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Research Article

Effect of *Averrhoa carambola* Linn. fruit on ethylene glycol induced Urolithiasis in rats

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ABSTRACT

Objective: Fruits of *Averrhoa carambola* Linn. (Oxalidaceae) are well known for their nutritional values. The fruits are traditionally used to treat indigestion, mouth ulcer, hemorrhoids, diarrhea, jaundice, malarial splenomegaly, skin rashes, toothache, respiratory problems and as diuretic in kidney and bladder related problems. The present study was focused to provide a pharmacological basis for the antiurolithiatic activity of methanolic *A. carambola* (MeAC) in ethylene glycol-induced calculi on the basis of investigation in rats.

***Methods*:** The antiurolithiatic activity of MeAC was explored in detail from the aspects of kidney weight, blood and urine parameter such as calcium, phosphate, urea, uric acid and creatinine levels. The obtained results showed that MeAC had protective effects on calculi formation and might possess antiurolithiatic efficacy. Daily treatment with 400 mg/kg of MeAC in ethylene glycol-treated rat for 13 days indicated that this drug had beneficial effects in urolithiasis. Antioxidant enzymes activities such as superoxide dismutase (SOD) and glutathione (GSH) levels significantly decreased in the kidney of calculi induced rats compared to controls.

***Results*:** MeAC in the calculi induced rats also ameliorated antioxidant enzymes activities and significantly decreased malonaldialdehyde (MDA) levels.

Conclusion: These findings suggest that MeAC might be of potential value in the therapy and protection of renal calculi.

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1. Introduction

Urolithiasis is the third most prevalent disorder of the urinary tract after urinary tract infections and benign prostatic hyperplasia. It has affected the human being since long times worldwide, sparing no geographical, cultural or racial groups (Moe, 2006). Urinary stones can persist, with serious medical consequences (such as variable degree of pain, hydronephrosis, infection and bleeding), throughout a patient's lifetime. The urinary stones are estimated to occur in approximately 12% of the world's population (Tania et al., 1999) and 11% population in India (Moe, 2006). Men are three times more likely to be affected than women. Development of kidney stones is a complicated process activated by genetic factors, dietary factors and lifestyle. Different types of calcium oxalate (monohydrate, dehydrate, and basic calcium, phosphate) (70-80%), magnesium ammonium phosphate (10-15%), uric acid (3-10%) and cystine 0.5-1% contributes in stone formation (Lorenz et al., 2013). Urolithiasis is a complex process, beginning with crystal nucleation, growth and aggregation, and ending with retention within the urinary tract. The mechanisms governing the induction of all of these processes remain speculative. The principle causative factor for the formation of stones is attributed to the super-saturation of precipitating salts. Recurrence of urolithiasis is most common (Bouanani et al., 2010). Reactive oxygen species (ROS) often seems to be responsible for cellular injury, therefore a reduction of renal oxidative stress could also be an effective therapeutic approach (Butterweck and Khan, 2009). Unfortunately, the available therapies like thiazide diuretics, alkali-citrate, extracorporeal shock wave lithotripsy and percutaneous nephrolithotomy remain costly, and in most cases are invasive in nature and results in numerous side effects. These modern medical treatments cause adverse effects such as loss of renal function, hemorrhage, hypertension, tubular necrosis and subsequent fibrosis of the kidney leading to cell injury and an increased rate of new stone occurrence (Prasad et al., 2007). Therefore, in recent years the researchers are looking for phytotherapeutic agents effective in urolithiasis (Bouanani et

al., 2010) as an alternative or adjunct therapy. Herbal drug therapy is the most trusted system of medicine in South East Asian countries like India where plants are the part of culture and life style. A variety of plants were previously investigated for their usefulness in urolithiasis without any adverse effects (Prasad et al., 2007). So, it is important to find out an alternative approach for the management of urinary stones, therefore phytotherapy is being sought.

A. carambola (Oxalidaceae) is a plant with diverse potentials. The plant is found throughout India, particularly in Gujarat and Maharashtra states. *A. carambola* is a small, evergreen, multi-stemmed tree 3-5 m high. Many secondary metabolites have been identified from plant such as quercetin-3-O-β-d-glucoside lupeol, anthraquinone glucoside, rutin, β-sitosterol. Anti-inflammatory, antimicrobial, antioxidant, anti ulcer effect (Dasgupta, 2013) etc. were reported for the plant but till today no investigation was performed for its antiurolithiatic activity. According to Ahmed and Singh, (2011) drinking of half tea glass of fruit juice supplemented with silver daily for five days remove kidney stone (Ahmed and Singh, 2011). In another reported remedy Citrus latipes (Swingle) Tanaka fruit extract mixed with that of A. carambola with a pinch of salt and honey is prescribed in urinary tract stones (Lokendrajit et al., 2011).

2. Material and Methods

2.1. Plant material

All the plant materials were collected from the local market of Varanasi, Uttar Pradesh, India. The plants were authenticated by Dr. Anil Kumar Singh, Professor, Department of Dravyaguna, Faculty of Ayurveda, Institute of Medical Sciences, Banaras Hindu University, Varanasi. The specimen of the same has been deposited in department.

2.1.1. Preparation of aqueous extract

The dried plant material was made into coarse powder using mechanical grinder, passed through mesh sieve (20 #). The powdered material was subjected to cold maceration using methanol for 7 days (shaking frequently for 6 h and was allowed to stand for 18 h) at room temperature. The content of flask was filtered through Whatmann No. 1 filter paper. Filtrate was then dried in rotary flash evaporator (Perfit India, Pvt. Ltd.) below 60°C under reduced pressure and dried extract was obtained. The extract was solubilized in 5% CMC and used for studying antiurolithiatic activity.

2.2. Animals

Adult male albino rats (170±30 g) were procured from the Central Animal House, Hygia Institute of Pharmaceutical Education and Research, Lucknow. The animals were kept in a temperature-controlled room (22±2°C) with humidity (55±10%) and 12 h light and 12 h dark cycle. The animals were provided with standard pelleted feed (Amrit Pvt. Ltd. Pune, India) and fresh water *ad libitum*. Rats were exposed to standard laboratory environment for at least one week before the experiment. The study has been approved by the Institutional Animal Ethical Committee (IAEC) of Committee (HIPER/2013-14/CAEC/24).

2.3. Chemicals and Reagent

Ethylene glycol (Merck Laboratories, Mumbai, India), Cystone tablets (The Himalaya Drug Company, Makali, Bangalore, India; Batch No. 19300746F; Mfg. Date: Sep.-2013; Exp. Date: Sep-2018), MgCl (E. Merck, Darmstadt, Germany), Citric acid trisodium salt (Sigma Chemical Co., USA) were used in the experiment. Standard estimation kits for calcium, phosphate, uric acid, urea and creatinine were obtained from Span Diagnostics Ltd. Udhna, Surat, India. All the other reagents used for analysis were of analytical quality.

2.4. Acute Oral toxicity

The acute oral toxicity study of methanolic extract of *A. carambola* fruit was carried out on overnight fasted rats by single dose administration per oral, as per the guidelines (OECD, 2008). The toxicity signs and symptoms or any abnormalities associated with extract administration were observed at periodic interval for 24 h. To ascertain any rate of mortality, the rats were kept under observation once a day for the next 14 days.

2.5. For antiurolithiatic activity

Thirty six male rats (150-200 g) were divided into six groups having six animals in each. Group 1 received only vehicle (5% CMC, 0.5 mL/kg); group 3 received Cystone (750 mg/kg body weight) and group 4, 5, 6 have been treated with MeAC at 100, 200 and 400 mg/kg b.w. Group 2 served as negative control group. All the test groups except group 1 received 0.75% ethylene glycol in distilled water for 28 days. The standard drug and test drugs were given from 15th day (Kalyani et al., 2010).

2.6. Assessment of antiurolithiatic activity

2.6.1. Collection and analysis of urine

On the completion of treatment, all animals were kept in individual metabolic cages (B.I.K. Industries Ltd., Mumbai, India). Animals had free access to drinking water during the urine collection period. Urine samples (24 h) were collected on 28th day. The pH of urine was measured immediately after the collection of urine using digital pH meter (Anonymous, 2002). Further microscopy of the urine was performed at 100× using compound microscope (Khan et al., 2011). A drop of concentrated Hydrochloric acid was added to the collected urine before being stored at 4°C. Urine was analyzed for the calcium (Simas et al., 2001), phosphate (Fossati et al., 1988), oxalate (Hodgkinson and William, 1972), urea (Lequang et al., 1987), uric acid (Hisham et al., 2013) and citrate (Seker et al., 2009) by using standard methods.

2.6.2. Serum analysis

After the experimental period, blood was collected from the retro orbital puncture under ether anesthesia and serum was separated by centrifugation at 10,000 rpm for 10 min and analyzed for calcium (Simas et al., 2001), phosphate (Fossati et al., 1988), urea (Lequang et al., 1987), uric acid (Hisham et al., 2013) and creatinine (Bowers, 1980).

2.6.3. Kidney homogenate analysis

The animals were sacrificed by cervical decapitation and the abdomen was cut open to remove both the kidneys from each animal. Isolated kidneys were cleaned off extraneous tissue and wet weight of kidney was measured (g/100 g b.w) followed by preservation in 10% neutral formalin. Left kidney was weighed on electronic weighing balance, and dried at 80^0C in a hot air oven and weight of kidney (g/100 g b.w.) was measured. A sample of 100 mg of the dried kidney was boiled in 10 mL of 1 N hydrochloric acid for 30 min. and homogenized. The homogenate was centrifuged at 2000 rpm for 10 min and the supernatant was separated. The calcium, phosphate and oxalate content in kidney homogenate were determined (Kalyani et al., 2010).

2.6.4. Enzyme assay

A portion of kidney was taken from all the groups, and a 30% w/v homogenate was prepared in 0.9% buffered KCl (pH 7.4) for the estimation of superoxide dismutase (SOD) (Kakkar et al., 1984), Glutathione (GSH) (Sedlak and Lindsay, 1968) and malondialdehyde (MDA) (Okhawa et al., 1979).

2.7. Statistical Analysis

Results were expressed as mean ± S.E.M. Differences among data were determined using one-way ANOVA followed by Tukey's Multiple Comparison Test (Graph Pad Prism software for windows, version 5.01). $p<0.05$ was considered statistically significant.

3. Results

3.1. Acute Oral Toxicity

Up to 5000 mg/kg dose of crude extract of *A. carambola* fruits (MeAC), no adverse effects were observed on the respiratory, circulatory, autonomic and central nervous systems and somatomotor activity. Also, the behavioural pattern was found to be normal throughout the study period. None showed any sign of abnormal change in skin, fur, eyes and mucous membranes. Tremors and convulsions were also not observed in any of the survivor. Thus, the extract was found to be safe upto 5000 mg/kg body weight. Doses of 100, 200 and 400 mg/kg b.w. were selected for the pharmacological studies on the basis of pilot study.

3.2. Urine microscopy

On microscopic examination at 100× using compound microscope, it was found that the urine of normal group was devoid of any crystals (Figure 1), while in calculi induced group, the urine sample showed abundant, large crystals of Calcium oxalate. In preventive regimen, the MeAC (400) showed better dissolution of the preformed crystals of Calcium oxalate as compared with MeAC (200) and MeAC (100). However, small fragments of crystals were seen in both the animal groups treated with MeAC (200) and MeAC (100). The result of MeAC (400) in preventive regimen was comparable to that of standard drug (cystone) treated group, which showed few crystals of calcium oxalate.

3.3. Urine Analysis

In the present study, urolithiasis was induced by the supplementation of normal commercial diet with glycolic acid for the 28 days. Table 1 indicates the various physical parameters that were measured including the volume of urine collected at the end of the treatment, the pH of urine and the weight of dry and wet kidney. The volume of urine was reduced in calculi induced animals as compared to normal control group. The treatment with MeAC (100, 200, 400 mg/mL) and standard drug Cystone increase the volume of urine in calculi induced animals when compared with control. MeAC 400 significantly increases the volume of urine when compared with normal animals as well as Cystone treated animals. The pH of urine of calculi induced animals were significantly ($p<0.05$) increased as compared to normal control rats which was slightly acidic. The treatment with MeAC 400 and Cystone significantly ($p<0.05$) decreases the pH of urine near to neutral in calculi induced animals. There was a significant ($p<0.05$) increase in the kidney weight (both dry and wet weight) of animals receiving 3% glycolic acid which was significantly ($p<0.05$) reduced by the treatment with Cystone and MeAC 400.

Table 1: Effect of MeAC on various physical parameters of urine

Group	Volume (mL/24 hr)	pH	Wet weight (g/100g) of kidney	Dry weight (g/100 g) of kidney
Normal	4.75±0.49	6.88±0.15	0.72±0.04	0.32±0.03
Calculi induced	12.61±0.69[a]	8.85±0.17[a]	1.39±0.09[a]	0.54±0.04[a]
Std Cystone	16.46±0.73[ab]	6.89±0.16[b]	0.81±0.05[b]	0.32±0.02[b]
MeAC 100	11.41±0.41[b]	8.31±0.26[a]	1.13±0.05[a]	0.45±0.02[a]
MeAC 200	18.28±0.83 [ab]	8.02±0.27[a]	0.97±0.10[b]	0.41±0.02[b]
MeAC 400	22.24±0.69[ab]	7.17±0.21[b]	0.78±0.03[b]	0.34±0.02[b]

All results are expressed as Mean ± S.E.M (n=6 in each group). Statistical comparison was made by one way ANOVA followed by the Tukey's multiple comparison tests. [a]$p<0.05$, statistically significance as compared to normal control. [b]$p<0.05$, statistically significance as compared to Ethylene glycol induced Calculi.

Table 2: Effect of MeAC on various parameters in urine

Group	Calcium (mg/24 hr)	Phosphate (mg/24 hr)	Oxalate (mg/24 hr)	Urea (mg/24 hr)	Uric Acid (mg/24 hr)	Citrate (mg/24 hr)
Normal	3.89±0.16	3.12±0.05	3.59±0.42	7.87±0.50	1.68±0.17	17.21±0.58
Calculi induced	16.97±0.61[a]	16.45±0.95[a]	12.03±0.42[a]	13.77±0.66[a]	5.76±0.54[a]	10.03±0.30[a]
Std Cystone	4.56±0.31[b]	4.73±0.29[b]	4.19±0.18[b]	8.18±0.46[b]	2.08±0.32[b]	21.18±0.64[ab]
MeAC 100	12.97±0.70[ab]	9.17±0.50[ab]	11.11±0.32[a]	13.61±0.73[a]	4.38±0.47[a]	11.33±0.58[a]
MeAC 200	8.11±0.38[ab]	7.12±0.60[ab]	8.17±0.34[ab]	11.69±2.01	4.11±0.21[ab]	13.32±0.66[ab]
MeAC 400	4.90±0.43[bd]	4.74±0.54[bd]	5.38±0.69[b]	9.65±0.30	2.45±0.23[b]	22.69±0.47[abde]

All results are expressed as Mean ± S.E.M (n=6 in each group). Statistical comparison was made by one way ANOVA followed by the Tukey's multiple comparison tests. [a]$p < 0.05$, statistically significance as compared to normal control. [b]$p < 0.05$, statistically significance as compared to Ethylene glycol induced Calculi.

Table 3. Effect of MeAC on various parameters in serum

	Calcium (mg/dL)	Phosphate (mg/dL)	Uric Acid (mg/dL)	Urea (mg/dL)	Creatinine (mg/dL)
Normal	7.27±0.32	4.12±0.29	4.34±0.23	38.04±1.01	0.88±0.08
Calculi induced	12.44±0.39[a]	11.26±0.44[a]	9.44±0.53[a]	88.71±0.83[a]	7.66±0.24[a]
Std Cystone	7.37±0.37[b]	5.09±0.14[b]	4.91±0.29[b]	37.85±1.60[b]	1.10±0.09[b]
MeAC 100	11.17±0.46[a]	10.37±0.33[a]	9.46±0.59[a]	69.37±3.20[ab]	7.85±0.27[a]
MeAC 200	10.84±0.54[a]	9.73±0.26[ab]	7.02±0.36[ab]	58.83±2.36[ab]	4.39±0.30[ab]
MeAC 400	7.83±0.37[b]	5.12±0.33[b]	5.04±0.31[b]	41.33±2.03[b]	1.37±0.22[b]

All results are expressed as Mean ± S.E.M (n=6 in each group). Statistical comparison was made by one way ANOVA followed by the Tukey's multiple comparison tests. [a]p < 0.05, statistically significance as compared to normal control. [b]p < 0.05, statistically significance as compared to Ethylene glycol induced Calculi.

Table 4. Effect of MeAC on various parameters in kidney homogenate

	Calcium (mg/g)	Phosphate (mg/g)	Oxalate (mg/g)	Uric Acid (mg/g)
Normal	3.20±0.21	3.51±0.15	0.92±0.09	1.85±0.19
Calculi induced	6.64±0.27[a]	6.79±0.35[a]	4.28±0.25[a]	4.64±0.28[a]
Std Cystone	3.69±0.17[b]	3.90±0.20[b]	1.11±0.09[b]	2.15±0.14[b]
MeAC 100	6.42±0.24[a]	6.12±0.35[a]	3.93±0.31[a]	3.99±0.22[a]
MeAC 200	5.47±0.18[ab]	5.59±0.24[ab]	2.96±0.13[ab]	3.80±0.15[a]
MeAC 400	4.02±0.20[b]	4.18±0.21[b]	1.44±0.19[b]	2.26±0.25[b]

All results are expressed as Mean ± S.E.M (n=6 in each group). Statistical comparison was made by one way ANOVA followed by the Tukey's multiple comparison tests. [a]p < 0.05, statistically significance as compared to normal control. [b]p < 0.05, statistically significance as compared to Ethylene glycol induced Calculi.

3.4. Urine biochemistry

The details of 24 h urinary excretion of stone forming constituents namely calcium, phosphorous and oxalate as well as urinary excretion of uric acid and urea are shown in table 2. The animals treated with Ethylene glycol showed significant (p<0.05) increase in the urinary excretion of calcium, phosphate, uric acid, urea and oxalate as compared to the normal control group due to the hyperoxaluria. The animals treated with standard drug Cystone and MeAC 400mg/kg showed significant (p<0.05) reversal of these changes as compared to the negative control group. MeAC in the dosages of 100 mg/kg p.o showed insignificant reduction in the urinary excretion of uric acid, urea and oxalate as compared to the negative control group. MeAC in the dosages of 200 mg/kg p.o showed significant reduction in the urinary excretion of calcium, phosphate, uric acid and oxalate as compared to the negative control group. MeAC showed dose dependent reduction in the urinary excretion of calcium and oxalate. Urinary citrate flow was decreased by Ethylene glycol treatment. However, supplementation with MeAC 200 and 400 significantly (p<0.05) maintained the elevated level of citrate and restores it near to normal value. The effect of MeAC 400 was similar to standard drug cystone.

3.5. Serum parameters

Effect of the MeAC on the various serum parameters such as calcium, inorganic phosphate, creatinine, uric acid and urea are summarized in the table 3. Renal stone induction caused impairment of renal functions, glomerular and tubular damage as reflected by significant (p<0.05) increase in the levels of serum creatinine, uric acid and urea in Ethylene glycol treated animals. The animals treated with standard drug Cystone and MeAC 400mg/kg showed significant (p<0.05) reversal of these changes as compared to the negative control group. MeAC in the dosages of 100 g/kg p.o., showed insignificant reduction in the urinary excretion of phosphate, uric acid, urea and oxalate as compared to the negative control group. MeAC showed dose dependent reduction in the serum uric acid, urea and creatinine. The serum calcium and inorganic phosphate were also significantly increased in calculi–induced animals. However, treatment with standard drug Cystone and MeAC 400 mg/kg significantly (p< 0.05) lowered the elevated serum level of calcium and inorganic phosphate in calculi induced animals.

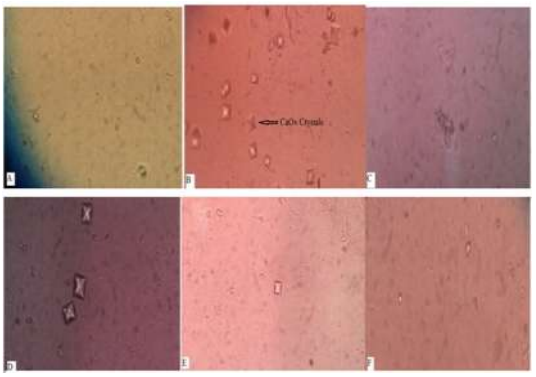

Figure 1. Urine Microscopy of unstained smear **[A]:** Normal; **[B]:** Calculi induced group (0.75% ethylene glycol); **[C]:** Standard drug treatment (ethylene glycol + Cystone 750 mg/kg bw); **[D]:** Test MeAC 100 (ethylene glycol + MeAC 100 mg/kg bw); **[E]:** Test MeAC 200 (ethylene glycol + MeAC 200 mg/kg bw); **[F]:** Test MeAC 400 (ethylene glycol + MeAC 400 mg/kg bw).

Table 5. *In vivo* antioxidant activity of MeAC

	LPO [MDA (nmol/g tissue)]	SOD (units/g tissue)	GSH (µg /g tissue)
Normal	12.94±1.22	36.39±0.47	2959.05±197.90
Calculi induced	86.54±2.87[a]	9.50±3.58[a]	724.19±289.21[a]
Std Cystone	17.01±0.44[b]	34.81±0.37[b]	2475.29±230.70[b]
MeAC 100	71.57±1.77[ab]	12.58±1.80[a]	1008.80±249.96[a]
MeAC 200	43.34±3.38[ab]	30.63±1.15[b]	1660.57±88.98[a]
MeAC 400	17.32±2.09[b]	33.02±0.34[b]	2683.14±128.11[b]

All results are expressed as Mean ± S.E.M (n=6 in each group). Statistical comparison was made by one way ANOVA followed by the Tukey's multiple comparison tests. [a]p < 0.05, statistically significance as compared to normal control. [b]p < 0.05, statistically significance as compared to Ethylene glycol induced Calculi.

3.6. Kidney homogenate biochemistry

The retention of crystalline components, namely calcium, phosphate and oxalate in various animal groups are shown in table 4. Results of in vivo antioxidant activity are summarized in the table 5. The calcium, phosphate, uric acid and oxalate level were significantly (p<0.05) increased in kidney homogenate of Ethylene glycol treated animals. However, treatment with standard drug Cystone and MeAC 400 mg/kg significantly (p< 0.05) maintained the levels of all parameters mentioned above. The MeAC (200 mg/kg) exhibit significant reduction in calcium, phosphate and oxalate level in kidney homogenate except uric acid level.

The animal treated with Ethylene glycol showed significant (p<0.05) increase of LPO as compared to the normal control group. The standard drug Cystone and MeAC showed signifi-

-cant (p<0.05) reduction of LPO as compared to the negative control group. The effect of MeAC was dose dependent. The animal treated with the Ethylene glycol showed significant (p<0.05) decrease in the activity of SOD as compared to the normal control group. The standard drug Cystone and animals MeAC (200 and 400 mg/kg, p.o) showed significant (p< 0.05) increase in the activity of SOD as compared to the negative control group. The animal treated with the Ethylene glycol showed significant (p<0.05) decrease in the level of GSH as compared to the normal control group. The standard drug Cystone and MeAC 400mg/kg, p.o showed significant (p< 0.05) increase in the level of GSH as compared to the negative control group.

4. Discussion and Conclusion

Male rats urinary system is very much similar to that of human, therefore only male rats were selected as experimental animals for anti urolithiatic activity. Moreover, female sex hormones were reported as inhibitor of kidney stone formation by increasing osteopontin expression in the kidney and decreasing urinary excretion of oxalate while testosterone promotes stone formation by suppressing osteopontin expression (Lee et al., 1996). The outcome of the present study confirmed the traditional use of *A. carambola* on scientific background. It indicates that *A. carambola* is capable of preventing crystal formation or growth, stone formation and crystalluria. This effect could be valuable in preventing the calculi formation by flushing out the smaller crystals from the kidney and reducing the probability of their retention in the urinary tract.

Ethylene glycol (EG), a metabolic precursor of oxalate is commonly used to induce urinary stone in experimental animals. Development of urinary calculi is multifaceted processes involving series of events viz. super-saturation, nucleation, crystal growth, aggregation of smaller crystals and adheration within the renal tubules. The MeAC treatment produced an increase in urine amount which may reduce super-saturation of urine, inhibit the nucleation and growth of crystals. Diuretic action of MeAC accelerate the process of excretion of stones with precipitating substances (calcium, phosphates and oxalates) which is one of the favorable requisition factors for stone formation by crystallization (Lee et al., 1996). Previously some diuretic drugs were also reported for antilithiatic activity (Kalyani et al., 2010; Patel et al., 2012). The pH was significantly affected by the MeAC treatment. calcium- and phosphate-containing calculi is proted by an alkaline urine while acidic urine favors uric acid or cystine containing stones (Wagner and Mohebbi, 2010). The mechanism of alkaline urine production after ethylene glycol treatment and its possible correlation with nephrolithiasis in this rat model remains unclear and needs further studies. Our results are in accordance with other studies, as shown by the significant increase in kidney weight (Fossati et al., 1988). In calculi induced group, precipitation and accumulation of stone forming ions leads to increase in kidney weight, which was almost normalized by treatment with standard drug and MeAC.

Biochemical estimation of certain minerals in urine is an esse-ntial feature in determining the calculi nature. In this study, 28 days lithogenic treatment with EG in rats produces renal calculi composed of mainly calcium oxalate (Patel et al., 2012). The mechanism may be an imbalance between physiological stone promoters (calcium, phosphates and oxalates) and inhibitors (citrate). Increased urinary concentration of calcium, phosphates and oxalates were observed in EG treated rats. These minerals facilitate nucleation and crystallization of CaOx. Higher concentration of oxalate in urine increases probability of stone formation by 15 folds (Karadi et al., 2006). In addition, not only calcium and oxalate excretion but also excretion of inorganic phosphate was an important contributing factor in urinary stone (Soundarajan et al., 2006). EG treated rats shows a decline in the amount of citrate excreted in urine. Hypocitraturia is the major metabolic abnormality in patients with renal stones. Investigations of citrate metabolism in stone formers have shown that tubular citrate re-absorption is the main mechanism regulating urinary citrate excretion (Soundarajan et al., 2006). In urine, citrate complexes with calcium, reducing the free calcium ion activity. The complex formation was pH dependent and observed above the pH 6.5 (Ghodkar, 1994).

EG treatment resulted into renal damage and decline in glomerular filtration rate (GFR). The two factors contribute in accumulation of nitrogenous waste products *viz*. urea, creatinine and uric acid in the blood (Tania et al., 1999). Hyperuricosuria decreases CaOx solubility through specific protein, moreover it reduces the inhibitory activity of glycos-amino-glycans (Selvam et al., 2001). Treatment with MeAC lowered the serum levels of these markers as well as excretion of uric acid, thus reduces the risk of stone formation. Due to deposition of stone forming minerals i.e. calcium and oxalate, their level in wet tissue increases in EG treated rats which was later revert back toward normal by the MeAC. It further reduces chance of renal cell injury induced by CaOx crystal. The effect of MeAC might be due to the increased bioavailability of NO (nitric oxide) which in turns activates GMP (Guanosine monophosphate) that controls the increase in intracellular calcium levels (Jaydip et al., 2010). Previously it was reported that plants containing pytochemicals which acts as NO donors enhance the intracellular calcium (Jaydip et al., 2010). Elevated urinary oxalate level in urine has been reported to induce lipid peroxidation and cause renal damage by reacting with polyunsaturated fatty acids in cell membrane (Karadi et al., 2006). The decrease in concentration of creatinine, urea and uric acid in serum and calcium, phosphate and oxalate in urine in MeAC treated rats were attributed to rich antioxidants (SOD and GSH) present in these extract as evident from decreased lipid peroxidation and increased levels of antioxidant (SOD and GSH) as compared to rats supplemented with a calculi-producing diet.

Stone matrix protein fractions nucleation and aggregation was promoted by certain circumstances which develop peroxidation and depletion of thiol content and leads to increase in the oxalate binding activity. This behavior is also associated with peroxidized mitochondria and nuclei suggesting that the peroxidation can be a causative factor for the initiation of stone formation (Ramezani et al., 2009).

The test drug *A. carambola* has been previously reported for its antioxidant activity especially due to presence of polyphenol-

-oxidase, proanthocyanidins, epicatechin and vitamin C (Leong and Shui, 2002) suggesting that it at least partially evolves the antioxidant mechanism to induce the antilithogenic effect. Stone formation process caused hypertrophy and extensive calcium oxalate crystal deposition in kidneys of untreated rats accompanied by oxidative damage as reflected from increased levels of markers of oxidative injury (MDA and protein carbonyl content) and decreased activities of antioxidant enzymes along with GSH levels in kidneys (Bashir et al., 2009). Therefore, these antioxidant enzymes compensate the enhanced oxidative stress in the kidney. The decreased activities of catalase in the nephrolithiasis in the present study may have led to more H_2O_2 accumulation in the kidney, resulting in more hydroxyl radical formation; because catalase is the only enzyme that regulates the potent hydroxyl radical (Selvam, 2002). MeAC may prevent the lipid peroxidation induced renal damage caused by calcium oxalate crystals deposition in the kidney. MeAC also significantly increases the levels of SOD and GSH levels in kidney as compared to the control animals, which suggests its efficacy in preventing free radical-induced damage. Previously potential antioxidants like *Punica granatum* fruits (Rathod et al., 2012) and Vitamin E were reported to prevent calcium oxalate crystal deposition in the kidney by preventing oxidative damage to the renal tissue induced by hyperoxaluria, leads to prevent the attachment of calcium oxalate crystal and subsequent development of the kidney stones (Rathod et al., 2012).

Moreover, deficiency of inhibitory factors and the presence of promoters in urine are considered to be the most important risk factors in the process of urinary stone disease (Selvam et al., 2001). When these conditions favor stone formation, the antiadherent layer of glycos-amino-glycans acts as a protective barrier against urinary stone disease. If this layer is damaged, i. e. due to bacterial infection, a calculi nucleus might develop leading to a full stone in the urinary tract. Antimicrobial as well as anti-inflammatory properties of the drug also favor its anti-lithogenic by protecting the anti-adherent glycosaminoglycans layer covering the epithelium of the collecting system (Ramezani et al., 2009) as reported previously for some other drugs (Kalyani et al., 2010; Rathod et al., 2012). Moreover some of the phytoconstituents of the plant may be responsible for its activity. It contains saponins and flavonoids (Thomas et al., 2008). Earlier reports show that flavonoids rich plant extracts and saponin derivatives posses anti-urolithiatic activity (Patel et al., 2012; Park *et al*., 2007). Other phyto-constituents present in plants were also reported for anti-urolithiatic activity like anthraquinone glycoside (Kalyani et al., 2010), rutin (Jaydip et al., 2010), lupeol (Anand et al., 1995) and quercetin (Park et al., 2007).

In this paper, we demonstrated the effectiveness of *A. carambola* in reducing oxalate deposition in the renal tissues and facilitate disintegration of formed stone ultimately thereby contributes in the prevention of recurrence of urolithiasis. This study is preliminary and requires further continuation for understanding the mechanisms through which *A. carambola* exerts its antiurolithiatic actions. The findings justify the traditional use of *A. carambola* in the treatment of renal calculi.

CONFLICT OF INTEREST

Authors report no conflict of interest.

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