over the world due to easy access, low cost and ancestral experience, safe and no undesirable side effects. They increases the resistance of body against infection to promote quick restoration of physiological functions after depleting diseases and claimed to improve physical and mental health³.

Traditional medicine is the synthesis of therapeutic experience of generations of practicing physicians of indigenous systems of medicine. Throughout the history of mankind, many infectious diseases have been treated with herbals. The traditional medicine is increasingly solicited through the tradipractitioners and herbalists in the treatment of infectious diseases.

An appropriate experimental urolithiasis model is of paramount importance for studying the pathogenesis of urinary tract stone, evaluating the relative importance of various lithogenic factors and assessing the efficacy of plant drug in preventing stone formation. Thus we have established an glycolic acid induced urolithiasis model in rat. The present investigation has been under taken to study the role of the ethanolic extract of *Saccharum spontaneum* as a remedy for glycolic acid induced calcium oxalate stone 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 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 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 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°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 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) 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 receive thiazide (150µg/ kg b.w.) by oral administration for 28 days at the rate of 1.0 ml / rat / day.

Collection of urine sample

Before the day of sacrifice the rats were placed in metabolic cages and urine was or 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°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 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 liver and kidney samples

The experimental animals were sacrificed, liver and kidney were removed immediately, washed with ice cold saline 10% 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 \pm 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 AND DISCUSSION

Tables 1 and 2 represent the activities of oxalate synthesizing enzymes such as lactate dehydrogenase (LDH) levels in serum, urine, kidney and liver and GAO in liver of control and experimental rats. The glycolic acid induced urolithiatic rats (group II) exhibited significant increase ($p < 0.05$) in liver GAO activity when compared to that of the normal control rats (group I). The LDH activity was monitored in urine, serum, kidney and liver.

Table 1 Effect of *S.spontaneum* root extract on LDH in serum and urine of control and experimental rats

Group	Serum [#]	Urine ^s	Kidney ^ψ	Liver ^ψ
I	150.41 ± 0.14	130.55 ± 0.16	2.31 ± 0.186	3.21 ± 0.19
II	200.05 ± 0.05 a*	190.54 ± 0.10 a*	5.11 ± 0.08 a*	6.74 ± 0.17 a*
III	152.09 ± 0.12 b* e ^{ns}	129.67 ± 0.17 b* e ^{ns}	2.36 ± 0.01 b* e ^{ns}	3.26 ± 0.05 b* e ^{ns}
IV	152.08 ± 0.08 c*f ^{ns}	129.59 ± 0.16 c*f ^{ns}	2.35 ± 0.024 c*f ^{ns}	3.24 ± 0.05 c*f ^{ns}
V	152.11 ± 0.014 d*	129.75 ± 0.01 d*	2.38 ± 0.15 d*	3.28 ± 0.02 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

μ moles of phenol liberated / L

\$ μ moles of pyruvate liberated / 24 hr urine

Ψ μ moles of phenol liberated / min/ mg protein

Table 2. Effect of *S.spontaneum* root extract on GAO in liver of control and experimental rats

Group	Liver Ψ (n moles of glyoxylate formed /min.mg protein)
I	3.35 \pm 0.17
II	6.25 \pm 0.22 a*
III	3.54 \pm 0.02 b* e ^{ns}
IV	3.52 \pm 0.13 c*f ^{ns}
V	3.59 \pm 0.09 d*

Values are expressed as mean \pm 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 Ψ n moles of glyoxylate formed/min/ mg protein

Liver and kidney act as the main sites of endogenous oxalate synthesis. The activities of oxalate synthesizing enzyme were assayed in the control and experimental groups and are presented in table. LDH, a cytosolic enzyme is a regulator of many biochemical reactions in the body tissues and fluids. The enzyme catalyses the coupling of oxidation and reduction of glyoxylate in the presence of pyridine nucleotide coenzyme, with the simultaneous formation of glycolate and oxalate. LDH is a bi substrate enzyme that operates in the presence of high ratio of $NAD^+/NADH$. The activity of LDH was significantly increased in the liver of ethylene glycol treated rats which may due to the substrate-mediated induction of the enzyme⁴.

Oxalate can exert derangement of electron transport chain and electron leak by 3 mechanisms. Increased oxalate releases arachidonic acid that uncouples electron transport and increases hydrogen peroxide production via complexes I and III in the mitochondrial respiratory chain⁵. Inhibition of ETC by the prevailing increased $NADH/NAD^+$ ratio by the increased activities of LDH and GAO (Muthkumar and Selvam, 1997) and Mitochondrial membrane damage is obvious in oxalate toxicity leading to impaired electron transport^{6,7}.

LDH is an oxalate synthesizing enzyme; its activity was increased on ethylene glycol administration. It was released into the blood serum and urine. This may be attributed to oxalate induced renal and hepatic cellular damage. Renal damage is particularly confined to the proximal

tubule, a part of the nephron closely involved in handling urinary oxalate. Further damage to proximal tubular epithelium is generally associated with the shedding of brush border membrane thereby causing crystal retention⁸.

Oxalate is synthesized by glycolic acid oxidase (GAO). It is present only in liver but not in kidney. Glycolic acid is synthesized from oxalate and to produce hydrogen peroxide and superoxide anions⁹. The increase in the activities of this enzyme in hyperoxaluric acid rats results in an increase in free radical and lipid peroxidation¹⁰.

They were significantly raised ($p < 0.05$) in the urolithic rats when compared with that of the normal control rats. Treatments with ethanolic root extract of *S.spontaneum* reduced the activities of GAO and LDH activities to near normal control rats. When *S.spontaneum* root extract treated rats group III-IV were compared with thiazide treated rats (Group V) and there was no significant difference in the levels of oxalate synthesizing enzymes between these groups of rats. Treatment with *S.spontaneum* root extract significantly decreased ($p < 0.05$) the levels and brought back the values to near normal levels in groups (group III- IV). Treatment with thiazide significantly decreased ($p < 0.05$) the levels and brought back the values to near normal range in group III rats.

Our results are in agreement with the findings of Prakasam and Kalaiselvi (2005) who reported an increased lactate dehydrogenase (LDH) activity in urolithiatic rats and their restoration to normal levels by L-arginine administration by decreasing the lactate dehydrogenase level in liver and kidney.

Our results are in accordance with that of Soundarajan *et al.* (2006) who showed the decreased level of GAO and LDH activity during *Aerva lanata* aqueous suspension with concomitant decrease in kidney oxalate level may prove beneficial as a prophylactic measure in preventing stone recurrence.

Soundarajan *et al.*, (2007). has reported an increased lactate dehydrogenase (LDH) activity in urolithiatic rats. Therapeutic treatment of *Aerva lanata* aqueous suspension had minimized the stress and thus decreased the enzymes related to stone synthesis and protected the cell integrity.

CONCLUSION

In conclusion, the presented data indicate that administration of the ethanolic extract of *S.spontaneum* to rats with glycolic acid induced lithiasis reduced and prevented the growth of urinary stones, supporting folk information regarding antiurolithiatic activity of the plant. Our results show that the anti urolithiatic effect of the plant may be due to its antioxidant, free radical

scavenging properties of the secondary metabolites present in the plant. These effects could conclude the antiurolithiatic property of *S.spontaneum*.

REFERENCES

1. Begun FP, Knoll CE, Gottlieb M, Lawson RK Chronic effects of focused electro hydraulic shock waves on renal function an hyper tension. J Urol 1991;145: 635-639.
2. Atmani F, Slimani Y, Mimouni M, Hach B. Prophylaxis of calcium oxalate stones by *Herniaria hirsuta* on experimentally induced nephrolithiasis in rats. BJU International 2003; 92(1): 137-140.
3. Ayoka RAO, Akomolafe O, Iwalewa EO, Ukponmwan OE. Studies on the anxiolytic effect of *Spondias mombin* L. (Anacardiaceae) extracts. Afr J Trad 2005; 2 (2): 153.
4. Pragasam V, Kalaiselvi P. Counteraction of oxalate induced nitrosative stress by supplementation of L-arginine, a potent antilithic agent. Clin Chem Acta 2005; 354(1-2):159 – 166.
5. Cocco T. Di M, Papa PS, Lorusso M. Arachidonic acid interaction with the mitochondrial electron transport chain promotes reactive oxygen species generation. Free Rad Biol Med 1999; 27(1-2):51-59.
6. Selvam R. Calcium oxalate stone disease: role of lipid peroxidation and antioxidants. Urol Res. 2003; 30(1):35-47.
7. Cao LC, Honeyman TW, Cooney R, Kennington L, Scheid CR, Jonassen JA. Mitochondrial dysfunction is a primary event in renal cell oxalate toxicity. Kidney Int 2004; 66(5): 1890-900.
8. Khan SR, Shevock PN, Hackett RL. Urinary enzymes and calcium oxalate nephroilthiasis. J Urol 1989; 142(3):846-849.
9. Santhoshkumar M, Selvam R. Supplementation of vitamin E and selenium prevents hyperoxaluria in experimental urolithic rats. J Nutri Biochem 2003; 14(6): 306 – 313.
10. Gutteridge JMC, Westermarck T, Halliwell B. Oxygen radical damage in biological system. Pub Med 1987; 92(4):193-198.
11. Soundarajan P, Mahesh R, Ramesh T, Begum VH. Effect of *Aerva lanata* on calcium oxalate urolithiasis in rats. Indian J Experimental Biology 2006; 44: 981-986.
12. Soundarajan, P., Mahesh,R., Ramesh, T. and Hazeena Begum,V. Biopotency of *Aerva lanata* Linn on Membrane Bound ATPases and Marker Enzymes in urolithic Rats. Int J Biological Chem 2007; 1(4): 221-228.