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Influence of *Cucumis Trigonus* R. Fruit extract on biochemical parameters in Urolithiasis induced Wistar albino 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 biochemical parameters urea, uric acid and creatinine in serum and urine of the urolithiatic rats. The ethanol extract (150 mg / kg b.w.) reduced the levels of elevated biochemical parameters in serum and urine significantly ($p<0.05$) when compared with the toxic groups. The results shown by the ethanol extract (150 mg / kg b.w.) was compared to standard thiazide drug treated group showing no significant difference ($p<0.05$) and thus exhibited potent antiurolithiatic activity.

Introduction

Cucumis trigonus Roxburghii of family Curcurbitaceae is a perennial scabrid 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 where as female flowers are solitary. Fruits are ellipsoid or sub-global, yellow or yellow with green stripes, seeds are white and ellipsoid. *Cucumis trigonus* is distributed throughout India and found in areas of Ceylon, Afghanistan, Persia and Northern Australia [1]. Roots, fruits and seeds are the 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 astringent.

Urolithiasis refers to the solid nonmetallic minerals in the urinary tract. Among the several types of kidney stones, the most common are calcium oxalate. The formation of these stones involves several physicochemical events, beginning with crystal nucleation, aggregation and ending with retention within the urinary tract [2]. It has been described as the third most common affliction of human urinary tract [3]. Some common causes are inadequate urinary drainage, foreign bodies in the urinary tract, microbial infections, diet with excess oxalates and calcium, vitamin abnormalities, viz. vitamin A deficiencies, vitamin D excess, metabolic diseases like hyperparathyroidism, cystinuria, gout and intestinal dysfunction [4].

Urea an organic compound, which serves an important role in the metabolism of nitrogen containing compounds and is the main nitrogen containing substance in the urine of mammals. The handling of urea by the kidneys is a vital part of human metabolism. It plays a role in the countercurrent exchange system of the nephrons that allows for re-absorption of water and critical ions from the excreted urine. Urea is reabsorbed in the inner medullary collecting ducts of the nephrons [5]. Some of the reabsorbed urea will eventually flow back into the thin ascending limb of the tubule through the collecting ducts into the excreted urine.

Uric acid is created when the body breaks down purine nucleotides. Uric acid is also associated with other medical conditions like ammonium acid urate kidney stones.

Uric acid is known to promote calcium oxalate crystal growth [6]. The predominance of uric acid crystals in calcium oxalate stones and the observation that uric acid binding proteins are capable of binding to calcium oxalate and modulate its crystallization also suggests its primary role in stone formation [7].

Creatinine, a breakdown product of creatinine phosphate in muscle. Creatinine is chiefly filtered out of the blood by the kidneys (glomerular filtration and proximal tubular secretion). If the filtering of the kidney is deficient, there is a rise in creatinine blood level. Therefore creatinine levels in blood and urine may be used to calculate the creatinine clearance, which reflects the glomerular filtration rate.

The present study is to investigate the modulatory activity of the ethanolic fruit extract of *Cucumis trigonus* R. towards these biochemical parameters in urolithiasis induced rats.

Materials and Methods

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 37ºC 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.

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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).

Experimental design of animals for *in vivo* studies

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

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 $0^{o}C$ 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 urea

Urea is estimated by using the method of Varley 1976. Diacetyl monoxime in the presence of acid, hydrolysis to produce the unstable compound diacetyl. This reacts with urea to produce a yellow diazone derivative. The color of this product becomes pink by addition of thiosemicarbazide which is measured colorimetrically at 520nm.

Estimation of uric acid

Uric acid is estimated by using the method of Caraway 1963. Uric acid is oxidized to allantoin and carbondioxide by phosphotungstic acid reagent in alkaline solution. Phosphotungstic acid is reduced in this reaction to tungsten blue, which is measured at 660nm.

Estimation of creatinine

Creatinine is estimated by using the method of Owen *et al*., 1954. Creatinine forms a coloured complex with picrate in alkaline medium. The rate of formation of the complex is measured at 540nm.

Statistical analysis

Results were expressed as mean ± SD of six animals in each group. Statistical significance was determined by one-way analysis of variance (ANOVA) and post hoc least-significant difference (LSD) test.

Results and Discussion

Serum biochemical parameters

Due to the presence of stones, there is a obstruction to the outflow of urine in urinary system and because of this reason the glomerular filtration rate (GFR) also decreases. Reduction in the GFR leads to accumulation of the waste products, particularly nitrogenous substances such as urea, creatinine and uric acid in blood occurs [11]. Table 2 represents the serum biochemical parameters like urea, uric acid and creatinine of control and experimental rats. From the results it is evident that the levels of urea, uric acid and creatinine significantly increased ($p<0.05$) in urolithiatic rats (group II), when compared to ethylene glycol intoxicated rats (group I).

Uric acid is an end product of purine metabolism produced within peroxisomes and excreted in urine and can crystallize to form stones. Pure uric acid stone formation is rare and mostly accompanied by urates, phosphates or calcium oxalate [12].

Group III rats treated with the *C. trigonus* extract showed a significant restoration of serum biochemical parameters when compared to ethylene glycol treated rats (group II), which might be an indication of recovery proving the antiurolithiatic potential of *C. trigonus*. Anbu *et al*., (2011) reported that the root extract of *Ichnocarpus frutescens* normalized the serum levels of urea, uric acid and creatinine in experimental animals. Our result are in accordance with that of Al-Attar (2010) who reported that Spirulina administration restore the levels of serum biochemical parameters in ethylene glycol induced nephrolithiasis in male rats.

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 of similarity of the antiurolithiatic activity of ethanolic extract and standard drug thiazide.

The results clearly prove that the ethanolic fruit extract normalized the levels of serum biochemical parameters, thus proving the antiurolithiatic property of the fruit extract.

Urine biochemical parameters

The urine biochemical parameters like urea, uric acid and creatinine of control and experimental rats are given in the table 3.

From the table 18, it is prevalent that the biochemical parameters were significantly increased on ethylene glycol intoxication when compared to control rats (Group I). Uric acid is known to promote calcium oxalate crystal growth [15]. The predominance of uric acid crystals in calcium oxalate stones and the observation that uric acid binding proteins are capable of binding to calcium oxalate and modulate its crystallization also suggests its primary role in stone formation [16].

Group III rats treated with the *C. trigonus* fruit extract showed a significant restoration of urinary biochemical parameters when compared to ethylene glycol treated rats (group II), which might be an indication of recovery proving the antiurolithiatic property of *C. trigonus*. Our findings were similar to that of McHarg *et al.* (2003) who showed that ingestion of cranberry juice significantly altered the urinary parameters in urolithiatic rats.

Selvam *et al.* (2001) showed that two siddha drugs. *Aerva lanata* and *Vediuppu chunnam* increased the urine volume by reducing the salt forming constituents - urea, uric acid and creatinine in urine. Our results agrees well with that of Chaitanya *et al*. (2010) who reported that *Macrotyloma uniflorum* seed extract restored the urinary biochemical parameters in ethylene glycol induced urolithiasis in rats.

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 of the similarity of the ethanolic extract with that of the standard drug thiazide From the above results, it is prevalent that the ethanolic fruit extract of *C. trigonus* normalized the levels of urinary biochemical parameters, thus proving the antiurolithiatic property of the fruit extract.

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Table 1. Experimental design of animals for *in vivo* studies

Group	Experimental design
I	Control rats - received normal pelleted diet.
II	Ethylene glycol intoxicated rats - Urolithiasis was induced by administrating 0.75% ethylene glycol (0.75ml ethylene glycol in 100 ml drinking water) to rats for 28 days.
III	Fruit extract treated rats - Urolithiasis induced rats received ethanolic fruit extract of *C.trigonus* (250 mg / kg b.w.) by oral administration for 28 days at a rate of 1.0 ml / rat / day.
IV	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.

Table 2. Effect of ethanolic fruit extract of *C. trigonus* on serum biochemical parameters of control and experimental rats

Groups	Urea ¤	Uric acid ¤	Creatinine ¤
I	11.41 ± 0.06	5.72 ± 0.05	0.97 ± 0.04
II	24.13 ± 0.11 a*	9.69 ± 0.25 a*	6.92 ± 0.08 a*
III	12.01 ± 0.10 b*	5.85 ± 0.07 b*	1.02 ± 0.03 b*
IV	12.52 ± 0.09 c*d^{ns}	5.97 ± 0.06 c*d^{ns}	0.99 ± 0.04 c*d^{ns}

Values are expressed as mean ± SD of six animals
Statistical comparisons are as in table 10
Units
¤ mg/dl
Experimental design
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
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

Table 3. Effect of ethanolic fruit extract of *C. trigonus* on Urine biochemical parameters of control and experimental rats

Groups	Urea€	Uric acid€	Creatinine€
I	24.58 ± 0.11	44.25 ± 0.05	145.56 ± 0.11
II	45.42 ± 0.11 a*	101.90 ± 0.25 a*	292.75 ± 0.26 a*
III	25.01 ± 0.10 b*	44.55 ± 0.11 b*	146.96 ± 0.03 b*
IV	25.82 ± 0.12 c*d^{ns}	45.58 ± 0.09 c*d^{ns}	148.45 ± 0.04 c*d^{ns}

Values are expressed as mean ± SD of six animals
Statistical comparisons are as in table 10
Units
€mg/24hr urine