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ORIGINAL RESEARCH ARTICLE

Antiurolithiatic Activity of Aqueous and Methanolic Extracts of *Tecoma stans Flowers in* Rats

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ABSTRACT

In the aboriginal system of medicine, the *Tecoma stans* flowrs. (Family- Bignoniaceae) are reported to be constructive in the management of urinary stones. Hence, in the present study the *Tecoma stans* flowers have been preferred for their antiurolithiatic activity on experimentally induced urolithiatic rats. Antiurolithiatic activity of aqueous and methanolic extracts of *Tecoma stans* (AETS & METS) was carried out on ethylene glycol (0.75% v/v) induced urolithiasis in rats. Treatment with aqueous extract (200mg/kg, p.o) and methanolic extract (250mg/kg, p.o) of of *T.stans* flowers significantly lowered (P<0.001) the increased levels of oxalate, calcium and phosphate in urine and also significantly reduced (P<0.001) their retention in kidney. The treatment with Aqueous extract and Alcoholic extract of Tecoma stans flowers significantly (P<0.001) lowered the elevated serum levels of Blood urea nitrogen, creatinine and uric acid in both regimens. The histopathological study of the kidney also supported the above results. The results were comparable to that of standard drug (Cystone). The presented data indicate that administration of AETS and METS to rats with experimentally-induced urolithiasis reduced and also prevented the formation of urinary stones, supporting folk information regarding antiurolithiatic activity of the plant. The reduction in the stone forming constituents in urine and renal tissue brought about by *T.stans* could contribute to its antiurolithiatic property.

Keywords: Calcium oxalate, ethylene glycol, urolithiasis, *Tecoma stans*.

INTRODUCTION

Urolithiasis is an extremely painful disease that afflicts the human population since ancient times [1]. The mechanism of calcium oxalate renal calculi formation has attracted the attention of medical scientists because of its widespread clinical occurrence and the difficulty of treatment. Hyperoxaluria is one of the main risk factors of human idiopathic calcium oxalate disease. Oxalate, the major stone-forming constituent, is known to induce lipid peroxidation which causes disruption of the cellular membrane integrity [2, 3]. Lipid peroxidation is a free radical induced process leading to oxidative deterioration of polyunsaturated lipids. This alters the membrane fluidity, permeability and thereby affects the ion transport across the cellular organelle [4, 5].

Calcium oxalate is one of the main constituents of deposits in urinary tract. Crystallisation of calcium oxalate is of particular interest not only from the theoretical point of view but also because of its biological importance. The exact mechanism of the initiation of the calcium oxalate stone formation is not completely understood. Factors leading to the nucleation, crystal growth and aggregation of various hydrates of calcium oxalate depend not only on the excess of calcium and oxalate concentrations but also on the presence of various foreign substances. A number of studies have been carried out to determine the effect of various additives such as metallic ions and their complexes [6], sodium dodecyl sulphate [7], α-ketoglutaric acid (a normal physiological constituent of urine) [8], plant extracts [9], maleic acid copolymer [10] and a protein from human kidney [11] on inhibition of calcium oxalate crystallisation.

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As far as urinary system is concerned; it is 3rd prevalent disorder which usually starts with obstruction and if left untreated results in severe complications like multiple infections and hemorrhage suggesting need of ideal medical care [12]. Pathophysiologically, urolithiasis occurs as a consequence of the breakdown of a delicate balance to be maintained by the kidneys i.e. excretion of materials that have a low solubility and conservation of water. These two opposing requirements must be balanced during adaptation to diet, climate and various activities. Whenever the urine becomes supersaturated with insoluble material, because excretion rates are excessive and/or reduced water conservation, crystals are formed, grow and aggregate to form a stones. The impact of these stones increases by many folds in the presence of other complaints like hypertension, obesity hepatic dysfunction etc [13-16]. The etiology of this disorder is multifactorial and is strongly related to dietary habits or practices [17].

The medical management of urolithiasis mainly involves techniques like extracorporeal shock wave lithotripsy and percutaneous nephrolithotomy, however, these treatment options are too costly and with these procedures recurrence is quite common [18]. The scientific documents revealed that The recurrence rate without preventive treatment is approximately 10% at 1 year, 33% at 5 year and 50% at 10 years suggesting its need, however continuation of therapy is adversely affected by wide range of undesirable side effects such as hemorrhage, hypertension, tubular necrosis and subsequent renal fibrosis etc [19,20]. The overall outcome gives clear cut indication for exploration of new remedy. In light of this, exploitation of natural sources has been assumed to be of greater potential. In the Indian traditional systems of medicine including Ayurveda, most of the remedies are derived from plants and their traditional applications are proved to be useful chiefly decreasing the recurrence rate of urolithiasis without causing any potential side effects [21]. However, the scientific documentation of their use is not well established through systematic scientific documentation making it worthwhile to explore. The study was designed with an objective to carry out *in vivo* anti-urolithiatic activity of aqueous and methanolic extract of *Tecoma stans* flowers.

MATERIALS AND METHODS

Animals:

Wistar albino rats of either sex weighing between 150 and 200 g were selected for acute toxicity studies and for the antiurolithiatic activity. The animals were acclimatized to standard laboratory conditions of temperature (22±3ºC) and maintained on 12:12 h light:dark cycle. They were provided with regular rat chow and distilled water *ad libitum.* The animal care and experimental protocols were in accordance with CPCSEA / IAEC.

Plant material:

The flowers of *Tecoma stans* were collected in the month of May 2011 from Rasipuram (Namakkal District) Tamil Nadu. A herbarium specimen of the plant was deposited in the Department of Pharmacognosy. The plant was identified by Dr.G.V.S.Murthy, Joint Director of the Botanical Survey of India, Southern circle, TNAU Campus, Coimbatore, who authenticated the plant from information available in the literature. The flower petals were dried in the shade and then powdered and 100 g of the dried powder was extracted with methanol using a soxhlet apparatus. The solvent was removed under reduced pressure and controlled temperature using a rotary flash evaporator. Suspension of AETS, METS in 2% (v/v) tween-80 was prepared for oral administration by gastric intubation method. Phytochemical screening of the extract revealed the presence of tannin, flavonoids, phenols, alkaloids, steroids,triterpenes and saponins.

Experiment design:

Ethylene glycol induced urolithiasis model in albino rats: [22-24]

Animals were divided in 7 groups containing six animals in each and kept in cages. All animals had free access to regular rat chow and drinking water *ad libitum* for 28 days. Renal calculi were induced in group II to VII by supplementing with 0.75%v/v ethylene glycol in drinking water *ad libitum*. Group IV to V were treated with both extracts starting from 15th day to 28th day (Curative regimen). Group VI to VII were treated with both extracts starting from 1st day to 28th day (Preventive regimen).

Assessment of antiurolithiatic activity:

Collection and analysis of urine:

All animals were kept in individual metabolic cages and urine samples of 24 h were collected on 28th day. Animals had free access to drinking water during the urine collection period. After urine collection, urine volume and pH of urine were measured [25]. A drop of concentrated hydrochloric acid was added to the urine before

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being stored at 4oC. Urine was analyzed for magnesium**,** phosphate, oxalate, calcium, and citrate.

Serum analysis:

After the experimental period, blood was collected by heart puncture under anesthetic condition. Serum was separated by centrifugation at 10,000×*g* for 10 min and analyzed for Uric acid, Blood urea nitrogen (BUN), Creatinine, Total protein.

Kidney homogenate analysis:

The abdomen was cut open to remove both kidneys from each animal. Isolated kidneys were cleaned off extraneous tissue and one of them was preserved in 10% neutral formalin. The other one was dried at 80ºC in a hot air oven. A sample of 100 mg of the dried kidney was boiled in10 ml of 1N hydrochloric acid for 30 min and homogenized. The homogenate was centrifuged at 2000×*g* for 10 min and the supernatant was separated [26] and analyzed for phosphate, oxalate, Calcium.

Histopathology: [27]

The abdomen was cut open to remove either kidney from each animal. Isolated kidneys were cleaned off extraneous tissue and preserved in 10% neutral formalin. One of the isolated kidneys was then embedded in paraffin using conventional methods and cut into 5 μm thick sections and stained using hematoxylin–eosin dye and finally mounted in diphenyl xylene. Then the sections were observed under microscope for histopathological changes in kidney architecture and their photomicrographs were taken.

Statistical analysis: [28]

The data obtained by the various parameters was statistically evaluated by one way analysis of variance (ANOVA) followed by Dunnett's Multiple Comparison Test using Graph Pad Prism software (GraphPad software Inc., Version 4.0.0.255). The mean values ± SEM were calculated for each parameter. The differences in biochemical parameters between the calculi induced group and standard drug treated group were considered as 100% and the changes in biochemical parameters by the plant extracts treated groups against the calculi induced group were analyzed accordingly. Level of significance was kept at $P<0.05$.

RESULTS AND DISCUSSION

Ethylene glycol induced urolithiasis resulted in significant elevation of urine and kidney calcium, oxalate, inorganic phosphate and serum blood urea nitrogen, creatinine, uric acid compared to normal control group. Treatment with cystone (750 mg/kg) and AETS-250 mg/kg; METS-200 mg/kg prevented the bio-chemical changes induced by ethylene glycol (Table 1,2 & 3).

In order to probe the possible mechanism by the plant *Tecoma stans* prevents renal damage caused by ethylene glycol, investigation on levels of various stone inhibitors like total protein, magnesium and citrate. There was signifi-cantly rise on total protein, magnesium and citrate after treatment with cystone and plant extracts (**Table 4**).

Administration of ethylene glycol significantly reduced the body weight, urine volume and pH of urine as compared to normal group. Rats treated with cystone and AETM & METS showed significant decreased in body weight, urine volume and pH of urine as compared to control group (**Table 5**).

The histopathological study of the kidney sections also supported the above results. In all the stone forming rats there was damage to the last part of the nephron, collecting system and peritubular interstitium as compared to the normal rat kidney architecture. The tubules appeared focally ecstatic and surrounded by inflammatory infiltration. Flattened epithelium with focal vacuolar degeneration and single cell necrosis bordered the tubules, which focally contained hyaline casts. Inflammatory infiltration was mainly composed of mature lymphocytes infiltrating tubular epithelium. Irregular crystals were present inside the tubules and in the peritubular interstitium, along the nephron and at papillary level. The extract treated groups showed normal histology of the kidney, and shows normal glomeruli, slight oedema of the tubular cells. The AETS & METS treated animals also showed the recovery; however, the renal tubular epithelial recovery was less significant compared to standard drug treated animals (**Table 6**).

Table 1: Changes in urinary excretion of stone forming constituents in control and experimental animals

Group	Dose (mg/kg)	Urine Parameters		
		Oxalate	Calcium	Phosphate
Normal	Vehicle	0.43 ± 0.62	1.16 ± 0.22	3.78 ± 0.07
Calculi Induced	Vehicle	3.18 ± 0.39a	3.92 ± 0.050a	7.31± 0.08a
Cystone Treated	750mg/kg	1.20 ± 0.19b	1.51 ± 0.11b	4.19 ± 0.19b
AETS	250 mg/kg	2.20 ± 0.28b	3.26 ± 0.15b	5.66 ± 0.03b
METS	200mg/kg	1.89 ± 0.11b	3.29 ± 0.24b	5.01 ± 0.08b

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Each value represents Mean±SEM., N=6; a compared to Normal ($P<0.001$); b compared to calculi induced ($P<0.001$).

Table 2: Changes in Kidney retention of stone forming constituents in control and experimental Animals

Group	Dose (mg/kg)	Kideny Parameters (Mean ± SEM)		
		Oxalate	Calcium	Phosphate
Normal	Vehicle	1.39 ± 0.06	3.24 ± 0.01	2.56 ± 0.031
Calculi induced	Vehicle	5.68± 0.02a	5.38± 0.03a	4.08 ± 0.03a
Cystone treated	750	2.18± 0.07b	3.53± 0.01b	2.74± 0.051b
AETS	250	3.35± 0.04b	4.67± 0.01b	3.46 ± 0.01b
METS	200	3.19± 0.06b	4.46± 0.01b	3.23 ± 0.03b

Each value represents Mean±SEM., N=6; a compared to Normal ($P<0.001$); b compared to calculi induced ($P<0.001$)

Table 3: Changes in serum parameters in control and experimental Animals

Group	Dose (mg/kg)	Serum Parameters (Mean ± SEM)		
		Bun	Creatinine	Uric Acid
Normal	Vehicle	23.35± 1.01	0.54 ± 0.02	2.27 ± 0.03
Calculi induced	Vehicle	37.39± 1.10a	0.99± 0.12a	4.39± 0.07a
Cystone treated	750	30.76 ± 0.8b	0.73 ± 0.01b	2.75± 0.02b
AETS	250	30.12 ± 0.8b	0.82 ± 0.01b	3.37± 0.03b
METS	200	42.07 ± 1.0b	0.83 ± .023b	3.21± 0.02b

Each value represents Mean±SEM., N=6; a compared to Normal ($P<0.001$); b compared to calculi induced ($P<0.001$)

Table 4: Changes in various inhibitors in control and experimental Animals

Group	Dose (mg/kg)	Inhibitory parameters (mean ± SEM)		
		Total protein	Magnesium	Citrate
Normal	Vehicle	2.98 ± 0.04	3.01 ± 0.03	51.88 ± 1.96
Calculi induced	Vehicle	1.41± 0.08a	0.63± 0.047a	48.71± 6.88a
Cystone treated	750	2.81± 0.08b	2.01 ± 0.129b	82.10± 13.27b
AETS	250	2.30 ± 0.10b	1.49 ± 0.157b	37.49 ± 2.81b
METS	200	2.11± 0.28b	1.39 ± 0.11b	53.44 ± 2.21b

Each value represents Mean±SEM., N=6; a compared to Normal ($P<0.001$); b compared to calculi induced ($P<0.001$)

Table 5: Change in physical parameters in ethylene glycol induced urolithiasis

Group	Dose (mg/kg)	Physical parameters (mean ± SEM)		
		% change in Body weight	Urine volume	pH
Normal	Vehicle	7.84 ± 0.36	14.05 ± 0.34	6.67 ± 0.16
Calculi induced	Vehicle	-7.64 ± 0.59a	10.47 ± 0.23a	5.90 ± 0.30a
Cystone treated	750	3.87± 0.40b	19.50 ± 1.01b	6.68 ± 0.19b
AETS	250	-4.61 ± 0.32b	13.80 ± 0.1b	6.05 ± 0.1b
METS	200	-4.09 ± 0.21b	17.05 ± 0.17b	6.47 ± 0.15b

Each value represents Mean±SEM., N=6; a compared to Normal ($P<0.001$); b compared to calculi induced ($P<0.001$)

Table 6: Histological features found from l.s of kidneys of different groups

Groups	Histological Feature					
	Tubular Congestion	Tubular Cast	Epithelial Disquamation	Glomerular Congestion	Blood Vessel Congestion	Inflammatory Cells
Normal	-	-	-	-	-	-
Calculi induced	++++	+++	+++	++++	++	+++
Cystone treated	++	+	+	-	-	+
AETS	+	-	+	+	+	++
METS	+	-	+	+	-	-

+++ = Presence of histological abnormality; - = Absence of histological abnormality

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