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Research Article

ANTI-UROLITHIATIC ACTIVITY OF DIFFERENT EXTRACTS OF *AGERATUM CONYZOIDES* (LINN.)

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ABSTRACT

Ageratum conyzoides Linn. (Asteraceae) is a soft hairy annual weed with powerful traditional uses. Aqueous, ethyl acetate and ethanolic extracts were prepared from the whole plant of *A. conyzoides* (L.). Urolithiasis was induced by using 5 % ethylene glycol in wistar albino rats. Rats were treated with different extracts of *A. conyzoides* (L.) at a dose level of 500 mg/kg bwt while Calcuri at same dose level was used as standard. The results of the study proved that all the plant extracts significantly reduced calcium and oxalate concentration in the excreted urine and the deposition of the same in the kidney, while the highest reduction in the calcium and oxalate in the urine and kidney was noted with the ethanol extract. Preliminary phytochemical analysis revealed the presence of a number of phytochemical constituents in different extracts which may help in the identification of plant. The present result advocates the anti-urolithiatic activities of different extracts of the plant.

Keywords: Anti-urolithiatic activity, *Ageratum conyzoides* (L.), Ethylene glycol, calcium oxalate, urine volume.

INTRODUCTION

Urolithiasis is the third most common disorder of the urinary tract is manifested by the formation of stones in the urinary tract. The overall prevalence of urolithiasis is ~12 % with a recurrence rate of 70-80 % in males, 47-60 % in females[1]. These stones are classified according to their chemical constituents, like calcium containing stones made of calcium oxalate monohydrate, calcium oxalate dihydrate and basic calcium phosphate, magnesium ammonium phosphate, uric acid and cystine. The most commonly occurring stones are calcium containing stones 75-90 % and magnesium ammonium phosphate 10-15 %[2,3]. Whenever the excretion rate is increased and the urine is supersaturated with insoluble materials, crystals are formed anywhere in the urinary tract as the course of repeated accumulation of salts. The process is accelerated by conditions like hepatic dysfunction, obesity and hypertension and is powerfully related to the dietary habits[4-8]. *Ageratum conyzoides* Linn. (Asteraceae) is a soft hairy annual weed grows up to a height of 90 cm with alternate or opposite leaves which are hairy on both sides. Traditionally roots or leaves are used for the treatment of various ailments. Roots are digestive, lithontriptic, appetizer used in conditions like dyspepsia, anorexia renal and vesicle calculi and pharyngopathy. Leaves are used to stop local bleeding and heal wounds and sores[9]. The literature survey about the plant support the anti inflammatory, analgesic and antimicrobial and wound healing properties of the plant[10-16]. The present study was undertaken to investigate the anti-urolithiatic activity of *Ageratum conyzoides* Linn.

MATERIALS AND METHODS

Preparation of plant extracts

The plant material was collected from Tirunelveli district, India and was identified. A voucher specimen was deposited in the herbarium of department of pharmacology, Sankaralingam Bhuvaneswari College of pharmacy, Sivakasi, India for future reference. The whole plant was washed under running tap water followed by distilled water to remove extraneous materials and shade dried. Coarsely powdered plant material was extracted with petroleum ether, ethyl acetate, ethanol and water successively under cold maceration procedure for 72 h. The resultant extracts were filtered concentrated in a rotary flash evaporator and was dried in a vaccum desiccator. The dried extracts were weighed and color, consistency and the percentage yield were noted for each extract. All extracts were subjected to preliminary phytochemical evaluation[17,18].

Animals

Wistar albino rats of either sex weighing 150-200 g maintained in the animal house of the Sankaralingam Bhuvaneswari College of Pharmacy were selected for the study. The females were nulliparous and non pregnant. All animals were kept in the standard environmental conditions of temperature 24 ± 1^{0}C, at 12: 12 dark light cycle and fed with commercial standard pellet diet supplied by Kamadhenu agencies, Bangalore, India and drinking water *ad libitum*. The animal experimental protocol for the present study was approved by our college Institutional Animal Ethical Committee [SBCP/ 2008-2009/ IAEC/ CPSCEA/ 10].

Ethylene glycol induced urolithiasis

Urolithiasis was induced by ethylene glycol in experimental animals by mixing 5 % Ethylene glycol (v/v) in drinking water for 7 days. Group I was the vehicle control group which received 0.5 % (w/v) gum acacia solution (5 ml/kg p.o). Group II-VI received 0.75 % (v/v) ethylene glycol in drinking water. Group III received standard drug calcuri (500 mg/kg, p.o.) and Group IV-VI received 500 mg/kg bwt of aqueous, ethyl acetate and ethanolic extracts of *A. conyzoides*

(L.) respectively. The drug treatment was continued for a period of 28 days.

Collection and analysis of urine

On the 28th day all animals were kept in individual metabolic cages with free access to drinking water and 24 hours urine samples were collected. The total volume and pH of the urine samples were noted. Various urine parameters such as oxalate, calcium and phosphate were performed.

Analysis of kidney biochemistry

All the experimental animals were sacrificed under ether anesthesia and kidney was carefully removed, washed in ice cold 0.15 M KCl and was homogenized in normal saline. The homogenate was centrifuged at 3000 rpm for 10 minutes and the supernatant was analyzed for the estimation of phosphate, calcium and oxalate.

Statistical analysis

Data were expressed as the mean ± standard errors for each group of animals. Statistical analysis was performed with one way analysis of variance (ANOVA) followed by Dunnett's t test.

Table 1: Preliminary phyto profile of different extracts of *A. conyzoide* (L.)

S. No	Extracts	Percentage yield	Color	Consistency
1	Petroleum ether	0.72 %	Greenish-black	Sticky
2	Ethyl acetate	2.86 %	Brownish-black	Sticky
3	Ethanol	5.02 %	Dark-brown	Sticky
4	Water	12.08 %	Black	Sticky

Table 2: Preliminary phytochemical analysis of different extracts of *A. conyzoides* (L.)

S. No	Phytoconstituents	Pet. ether	Ethyl acetate	Ethanol	Aqueous
1	Alkaloids	-	-	-	-
2	Carbohydrate	-	+	+	+
3	Glycoside	-	-	+	+
4	Fixed oil and Fat	+	+	-	-
5	Saponins	-	+	+	+
6	Tannins and Phenolic compounds	-	-	+	+
7	Proteins and Amino acid	+	-	+	+
8	Gums mucilage	-	-	+	+
9	Flavonoids	+	+	+	+
10	Lignin	-	-	+	+
11	Steroids	+	+	-	-

- : negative, +: Positive

Table 3: Effect of various extracts of *A. conyzoide* (L.) on urine parameters in ethylene glycol induced urolithiasis in albino rats

Groups	Treatment	Calcium (mg/dl)	Oxalate (mg/dl)	Phosphate (mg/dl)	Urine volume(ml)
I	Positive Control (NS)	0.205 ± 0.192	2.188 ± 0.024	2.082 ± 0.126	8.685 ± 0.342
II	Negative Control (5 % EG)	0.878 ± 0.053	7.8 ± 0.138	5.15 ± 0.173	3.77 ± 0.042
III	Standard (Calcuri)	0.308 ± 0.126^{c}	2.388 ± 0.190^{b}	2.815 ± 0.173^{c}	7.9 ± 0.052^{a}
IV	Aqueous extract (500 mg/ kg bwt)	0.803 ± 0.310^{a}	7.28 ± 1.467^{a}	4.7 ± 0.337^{a}	6.8 ± 0.365^{b}
V	Ethyl acetate extract (500 mg/ kg bwt)	0.305 ± 0.024^{c}	2.175 ± 0.526^{b}	2.718 ± 0.024^{c}	5.22 ± 0.037^{c}
VI	Ethanolic extract (500 mg/ kg bwt)	0.123 ± 0.021^{b}	1.523 ± 0.040^{b}	1.218 ± 0.021^{b}	7.775 ± 0.342^{a}

Values are mean ± S.E.M., n = 6, One way ANOVA followed by Dunnet's *t*-test, $^{a}p < 0.001$, $^{b}p < 0.01$, $^{c}p < 0.02$, $^{d}p < 0.05$., NS – Normal saline, EG – Ethylene glycol

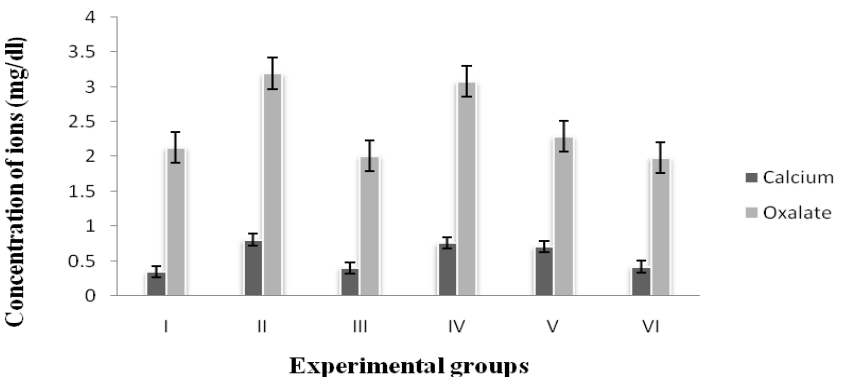

Figure 1: Effect of *Ageratum conyzoides* Linn. on calcium and oxalate deposition in kidney homogenate

RESULT AND DISCUSSION

The percentage yield of *Ageratum conyzoides* Linn. were 0.72 %, 2.856 %, 5.018 % and 12.08 % w/w for petroleum ether, ethyl acetate, ethanol and aqueous extracts respectively. The color and consistency of the various extracts ranged from greenish black, brownish black, dark-brown color and black for petroleum ether, ethyl acetate, ethanol and aqueous extracts respectively and all the extracts obtained were sticky in consistency (Table 1). Preliminary phytochemical analysis revealed the presence of phytoconstituents such as alkaloids, carbohydrate, glycoside, fixed oil, saponins, tannins, phenolic compounds, proteins and amino acid, gums, mucilage, flavonoids, lignin, steroids etc (Table 2). A number of animal models have been used for the study of nephrolithiasis. 5 % ethylene glycol (2 ml/rats/p.o for seven days) induced hyperoxaluria model in rats was used in the present study because of close resemblance of rat urinary system to that of humans. In the present study urine output was significantly decreased in all the ethylene glycol treated rats when compared to the negative control animals. The obstruction of urine excretion is due to nidus formation, crystal aggregation and calcium oxalate salt saturation. The origin of calculus is attributed to a scarcity of crystallization inhibitors (nucleation inhibitors) and/ or an increase of promoters[19]. The mechanisms involved in the formation of calcific stones are not clear but it is generally agreed that urolithiasis is a complex process involving events leading to crystal nucleation, aggregation and growth of insoluble particle[4]. Administration of aqueous, ethyl acetate and ethanolic extracts of *A. conyzoides* L. (500 mg/kgbwt/day) and calcuri (500 mg/kg bwt/day) to the animals increased urine output (Table 3). In the calcuri treated group significantly increased urine output (7.9 ± 0.052) was noted on the 28th day. Ethanolic extract (7.775 ± 0.342) also put forth a significant increase in the urine volume followed by aqueous extract (6.8 ± 0.365) while the ethyl acetate extract (5.22 ± 0.037) exerted least urine volume among the test drugs but was significantly higher than that observed in the positive control group. Ethanol extracts of *A. conyzoide* significantly reduced the calcium (0.123 ± 0.021) and oxalate (1.523 ± 0.040) levels in the urine followed by ethyl acetate extract (2.175 ± 0.526 mg/dl of oxalate and 0.305 ± 0.024 mg/dl calcium). Changes in urinary chemistry such as hyper calciuria and hyperoxaluria, leads to the urinary super saturation followed by aggregation of crystals subsequently leads to stone formation[20]. Administration of aqueous extract, ethyl acetate extract and ethanolic extract of *A. conyzoides* (L.) at a dose 500 mg/kg bwt/day statistically reduced calcium (50 %) and oxalates (37 %) deposition in the kidney when compared to the normal groups (Figure 1). Similar results were also reported with water extracts of *Spirulina* which could offer a significant decrease in the calcium, sodium and chloride levels in the kidney[21]. An increase in the calcium and oxalate deposition in the kidney was observed in the 5 % ethylene glycol treated groups may be due to effective renal re-absorption. The biochemical mechanisms for this process are related to an increase in the urinary concentration of oxalate. Stone formation in ethylene glycol fed animals is caused by hyperoxaluria, which causes increased renal retention of oxalate[20]. The appearance of calcium oxalate in renal tubules following ethylene glycol injection will lead to necrosis of tubular cells, which results in exposure of tubular, basal lamina and finally results in the formation of luminal cellular debris. The calcium oxalate crystals do causes cytolysis of polymorphonuclear leukocytes following phagocytosis and may be destructive to renal epithelium. Ethylene glycol challenge brings about rapid increase in urinary excretion of calcium oxalate and formation of crystals takes place in kidneys. These crystals deposit progressively in the cortex, medulla and renal tubules. In the present study, the increased severity of kidney crystal deposition with ethylene glycol correlated well with increased calculi oxalate concentration in kidney. In conclusion, different extracts of *A. conyzoide* (L.) significantly attenuated the urinary excretion of calcium and oxalate without affecting the phosphate concentration and calcium oxalate deposition in kidney suggested their beneficial effects against calcium oxalate deposition in urolithiasis.

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