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Antiurolithiatic Property of *Moringa oleifera* Root Bark

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Abstract

In spite of tremendous advances in the field of medicine, there is no truly satisfactory drug for the treatment of renal calculi. In the present study, the efficacy of the root bark of *Moringa oleifera* Lam. (Moringaceae) as an antiurolithiatic agent was investigated using an experimentally induced urolithiatic rat model. Hyperoxaluria was induced in rats using 0.75% ethylene glycol in water. Aqueous (AqE) (200 mg kg^{-1} body weight) and alcoholic extracts (AlcE) (200 mg kg^{-1} body weight) of the root bark of *M. oleifera* were given orally in curative and preventive regimens over a period of 28 days. Both the extracts significantly ($P < 0.001$) lowered the urinary excretion and kidney retention levels of oxalate, calcium and phosphate. Moreover, elevated serum levels of urea nitrogen, creatinine and uric acid were significantly ($P < 0.001$) reduced by the extracts. The results were comparable with the standard drug, cystone (750 mg kg^{-1} body weight). The reduction of stone forming constituents in urine and their decreased kidney retention reduces the solubility product of crystallizing salts such as calcium oxalate and calcium phosphate, which could contribute to the antiurolithiatic property of root bark of *M. oleifera*.

Keywords: Cystone, ethylene glycol, hyperoxaluria, *Moringa oleifera* Lam., urolithiasis.

Introduction

Urinary stone disorder (urolithiasis) has afflicted humankind since antiquity and can persist with serious medical consequences throughout a patient's lifetime. Presently, urolithiasis is the third most common disorder of the urinary tract, the others being frequently occurring urinary tract infections and benign prostatic hyperplasia (Hiatt & Friedman, 1982). The major factors responsible for urolithiasis are super-saturation of urine with the offending salt and crystallization (Balaji & Menon, 1997). The majority of urinary calculi are made up of calcium phosphate, calcium oxalate, uric acid (urates) or magnesium ammonium phosphate. Many remedies have been employed through the ages to treat urolithiasis. In most cases, the management of urolithiasis involves both surgical and medical approaches, i.e., percutaneous nephrolithotomy (PCNL), extracorporeal shock wave lithotripsy (ESWL) and antibiotics (Rivers et al., 2000). However, these treatments are relatively costly, painful and require expert hands with availability of appropriate equipments. This has stimulated research on traditional remedies showing antiurolithiatic activity.

A number of vegetable drugs have been used in India, which claim efficient cure of urinary stones. In *Charaka Samhita*, an ayurvedic compendium of India, the aqueous extract of roots of *Moringa oleifera* Lam. (Moringaceae) is reported to be useful in the treatment of urinary stones. *M. oleifera*, commonly known as drumstick or horseradish, is indigenous in the southern foothills of the Himalaya and sub-Himalayan tract from the Chenab eastwards to Sarda and Oudh forests, and cultivated all over the plains of India. The root bark is used as aphrodisiac, alexeteric, anthelmintic, analgesic, and also for heart complaints, eye diseases, inflammation, dyspepsia, etc. Juice of roots is prescribed with milk as antilithic (Chopra et al., 1982; Nadkarni, 1976), though the rationale behind its use is not scientifically established. Based on this information and in continuation of our search on herbal drugs (Karadi et al., 2006), an attempt is made in the present study to exploit the antiurolithiatic properties of *M. oleifera* using an experimental hyperoxaluric model of albino rats.

Materials and Methods

Plant material

The fresh roots of *M. oleifera* were collected from local areas of Pune, Maharashtra, India and were authenticated

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by Dr. V. Prasad, Botanical Survey of India (BSI), Pune, India. A voucher specimen of the plant was deposited in the BSI herbarium (BSI/WC/2005/Tech/997). The root bark was separated, dried, and ground to yield coarse powder.

Preparation of extracts

The aqueous extract (AqE) of root bark was prepared using chloroform water (Indian Pharmacopeia) by maceration method for 24 h at room temperature and the alcohol extract (AlcE) was made using 70% alcohol by Soxhlet method. The extracts were concentrated under vacuum and dried over anhydrous sodium sulphate.

Experimental protocols and chemicals

Wistar albino mice of either sex weighing between 25 and 30 g were selected for acute toxicity studies while healthy adult male Wistar albino rats weighing between 150 and 200 g were used for antiurolithiatic activity. The animals were acclimatized to standard laboratory conditions (temperature: 25 ± 2°C) and maintained on 12:12 h light:dark cycle. They were provided with regular rat chow (Lipton India Ltd., Mumbai, India) and water ad libitum. The animal care and experimental protocols were in accordance with the Institutional Animal Ethical Committee (IAEC). Ethylene glycol (AR grade) was purchased from Merck Laboratories, Mumbai, India. Cystone tablets were procured from the Himalaya Drug Company, Bangalore, India. All other chemical reagents and solvents were of analytical grade.

Acute toxicity studies

The acute oral toxicity study was carried out as per the guidelines set by the Organization for Economic Co-operation and Development (OECD) received from the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). One tenth of the median lethal dose (LD_{50}) was taken as an effective dose (Handa & Anupama, 1990).

Antiurolithiatic activity

Ethylene glycol induced urolithiasis model in albino rats

The method of Atmani et al. (2003) was used to assess the antiurolithiatic activity in albino rats. Animals were divided into seven groups each containing six. Group I served as control and received regular rat food and water *ad libitum*. Ethylene glycol (0.75%) in water was fed to group II to VII for induction of renal calculi until day 14. Group III received the standard antiurolithiatic drug, Cystone (750 mg kg^{-1} body weight), from day 15 until day 28. Group IV and V served as curative regimen (CR). Group IV received AqE (200 mg kg^{-1} body weight) and group V received AlcE (200 mg kg^{-1} body weight) from day 15 until day 28. Group VI received AqE (200 mg kg^{-1} body weight) and group VII received AlcE (200 mg kg^{-1} body weight) from day 1 until day 28 and served as preventive regimen (PR). These extracts were administered once a day by the oral route.

Collection and analysis of urine

Animals were kept in separate metabolic cages and urine samples of 24 h were collected on day 28. A drop of concentrated hydrochloric acid was added to the urine before being stored at 4°C. Urine was analyzed for calcium, phosphate and oxalate content (Mustafa & Medeiros, 1985; Fiske & Subbarow, 1925; Hodgkinson & Williams, 1972).

Serum analysis

After the experimental period, blood was collected from the retro-orbital under anaesthetic conditions and the animals were sacrificed by cervical decapitation. Serum was separated by centrifugation at 10,000 rpm for 10 min and analyzed for creatinine, urea nitrogen and uric acid (Raghuramulu et al., 1983; Caraway, 1963).

Kidney homogenate analysis

The abdomen was cut open to remove both kidneys from each animal. Isolated kidneys were cleaned of extraneous tissue and dried at 80°C in a hot-air oven. A sample of 100 mg was boiled in 10 ml of 1 N hydrochloric acid for 30 min and homogenized (Chow et al., 1975). The homogenate was centrifuged at 2,000 rpm for 10 min and the supernatant was analyzed for calcium, phosphate, and oxalate contents.

Statistical analysis

Data were expressed as mean ± SD. Statistical significance between the data of different groups was evaluated using one-way analysis of variance (ANOVA) followed by Student's Newman-Keul's test (Graphpad Prism software for Windows, Version 4.10.1998). The minimum level of significance was set at $P < 0.05$.

Results

From the acute toxicity studies, the LD_{50} cut-off dose for AqE and AlcE of root bark of *M. oleifera* was found to be 2,000 mg kg^{-1} body weight. Hence, the therapeutic dose was taken as 200 mg kg^{-1} body weight for both extracts.

In the present study, hyperoxaluria was induced by feeding rats with ethylene glycol. The urinary excretion of oxalate increased significantly ($P < 0.001$) in calculi-induced rats (Group II) as compared to control (Group I) (Table 1). A gradual increase in calcium and phosphate excretion was also seen in Group II. The deposition of oxalate,

Table 1. Effect of aqueous and alcoholic extract of *Moringa oleifera* Lam. root bark in experimental hyperoxaluria.

Parameter (unit)	Group I Control	Group II Calculi induced	Group III Cystone treated	Curative regimen		Preventive regimen	
				Group IV AqE	Group V AlcE	Group VI AqE	Group VII AlcE
Urine (mg/dl)							
Oxalate	0.37 ± 0.03	$3.64 \pm 0.11^{*a}$	$0.53 \pm 0.04^{*a,b}$	$0.88 \pm 0.04^{\dagger f*b,c}$	$1.29 \pm 0.07^{*b,c,g}$	$0.79 \pm 0.04^{\dagger d*b,c}$	$1.08 \pm 0.08^{*b,c,e}$
Calcium	1.27 ± 0.07	$4.51 \pm 0.10^{*a}$	$1.50 \pm 0.06^{*a,b}$	$1.77 \pm 0.08^{\dagger f*b,c}$	$1.91 \pm 0.07^{*b,c}$	$1.68 \pm 0.08^{\dagger d*b,c}$	$1.87 \pm 0.05^{*b,c}$
Phosphate	3.64 ± 0.04	$7.29 \pm 0.06^{*a}$	$3.81 \pm 0.09^{*a,b}$	$4.05 \pm 0.06^{*b,c}$	$4.13 \pm 0.07^{\dagger g*b,c}$	$3.98 \pm 0.08^{*b,c}$	$4.21 \pm 0.05^{\dagger e*b,c}$
Kidney (mg/g)							
Oxalate	1.41 ± 0.06	$5.73 \pm 0.06^{*a}$	$1.61 \pm 0.06^{*a,b}$	$1.84 \pm 0.04^{\dagger f*b,c}$	$2.13 \pm 0.08^{\ddagger g*b,c}$	$1.76 \pm 0.05^{\dagger d*b,c}$	$2.07 \pm 0.09^{\ddagger e*b,c}$
Calcium	3.23 ± 0.04	$4.79 \pm 0.16^{*a}$	$3.42 \pm 0.07^{*a,b}$	$3.69 \pm 0.05^{\dagger f*b,c}$	$4.11 \pm 0.03^{\ddagger g*b,c}$	$3.59 \pm 0.07^{\dagger d*b,c}$	$3.97 \pm 0.04^{\ddagger e*b,c}$
Phosphate	2.35 ± 0.03	$3.74 \pm 0.10^{*a}$	$2.52 \pm 0.07^{*a,b}$	$2.75 \pm 0.04^{\dagger f*b,c}$	$2.91 \pm 0.04^{*b,c}$	$2.66 \pm 0.03^{\dagger d*b,c}$	$2.86 \pm 0.06^{*b,c}$
Serum (mg/dl)							
BUN	37.61 ± 0.15	$49.97 \pm 0.48^{*a}$	$39.30 \pm 0.48^{*a,b}$	$40.80 \pm 0.29^{\dagger f*b,c}$	$42.98 \pm 0.23^{*b,c,g}$	$40.36 \pm 0.13^{\dagger d*b,c}$	$41.12 \pm 0.21^{*b,c,e}$
Creatinine	0.75 ± 0.01	$0.94 \pm 0.03^{*a}$	$0.81 \pm 0.02^{*a,b}$	$0.85 \pm 0.01^{*b,c}$	$0.91 \pm 0.02^{\ddagger b*c}$	$0.85 \pm 0.01^{*b,c}$	$0.91 \pm 0.02^{\ddagger b*c}$
Uric acid	1.49 ± 0.07	$3.64 \pm 0.11^{*a}$	$1.71 \pm 0.04^{*a,b}$	$1.89 \pm 0.07^{*b,c}$	$2.12 \pm 0.04^{*b,c}$	$1.88 \pm 0.09^{*b,c}$	$2.06 \pm 0.07^{cb,c}$

Values for urine parameters are assessed in 24 h urine sample; BUN, blood urea nitrogen.
All values are expressed as mean ± SD for six animals in each group.
Comparisons are made: [a] with Group I; [b] with Group II; [c] with Group III; [d] with Group IV; [e] with Group V; [f] with Group VI; [g] with Group VII.
Symbols represent statistical significance: $^{\dagger}P < 0.05$, $^{\ddagger}P < 0.01$, $^{*}P < 0.001$.

calcium, and phosphate in the renal tissue also increased in the calculi-induced rats (Group II).

AqE and AlcE of root bark significantly ($P < 0.001$) reduced the urinary excretion as well as kidney retention of oxalate, calcium and phosphate in both CR and PR when compared to treatment with cystone (Table 1). But the reduction in stone-forming constituents produced by AqE and AlcE in CR (Groups IV and V, respectively) was less significant ($P < 0.05$) as compared to PR (Group VI and VII, respectively). The stones formed in the kidneys were found to cause extensive damage and this was indicated by elevated levels of blood urea nitrogen (BUN), creatinine, and uric acid (Group II). The treatment with AqE (Group IV and VI) and AlcE (Group V and VII) significantly ($P < 0.001$) reduced the serum levels of creatinine, uric acid and BUN in both regimens. The reduction in BUN exhibited by AqE in CR (Group IV) was less significant ($P < 0.05$) as compared to PR (Group VI) but AlcE produced a significant lowering of BUN in both regimens. The reduction in serum levels of creatinine and uric acid, exhibited by AqE and AlcE in CR (Group IV and V, respectively) was not significant ($P > 0.05$) in comparison to that of PR (Group VI and VII, respectively). The prophylactic treatment (Group VI and VII) was highly significant ($P < 0.001$) for both extracts as compared to the curative treatment (Group IV and V) while the overall effect exhibited by AqE in both regimens (Group IV and VI) was more pronounced than that of AlcE (Group V and VII).

Discussion

In the present study, male rats were selected to induce urolithiasis because the urinary system of male rats resembles that of humans (Vermeulen, 1962) and also earlier studies have shown that the amount of stone deposition in female rats was significantly less (Prasad et al., 1993). Urinary super-saturation with respect to stone-forming constituents is generally considered to be one of the causative factors in calculogenesis. Administration of ethylene glycol (0.75% v/v) to young male albino rats for 14 days results in the formation of renal calculi because of an increase in the urinary concentration of oxalate (Huang et al., 2002; Selvam et al., 2001). Increased urinary calcium is a factor favoring the nucleation and precipitation of calcium oxalate or apatite (calcium phosphate) from urine and subsequent crystal growth (Lemann et al., 1991). AqE and AlcE of *M. oleifera* Lam. root bark lowered the levels of oxalate as well as calcium excretion (Table 1).

A gradual increase in urinary phosphate was observed in calculi-induced rats (Group II). Increased urinary phosphate excretion along with oxalate stress seems to provide an environment appropriate for stone formation by forming calcium phosphate crystals, which epitaxially induces calcium oxalate deposition (Roger et al., 1997). Treatment with root bark extracts restored the urinary phosphate level thus reducing the risk of stone formation.

In urolithiasis, the glomerular filtration rate (GFR) decreases due to obstruction to outflow of urine by stones in urinary system. Due to this, the waste products, particularly nitrogenous substances such as urea, creatinine, and uric acid are accumulated in blood. Also, increased lipid peroxidation and decreased levels of antioxidant potential have been reported in the kidneys of rats supplemented with a calculi-producing diet (Sumathi et al., 1993). Oxalate has been shown to induce lipid peroxidation and cause renal tissue damage by reacting with polyunsaturated fatty acids in cell membrane (Ernster & Nordenbrand, 1967). In

calculi-induced rats (Group II), marked renal damage was seen as indicated by the elevated serum levels of creatinine, uric acid and BUN. The treatment with AqE (Group IV and VI) and AlcE (Group V and VII) significantly ($P < 0.001$) reduced the serum levels of creatinine, uric acid and BUN in both regimens. Ruckmani et al. (1997, 1998) have shown root bark of *M. oleifera* to possess significant diuretic and hepatoprotective properties. The positive effects seen with root bark extracts in the present study may be due to diuresis, which hastens the process of dissolving the preformed stones and prevents new stone formation. The significant lowering of serum levels of accumulated waste products could be attributed to the anti-lipid peroxidative property.

The phytochemical investigation revealed the presence of saponin glycosides in AqE of root bark of *M. oleifera.* Lupeol, a sapogenin isolated from the bark of *Crataeva nurvala* Buch Ham (Capparidaceae) was shown to possess antiurolithiatic activity (Baskar et al., 1996). Similarly, lupeol was found to inhibit kidney stone formation in glycolic acid induced renal lithiasis (Vidya & Varalakshmi, 2000) and in experimental hyperoxaluria (Buhler et al., 1991). Moreover, the urinary volume was markedly increased when lupeol was used as an antilithic agent (Malini et al., 1995). Similar effects might have been observed on administration of root bark extracts of *M. oleifera* in the present study accounting for the antiurolithiatic activity of the drug.

In conclusion, the presented data supports the folklore usage of *M. oleifera* in the treatment of patients suffering from kidney stones. The mechanism underlying this effect is still unknown but is apparently related to diuresis and lowering of urinary concentrations of stone forming constituents. The protective effect against oxalate-induced lipid peroxidation may be contributory to the recovery of renal damage.

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