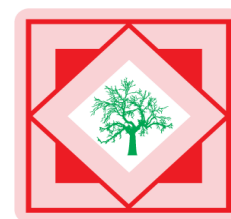




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Antiurolithic activity of *Ceropegia bulbosa* extract in rats

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ABSTRACT

In present study antiurolithic activity of hydroalcoholic extract of leaves of *Ceropegia bulbosa* was investigated in animal models of urolithiasis. Urolithiasis was induced by Ethylene glycol (0.75 % v/v). Investigation was done on the basis of elevated level of oxalate, phosphate, calcium, uric acid, BUN and creatinine due to urolithiasis. At 100, 200 and 400 mg/kg dose extract significantly protected animal's kidney from urolithiasis.

Key Words: *Ceropegia bulbosa*, urolithiasis, ethylene glycol.

INTRODUCTION

Herbal medicines are considered as the basis of therapy throughout the world since the ancient time of mankind and are still widely used. Recognition of their therapeutic uses is still growing, although this varies widely in different countries. Huge segment of inhabitants in developing countries still rely on the conventional practitioners, medicinal plants and herbal medicines for their prime care [1]. During past decades, public curiosity in natural therapies has augmented to a great extent in developed countries, with mounting use of medicinal plants and herbal medicines. Medicinal plants and derived medicine are widely used in traditional cultures all over the world [2]. Nearly 80% of the world populations depend on herbal and/or alternative medicine for their primary health care [3].

Urolithiasis is the development of stones in the urinary tract. This may lead to pain and bleeding. It is considered as the third most common affliction of the urinary tract. In most of the types of stones that are formed, the most frequent are calcium oxalate. As per clinical and epidemiological studies, calcium oxalate followed by calcium phosphate is the most commonly encountered crystalline components found in urolithiasis [4].

From the every beginning, herbal healing has been a preferred one for traditional healers. Conventional medicine has derived numerous of its drugs from herbal origin. According to the World Health Organization, approximately 75% of the global population, most of the developing world, depends on botanical medicines for their basic healthcare needs. Substances first isolated from plants account for approximately 25% of the western pharmacopoeia, with another 25% derived from modification of chemicals first found in natural products. Many plants like *Herniaria hirsute*, *Amni visnaga*, *Tribulus terrestris*, *Bergenia ligulata*, *Dolichos biflorus*, *Aerva lanata*, *Vediuppu chunnam*, *Raphanus sativus*, *Achyranthus Aspera*, *Quercus salicina*, *Phyllanthus niruri*, *Cranberry juice*,

Cynodon dactylon, Grapefruit juice, *Paronychia argentea*, Lemonade juice, *Pyracantha crenulata*, *Trachyspermum ammi*, *Moringa oleifera*, *Costus spiralis* are used as antiurolithic agent [5].

Ceropegia bulbosa (Asclepiadaceae) is a slender fleshy twining herb found in India. Traditionally leaves of *Ceropegia bulbosa* is used by local healers of Orissa state of India for treatment of kidney stones. Still no scientific report is available which proves its antilithiatic property. Thus present study was designed to investigate antiurolithic activity of hydroalcoholic extract of leaves of *Ceropegia bulbosa* in animal model of urolithiasis.

MATERIALS AND METHODS

Chemical and reagents

All chemicals used were of analytical grade. Cystone (Himalaya Drugs Co. Bangalore), Ammonium chloride and ethylene glycol (CDH, Mumbai) were purchased. Kits used in the study for determination of BUN, uric acid, and creatinine were purchased from SPAN Diagnostics, Gujarat.

Plant material and extraction

Ceropegia bulbosa leaves were collected locally from region near Bhubaneswar, Orissa, India. Plant herbarium was also prepared and submitted and authenticated by Dr. Ziaul Hasan, Botanist, Department of Botany, Safia Science College. Plant was dried under shade and crushed using commercial grinder at University department of Pharmaceutical Sciences, Utkal University, Bhubaneswar, Orissa. Dried plant material was extracted using defated using petroleum ether (40:60) by maceration for seven days with continuous stirring intermittently. After defating dried plant material was soaked with 70 % alcoholic solution for maceration with continuous stirring up to seven days. Extract (CBE) was dried using rotary vacuum evaporator and kept in air tight container till any further use.

Animals

Albino wistar rats weighing 200 ± 30 of either sex were selected at random from animal house of PBRI, Bhopal, India. Animals were further randomly divided into various treatment groups and kept in propylene cage with sterile husk as bedding. Animals were housed in relative humidity of 30.7 % at 22 ± 2 °C and 12:12 light and dark cycle. Animals were fed with standard pellets (Golden feeds, New Delhi, India) and water was available *ad libitum*. All animal experiments were approved by Institutional Animal Ethics Committee (IAEC) of PBRI, Bhopal (CPCSEA Reg No. - 1283/c/09/CPCSEA).

Ethylene glycol-induced urolithiasis

The albino rats were divided in six groups each of six animals. The animals of group I received vehicle, Group II received ethylene glycol (0.75% v/v) for 28 days, Group III, IV and V received ethylene glycol for 28 days and CBE (100, 200 and 400 mg/kg) from 15th to 28th day and Group VI received ethylene glycol for 28 days and cystone (750 mg/kg) from 15th to 28th day,

One day after (on day 29), blood was collected from retro-orbital puncture and serum was separated by centrifugation at 10000 rev/min for 10 min and analyzed for uric acid, creatinine, and blood urea nitrogen (BUN) [6]. Animals were sacrificed by cervical dislocation and kidneys were removed. It was used for estimation of phosphate [7], calcium [8], and oxalate [9].

Collection and analysis of urine

Rats were kept separately in metabolic cages and urine samples of 24 h were collected on 28th day. A drop of concentrated hydrochloric acid was added to the urine. Urine samples were analyzed for calcium, phosphorus, and oxalate content.

Statistical analysis

The data were presented as Mean \pm SD. Data was analyzed by one-way ANOVA followed by student's Newman Keul's test. $P < 0.05$ was considered statistically significant.

RESULTS

In preset study it was observed that administration of 0.75 % ethylene glycol (EG) solution significantly increased ($P<0.05$) level of oxalate, calcium, phosphate in kidney and urine (Table 1 and 2). It also increased level of creatinine, uric acid and BUN (Table 3). It was revealed that at 100mg/kg hydoroalcoholic extract of whole herb of *Ceropegia bulbosa* (CBE) significantly lowered ($P<0.05$) level of oxalate and calcium in kidney, but its effect on phosphate at 100mg/kg was not significant (Table 1). At 200mg/kg and 400mg/kg effect of CBE was found to be significant on reduction of oxalate, calcium and phosphate level in kidney (Table 1).

Table 1: Effect of CBE on stone forming constituents in kidney in EG induced urolithiasis

Group No.*	Treatment	Level in kidney (mg/g) [#]		
		Oxalate	Calcium	Phosphate
I	Vehicle only	1.36±0.12	2.88±0.22	2.01±0.12
II	Vehicle + EG	5.06±0.16 ^a	4.54±0.32 ^a	3.66±0.24 ^a
III	CBE 100 mg/kg + EG	4.46±0.31 ^b	4.09±0.07 ^b	3.46±0.05
IV	CBE 200 mg/kg + EG	2.09±0.09 ^b	3.62±0.14 ^b	3.00±0.22 ^b
V	CBE 400 mg/kg + EG	1.85±0.05 ^b	3.10±0.27 ^b	2.66±0.26 ^b
VI	Cystone 750 mg/kg + EG	1.48±0.06 ^b	3.01±0.14 ^b	2.15±0.27 ^b

*Each group consist of sex animals

#Data presented in mean±SD

^a $P<0.05$ as compared to vehicle treated group

^b $P<0.05$ as compare to vehicle + EG treated group

For estimation of effect of CBE on excreted amount of oxalate, calcium and phosphate in urine EG induced lithiasis level of these three components was also measured in urine. Observations of effect of CBE at 100, 200 and 400mg/kg are mentioned in Table 2. It was observed that at all three selected doses level CBE significantly decreased ($P<0.05$) oxalate, calcium and phosphate level in urine.

Table 2: Effect of CBE on stone forming constituents in urine in EG induced urolithiasis

Group No.*	Treatment	Level in urine (mg/dl) [#]		
		Oxalate	Calcium	Phosphate
I	Vehicle only	0.35±0.08	1.2±0.05	3.35±0.33
II	Vehicle + EG	3.39±0.30 ^a	4.3±0.18 ^a	6.27±0.21 ^a
III	CBE 100 mg/kg + EG	3.00±0.09 ^b	3.47±0.24 ^b	5.72±0.17 ^b
IV	CBE 200 mg/kg + EG	0.66±0.03 ^b	1.40±0.02 ^b	3.76±0.03 ^b
V	CBE 400 mg/kg + EG	0.59±0.03 ^b	1.39±0.01 ^b	3.6±0.08 ^b
VI	Cystone 750 mg/kg + EG	0.51±0.05 ^b	1.36±0.03 ^b	3.51±0.30 ^b

*Each group consist of sex animals

#Data presented in mean±SD

^a $P<0.05$ as compared to vehicle treated group

^b $P<0.05$ as compare to vehicle + EG treated group

BUN, uric acid and creatinine are important markers for assessment of effect on kidney function. In EG treated group it was observed that, it significantly increased level of all these three components (Table 3). At 100mg/kg effect of CBE significantly decreased ($P<0.05$) level of BUN, uric acid and creatinine. At 200mg/kg and 400mg/kg CBE significantly decreased level of BUN, uric acid and creatinine (Table 3).

Table 3: Effect of CBE on serum parameters in EG induced urolithiasis

Group No.*	Treatment	Serum parameters(mg/dl) [#]		
		BUN	Uric acid	Creatinine
I	Vehicle only	36.09±1.04	1.45±0.05	0.70±0.07
II	Vehicle + EG	51.12±2.30 ^a	3.68±0.10 ^a	1.07±0.13 ^a
III	CBE 100 mg/kg + EG	48.61±2.09	3.12±0.08 ^b	0.99±0.04
IV	ACE 200 mg/kg + EG	41.89±1.30 ^b	1.89±0.07 ^b	0.85±0.02 ^b
V	CBE 400 mg/kg + EG	39.72±2.77 ^b	1.78±0.04 ^b	0.80±0.03 ^b
VI	Cystone 750 mg/kg + EG	37.53±1.70 ^b	1.68±0.05 ^b	0.79±0.03 ^b

*Each group consist of sex animals

[#]Data presented in mean±SD

^aP<0.05 as compared to vehicle treated group

^bP<0.05 as compare to vehicle + EG treated group

DISCUSSION

Ayurveda is the most ancient health care system and is practiced widely in India, Srilanka and other countries [10]. It is commonly considered that herbal drugs are cheaper and safer as compared to synthetic drugs [11]. In present study one of the traditionally used herbs, *Ceropegia bulbosa* was investigated for its antiurolithic activity in animal model of urolithiasis. In ethylene glycol (EG) administered groups oxalate and calcium excretion progressively increased, and even the level of these components in kidney was also found to high. It has been reported that when rats were treated with ethylene glycol and ammonium oxalate, level of these components increased upto significant level [12]. It is established that hyperoxaluria is a far more significant threat factor in the pathogenesis of renal stones than hypercalciuria. Increased excretion of calcium has been reported in humans as well as rats [13]. An increased urinary calcium concentration is a factor favouring the nucleation and precipitation of calcium oxalate (calcium phosphate) from urine and subsequent crystal growth. A gradual increase in urinary phosphorus and uric acid excretion is observed in EG treated animals. Increased excretion of phosphorus and uric acid has been reported in stone formers [14] in hyperoxaluric rats. Increased urinary phosphorus excretion along with oxalate stress seems to provide an environment suitable for stone formation by forming calcium phosphate crystals, which induces calcium oxalate deposition. Uric acid interferes with calcium oxalate solubility [15] and it binds and reduces the inhibitory activity of glycosaminoglycans [16]. 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 [17]. In present study CBE treatment restores oxalate and calcium and phosphorus levels, to normal thus reducing the risk of stone formation. In extract treated groups it was also found that level of BUN, uric acid and creatinine was comparable to control group.

CONCLUSION

From study it was concluded that hydroalcoholic extract of leaves of *Ceropegia bulbosa* possess significant antiurolithic activity.

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