Anti-Urolithiatic Activity of Ethanolic Extract of Seeds of *Benincasa Hispida (Thumb)*

Patel RK[1]*, Patel SB[1], Shah JG[1]

[1]Department of Pharmacology, Indukaka Ipcowala College of Pharmacy, New Vallabh vidyanagar, Anand, Gujarat, India.

***Address for correspondence:**

Assistant Professor

Ravindra K. Patel.

Department of Pharmacology.

Indukaka Ipcowala College of Pharmacy,

New Vallabhvidya nagar,

Anand, Gujarat, India.

E-mail: ravistar_23@yahoo.com

Summary

Background: *Benincasa hispida* Thumb. (Cucurbitaceae) is widely used plant in South Asia, mainly India and Pakistan, as a traditional medicine for treatment of urolithiasis**. Objectives:** The present study was designed to investigate the ameliorating effect of ethanolic extract of *Benincasa hispid*a seeds (BHE) in hyperoxaluria and renal cell injury. **Materials and Methods:** Wistar albino rats were given 0.75% v/v ethylene glycol in drinking water to induce chronic hyperoxaluria and simultaneously BHE was given to nephrolithiasic treated rats at the dose of 250 and 500 mg/kg b.w. orally for 35 days. Urinary volume, oxalate, calcium, total protein, serum Creatinine, sodium, calcium, Phosphorus was evaluated. **Results:** Ethylene glycol feeding resulted in hyperoxaluria as well as increased renal excretion of calcium. Supplementation with BHE significantly reduced the elevated urinary oxalate, showing a regulatory action on endogenous oxalate synthesis. BHE significantly lowered the urinary excretion and kidney retention levels of oxalate, protein and calcium. Moreover, elevated serum levels of sodium, Creatinine and calcium, phosphorus were significantly reduced by the extracts **Conclusions:** The reduction of stone forming constituents in urine and their decreased kidney retention reduces the solubility product of crystallizing salts such as calcium oxalate and calcium phosphate, which could contribute to the anti-urolithiatic property of seeds of *Benincasa hispida*

KEYWORDS : *Benincasa Hispida*, Winter melon, Hyperoxaluria; Urolithiasis; Ethylene glycol

Introduction

Urinary calculi are the third prevalent disorder in the urinary system. Approximately 80% of these calculi are composed of calcium oxalate and calcium phosphate. Urinary calculi may cause obstruction, hydronephrosis, infection and hemorrhage in the urinary tract system [1]. Surgical operation, lithotripsy and local calculus disruption using high-power laser are widely used to remove the calculi. However, these procedures are highly costly and with these procedures recurrence is quite common [2]. The recurrence rate without reventivetreatment is approximately 10% at 1 year, 33% at 5 year and 50% at 10 years [3]. Many remedies have been employed through the ages to treat urolithiasis. In most cases, the management of urolithiasis involves both surgical and medical approaches, i.e., percutaneous nephrolithotomy (PCNL), extracorporeal shock wave lithotripsy (ESWL) and antibiotics [4].However; these treatments are relatively costly, painful and require expert hands with availability of appropriate equipments. This has stimulated research on traditional remedies showing anti-urolithiatic activity. These plant products are reported to be effective in decreasing the recurrence rate of renal calculi with no side effects [2].

Benincasa hispida (Ash gourd, Family: Cucurbitaceae) is a commonly used vegetable, which has found mention in 'Charaka Samhita' for its medicinal properties. It is the main ingredient in 'Kusumanda Lehyam" used in Ayurvedic system of medicine as a rejuvenating agent and recommended for various ailments like epilepsy, constipation, piles, dyspepsia, syphilis and diabetes Fruit has also been used in India to treat disorders of the GI tract, respiratory tract, urinary tract and diabetes mellitus [5,6] . It is known to render protection against histamine induced bronchospasm [7]. A multitude of studies have shown that the antioxidant status of an individual was compromised in most of these disorders. However, no scientific study has been reported regarding the anti-urolithiatic property of the ethanolic extract of *Benincasa hispida* seeds (BHE). In this study, we investigated the protective effect of the BHE against ethylene glycol induced.

Materials and Methods

Plant material and preparation of extract

The dried seeds of *Benincasa hispida* were received from Kondappanaikanpatti Salem, Tamil Nadu. Dr. Marimuthu Govt. Arts and Science College, authentified plant. The seeds were coarsely powdered and packed into Soxhlet column and extracted with 95 % v/v ethanol in water at 75–79 ^{0}C for 22 h. The yield of the extract 7.8% w/w was stored in a refrigerator at 4^{0}C, until use for the biological testing

Chemicals and reagents

cystone (Himalaya drug company, Bangalore), ethylene glycol (EG) being a chief substitute for alcohol, EG is commonly used as anti freeze in cooling systems of automobiles, aircrafts and has wide industrial application. Many accidental deaths due to its poisoning have been reported [8].toxicity from EG is produced from the metabolites such as Glyceraldehydes and oxalate, producing wide spread tissue injury in the kidney. Patients die of acute renal failure due to EG toxicity [9]. All other chemicals and reagents used were analytical grade and procured from approved chemical suppliers.

Animals

Male Wistar albino rats weighing between 150–200 g each were used for this experiment. They were procured from Sri Venkateswara Enterprises, Bangalore, India. They were housed in polypropylene cages and maintained at 27 ± 2^0C relative humidity 65 ± 10% under 12 h light/dark cycles. The animals were given a standard diet supplied by Hindustan Lever Ltd, Bangalore, India. The study protocol was approved from the Institutional Animal Ethics Committee (Ref. No.: IAEC/Pcology/08/2006) constituted in accordance with the rules and guidelines of the CPCSEA (Committee for the purpose of Control and Supervision of Experiments on Animals), India.

Antiurolithiatic activity of Ethanolic extract of seeds of *Benincasa hispida*

Ethylene glycol and ammonium chloride induced hyperoxaluria model [10-11] was used to induce urolithiasis. Thirty animals were randomly divided into five groups containing six animals in each. Group I served as a vehicle treated control and maintained on regular rat food and drinking water ad libitum. Ethylene glycol (0.75%) in drinking water was fed to groups II-V for induction of renal calculi until the 34th day. As well as ethylene glycol, groups 2-6 also received the following treatments:

Groups III received standard antiurolithiatic drug, cystone (750 mg/kg body weight).
Group IV received ethanolic extract of *Benincasa hispida* seeds (250 mg/kg body weight)
Group V received ethanolic extract of *Benincasa hispida* seeds (500 mg/kg body weight).

All extracts were given once daily by oral route.

Collection of urine and serum analysis

All animals were kept in individual metabolic cages. Urines samples for a 24 h period were collected on 34th day of the protocol. Urine was analyzed for calcium, [12] oxalates [13] and total proteins. [14] On the 35th day all animals were sacrificed, blood samples were taken and analyzed for sodium, calcium, Creatinine and phosphorus.

Statistical analysis

The results were expressed as mean ± standard error of the mean (SEM). The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Dunnett's comparison test ($p < 0.05$ was considered significant).

Results

In the present study, chronic administration of 0.75% (v/v) ethylene glycol aqueous solution to male Wistar rats resulted in hyperoxaluria. Oxalate, calcium and total protein excretion were significantly increased in calculi-induced animals (Table 1, group-II).

Table 1: Effect of ethanolic extracts of seeds *Benincasa hispida* on urine parameters in nephrolithic rats

Group	Treatment	Dose (mg/kg)	Calcium	Total protein	Oxalate
I	Normal Control	-	22.13±0.47	76.16±0.78	6.48±2.31
II	Lithiotic Control	1% EG solution	41.93±0.15[a]	197.66±1.85[a]	15.43±1.41[a]
III	Drug Control(Cystone)	500	17.36±0.67[b]	66.50±0.04[b]	7.65±2.19[b]
IV	Ethanolic Extract	250	18.55±0.19[b]	142.00±0.14[b]	12.03±0.01[b]
V	Ethanolic Extract	5oo	17.65±0.73[b]	131.00±1.15[b]	8.08±1.21[b]

Values are expressed in mean ± SEM for 6 animals.
[a]P<0.05 Vs Normal control.
[b]P<0.05 Vs Lithiotic control

The deposition of the crystalline components in the renal tissue (sodium, calcium, Creatinine and phosphorus) was increased in the stone forming rats (Table 2, group II). The ethanolic extract of seeds *Benincasa hispida* significantly (P <0.05) reduced the renal content of these stone forming constituents in regimens (Table 2, groups IV and V).

Table 2: Effect of ethanolic extracts of seeds *Benincasa hispida* on blood parameters in nephrolithic rats

Group	Treatment	Dose (mg/kg)	Sodium	Calcium	Creatinine	phosphorus
I	Normal Control	-	142.66 ±0.49	19.41±0.06	0.53±0.06	3.45±0.07
II	Lithiotic control	1% EG	139.16±2.21[a]	35.38±0.33[a]	0.75±0.05[a]	5.91±0.09[a]
III	Drug control (cystone)	500	142.16±0.30[a]	20.55±0.09[b]	0.36±0.04[b]	4.30±0.03[b]
IV	Ethanolic Extract	250	140.5±0.42[b]	20.84±0.42[b]	0.28±0.04[b]	4.25±0.14[b]
V	Ethanolic Extract	500	140.83±0.47[b]	14.64±0.15[b]	0.26±0.03[b]	4.23±0.27[b]

Values are expressed in mean ± SEM for 6 animals.
[a]P<0.05 Vs Normal control.
[b]P<0.05 Vs Lithiotic control

Discussion

In the present study, male rats were selected to induce urolithiasis because the urinary system of male rats resembles that of humans. [15] Earlier studies have also shown that the amount of stone deposition in female rats was significantly less than in male rats.[16]Urinary supersaturating with respect to stone-forming constituents is generally considered to be one of the causative factors in calculogenesis. Evidence in previous studies indicated that in response to 14 day period of ethylene glycol (0.75%, v/v) administration, young male albino rats form renal calculi composed mainly of calcium oxalate.[17-18] The biochemical mechanisms for these processes are related to increases in the urinary concentration of calcium and oxalate. Stone formation in ethylene glycol fed animals is caused by hyperoxaluria, which causes increased renal retention and excretion of oxalate. [19]

Our study demonstrated that calcium excretions are progressively increased in calculi-induced animals (Group II). Since it is accepted that hyperoxaluria is a far more significant risk factor in the pathogenesis of renal stones than hypercalciuria.[20] Increased urinary calcium is a factor favoring the nucleation and precipitation of calcium oxalate or apatite (calcium phosphate) from urine and subsequent crystal growth.[21] However, ethanolic extracts of. *Benincasa hispida* seeds lower the levels of oxalate.

An increase in urinary phosphate was observed in calculi induced rats (Group II). Increased urinary phosphate excretion along with oxalate stress seems to provide an environment appropriate for stone formation by forming calcium phosphate crystals, which epitaxially induces calcium oxalate deposition.[22] Treatment of *Benincasa hispida* seed extracts restores phosphate levels, thus reducing the risk of stone formation.

In urolithiasis, the glomerular filtration rate (GFR) decreases due to an obstruction to the outflow of urine by stones in urinary system. Due to this, the waste products (particularly nitrogenous substances such as urea, creatinine and uric acid) get accumulated in blood.[23] Also, increased lipid peroxidation and decreased levels of antioxidant potential have been reported in the kidneys of rats supplemented with a calculi-producing diet.[24-25] In this context, oxalate has been reported to induce lipid peroxidation and to cause renal tissue damage by reacting with polyunsaturated fatty acids in the cell membrane.[26] In calculi-induced rats (Group II), marked renal damage was seen as indicated by the elevated serum levels of creatinine. However, the curative and prophylactic treatment with ethanolic extract of *Benincasa hispida* seeds causes diuresis[27] and presumably hastens the process of dissolving the preformed stones and the prevention of new stone formation in urinary system. The significant lowering of serum levels of accumulated waste products is attributed to the enhanced GFR and the anti-lipid peroxidative property of *Benincasa hispida.*

.In conclusion, the presented data indicate that administration of the Ethanolic extract of *Benincasa Hispida* seeds to rats with ethylene glycol induced lithiasis, reduced and prevented the growth of urinary stones, supporting folk information regarding antiurolithiatic activity of the plant. The mechanism underlying this effect is still unknown, but is apparently related to increased diuresis and lowering of urinary concentrations of stone forming constituents. These effects could conclude the antiurolithiatic property of *Benincasa Hispida.*

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