

Original Article

INVESTIGATION OF ANTI-UROLITHIATIC ACTIVITY OF BRASSICA OLERACEA GONGYLODES AND DESMOSTACHYA BIPINNATA IN EXPERIMENTALLY INDUCED UROLITHIASIS IN ANIMAL MODELS

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ABSTRACT

Objectives: The present study was undertaken to evaluate the effects of some traditional medicinal plants i.e., *Brassica oleracea Gongyloides* and *Desmostachya bipinnata* in combination and in alone on experimentally-induced (urolithiasis) kidney stones.

Methods: Urolithiasis in animals was induced experimentally by administration of ethylene glycol (0.75% v/v) with ammonium chloride (1% w/v) in drinking water for ten days. The aqueous extract of both plants were administered alone and in combination to urolithiasis induced test group rats at a dose of 400 mg/kg respectively for 10 days. After 10 days, renal function parameters measured such as increase in the urine urea, uric acid, and calcium and creatinine levels, which reflect the deposition of calcium oxalate in the kidneys.

Results: The serum analysis showed significant increase in the serum uric acid, serum creatinine, blood urea and calcium in urolithiasis control group rats. In addition, vehicle treated induction control group rats showed significant increase in the biochemical parameters.

Conclusion: Daily oral treatment with all most all extracts not significantly decreased the quantity of calcium oxalate deposited in the kidneys but also reverted all the biochemical changes induced by calcium oxalate urolithiasis thus supporting its traditional claim. Aqueous extract of both plants in combination and in alone was found to be significant when compared with standard and control group.

Keywords: Urolithiasis, Creatinine, *Desmostachya bipinnata*, *Brassica oleracea Gongyloides*,

INTRODUCTION

Urolithiasis is the process of forming a stone in the kidney or in the urinary tract. The development of the stones is related to decreased urine volume or increased excretion of stone-forming components such as calcium, oxalate, urate, cystine, xanthine, and phosphate [1-3]. The blood in the urine and pain in the abdomen, flank, or groin were seen during the Urolithiasis. Kidney stones occur in 1 in 20 people at some time in their life. Urinary composition determines stone formation based on three factors: exceeding the formation product of stone forming components, the quantity of inhibitors (e.g., citrate, glycosaminoglycans, etc.) and promoters (e.g., sodium, urates, etc.) in the urine. The stones form in the urine collecting area (the pelvis) of the kidney and may range in size from tiny to the size of the renal pelvis itself. Currently no allopathic medicines are available for urolithiasis [4]. Surgery, lithotripsy, and local calculus disruption using a high power laser are used to treat calculi [5-7]. However, these procedures are expensive and recurrence is quite common.

In recent times, focus on plant research has increased all over the world and a large body of evidence has collected to show immense potential of medicinal plants used in traditional systems [8-10]. *Brassica oleracea Gongyloides* is a biennial vegetable crop belonging to the family Brassicaceae. *B. oleracea* has become established as an important human food crop plant, used because of its large food reserves, which are stored over the winter in its leaves [11-13]. It is rich in essential nutrients including Vitamin C. *Desmostachya bipinnata* belonging to family Poaceae, has many therapeutic benefits such as its use in tribal area as dysentery, menorrhagia, diuretic, fodder for domestic livestock.

The present study is to evaluate the antiurolithiatic activity of an aqueous extract of *Brassica oleracea Gongyloides* and *Desmostachya bipinnata* in combination and in alone.

MATERIALS AND METHODS

Plant Material

Brassica oleracea Gongyloides and *Desmostachya bipinnata* were collected from local area and authenticated from the department of Pharmacognosy, GCOP, R.R. Dist. The aerial parts of the plants were dried under shade at room temperature, later chopped and grounded into coarse powder [14-18]. The powdered materials were used for extract preparations.

Chemicals

Ethylene glycol was obtained from Merck Ltd. Mumbai, India. All other chemicals and reagents used were analytical grade and procured from approved chemical suppliers.

Animals

Albino wistar rats (200-250 g) were purchased and maintained under standard environmental laboratory conditions and fed [19-22] with laboratory diet and water *ad libitum* and the protocol was approved by the institutional animal ethical committee.

Experimental design

Thirty six healthy adult wistar albino strain rats of either sex weighing 200- 250 g were randomly selected and then divided into six groups with 6 animals in each group. The treatment period was considered for 10 days. Group-I served as normal received drinking water, Group-II served as Urolithiatic control received Drinking water containing 0.75 % [v/v] ethylene glycol and 2% [w/v] ammonium chloride, Group-III served as standard received Cystone 5ml/kg body weight per oral & drinking water containing 0.75 % [v/v] ethylene glycol and 2% [w/v] ammonium chloride, Group-IV received Aqueous extract of *Brassica oleracea Gongyloides*, Group-V served as test received Aqueous extract of *D. bipinnata* and Group-VI received combination of both plant extracts at 400 mg/kg body weight per oral route & drinking water containing 0.75 % [v/v] ethylene glycol and 2% [w/v] ammonium chloride [23-25].

Assessment of Anti-Urolithiatic activity

Formation of crystalluria and stone formation was verified by different biochemical marker analysis of urine and serum. At the end of the experiment, all animals kept in individual metabolic cages and 24-hour urine samples were collected and measured on the 10th day. Animals had free access to drinking water during the urine collection period [26-27]. The urine was analysed for calcium, magnesium, phosphate, urea, uric acid, oxalate and citrate.

Collection of Blood sample

On the 10th day, the animals were anesthetized and blood was collected from the retro-orbital sinus under mild anaesthesia. Serum was separated by centrifugation at 15,000 rpm for 20 minutes and analyzed for calcium, oxalate, magnesium, phosphate, urea, uric acid and creatinine [28].

Statistical analysis

The values Mean±SEM are calculated for each parameter. For determining the significant inter group difference each parameter was analysed separately and one-way analysis of variance was carried out.

RESULTS AND DISCUSSION

In the present study, Ammonium chloride (2%) + Ethylene glycol (0.75%) induced urolithiasis resulted in significant elevation of serum

and urine analysis, body weight and kidney weight were compared to normal control group [29]. Treatment with cystone (750 mg/kg) and test sample 400 mg/kg prevented the bio-chemical changes induced by Ammonium chloride (2%) + Ethylene glycol (0.75%).

Urine Analysis

The urine creatinine, uric acid, urea, and calcium (mg/dl) level of normal, control & treated was found to be significant. Combination of the both the plant extracts showed significant when compared to alone as shown in Table-1.

Serum Analysis

The serum creatinine, urea, uric acid and calcium (mg/dl) level of normal, control & treated was found to be significant. Combination of the both the plant extracts showed significant when compared to alone as shown in Table-2.

Calcium, urea, uric acid, and creatinine values of serum of rats are decreased in serum may be drug effects metabolism of calcium, urea, uric acid and creatinine. May it effect oxalate formation as it is main constituent in inducing urolithiasis. Intense pain may lead to decrease in the food consumption which may further result into decrease in the body weight [30-31]. As in urolithiasis induced control group kidney weight is increased because of accumulation of urine in kidneys as obstruction in excretion of urine.

Table 1: Effect of aqueous extracts of *D. Bipinnata* & *B.oleracea Gongylodes* extracts on urine parameters

S. No.	Groups	Urea (mg/dl)	Uric acid (mg/dl)	Calcium (mg/dl)	Creatinine (mg/dl)
1.	Normal	75.9±0.2	3.5±0.1	4.2±0.2	0.3±0.3
2.	Control	82.2±0.1	7.9±0.2	7.8±0.3	9.5±0.1
3.	Standard	90.1±0.2	3.6±0.1	5.5±0.1	3.9±0.2
4.	<i>D. Bipinnata</i>	45.2±0.1	6.9±0.2	7.0±0.2	5.2±0.1
5.	<i>B.oleracea Gongylodes</i> ,	64.8±0.2	6.3±0.1	7.4±0.1	6.1±0.2
6.	<i>D. Bipinnata</i> + <i>B.oleracea Gongylodes</i> ,	70.2±0.2	5.5±0.1	6.6±0.1	5.7±0.1

The values are expressed as mean ±SEM, n=6 in each group.

Table 2: Effect of aqueous extracts of *D. Bipinnata* & *B.oleracea Gongylodes* extracts on serum parameters

S. No.	Groups	Urea (mg/dl)	Uric acid (mg/dl)	Calcium (mg/dl)	Creatinine (mg/dl)
1.	Normal	65.3±0.2	6.3±0.1	0.9±0.3	3.18±0.15
2.	Control	129.2±0.1	4.1±0.2	7.8±0.2	0.69±0.12
3.	Standard	53.4±0.2	9.5±0.2	1.3±0.1	0.60±0.23
4.	<i>D. Bipinnata</i>	108.7±0.4	3.5±0.2	4.2±0.3	0.71±0.28
5.	<i>B.oleracea Gongylodes</i> ,	94.5±0.1	3.1±0.1	2.1±0.2	0.58±0.67
6.	<i>D. Bipinnata</i> + <i>B.oleracea Gongylodes</i> ,	70.1±0.2	2.8±0.2	3.5±0.1	0.62±0.41

The values are expressed as mean ±SEM, n=6 in each group.

Table 3: Effect of aqueous extracts of *D. Bipinnata* & *B.oleracea Gongylodes* extracts on total body weight and kidney weight

S. No.	Groups	Body weight		Kidney weight(gm)
		Initial (gm)	Final (gm)	
1.	Normal	205±4.5	215±3.5	0.58±0.01
2.	Control	220±6.0	198±3.5	0.81±0.03
3.	Standard	200±3.5	205±3.0	0.49±0.02
4.	<i>D. Bipinnata</i>	225±2.5	215±4.5	0.63±0.02
5.	<i>B.oleracea Gongylodes</i> ,	200±3.5	210±1.5	0.52±0.03
6.	<i>D. Bipinnata</i> + <i>B.oleracea Gongylodes</i> ,	230±1.5	240±2.5	0.50±0.03

The values are expressed as mean ±SEM, n=6 in each group.

Change in body weight

The change in body weight (%) of normal, control & treated was found to be significant. Combination of the both the plant extracts showed significant when compared to alone as shown in Table-3.

Anti urolithiatic activity may be due to diuretic effect and antioxidant effects of flavonoids are already reported and thus it may protect urolithiasis is by protecting from peroxidation of kidney apoptosis [32]. Thus by consideration of urine and serum parameters combination of *Brassica oleracea Gongylodes* and *Desmostachya bipinnata* has anti urolithiasis effect than alone used.

CONCLUSION

The results of the present study have shown that the urinary stones could be dissolved with aqueous extract of *Brassica oleracea Gongyloides* and *D. Bipinnata*. The mechanism underlying this effect is still unknown, but is apparently related to diuresis and lowering of urinary concentrations of stone forming constituents. Further studies are needed to identify the active principle(s) responsible for the antiurolithiatic effect and evaluate its mechanism of action in different model.

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