ANTIUROLITHIC ACTIVITY OF *AGERATUM CONZOIDES* EXTRACT IN RATS

Mohd. Azaz Khan.[1]*, Debasish Pradhan[1]

[1] University Department of Pharmaceutical Sciences, Utkal University, Vanivihar, Bhubanehwar, 751004, Orissa, India
* Corresponding Author – azazkhan23@gmail.com

Summary

In present study Antiurolithic activity of hydroalcolohlic extract of whole plant of *Ageratum conzoides (ACE)* was investigated in rats. Urolithiasis was induced in animals by using ethylene glycol (0.75 % v/v). Investigation was done on the basis of estimation of stone forming constituents oxalate, calcium, and phosphate, in kidney and urine. Effect on BUN, uric acid, and creatinine level was also analyzed to ascertain effect on kidney function test. At all selected dose of 100 mg/kg, 200 mg/kg and 400 mg/kg extract decreased level stone forming constituents and it also significantly decreased level of BUN, uric acid and creatinine as compared to control group. From present study it was revealed that ACE possesses significant antiurolithic activity.

Key Words: *Ageratum conzoides,* Hydroalcoholic, Antiurolithic, Ethylne glycol,

Introduction

Since ancient times plants are used as rich source of medicine. Scientific exploration of traditional knowledge of use of herbs in treatment of various ailments is one of the thrust areas of research. Ayurveda, Siddha, Unani and Folk medications are the main systems of indigenous drugs. Researchers are providing evidence and research, in validating efficacy and safety of utilizing traditional awareness for health and healing.

Urinary stones affect 10–12% of the population in industrialized countries [1]. The incidence of urinary stones has been increasing over the last years while the age of onset is decreasing [2]. The etiology of this disorder is multifactorial and is strongly related to dietary lifestyle habits or practices [3]. Although there are a few recent reports of beneficial effects of medical treatments in enhancing clearance of stones in the distal ureters [4], de facto there is still no satisfactory drug to use in clinical therapy, especially for the prevention or the recurrence of stones. In this regard, many plants have been traditionally used to treat kidney stones and have been shown to be effective.

In ayurveda and folklore medicine many herbs are used in management of urolithiasis. Many researchers through out the globe are working on ascertaining potential of herbs in urolithiasis. Some of reported plants from India for anti-urolithic potential are Sesbania grandiflora (L.) Pers. [5]; Aerva lanata(L.) Juss. Ex. Schult. [6] Moringa oleifera Lam. [7]; Asparagus racemosus Willd. [8] Rotula aquatica Lour. [9]; Cyclea peltata (Lam.) Hook.f.& Thoms. [10] Tribulus terrestris L. [11]; Musa sapienta L. (banana stem) [12] Ammannia baccifera L. [13]; Mimosa pudica L. [14] Crataeva nurvala Buch-Ham. [15].

Ageratum conyzoides L., Asteraceae, is an annual herbaceous plant. *A. conzyoide* have many bioactive secondary metabolites which include flavonoids, alkaloids, cumarins, essential oils, and tannins. Pharmacologically it possess antibacterial activity [16], analgesic activity [17], anti-inflammatory [18], muscle relaxant and anti spasmodic activity [19]. Plants have been used traditionally to treat colic, colds and fevers, diarrhea, rheumatism, spasms, or as a tonic [20-26].

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

Ageratum conzoides whole plants 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 % ethanolic solution for maceration with continuous stirring up to seven days. Hydroalcoholic extract of A. conzoides (ACE) was dried using rotary vaccum 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 Instutional 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 ACE (100, 200 and 400 mg/kg) from 15^{th} to 28^{th} day respectively and Group VI received ethylene glycol for 28 days and cystone (750 mg/kg) from 15^{th} to 28^{th} day,

One day after (on day 29), blood was collected from retro-orbital puncture and serum was separated by centrifugation at 10000 rpm for 10 min and analyzed for uric acid, creatinine, and blood urea nitrogen (BUN) [26]. Animals were sacrificed by cervical dislocation and kidneys were removed and were used for estimation of phosphate [27], calcium [28], and oxalate [29].

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 ± 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 hydoralcoholic extract of whole herb of *Digera muricata* (DME) 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 DME was found to be significant on reduction of oxalate, calcium and phosphate level in kidney (Table 1).

Table 1: Effect of ACE 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	ACE 100 mg/kg + EG	3.62±0.38[b]	3.98±0.27[b]	3.41±0.33
IV	ACE 200 mg/kg + EG	2.26±0.22[b]	3.71±0.08[b]	3.08±0.13 [b]
V	ACE 400 mg/kg + EG	2.13±0.36[b]	3.13±0.21[b]	2.70±0.33 [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 DME 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 DME at 100, 200 and 400mg/kg are mentioned in Table 2. It was observed that at all three selected doses level DME significantly decreased ($P<0.05$) oxalate, calcium and phosphate level in urine.

Table 2: Effect of ACE 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	ACE 100 mg/kg + EG	1.34±0.09 [b]	2.01±0.14 [b]	4.08±0.16 [b]
IV	ACE 200 mg/kg + EG	0.75±0.06 [b]	1.43±0.03 [b]	3.81±0.18 [b]
V	ACE 400 mg/kg + EG	0.59±0.03 [b]	1.39±0.02 [b]	3.73±0.13 [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, 200mg/kg and 400mg/kg DME significantly decreased level of BUN, uric acid and creatinine (Table 3).

Table 3: Effect of ACE 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	ACE 100 mg/kg + EG	44.06±2.17[b]	2.11±0.08 [b]	0.91±0.07 [b]
IV	ACE 200 mg/kg + EG	42.48±2.27 [b]	2.08±0.06 [b]	0.86±0.04 [b]
V	ACE 400 mg/kg + EG	41.59±2.48 [b]	1.83±0.07 [b]	0.84±0.04 [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
[a]P<0.05 as compared to vehicle treated group
[b]P<0/05 as compare to vehicle + EG treated group

Discussion

Formation of calculi is associated with supersatuartion of urine with stone forming constituents. Reserachers have proved that repeated administration ethylene glycol (0.75% v/v) cause generation of kidney stone and the most important cause for it was found to be presence of calcium oxalate (30, 31). Increase in the urinary concentration of oxalate is considered as one of the major cause responsible for formation of stone. Stone formation in ethylene glycol administered animals is caused by hyperoxaluria, which enhances renal retention and excretion of oxalate (30). Similar results have been obtained when rats were treated with ethylene glycol and ammonium oxalate (32, 33). In present study it was observed that ACE significantly decreased level of oxalate, calcium and phosphate in kidney as well as urine at selected dose.

Hence, it is providing protection against urolithiasis by decreasing level of causative factor for calculi in kidney. Decrease in glomerulas filtration due to obstruction generated in kidney cause accumulation of waste product in blood, thus level of waste components like BUN, uric acid and Creatinine increases in blood. In this investigation it was observed that level of BUN, uric acid and Creatinine in ACE treated group at selected dose was comparable to that of negative control group (vehicle treated). Also, increased lipid peroxidation and decreased levels of antioxidant potential have been reported in the kidneys of rats supplemented with a calculi-producing diet. *Ageratum conzoides* is rich in various components that possess significant antioxidant activity. Thus ACE would also be producing protective effect due to its antioxidant potential. Extract was found be effective in normalising all tested parameters which are having direct or indirect effect on urolithiasis.

Conclusion

From present study it can be concluded that hydroalcoholic extract of *Ageratum conzoides* (ACE) possess significant antiurolithic activity.

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