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Effect of *Cucumis Trigonus* on Mineral Constituents of Urolithatic Rats

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ABSTRACT

Plants are utilized as therapeutic agents since time immemorial in both organized (Ayurveda, Unani) and unorganized (folk, tribal, native) form. The ethanolic fruit extract of *Cucumis trigonus* Roxb of family Cucurbitaceae was used to treat the urolithiasis induced by ethylene glycol. On this course, the extract also repairs the changes that happened in the mineral constituents like calcium, magnesium, phosphorus and oxalate in serum and urine of the urolithatic rats. The ethanolic fruit extract (150 mg / kg b.w.) elevated the levels of reduced mineral parameters like calcium, magnesium and phosphorus and reduced the level of oxalate in serum and reduced the levels of calcium, phosphorus and oxalate and elevated the level of magnesium in urine significantly ($p < 0.05$) when compared with the toxic groups. The results shown by the ethanolic fruit extract (150 mg / kg b.w.) was compared to standard thiazide drug treated group, showing no significant difference ($p < 0.05$) and thus it proves that the ethanolic fruit extract of *Cucumis trigonus* exhibits potent antiurolithatic activity.

Keywords: *Cucumis trigonus*, mineral constituents, thiazide, ethylene glycol.

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INTRODUCTION

The urinary system is a group of organs in the body concerned with filtering out excess fluid and other toxic substances from the bloodstream. The substances are filtered out from the body in the form of urine. Urine is a liquid produced by the kidneys, collected in the bladder and excreted through the urethra and is used to extract excess minerals or vitamins as well as blood corpuscles from the body. One aspect of its function is to get rid of the body's waste products that accumulate as a result of cellular metabolism. Because of this, the urinary system is sometimes referred to as the excretory system ¹. The primary functions of kidneys are to maintain a stable internal environment for optimal cell and tissue metabolism. They separate urea, mineral salts, toxins and other waste products from the blood ².

Urolithiasis refers to calcifications that form in the urinary system, primarily in the kidney or ureter, and may also form in or migrate into the lower urinary system like bladder or urethra. Kidney stones are formed within the kidney, within the ureter or in the bladder ³. Stone formation is also caused by hyperoxaluria, which leads to increased renal retention and excretion of oxalate. Magnesium one of the inhibitors of stone formation, reduces the super saturation of calcium oxalate by reducing the saturation of calcium oxalate and the growth of calcium oxalate crystals.

Hyperoxaluria is the main initiating factor of human idiopathic calcium oxalate stone disease. The study of urinary chemistry with respect to the stone forming minerals will provide a good indication of the risk of stone formation. In the present study, observed hypercalciuria in urine of ethylene glycol intoxicated rats might be a factor favouring the nucleation and precipitation of calcium oxalate from urine and subsequent crystal growth ⁴.

Cucumis trigonus Roxburghii of family Curcubitaceae is a perennial scandent monoecious tendrillar herb with slender angled stem, leaves deep palmately five lobed, hispid on the nerves beneath and rounded at the apex. Male flowers are small and are found in clusters whereas female flowers are solitary. Fruits are ellipsoid or sub-globose, yellow or yellow with green stripes and seeds are white and ellipsoid ⁵. The plant is distributed throughout India and found in areas of Ceylon, Afghanistan, Persia and Northern Australia⁶. Roots, fruits and seeds are extensively used medicinal parts of the plant. Roots are purgative and liver tonic. Fruits are used for stomachic, ascites, anemia and constipation and acts as a diuretic. Seeds have unsaturated lipids as major constituents and acts as a coolant and astringent⁷.

The present study is to investigate the modulatory activity of the ethanolic fruit extract of *Cucumis trigonus* R. towards the mineral constituents in the serum and urine of urolithiasis induced rats.

MATERIALS AND METHODS

Collection of the plant material

Cucumis trigonus Roxb. fruits were collected from Kovanur foot hills of Coimbatore district, Tamil Nadu, India during the month of July to August, 2009. 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 herbarium centre, Department of Botany, Kongunadu Arts and Science College, Coimbatore. Voucher number is KASCH Acc.No.: 607

Preparation of ethanolic fruit extract for *in vivo* studies

Fruits of the plants were washed, shade dried, powdered and stored in tight containers under refrigeration. 100g of *C.trigonus* 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 48hr. 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 18g, and the residue was suspended in water and administered orally to the experimental rats.

Selection of animals for toxicity studies

Healthy 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).

Induction of experimental urolithiasis

Calcium oxalate urolithiasis was induced in experimental animals. 0.75% (0.75 ml of ethylene glycol in 100ml of drinking water) of rats for a period of 28 days for the production of calcium oxalate stone in rats.

Experimental design of animals for *in vivo* studies

The experimental design of animals is given in table1 for *in vivo* studies

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

Group II: **Urolithiasis induced rats** - received 0.75% ethylene glycol in water for 28 days

Group III: **Plant drug treated rats** - urolithiasis induced rats received *C.trigonus* fruit extract (150 mg / kg body weight) by oral administration for subsequent 28 days at a rate of 1.0 ml / rat / day

Group IV: **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

Collection of urine sample

Before the day of sacrifice the rats were placed in metabolic cages, urine was collected for 24 hr., and 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 at 3000rpm for 10 min. and any sediment present was discarded. It was used for further analysis.

Collection of serum sample

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

Estimation of calcium

At alkaline pH, calcium binds with orthocresolphthalein complexone (OCPC) to form a bluish-purple complex. The intensity of the colour so formed is proportional to calcium concentration and is measured at 548nm. Interference from magnesium is overcome by the presence of 8-hydroxyquinoline in reagent 1, which binds free magnesium ions.

Estimation of magnesium

Magnesium is analysed by calmagite method. Magnesium present in the sample complexes with titan yellow in alkaline medium to give a red color which is read at 540nm.

Estimation of phosphorus

Phosphorus estimation was done by the method as in Raghuramulu *et al.* , 1983. Phosphorus reacts with molybdate to form phosphomolybdate. The increase in absorbance, due to formation of this complex is measured at 340nm and is proportional to the concentration of phosphorus in the sample.

Estimation of oxalate

The method used for estimating the level of oxalate is as in Hodgkinson and Williams, 1972. 0.1 ml of aliquot was taken in a boiling tube and made up to 1.0 ml with water. One ml of 4N sulphuric acid was added. Then added a piece of freshly cleaned zinc. Heated in a boiling water bath for 30 min. Removed zinc, washed with 0.5 ml of 1% chromotropic acid solution. Standards in the range of 20-200µg were taken and treated as above. Added 5.0 ml of concentrated sulphuric acid to both standard and test solutions. Heated for 30 min. in a boiling water bath. Added 20 ml of 10N sulphuric acid to all the tubes through their slides slowly, with constant shaking. Cooled and read at 570 nm. The colour was stable for several.

The amount of oxalate present in serum sample was expressed as mg/dl.

The amount of oxalate present in urine sample was expressed as mg/day.

RESULTS AND DISCUSSION

Serum mineral constituents

Oxalate is one of the major stone-forming constituents is known to induce lipid peroxidation, which causes disruption of the membrane integrity is found to be increased in stone formation. It alters membrane fluidity, membrane permeability and thereby affects the ion transport across the cellular organelle¹⁰.

From the figure 1 and 2, it is evident that the level of serum mineral constituents calcium, magnesium and phosphorus were significantly decreased whereas, the level of oxalate were significantly increased ($P < 0.05$) in group II ethylene glycol induced urolithiatic rats when compared to the normal control rats.

The level of serum calcium and phosphorus levels in the urolithiatic rats treated with ethylene glycol is decreased and an increase in the level of oxalate. The decrease of serum calcium concentration indicates an increase of urinary calcium and calcium oxalate stone formation¹¹.

Stone formation is also caused by hyperoxaluria, which leads to increased renal retention and excretion of oxalate. Magnesium one of the inhibitors of stone formation, reduces the super

saturation of calcium oxalate by reducing the saturation of calcium oxalate and the growth of calcium oxalate crystals.

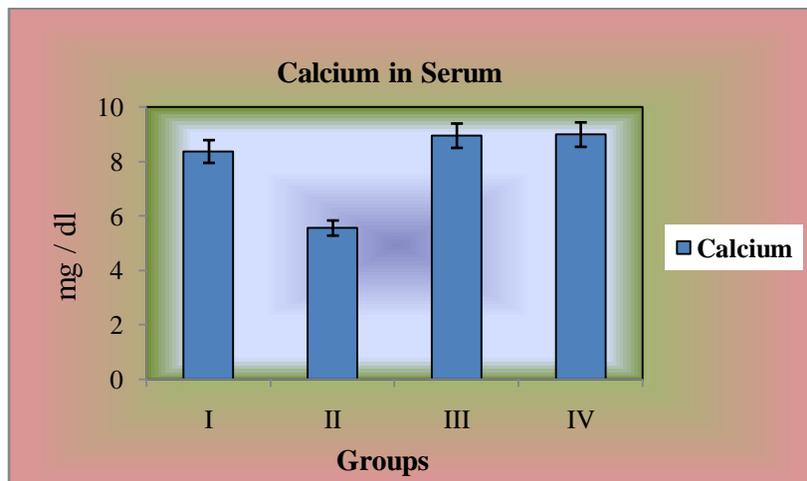


Fig. 1. Effect of ethanolic fruit extract of *C. trigonus* on calcium in serum of control and experimental rats

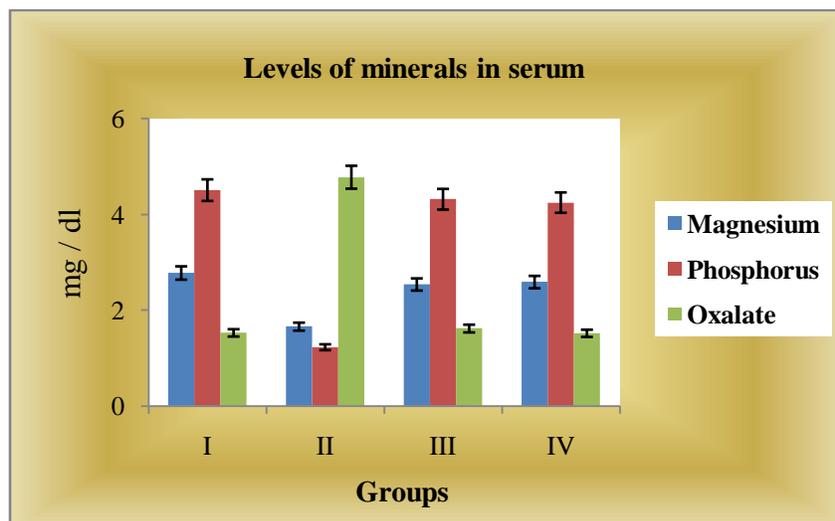


Fig. 2. Effect of ethanolic fruit extract of *C. trigonus* on minerals in serum of control and experimental rats

The animals treated with the ethanolic extract showed a significant alteration of serum mineral constituents when compared to ethylene glycol treated animals, which might be an indication of the protective effect of *C. trigonus* fruit extract.

The aqueous extract of *Melia azedarach* Linn. normalized calcium oxalate and magnesium levels in serum of urolithiatic rats¹². *Cyclea peltata* root powder increased serum magnesium and phosphorous levels in urolithiatic rats¹³.

Our results coincides with that of¹⁴ who showed that root woods of *Moringa oleifera* Lam. reduced the oxalate level in serum of ethylene glycol induced urolithiatic rats.

There is no significant difference in the levels of mineral constituents when the ethanolic fruit extract treated rats (group III) is compared with the thiazide treated rats (Group IV). This result gives a supportive evidence for the antiurolithiatic activity of ethanolic fruit extract of *C. trigonus* which is similar to that of standard drug thiazide.

Urinary mineral constituents

The urinary mineral constituents – calcium, magnesium, phosphorus and oxalate in the control and experimental rats are shown in table 1. It is clearly shown that the 24 hr. urinary calcium, phosphorus and oxalate excretions were significantly increased, where as the magnesium levels was significantly decreased in ethylene glycol induced group II rats when compared with the control rats.

Table 1. Effect of ethanolic fruit extract of *C. trigonus* on mineral constituents in urine of control and experimental rats

Groups	Calcium ^{μψ}	Magnesium ^{μψ}	Phosphorus ^{μψ}	Oxalate ^{μψ}
I	1.65 ± 0.05	5.43 ± 0.26	3.15 ± 0.05	0.78 ± 0.02
II	6.46 ± 0.07 a*	1.12 ± 0.46 a*	9.54 ± 0.04 a*	6.56 ± 0.02 a*
III	1.82 ± 0.08 b*	5.23 ± 0.35 b*	3.77 ± 0.07 b*	0.98 ± 0.02 b*
IV	1.88 ± 0.07 c*d ^{ns}	5.12 ± 0.25 c*d ^{ns}	3.95 ± 0.05 c*d ^{ns}	1.12 ± 0.01 c*d ^{ns}

Values are expressed as mean ± SD of six animals

Comparison between the groups

‘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 IV and III

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

Units: ^{μψ}mg/24 hr. urine

Hypercalciuria in ethylene glycol induced urolithic rats favouring the nucleation and precipitation of calcium oxalate of apatite (calcium phosphate) from urine and finally leads to subsequent crystal growth ¹⁵.

Magnesium is considered as a potent inhibitor of calcium oxalate crystallization *in-vitro*, and binds to oxalate to form a soluble complex, consequently reducing the concentration available for calcium oxalate precipitation ¹⁶.

Urinary magnesium was significantly lowered in ethylene glycol intoxicated rats. Magnesium complexes with oxalate, thus reducing calcium oxalate supersaturation in urine and, as a consequence growth and nucleation rate of calcium oxalate crystals is reduced ¹⁷. Low magnesium levels also encountered in stone formers as well as stone forming rats ¹⁸.

Increased excretion of phosphorus has been reported in stone formers and hyperoxaluric 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 calcium oxalate deposition¹⁹.

Hyperoxaluria is another major risk factor in the pathogenesis of urolithiasis than hypercalciuria. It has been reported that oxalate plays an important role in stone formation and has about 15 fold greater effect than urinary calcium. This indicates that its pentacyclic triterpenes mainly butelin act as inhibiting some steps of oxalate synthesis from glycolic acid²⁰.

Group III rats treated with the *C. trigonus* extract showed a significant restoration of urinary mineral constituents when compared to ethylene glycol treated rats (group II), which might be an indication of recovery due to the administration of fruit extract of *C. trigonus* having antiurolithiatic activity.

Our results coincides with that of¹³ who showed that decoction of *Rotula aquatica* altered the excretion of calcium and oxalate in urine and confirmed the stone inhibitory effect in male wistar rats.

Rosa canina found to have significant activity on calcium oxalate urolithiasis in altering urinary mineral constituents²¹. The aqueous extract of *Phyllanthus niruri* acted as endogenous inhibitors of magnesium in urolithiatic rats²².

Tephrosia purpurea Linn. root extract restored the urinary mineral constituents in ethylene glycol induced urolithiasis in rats²³. NONI- herbal formulation restored the urinary mineral constituents in ethylene glycol induced nephrolithiasis in albino rats¹⁶.

When *C. trigonus* extract treated rats (Group III) were compared with thiazide treated rats (Group IV), there was no significant difference between these groups of rats. This result gives a supportive evidence for the antiurolithiatic activity of ethanolic extract which is similar to standard drug thiazide.

From the above results, it is prevalent that the ethanolic fruit extract of *Cucumis trigonus* normalized the levels of urinary electrolytes in ethylene glycol induced urolithiasis.

CONCLUSION

Our results highlight that *C. trigonus* is the most effective drug in inhibiting stone formation and maintaining the level of mineral constituents in the body tissues and liquids and also heals the renal damage caused by the oxalate toxicity, thus confirming its antiurolithiatic property.

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