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Protective effect of methanolic extract of *Abelmoschus moschatus* seeds against calcium oxalate urolithiasis in rats

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ABSTRACT

This study was undertaken to evaluate the antiurolithiatic effect of methanolic extract of Abelmoschus moschatus seeds (MAM) against calcium oxalate urolithiasis using male Wistar rats. Urolithiasis was induced by administration of 0.75 % v/v ethylene glycol with 1 % w/v ammonium chloride in drinking water for three days followed by only 0.75 % v/v ethylene glycol for next 25 days. Antiurolithiatic activity was evaluated at three doses of MAM (viz., 100, 200 and 400 mg/kg) in curative and preventive regimen by estimating histological changes in kidney tissues and biochemical changes in urine, serum and kidney tissue homogenate. Ethylene glycol-ammonium chloride feeding caused alteration in volume and levels of calcium, oxalate, total protein, phosphate, uric acid, magnesium, citrate in the urine. The MAM treatment also increased urine volume but less than calculi-induced animals. The MAM supplementation significantly prevented biochemical changes in the urine dose-dependently. Supplementation with MAM prevented the elevation of serum creatinine, uric acid and blood urea nitrogen levels. The increased calcium, oxalate and phosphate levels in the kidney tissue of lithiatic rats were significantly reduced by the MAM treatment. The MAM supplementation also caused significant decrease in accumulation of calcium oxalate deposits, histological changes and lipid peroxidation activity in the kidney tissue. These results indicate that administration of Abelmoschus moschatus seed extract reduced and prevented the growth of urinary stones. The possible mechanism underlying this effect is mediated collectively through diuretic, antioxidant, anti-inflammatory properties and lowering the concentration of urinary stone-forming constituents.

Key words: *Abelmoschus moschatus*, Ethylene glycol, Urolithiasis

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INTRODUCTION

Urolithiasis is the third prevalent disorder of the urinary system which is approximately 2-3 % in the general population. Urinary calculi may cause serious medical consequences such as extreme obstruction, hydronephrosis, infection and hemorrhage in the urinary tract system [1]. Surgical operation, lithotripsy and local calculus disruption using high-power laser are commonly used techniques to remove the calculi. However, these procedures may cause serious complications such as acute renal injury, decrease in renal function and an increase in stone recurrence [2]. The recurrence rate without preventive treatment is approximately 10 % at 1st year, 33 % at 5th year and 50 % at 10th years indicating the need to develop suitable alternative therapy [3].

Medicinal plants are always remained important source of drugs. Some medicinal plants and proprietary composite herbal preparations are reported to be effective in the treatment as well as prevention of recurrence of renal calculi

with minimal side effects [4]. In Indian indigenous system of medicine, several plants including *Abelmoschus moschatus* seeds are claimed to be useful for the renal calculi [5]. *Abelmoschus moschatus* (family: Malvaceae) is a medicinal plant, found wild all over the hilly regions of Deccan and Karnataka and also at the foothills of the Himalayas [6]. The plant has been reported to possess diuretic [7, 8], antioxidant activity and free-radical scavenging [9], antiproliferative [9], antimicrobial [9-11], antilithiatic [12], hepatoprotective [13], memory strengthening [14], antidiabetic [15], hemagglutinating [16], anti-ageing [17], anti-depressant, anxiolytic, anticonvulsant, hypnotic and muscle relaxant [18] properties. The objective of this study was to evaluate the antiurolithiatic activity of *Abelmoschus moschatus* seed extract against calcium oxalate urolithiasis and its possible underlying mechanisms using male Wistar albino rats.

EXPERIMENTAL SECTION

Animals:

Male Wistar albino rats weighing between 150-200 g were used for this study. They were procured from National Institute of Biosciences, Pune, India. The animals were acclimatized for ten days under standard laboratory conditions (Temperature: 25±2°C, Relative humidity 65±10 % under 12 h light/dark cycles) in the animal house of Maharashtra Institute of Pharmacy, Pune which is approved by CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals), India. The animals fed with standard diet supplied by Nutrivet Life Sciences, Pune, India. The study protocol was approved from the Institutional Animal Ethics Committee (Ref. No.: MIP/IAEC/2013-14/M1/Appr/004) of the institute.

Chemicals and apparatus:

Ethylene glycol (Qualigens Fine Chemicals, India; B. No.: 75316806-2), Ammonium chloride (Analab Fine Chemicals, India; B. No.: RKC221713) of analytical grade, procured from approved vendors were used for the study. Apparatus such as the metabolic cages (New Neeta Chemicals, India), cold centrifuge (BioEra, India) and UV-spectrophotometer (LabIndia, India) were used for this study.

Collection and extraction of plant material:

The capsules of *Abelmoschus moschatus* were collected in the month of November from local region of Thane, Maharashtra, India. It was authenticated by Dr. A. S. Upadhye, Scientist, Agharkar Research Institute, Pune, India. The capsules were dried under shade. Seeds were separated and dried under shade for 10-12 days. The dried seeds of *Abelmoschus moschatus* were coarsely powdered, packed into soxhlet column and extracted with 70 % v/v methanol in water at 65-70°C for 22 h. This methanolic extract (MAM) was then evaporated at 45°C and then dried in oven. The dried extract was stored in airtight container.

Preliminary phytochemical analysis:

The MAM was subjected to qualitative analysis of the various phytoconstitutents using well established procedure [19].

Experimental Design:

Ethylene glycol and ammonium chloride induced hyperoxaluria model was used to induce urolithiasis in rats [20]. The three dose levels of MAM (100, 200 and 400 mg/kg) were used for the evaluation of antiurolithiatic effect [21]. Animals were randomly divided into eight groups containing six animals in each. Group I served as vehicle control which were maintained on standard food and drinking water *ad libitum* and received 5 % w/v gum acacia solution (5 ml/kg, p.o.). All remaining groups received calculi inducing treatment for the period of 28 days, comprised of 0.75 % v/v ethylene glycol with 1 % w/v ammonium chloride in drinking water *ad libitum* for the period of 3 days followed by only 0.75 % v/v ethylene glycol for next 25 days. Group II served as lithiatic control and received 5 % w/v gum acacia solution (5 ml/kg, p.o.). Groups III, IV and V served as preventive treatment groups and received MAM at doses of 100, 200 and 400 mg/kg respectively from 1st day to 28th day of calculi induction. Groups VI, VII and VIII served as curative treatment groups and received MAM at doses of 100, 200 and 400 mg/kg respectively from 14th day to 28th day of calculi induction. The MAM was suspended in distilled water using 5 % w/v gum acacia and given once daily by oral route (5 ml/kg body weight).

Collection and analysis of urine:
On 0, 14 and 28th day of calculi induction, all animals were kept in individual metabolic cages and 24 h urine samples were collected. The collected urine samples were analyzed for its volume, calcium, oxalate [22], phosphate [23], magnesium [24, 25], uric acid [26], citrate [27] and total protein [28] contents. The urine calcium level was estimated using kit by Beacon Diagnostic Pvt. Ltd., India. The 28th day urine samples were also subjected to crystalluria study to estimate presence of calcium oxalate crystals in 3 h urine sample by viewing under light microscope.

Serum analysis:
After 28th day urine collection period, blood was collected from retro-orbital sinus under ether anesthesia. Serum was separated by centrifugation at 10,000 × g for 10 min and analyzed for creatinine, uric acid, blood urea nitrogen (BUN) spectrophotometrically by using diagnostic kit from Beacon Diagnostic Pvt. Ltd., India.

Kidney histopathology and homogenate analysis:
After blood collection, all animals were sacrificed by cervical dislocation. The abdomen was cut open to remove both kidneys from each animal. Isolated kidneys were cleaned-off extraneous tissue, rinsed in ice-cold physiological saline and used for histopathology and homogenate analysis.

The left kidney was finely minced and 20 % homogenate was prepared in Tris-Hcl buffer (0.02 mol/l, pH 7.4). The kidney homogenate was used to analyze tissue calcium, oxalate [22], phosphate [23] and lipid peroxidation inhibition activity [29]. Calcium in tissue homogenate was estimated using kit by Beacon Diagnostic Pvt. Ltd., India. The right kidney was fixed in 10 % neutral buffered formalin, processed in a series of graded alcohol and xylene, embedded in paraffin wax, sectioned at 5 µm and stained with Hematoxylin and Eosin for examination under light microscope to estimate damage index (DI). The histological changes such as congestion, hemorrhages, focal tubular swelling, granular and vacuolar changes in cytoplasm, tubular degeneration, sloughing of tubular epithelium, cystic tubular changes, necrotic changes of tubular epithelium, infiltration of mononuclear cells, glomerular changes and interstitial fibrosis were determined as a damage index with semiquantitative grading system as grade 0-no visible changes, grade 1-minimal changes (<25 %), grade 2-mild changes (25 % to 50 %), grade 3-moderate changes (51 % to 75 %) and grade 4-severe changes (>75 %). The kidney section was also stained by Pizzolato's method [30], which selectively stains calcium oxalate crystals, and observed under light microscope to estimate total number of calcium oxalate deposits.

To estimate DI and number of calcium oxalate deposits, a saggital section of each renal specimen was divided into 8 equal sized regions by four virtual lines according to previously reported method [31]. Each region was observed under light microscope and DI or calcium oxalate deposits were counted. The DI was reported as average of the eight readings, whereas sum of the eight readings was reported as total number of calcium oxalate deposits for each specimen.

Statistical analysis:
Results were expressed as mean±standard error of mean (SEM). The statistical significance was assessed using one-way analysis of variance (ANOVA) followed by Dunnett's comparison test and $p<0.05$ was considered significant.

RESULTS AND DISCUSSION

The yield of MAM was found to be 16.67 % w/w. Preliminary phytochemical analysis revealed the presence of carbohydrates, proteins and amino acids, flavonoids, tannins, saponins and phenolic compounds in the MAM.

A number of animal models using rats have been used to induce calcium oxalate urolithiasis [32]. The most commonly employed method is to provide ethylene glycol and ammonium chloride in drinking water to rats. Therefore, we evaluated the antiurolithiatic potential of *Abelmoschus moschatus* on calcium oxalate urolithiasis using this model. The biochemical mechanism of ethylene glycol and ammonium chloride-induced lithiasis is related to an increase in the urinary concentration of oxalate. Ethylene glycol is readily absorbed along the intestine and is metabolized in the liver to oxalate that further leads to hyperoxaluria. The oxalate precipitates in the urine as calcium oxalate due to its poor solubility. High oxalate levels and calcium oxalate crystals especially in nephron damages epithelial cells, leading to heterogeneous nucleation followed by aggregation of crystals [33, 34]. Furthermore, ammonium chloride has been reported to accelerate lithiasis [35].

Table 1: Effect of *Abelmoschus moschatus* extract on urine volume, oxalate, total protein, phosphate and uric acid levels in urolithiasis induced rats

Days	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII	Group
Volume (ml/24 h)								
0	7.33±0.36	8.20±0.55	8.35±0.51	8.47±0.53	8.37±0.40	8.38±0.49	9.43±0.33	7.93±0.46
14	7.20±0.25	19.48±1.39^{c}	9.63±0.86^{c}	12.27±1.06^{c}	14.83±1.36^{a}	19.72±1.12	20.07±1.41	19.65±1.28
28	7.58±0.27	19.75±1.41^{c}	11.67±0.72	14.05±0.77^{c}	16.85±1.07^{c}	10.93±0.61^{c}	12.15±0.88^{c}	15.92±0.93^{a}
Oxalate (mg/24 h)								
0	5.14±0.22	5.41±0.29	5.08±0.26	5.04±0.24	4.88±0.22	5.01±0.31	5.14±0.24	5.26±0.29
14	5.23±0.28	8.29±0.36^{c}	7.04±0.21^{a}	6.51±0.32^{c}	5.98±0.14^{c}	8.01±0.37	8.27±0.32	8.11±0.29
28	5.21±0.25	10.22±0.33^{c}	8.68±0.23^{b}	7.84±0.32^{c}	5.98±0.14^{c}	9.09±0.28^{a}	8.75±0.26^{b}	8.41±0.29^{c}
Total protein (mg/24								
0	5.86±.27	5.81±0.27	5.96±0.33	5.74±0.32	6.03±0.28	5.87±0.32	5.47±0.31	5.64±0.33
14	5.71±0.37	11.00±0.64^{c}	8.22±0.43^{b}	7.48±0.48^{c}	6.68±0.40^{c}	11.16±0.66	11.10±0.53	10.84±0.52
28	5.97±0.35	12.99±0.73^{c}	10.08±0.68^{b}	9.54±0.58^{c}	8.44±0.54^{c}	10.33±0.68^{a}	9.85±0.61^{b}	8.88±0.61^{c}
Phosphate (mg/24 h)								
0	5.56±0.23	5.61±0.20	5.24±0.17	5.44±0.20	5.82±0.19	5.93±0.19	5.59±0.24	5.80±0.28
14	5.57±0.18	6.69±0.29^{a}	6.29±0.22	6.08±0.22	5.87±0.20	6.63±0.26	6.81±0.27	6.74±0.25
28	5.84±0.19	7.56±0.21^{c}	6.42±0.20^{b}	6.13±0.19^{c}	5.90±0.14^{c}	6.50±0.26^{a}	6.31±0.21^{b}	6.07±0.25^{c}
Uric acid (mg/24h)								
0	1.40±0.04	1.47±0.07	1.42±0.04	1.47±0.05	1.47±0.05	1.53±0.04	1.48±0.05	1.42±0.06
14	1.36±0.06	2.11±0.15^{b}	1.93±0.13	1.78±0.14	1.66±0.14	2.08±0.10	2.01±0.13	2.04±0.11
28	1.38±0.04	2.51±0.07^{c}	2.12±0.06^{c}	1.99±0.05^{c}	1.79±0.05^{c}	2.18±0.05^{b}	2.06±0.05^{c}	1.89±0.05^{c}

Values are expressed as mean±SEM, n=6, Group I: Vehicle control; Group II: Lithiatic control; Group III, IV and V: Preventive treatment with MAM at 100, 200 and 400 mg/kg respectively; Group VI, VII and VIII: Curative treatment with MAM at 100, 200 and 400 mg/kg respectively, p-values: ^{a}<0.05; ^{b}<0.01; ^{c}<0.001, Values of Group-II were compared with Group-I and those of Group-III to Group-VIII with Group-II.

Table 2: Effect of *Abelmoschus moschatus* extract on urinary calcium, magnesium and citrate levels in urolithiasis induced rats

Days	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII	Group VIII
Calcium (mg/24 h)								
0	0.60±0.02	0.58±0.03	0.55±0.03	0.60±0.01	0.59±0.03	0.60±0.03	0.56±0.03	0.51±0.01
14	0.58±0.03	0.24±0.01^{c}	0.41±0.03^{c}	0.43±0.02^{c}	0.48±0.02^{c}	0.23±0.02	0.24±0.01	0.25±0.02
28	0.60±0.02	0.16±0.01^{c}	0.23±0.01^{a}	0.28±0.01^{c}	0.36±0.02^{c}	0.21±0.01^{a}	0.25±0.01^{c}	0.34±0.01^{c}
Magnesium (mg/24 h)								
0	2.37±0.09	2.26±0.11	2.61±0.11	2.64±0.13	2.73±0.12	2.51±0.09	2.37±0.10	2.45±0.12
14	2.71±0.08	1.65±0.17^{b}	2.25±0.09^{b}	2.42±0.11^{c}	2.51±0.09^{c}	1.60±0.10	1.66±0.16	1.69±0.20
28	2.67±0.11	1.46±0.04^{c}	2.06±0.08^{c}	2.27±0.09^{c}	2.41±0.10^{c}	1.97±0.07^{b}	2.18±0.10^{c}	2.34±0.10^{c}
Citrate (mg/24h)								
0	5.57±0.13	5.77±0.25	5.96±0.22	5.95±0.20	5.66±0.23	5.82±0.25	5.56±0.24	5.64±0.22
14	5.90±0.23	3.49±0.32^{c}	3.80±0.34	4.07±0.33	4.41±0.26	3.28±0.36	3.17±0.28	3.53±0.40^{a}
28	5.83±0.24	2.31±0.08^{c}	4.14±0.17^{c}	4.47±0.17^{c}	4.80±0.19^{c}	3.35±0.19^{b}	3.79±0.19^{c}	4.58±0.21^{c}

Values are expressed as mean±SEM, n=6, Group I: Vehicle control; Group II: Lithiatic control; Group III, IV and V: Preventive treatment with MAM at 100, 200 and 400 mg/kg respectively; Group VI, VII and VIII: Curative treatment with MAM at 100, 200 and 400 mg/kg respectively, p-values: ^{a}<0.05; ^{b}<0.01; ^{c}<0.001, Values of Group-II were compared with Group-I and those of Group-III to Group-VIII with Group-II.

Male rats were selected to induce urolithiasis because the urinary system of male rats has more resemblance to that of humans. In addition, earlier studies have reported that the amount of stone deposition in female rats was significantly less compared to male rats [36].

Consistent with previous reports [20, 37], urine volume was significantly (p<0.001) increased by calculi-inducing treatment to rats (Table 1). Treatment of animals with MAM in curative as well as preventive regimen also caused significant increase in urine volume in dose dependent manner. However, this increased urine volume in MAM-treated animals was significantly less than that of calculi-induced animals. This may be due to diuretic effect of MAM [7, 8] which reduced calcium oxalate supersaturation in the urine and thereby stone formation.

As reported in some previous reports [20, 37, 38], calculi-inducing treatment caused significant (p<0.001) increase in oxalate excretion in the urine (Table 1). This increased urinary oxalate levels was significantly decreased by treatment with all doses of MAM in curative as well as preventive regimen in dose dependent manner.

Table 3: Effect of *Abelmoschus moschatus* extract on serum and kidney parameters in urolithiasis induced rats

Days	Group I	Group II	Group III	Group IV	Group V	Group VI	Group VII	Group VIII
Serum								
Creatinine (mg/dl)	0.70±0.03	1.77±0.08^{c}	1.47±0.06^{b}	1.34±0.06^{c}	1.15±0.05^{c}	1.52±0.07^{a}	1.42±0.06^{b}	43.78±0.86^{c}
Uric acid (mg/dl)	1.49±0.04	5.30±0.13^{c}	4.67±0.08^{c}	4.45±0.08^{c}	4.19±0.07^{c}	4.89±0.10^{a}	4.78±0.09^{b}	4.52±0.08^{c}
BUN (mg/dl)	37.57±0.71	51.21±1.34^{c}	44.22±0.99^{c}	43.78±0.86^{c}	42.90±0.82^{c}	46.26±1.09^{a}	45.29±1.03^{b}	44.08±0.91^{c}
Kidney								
Calcium (mg/g)	0.14±0.01	0.33±0.02^{c}	0.23±0.02^{c}	0.20±0.01^{c}	0.17±0.01^{c}	0.25±0.02^{b}	0.22±0.01^{c}	0.18±0.01^{c}
Oxalate (mg/g)	1.34±0.10	5.86±0.18^{c}	4.36±0.14^{c}	3.55±0.13^{c}	2.47±0.11^{c}	5.04±0.16^{b}	4.17±0.14^{c}	3.05±0.13^{c}
Phosphate (mg/g)	2.36±0.10	3.81±0.15^{c}	3.12±0.12^{b}	2.97±0.12^{c}	2.68±0.11^{c}	3.22±0.13^{a}	3.08±0.12^{b}	2.85±0.12^{c}
Lipid peroxidation (%)	30.26±0.82	100.00±4.56^{c}	74.58±3.03^{c}	66.14±1.98^{c}	62.12±2.01^{c}	79.28±3.20^{c}	73.10±2.58^{c}	68.11±2.31^{c}
CaOx deposits (No.)	0.00±0.00	90.85±2.76^{c}	42.96±2.08^{c}	20.46±1.94^{c}	9.08±1.35^{c}	56.90±2.87^{c}	31.00±2.20^{c}	12.02±1.33^{c}
Damage index (Arbitrary units)	0.00±0.00	3.02±0.09^{c}	1.92±0.09^{c}	1.52±0.05^{c}	0.33±0.04^{c}	2.17±0.08^{c}	1.69±0.16^{c}	1.04±0.06^{c}

Values are expressed as mean±SEM, n=6, Group I: Vehicle control; Group II: Lithiatic control; Group III, IV and V: Preventive treatment with MAM at 100, 200 and 400 mg/kg respectively; Group VI, VII and VIII: Curative treatment with MAM at 100, 200 and 400 mg/kg respectively, p-values: ^{a}<0.05; ^{b}<0.01; ^{c}<0.001, Values of Group-II were compared with Group-I and those of Group-III to Group-VIII with Group-II.

Hyperoxaluria is a more significant risk factor in the pathogenesis of renal stone and reduced rate of alteration of urinary oxalate by MAM treatment indicates that MAM act by inhibiting some steps of oxalate synthesis.

Consistent with previous reports [20, 36], urinary total protein excretion was significantly (p<0.001) increased in calculi-induced animals compared to vehicle treated animals (Table 1). This increased urinary total protein excretion was significantly decreased by treatment with all doses of MAM in curative as well as preventive regimen in dose dependent manner. Supersaturation of urinary colloids results in precipitation of crystal initiation particle which when trapped acts as a nidus leading to subsequent crystal growth. This process is associated with proteinuria that reflects proximal tubular dysfunction [36]. Treatment of MAM showed significant reduction in the protein excretion and thus might have prevented the nidus formation of crystal formation.

As reported in previous reports [20, 37], urinary phosphate excretion was significantly (p<0.001) increased by calculi-induced treatment to rats (Table 1). This increased urinary phosphate excretion was significantly decreased by MAM treatment in both curative as well as preventive regimen in dose dependent manner. Previous study reports showed that increased urinary phosphate excretion along with oxalate stress provides an environment suitable for stone formation by forming calcium phosphate crystals, which induces calcium oxalate deposition [36]. In the present study, the MAM treatment found to decrease the rate of urinary phosphate excretion and thereby reduced the risk of stone formation.

Consistent with previous reports [20, 36], urinary uric acid excretion was significantly (p<0.001) increased in calculi-induced animals compared to vehicle treated animals (Table 1). Increased uric acid excretion has been reported in kidney stone patients and hyperoxaluric rats. Uric acid reported to interferes with calcium oxalate solubility. It also binds and reduces the inhibitory activity of glycosaminoglycans [20, 36]. 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 [36]. In the present study, the MAM treatment caused significant decrease in the urinary uric acid excreation and thereby reduces the risk of stone formation.

As reported in previous reports [20, 37], urinary calcium excreation was significantly (p<0.001) decreased by calculi-inducing treatment to rats (Table 2). Urinary calcium is utilized in calcium oxalate nucleation and crystal growth process and this may result in decreased urinary calcium excreation. This decreased urinary calcium excreation was significantly increased by MAM treatment suggesting that MAM interferes with nucleation and/or growth of calcium oxalate crystals.

Consistent with previous reports [20, 37], urinary magnesium excretion was significantly (p<0.001) decreased in calculi-induced animals compared to vehicle treated animals (Table 2). Magnesium is one of the urinary inhibitors of crystallization. Low levels of magnesium are also encountered in stone-forming rats as well as in patients with renal stones. Furthermore, magnesium levels also return to normal on drug treatment in renal stone patients [36]. Diets high in magnesium have been found to protect against deposition of calcium oxalate in the kidneys of vitamin B_6 deficient rats. Magnesium reported to form complex with oxalate and reduce the supersaturation of calcium

oxalate by reducing the saturation of calcium oxalate and as a consequence reduced the growth and rate of nucleation of calcium oxalate crystals [20, 36]. In the present study, the MAM treatment caused significant increase in the urinary magnesium excretion and thus reduced the growth of calcium oxalate crystals. The crystallization inhibitory potential of MAM, as evident from the examination of the crystalluria, thus could be the result of increased magnesium content of the urine of MAM-treated animals.

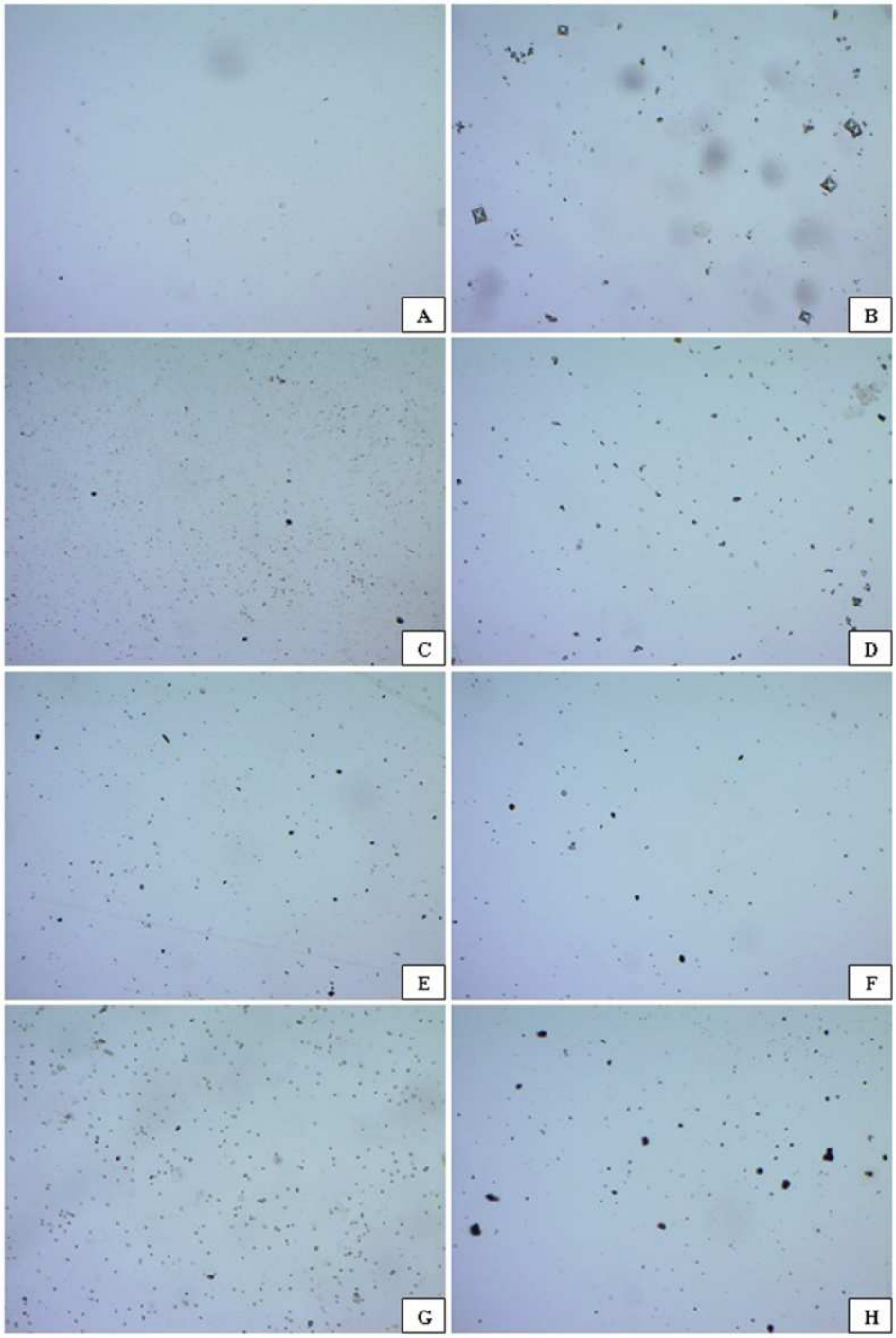

Fig 1: The calcium oxalate crystals, viewed under light microscope (400x), in 3 h urine from animals of (A) vehicle control, (B) lithiatic control; (C, D and E) preventive treatment with MAM at 100, 200 and 400 mg/kg respectively; (F, G and H) curative treatment with MAM at 100, 200 and 400 mg/kg respectively

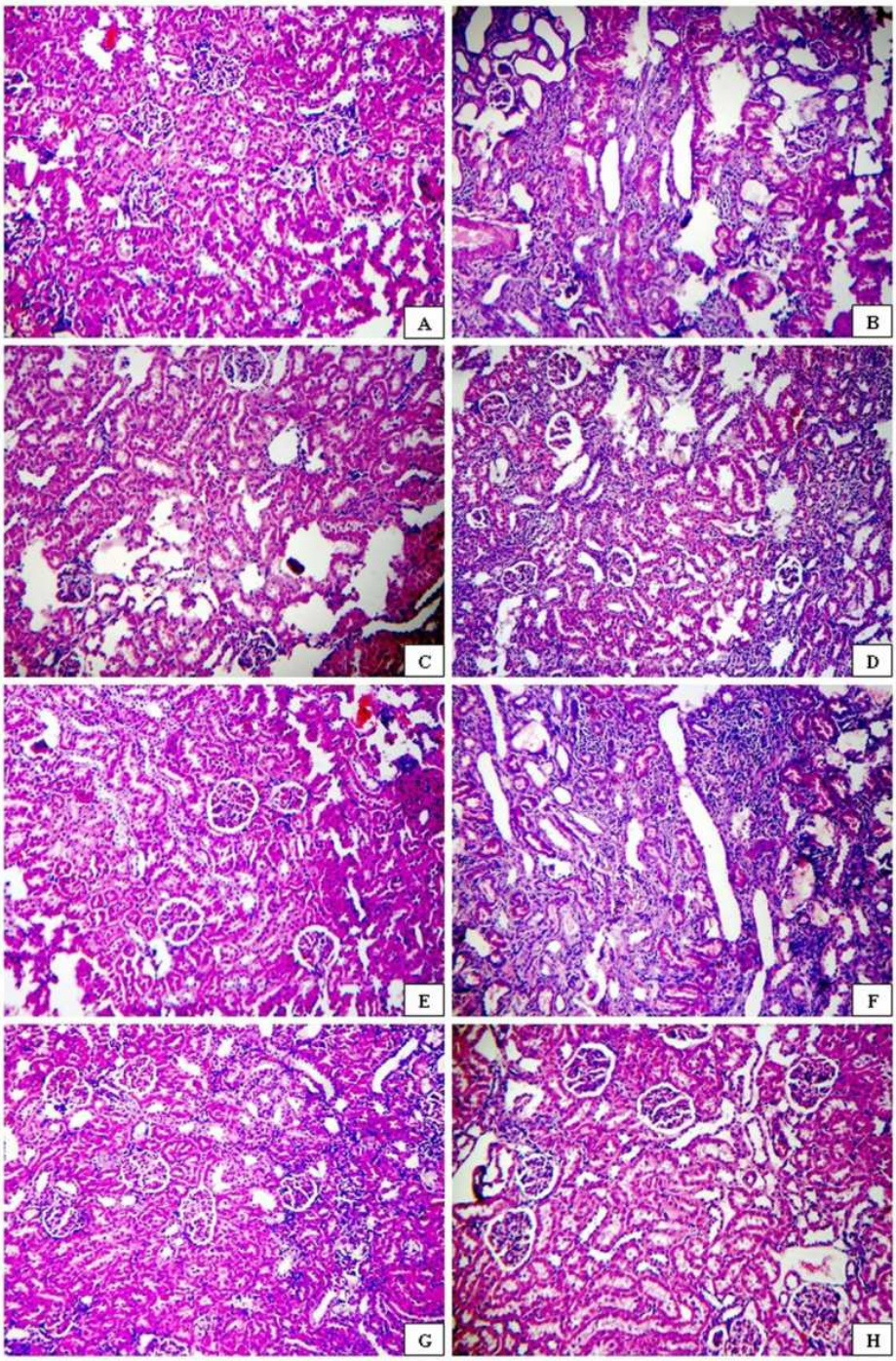

Fig 2: Microscopic images of kidney sections under light microscope (100x) after Hematoxylin and Eosin staining from animals of (A) vehicle control; (B) lithiatic control; (C, D and E) preventive treatment with MAM at 100, 200 and 400 mg/kg respectively; (F, G and H) curative treatment with MAM at 100, 200 and 400 mg/kg respectively

As reported in previous reports [20], urinary citrate level was significantly (p<0.001) decreased by calculi-inducing treatment to rats (Table 2). Hypocitraturia is the major metabolic abnormality in patients with renal stones. In the present study, the MAM treatment caused significant increase in citrate excretion in both curative as well as preventive regimen in dose dependent manner. This indicates that MAM interferes with tubular citrate reabsorption which is reported as the main mechanism regulating citrate excretion in lithiatic patients [39].

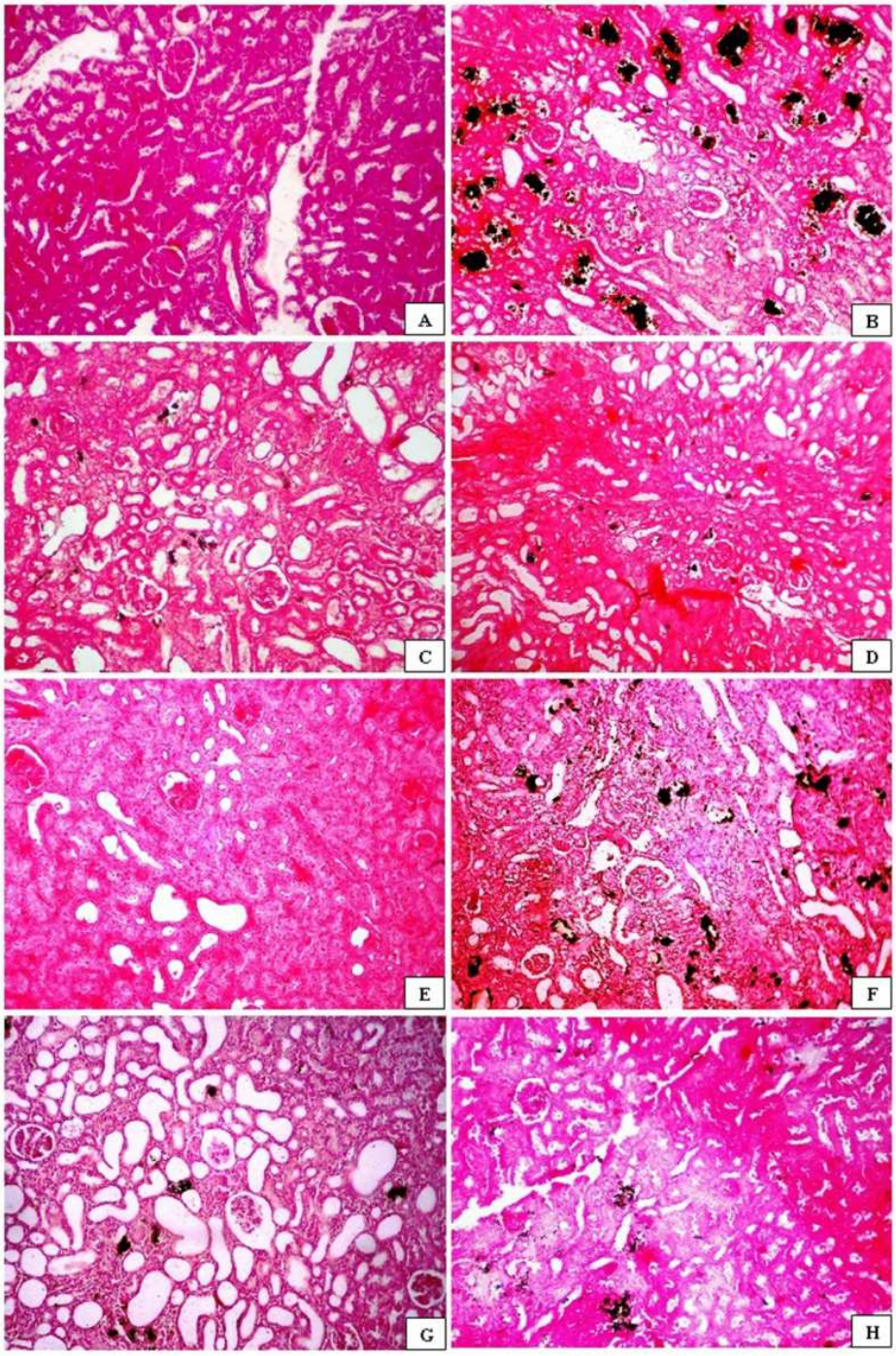

Fig 3: Microscopic images of kidney sections under light microscope (100x) after Pizzolato's staining from animals of (A) vehicle control; (B) lithiatic control; (C, D and E) preventive treatment with MAM at 100, 200 and 400 mg/kg respectively; (F, G and H) curative treatment with MAM at 100, 200 and 400 mg/kg respectively

In urine microscopy, no any calcium oxalate crystals were seen in the urine of vehicle control animals (Fig. 1). Calculi-inducing treatment resulted in appearance of dipyramid shaped calcium oxalate crystals in the urine. This calcium oxalate crystalluria was decreased in the MAM-treated animals in dose-dependent manner.

Consistent with previous reports [20], serum levels of creatinine, uric acid and BUN were significantly (p<0.001) increased in the calculi-induced animals compared to vehicle treated animals (Table 3). In addition, increased lipid

peroxidation was also observed in the kidneys of calculi-induced animals (Table 3). Elevated urinary oxalate level has been reported to induce lipid peroxidation and cause renal damage by reacting with polyunsaturated fatty acids in cell membrane [20, 40]. This renal damage was indicated by the elevated serum levels of creatinine, uric acid and BUN which are markers of glomerular and tubular damage. In the present study, MAM treatment showed to prevent elevation of serum levels of these markers as well as lipid peroxidation of kidney tissue. This indicates that MAM act by inhibiting the lipid peroxidation and thereby reduces the extent of tubular dysfunction.

Consistent with previous reports [36, 37], renal calcium, oxalate and phosphate levels were significantly (p<0.001) increased by calculi-inducing treatment to rats (Table 3). These elevated levels of calcium, oxalate and phosphate in kidney tissue were significantly decreased by treatment with all doses of MAM in curative as well as preventive regimen in dose dependent manner. This may be due to protective effect of MAM which inhibits retention of stone in the renal tubules.

Kidneys of calculi-induced animals showed marked histological changes such as accumulation of calcium oxalate crystals, dilatation of tubules, interstitial fibrosis and dense infiltration of mononuclear cells (Fig. 2). This resulted in significant (p<0.001) increase in the damage index of kidneys of calculi-induced animals as compared to vehicle-treated animals (Table 3). All these histological changes and damage index were significantly (p<0.001) reduced in the MAM-treated animals of both curative and preventive regimen in dose dependent manner. The Pizzolato's staining method revealed the presence of calcium oxalate deposits (stained black) in tubules of all regions of kidney (cortex, medulla and papilla) of calculi-induced animals. However, such deposits were small and less abundant in kidneys of animals treated with MAM as compared to those in the calculi-induced kidneys (Fig. 3). This may be in part due to potent antioxidant capacity [9] of the plant.

CONCLUSION

The present investigation supports the use of *Abelmoschus moschatus* in folk medicine against urolithiasis. It is concluded that administration of *Abelmoschus moschatus* seed extract reduced and prevented the growth of urinary stones. It also seems that the preventive effect is more effective than its curative treatment. The possible mechanism underlying this effect is mediated collectively through diuretic, antioxidant, anti-inflammatory properties and lowering the concentration of urinary stone-forming constituents. The further phytochemical exploration is required to establish exact mechanism of action.

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