Antiurolithiatic Activity of Ethanolic Extract of *Cucumis Sativus* Fruit In Wistar Rats

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Abstract-The present study explores the antiurolithiatic activity of an ethanolic extract of the *Cucumis Sativus* (CS) fruit in Wistar rats. Ethylene glycol (0.75% v/v) induced calculi in Wistar rats. They have assessed various parameters like biochemical estimations of serum, urine, and kidney tissue homogenate and histopathological studies of a kidney. The treatment with doses of 200 mg/kg and 400 mg/kg of ethanolic extract of CS exerts dose-dependent anti-urolithic activity. A significant reduction in the deposition of kidney stone-forming constituents like calcium, oxalate, and phosphate was found in the kidneys. The Wistar rat's kidney sections showed the presence of crystals, dilatation of tubules tubulorrhexis, and the epithelial recovered.

Keywords: *Cucumis sativus,* Antiurolithiatic activity, Allopurinol, and Ethylene glycol (0.75 % v/v)

1. INTRODUCTION

In the traditional systems of medicine, most of the remedies were taken from plants, and they were proven to be helpful; the rationale behind their use is not well established through systemic pharmacological and clinical studies except for some composite herbal drugs and plants [(1)]. Urolithiasis is one of the most common diseases of the urinary tract, which has been afflicting humankind since antiquity. Urolith formation is a multifactorial process. Several pharmacological investigations on the medicinal plants used in traditional antiurolithiatic therapy have revealed their therapeutic potential in the *in-vivo* or *in vitro* models [(2)].

The cucumber is the edible fruit of *Cucumis sativus*, which belongs to the gourd family Cucurbitaceae [(3)]. The present study was designed to evaluate the anti-urolithiasis activity of ethanolic extract of the fruit of *Cucumis sativus* in ethylene glycol-induced urolithiasis in Wistar rats.

2. MATERIALS AND METHODS

2.1. Extraction of plant material

Cucumis Sativus fruits were purchased from local areas. Afterward, they were washed in running tap water to remove dirt and cut into pieces. These cut pieces were then shed, dried, and milled into a coarse powder using a grinder. 50g of the *Cucumis sativus* pulp powder was macerated in 95% ethanol for 48 hours, which was then filtered. The filtrate was concentrated using a rotatory evaporator and dried using a laboratory oven at 50°C. Part of the extract was used for phytochemical analysis, while the remainder was preserved in a refrigerator for further usage [(4)].

2.2. Experimental animals

Wistar rats (180–200 gm) were selected for the experimental study. The animals were procured from the animal house of Sainath Agencies, Hyderabad. The animals were kept in polypropylene cages and maintained under laboratory conditions of temperature (21.5 ± 22°C), humidity (60 ± 1%), and a 12-hour light/dark cycle. They were allowed free access to food (standard pellets) and water *ad libitum*. The IAEC of CPCSEA provided ethical committee clearance (IAEC/08/CCPER/CPCSEA2022).

2.3. Experimental design

The Wistar rats were divided into 5 groups, with 6 animals in each group (n=6). Group, I was treated with a vehicle solution. Groups II, III, IV & V were supplemented with 0.75% v/v ethylene glycol in drinking water *ad libitum* to produce renal calculi. Group II was not received plant extract. Group III was treated with a standard drug (Allopurinol 50mg/kg). Group IV was treated with plant extract from the 1st day to the 28th day (200mg/kg; bw), and Group V was treated with plant extract starting from the 15th day to the 28th day (400mg/kg; bw) [(5)].

2.4. Analysis of urinary parameters

The urine samples were collected on the 28th day for 24hrs by using metabolic cages. Samples were stored at 4°C. Before being kept, a drop of Con. HCl was added to the urine. These stored samples were analyzed for urinary calcium, phosphate, and oxalate content [6].

2.5. Analysis of serum parameters

After the 28 days experimental period, blood was collected from the retro-orbital plexus under anesthetic conditions. Collected Blood samples were subjected to centrifugation to collect the serum at 10,000 rpm for 10min and analyzed for serum creatinine, uric acid, and blood urea nitrogen [7].

2.6. Isolation and analysis of kidney tissue homogenate

Animals were sacrificed by cervical dislocation under anesthetic conditions. The abdomen was cut open to remove a kidney from each animal. Isolated kidneys were cleaned off extraneous tissue, and one kidney was dried at 80°C in a hot air oven. A sample of 100mg of the dried kidney was boiled in 10 ml of 1 N HCl for 30 min. Then the kidneys were homogenized and obtained homogenate subjected to centrifugation at 2,000 rpm for 10 min. Supernatant separated & analyzed for calcium, phosphate, and oxalate content in kidney homogenate [8].

2.7. Histopathology study of kidney

Another isolated kidney was preserved in 10% neutral formalin. The specimens were dehydrated in descending grades of Ethanol, cleared in xylene, and embedded in paraffin wax. Sections of 4 -5 µm thickness were prepared and stained with Haematoxylin and Eosin (H & E), then examined microscopically [9].

2.8. Statistical analysis

The results of the biochemical estimations were reported as Mean ± SEM. The anti-urolithic activity of ethanolic extract of the fruit of *Cucumis sativus* was analyzed by one-way analysis of variance (ANOVA) followed by Dunnett's test, 'P' value <0.05 was considered as statistically significant [10].

3. RESULTS AND DISCUSSION

The preliminary phytochemical analysis of the ethanolic extract of the fruit of *Cucumis sativus* indicated the presence of various active constituents like steroids, carbohydrates, glycosides, tannins, triterpenoids, flavonoids, and alkaloids.

3.1. Biochemical estimation of serum parameters

The serum uric acid, BUN, and serum creatinine were remarkably increased in calculi-induced animals (Group II). On treatment, with alcoholic fruit extract of *Cucumis sativus* (200 mg/kg and 400 mg/kg), the elevated serum levels of creatinine, uric acid, and BUN were significantly (P<0.001) reduced; However, the results were significantly (P<0.001) comparable to those of Allopurinol treated Wistar rats (Group III). The results are shown in Fig 1.

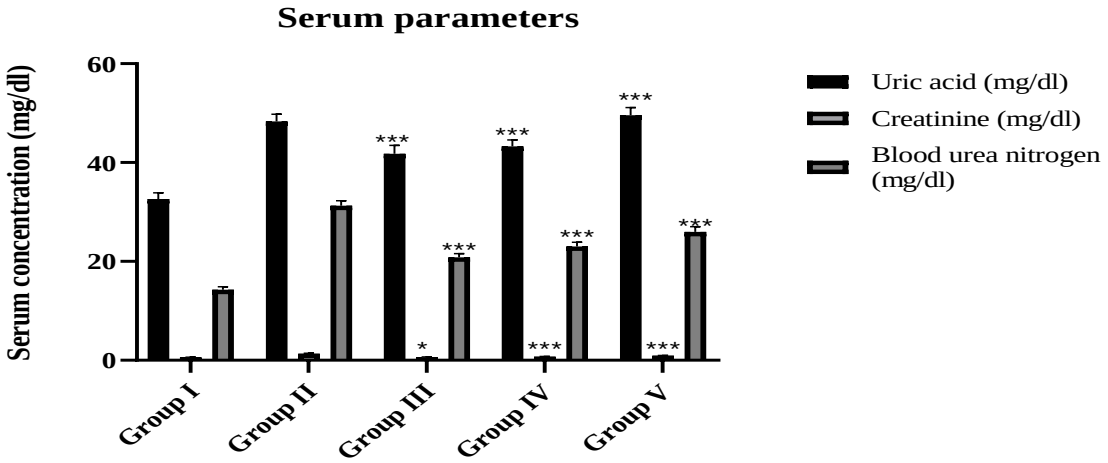

Fig 1: Effect of ethanolic extract of fruit of CS on serum parameters

3.2. Biochemical analysis of urinary parameters

The urinary excretion of calcium, oxalate, and phosphate levels in normal and experimental Wistar rats, urine should be collected for 24 hrs. Significantly increased oxalate excretion was found in Group II Wistar rats compared to Group I (control). This condition (hyperoxaluria) results from the chronic feeding of ethylene glycol (0.75% v/v). A significant ($p<0.001$) urinary excretion of calcium and phosphate were also found in Group II (calculi-induced) Wistar rats. Elevated levels of urinary calcium, oxalate, and phosphate were significantly lowered in the treatment of ethanolic fruit extract (200 mg/kg and 400 mg/kg) of *Cucumis sativus*. These obtained results (Fig 2) were significantly ($p<0.001$) comparable to the Group III (Allopurinol treated) Wistar rats.

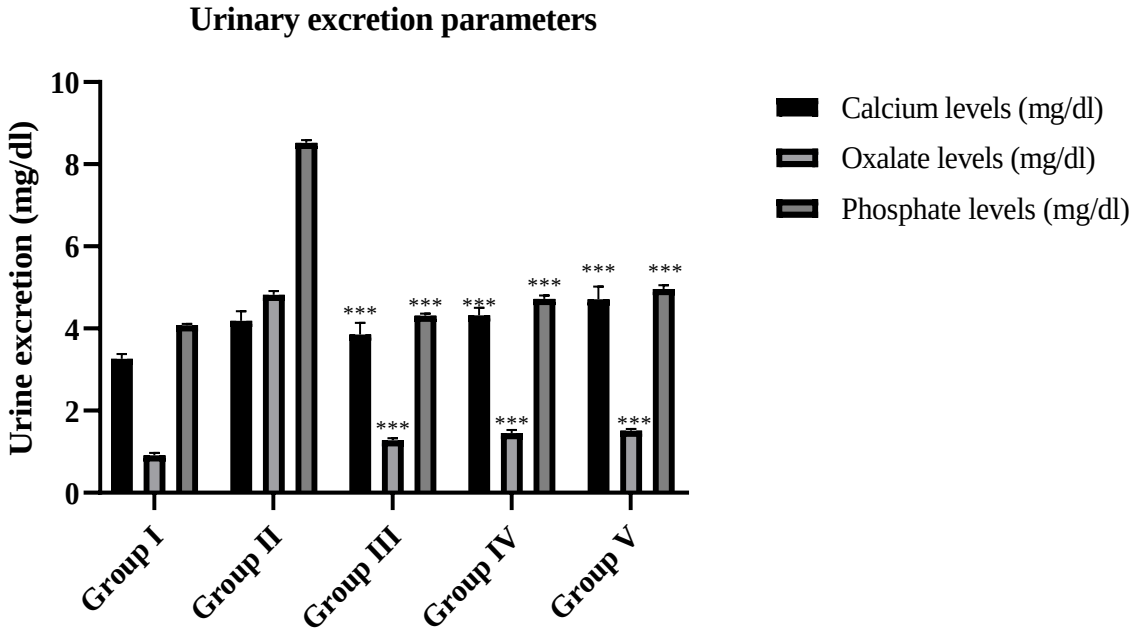

Fig 2: Effect of ethanolic extract of fruit of CS on urine excretion

$P<0.05^{*}$, $^{**}P<0.01$, $^{***}P<0.001$ (ANOVA) with Dunnet's t-test

Treatment with ethanolic extract of *Cucumis sativus* markedly reduced the excretion of calcium, oxalate, and phosphate in 200mg/kg and 400 mg/kg compared to the caliculi-induced group. It lowered the levels of oxalate and calcium in the urine and even their retention in the kidney.

3.3. Kidney homogenate analysis

The hyperoxaluric condition was observed because of the significant ($p<0.001$) deposition of oxalate crystals in Group II (caliculi-induced) Wistar rats as compared to Group I (control). An important ($p<0.001$) deposition of calcium phosphate was also found in Group II (calculi-induced) animal kidney homogenate. A significant ($p<0.001$) reduction in the deposition of kidney stone-forming constituents (calcium, oxalate, and phosphate) was found in the kidneys of 200mg/kg of *Cucumis sativus* (Group IV) and 400mg/kg of *Cucumis sativus* (Group V) Wistar rats. These obtained results were significantly ($p<0.001$) comparable to Group III (Allopurinol treated) animals.

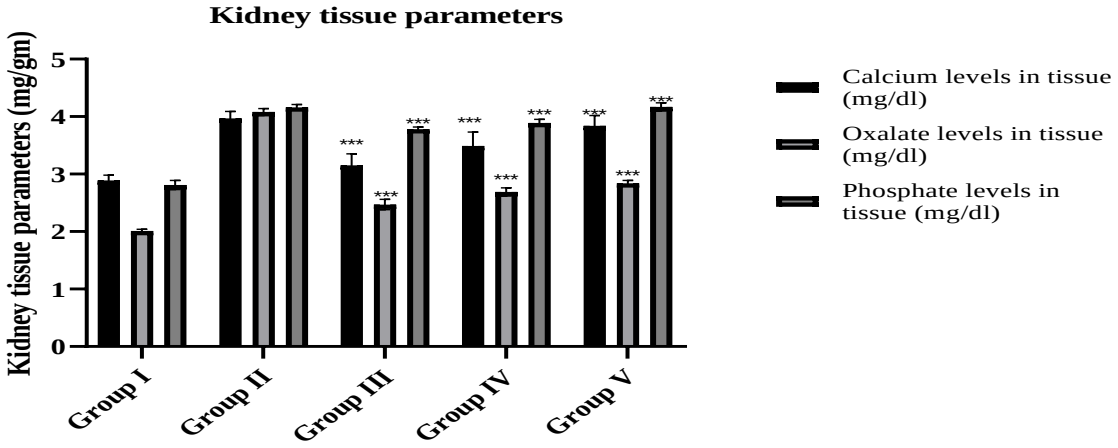

Fig 3: Effect of ethanolic extract of fruit of CS on kidney tissue

$P<0.05^{*}$, $^{**}P<0.01$, $^{***}P<0.001$ (ANOVA) with Dunnet's t-test

3.4. Histopathology studies

The histopathology studies of kidney sections were found to be Group I (Normal) Wistar rats kidneys showed the normal structure of the cortex, medulla, and collecting system. Group II (Calculi induced) Wistar rat's kidney sections showed irregular polymorphic highly refractive crystals in most tubules, predominantly in the cortex. Tubulorrehix was noted, with foci of necrosis. Group III (Allopurinol treated) Wistar rat's kidneys showed dilatation of tubules and dilated blood vessels and showed slight recovery, and edema of the tubular cells was observed. Group IV, V (200 mg/kg & 400 mg/kg) Wistar rats kidney sections showed the presence of crystals, dilatation of tubules tubulorrhexis, and the epithelial recovery was less significant compared to Group III (Allopurinol treated) Wistar rats.

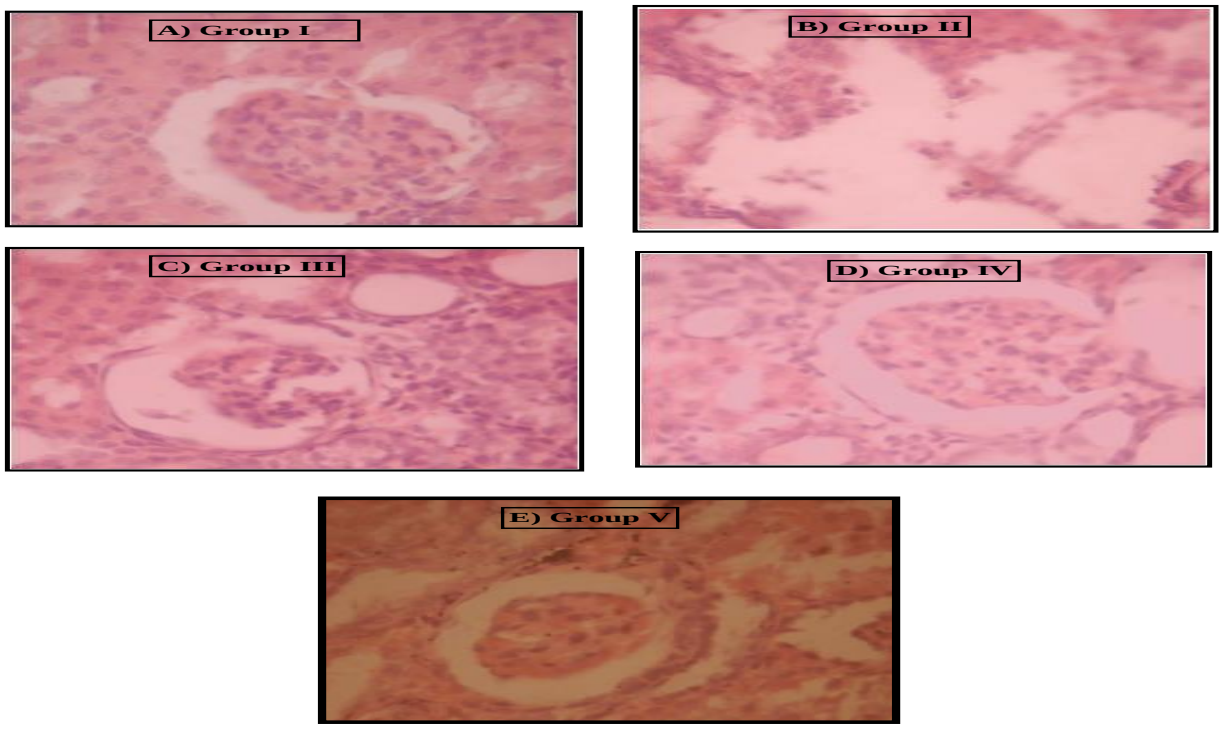

Fig 3: Histopathology of kidney of A) Normal group; B) Calculi induced; C) Allopurinol treated; D) Ethanolic extract of CS (200 mg/kg, BW); E) Ethanolic extract of CS (400 mg/kg, BW); (40X)

CONCLUSION

In the present study, an effort has been made to establish the scientific validity of the antiurolithiatic property of the plant *Cucumis sativus*. The ethanolic extract of CS was evaluated for antiurolithiatic in Wistar rats. A calcium oxalate calculus was induced by employing ethylene glycol (0.75% v/v). The parameters considered are serum creatinine, BUN, uric acid in serum, urinary excretion of calcium and oxalate, phosphate, and their deposition in the kidney were evaluated. The results obtained in the study prove the efficacy of the ethanolic extract of *Cucumis sativus* fruit as antiurolithiatic in the indigenous system of medicine.

REFERENCE

1. Evans, D., Griesbach, s. (1984). Regeneration. competence of cucumber handbook of plant cell culture volume1 ed. McMillian, New York,Us, pp 806 -815.
2. Heidari, H., Kamalinejad, M., Eskandari, M. (2012). Hepatoprotective activity of Cucumis sativus against cumene hydroperoxide induced-oxidative stress. Res. Pharm. Sci., 7(5), S936-S939.
3. Krauze-Baranowska, M.A., Wojciech, C. (2001). Flavonoids from some species of the genus Cucumis. Biochemical Systematics and Ecology, 29(3), 321-324.
4. Baxmann, AC., Mendonca, CD. and Heliberg, IP. (2003): Effect of vitamin C supplements on urinary oxalate and pH in calcium stone-forming patients. *Kidney Int*. 63: 1066-71.
5. Christiana, A.J., Ashok, K., Packia Lakshmi, M., Tobin, G.C., Preethi. and Murugesh, N. (2005): Antilithiatic activity of *Asparagus racemosus* Willd on ethylene glycol-induced lithiasis in male albino Wistar rats. Exp. Clin. Pharmacol. 27: 633-638.
6. Finlayson, B. (1978): Physicochemical aspects of urolithiasis, Kidney Int 13 pp. 344-360.
7. Breslau, NA., Brinkley, L., Hill, KD. and Pak CYC. (1988): Relationship of animal protein rich diet to kidney stone formation. J Clin Endocrinol Metab. 66:140-146.
8. Kagawa, M., Yamamoto, A., Ogawa, K., Shirai, T. and Fukushima, S. (1992): Uracil-induced urolithiasis in the urinary bladder of rats is irritation-dependent. Toxicol. Lett. (61): 21-26.
9. Prasad, K. V. S. R. G., Bharathi, K. and Srinivasan, K.K. (1994): Evaluation of *Ammania baccifera Linn*. For antiurolithiatic activity in albino rats. Ind. J. Expt. Biol. (32): 311-313.
10. Selvam, R., Kalsaiselvi, P., Govindaraj, A., Balamudrugan, V. and Satish Kumar, A.S. (2001): Effect of *Aereva lanata* leaf extract and *Vediuppa chunam* on the urinary risk factors of calcium oxalate urolithiasis during experimental hyperoxaluria. Pharmacol. Res. (43): 89-93.