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EFFECT OF METHANOLIC LEAF EXTRACT OF *COCHLOSPERUM VITIFOLIUM* ON GLYCOLIC ACID INDUCED UROLITHIASIS IN RATS

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ABSTRACT

The effect of the alcoholic extract of *Cochlosperum vitifolium* (Cochlospermaceae) against glycolic acid induced urolithiasis urolithiasis in albino rats is summarized in this study. Lithiasis was induced in rats by fed with a calculi -producing diet (CPD: commercial diet mixed with 3% glycolic acid) for 28 days and was manifested by high urinary calcium, phosphate, oxalate, protein, and low urinary magnesium content. Therapeutic treatment with plant extract (300 and 600 mg/kg b.wt.dose -1 day-1oral-1) has significantly ameliorated to near normalcy in the curative group. It also increased the urine volume, thereby reducing the tendency for crystallization. These results of the present study concluded that *C.vitifolium* can play an important role in the prevention of disorders associated with kidney stone formation.

INTRODUCTION

Urolithiasis can be produced in rats by induction of acute or chronic hyperoxaluria by using a variety of agents such as ethylene glycol, sodium oxalate, ammonium oxalate, hydroxyl-L-proline and glycolic acid. Kidney being the principal target for EG induced toxicity. Its administration to the experimental animals for 28 days resulted in substantial excretion of oxalate and deposition of micro crystals in kidney. EG is broken down in vivo into four organic acids viz., glycolaldehyde, glycolic acid, glycooxalic acid and oxalic acid leading to hyperoxaluria which is the main initiative factor for lithiasis. Oxalate urolithiasis was produced by the addition of 3% glycolic acid to the diet for a period for 4 weeks. In addition, oxalate precipitates as a calcium oxalate crystals in kidney. Oxalate metabolism is considered almost identical between rats and humans. Hence, rats are the most frequently used animals in models of calcium oxalate deposition in the kidneys, a process that mimics the etiology of kidney stone formation in human. Therefore in the present study, glycolic acid was preferred to induce lithiasis. *Cochlosperum vitifolium* is a traditional herb, it has excellence medicinal value; has been advocated in the treatment gynaecological troubles, respiratory disease. Leafs are used as galactagogue and diuretic and in ayurveda system leafs are also used as astringent, emollient, refrigerant, diuretic, purgative, tonic, and aphrodisiac and useful in treatment of dyspepsia, burning sensation, piles and sexual weakness. 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.

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MATERIALS AND METHODS

Collection of plant material

Cochlosperum vitifolium was collected from Tirumala hills, India during the month of September to November 2013. The plant was identified and authenticated by taxonomist Dr. K.Madhava chetty Assistant Professor, Department of Botany, SV University, Tirupathi India. Voucher specimen was deposited in herbarium centre.

Preparation of the methanolic leaf extract for in vivo Studies

Leafs of the plants were washed, shade dried, powdered and stored in tight containers under refrigeration. 100g of *C.vitifolium* 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 in adult male wistar albino rats weighing about 150 to 300 g were collected from animal breeding centre. 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 ± 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. 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) for28 days.
Group III : Leaf extract treated rats - Urolithiasis induced rats received methanolic leaf extract of *C.vitifolium* (300 mg / kg b.w.) by oral administration for 28 days at a rate of 1.0 ml / rat / day.
Group IV : Leaf extract treated rats - Urolithiasis induced rats received methanolic leaf extract of *C.vitifolium* (600 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 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 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.

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

Table 1 represents the serum mineral constituents -calcium, magnesium, phosphorus and oxalate in control and experimental rats. Calcium, phosphate and oxalate play a vital role in renal calculogenesis. In glycolic acid induced rats, the levels of serum calcium, oxalate and phosphorus were significantly ($P<0.05$) increased in group II rats whereas the level of magnesium significantly decreased. When

C.vitifolium leaf extract treated rats (Group III and IV) were compared with thiazide treated rats (Group V) and there was no significant difference in the levels of parameters tested between these groups of rats. This result gives a supportive evidence for the antiurolithiatic activity of methanolic leaf extract *C.vitifolium*, which is similar to that of standard drug thiazide.

Table 1. Effect of *C.vitifolium* leaf extract of on calculi-forming constituents in serum of control and experimental rats

Group	Calcium mg	Oxalate mg	Magnesium mg	Phosphorus mg
I	8.47 ± 0.11	1.75 ± 0.21	2.77 ± 0.19	5.12 ± 0.54
II	11.24 ± 0.06 a*	4.77± 0.18 a*	1.45 ± 0.13 a*	7.25 ± 0.16 a*
III	8.52 ± 0.03 b* ens	1.79 ± 0.02 b* ens	2.49 ± 0.17 b* ens	5.29 ± 0.024 b*ens
IV	8.92 ± 0.08 c*fns	1.75 ± 0.21 c*fns	2.54 ± 0.01 c*fns	5.28 ± 0.052 c*fns
V	8.84 ± 0.01 d*	1.82 ± 0.014 d*	2.44 ± 0.14 d*	5.32 ± 0.02 d*

DISCUSSION

An increased urinary calcium concentration is a factor favoring nucleation and precipitation of CaOx or apatite (calcium phosphate) from urine and subsequent crystal growth. This fact, combined with the increased urinary calcium leads to their supersaturation in urine and finally stone formation. One mechanism currently proposed as an important to prevent the formation of urinary stone is the presence of substance in urine that prevents calcium salt crystallization 7.By inhibiting calcium excretion the drug decreases the supersaturation of the urine with respect to CaOx and thereby decreased the risk of stone formation. Apart from urinary calcium excretion, decrease in serum calcium was evident in urolithiatic rats 8,9.The increase in calcium and phosphate excretion could be due to defective tubular reabsorption in the kidneys. The treatment with *C.vitifolium* leaf extract markedly reduced and normalized the levels of these ions, showing the protective effect of *C.vitifolium* extract against urolithiasis. On the contrary *C.vitifolium* extract treatment significantly ($P<0.05$) increased the serum magnesium levels. Review of literature suggest that inhibitor effect of magnesium on crystallization in urine and formation of calcium oxalate crystals by virtue of ability of magnesium to form a complex with free oxalate in the urine thereby forming a soluble complex thus reducing the availability of free oxalate to complex with calcium. Accordingly in group II, the magnesium level in serum was decreased which is a common feature in urolithiasis. Urinary phosphorus was observed in calculi induced 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 7.Treatment with methanolic extract of *Cochlosperum vitifolium* restored phosphorus levels significantly, thus reduced the risk of stone formationNormal urine contains many organic and inorganic inhibitors of crystallization; magnesium is one such well known inhibitor 6. Magnesium complexes with oxalate, thus reducing CaOx supersaturation in urine as consequence decrease the growth and nucleation rates of calcium crystals 12, 13.Review of literature suggest that inhibitor effect of magnesium on crystallization in urine and formation of calcium oxalate crystals by virtue of ability of magnesium to form a complex with free oxalate in the urine thereby forming a soluble complex thus reducing the availability of free oxalate to complex with calcium [1,2].

Accordingly in group II, the magnesium level in urine was decreased which is a common feature in urolithiasis. Our findings were similar to that of Gilhotra umesh and Christina (2011) who reported that Rotula aquatica Lour extract reduced the urinary calcium and oxalate excretion in the urolithiatic rats and hence minimize the conditions favorable for crystal growth. Our results coincides with that of Srithar et al. (2011) who showed that leaf extract of Crataeva magna altered the excretion of calcium and oxalate in urine and confirmed the stone inhibitory effect. Bahuguna et al. (3009) showed that aqueous and alcoholic flower extracts of Jasminum Auriculatum Vahl. reversed the levels of calcium, oxalate and phophorus to normal values in both curative and preventive regimens and prevent the formation of stones in the urinary system. Freitas et al. (3002) showed that aqueous extract of Phyllanthus niruri acted as endogenous inhibitors of magnesium in urolithiatic rats.Our results are in agreement with the findings of sathya et al. (2011) who reported that Acalypha indica methanolic extract lowered the excretion of calcium and phosphorus in urolithiatic rats and thus reduce the risk of stone formation [3,4].

CONCLUSION

The results of the present study have shown that the urinary stones could be dissolved with methanolic extract of *C.vitifolium*. The recurrence of stones could also be prevented to a greater extent. The antiurolithiatic activity of this plant can be attributed to its ability to reduce the super saturation of urine with calculogenic

ions, diuretic property and anti oxidant potential.

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