Antiurolithiatic Activity Studies of Momordica charantia Linn. Fruits

Biren N. Shah, Khodidas D. Raiyani* and D. C. Modi

Department of Pharmacognosy, Vidyabharti Trust College of Pharmacy, Unrakh, Gujarat, India,
*Corresponding author: Phone No.: 9825669377 E-mail: khodidas_raiyani@yahoo.com

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ABSTRACT
In the indigenous system of medicine, the fruits of Momordica charantia Linn. (Family- Cucurbitaceae) are reported to be useful in the treatment of urinary stones. Hence, in the present study the fruits of Momordica charantia Linn. have been selected for their antiurolithiatic activity on experimentally induced urolithiatic rats. Antiurolithiatic activity of fruit extract of M. charantia Linn. was carried out on ethylene glycol (0.75% v/v) induced urolithiasis in rats. Treatment with Aqueous Extract (200mg/kg, p.o) and Alcoholic Extract (250mg/kg, p.o) of fruits of M. charantia Linn. 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 fruits 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 M. charantia Linn. fruits extracts 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 M. charantia Linn. could contribute to its antiurolithiatic property.

KEY WORDS: Momordica charantia Linn., Urolithiasis, Ethylene glycol, calcium oxalate.

INTRODUCTION
Urolithiasis (Renal Stone) denotes stones originating anywhere in the urinary tract, including the kidneys, ureters and bladder. The formation of stones in the urinary tract affects 5–10% of the population in Europe and North America [1, 2, 3]. In most populations the occurrence of urolithiasis in men is two to three times higher than in women as testosterone enhances whereas estrogen inhibits calcium oxalate stone formation [4]. The formation of calcium oxalate stone is a multi-step process and includes nucleation, crystal growth, crystal aggregation and crystal retention which further result in precipitation of certain substances within urine [5]. This process is favored in the presence of a supersaturated milieu which is necessary for precipitating crystal. Thus supersaturation acts as a driving force for stone formation [6]. Additionally another theory of stone formation was identified and concluded as an imbalance between promoter (calcium, oxalate, uric acid, inorganic phosphate etc) and inhibitors (citrates, magnesium, potassium, pyrophosphate and urinary glycoprotein etc). Furthermore, reactive oxygen species or free radicals (species with one or more unpaired electrons) generated due to oxidative stress, damages epithelium of kidney or bladder, thereby producing a favorable environment for crystal attachment to surface [7, 8]. As a result of these, the stone may not be able to travel through the ureter, causing pain and possibly an obstruction, blocking the flow of urine out of the kidney [9]. Severe pain or aching in the back on one or both sides, sudden spasms of excruciating pain (renal or uterine colic), bloody, cloudy or smelly urine, feeling of being sick, a frequent urge to urinate, or a burning sensation during urination, fever and chills, etc are commonly observed symptoms in the patients.

Patients suffering from diseases like hyperparathyroidism, renal tubular acidosis, cystinuria, hypercalcuiuria, hyperoxaluria, crohn’s disease etc. are more prone to stone formation. Drugs like acetazolamide, vit D supplements, calcium supplements, sulfonamide, indinavir, triamterene enhances chances of stone formation [10]. It was also found that there is strong correlation of genetic defect with stone formation [11]. Decreased in urine volume, increased amount of solute in urine, change in urinary pH and infection may also lead to stone formation [12].

In India, medicinal plants have a good contribution to the development of ancient Indian medicines. One of the earliest treaties on Indian medicine, the Charak Samhita (1000 B.C.), records the use of over 340 drugs of vegetable origin. Most of these continue to be gathered from wild plants to meet the demand of the medical profession. Thus, despite the rich heritage of knowledge on the use of the plant drugs, little attention had been paid to grow them as field crops in the country till the latter part of the nineteenth century. Momordica charantia Linn. is a monoeocious climber found throughout India often under cultivation, up to an altitude of 1500 m. stem slender, more or less pubescent; leaves sub-orbicular, 5-7 lobed pubescent or sub-glabrous; flowers yellow, solitary [13]. Fruits are green coloured, 5-15 cm. long, pendulous, fusiform usually pointed or beaked, ribbed and bearing numerous triangular tubercles giving it the appearance of a crocodile’s back, 3-valved at the apex when mature. Seeds 8-13 mm. long, compressed, corrugate on the margin, sculptured on both faces [14].

The fruits and leaves contain charantin, a steroidal saponin.Also fruits contain a cathartic principle called mormordin.Carbohydrate (10%), mineral matter (1.5%) and ascorbic acid (88-188mg/100g).Additionally, alkaloids, glycoside, saponins and mucilage are other contents of karela. It is used as Appetizer, digestive, cholagogue, purga-
Serum analysis.

phosphate, oxalate, calcium, and citrate.

tracts starting from 15th day to 28th day (Curative regimen).

ter supplementing with 0.75% v/v ethylene glycol in drinking wa-

days. Renal calculi were induced in group II to VII by sup-

mals in each and kept in cages. All animals had free access
to regular rat chow and drinking water

Experiment design

The fresh fruits of Momordica charantia Linn. were collected from local areas of Surat, Gujarat, India, during March-2010 and authenticated by H.B.Singh, Head of Raw Materials Herbarium & Museum, NISCAIR, New Delhi.

Preparation of extract

The aqueous extract (AqE, 10%, w/v) of fruits was prepared using chloroform water, I.P., by maceration method for 7 days at room temperature (yield 29%, w/w) and the alcoholic extract (AlcE, 10%, w/v) was prepared using 70% (v/v) alcohol by soxhlet method at a temperature of 60–70 ºC (yield 09%, w/w). The extracts were concentrated under vacuum. A suspension of AqE and AlcE in 2% (v/v) tween-80 was prepared for oral administration by gastric intubation method.

Experiment design

Ethylene glycol induced urolithiasis model in albino rats [15, 16, 17].

Animals were divided in 7 groups containing six ani-
mals 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 wa-
ter ad libitum. Group IV to V were treated with plant ex-
tracts starting from 15th day to 28th day (Curative regimen).

Assessment of antiurolithatic 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. Ani-
mals had free access to drinking water during the urine collection period. After urine collection, urine volume and pH of urine were measured [18]. A drop of concentrated hydrochloric acid was added to the urine before being stored at 4°C. Urine was analyzed for magnesium, phospho-

date, oxalate, calcium, and citrate.

Serum analysis.

After the experimental period, blood was collected by heart puncture under anesthetic condition. Serum was sepa-
rated by centrifugation at 10,000×g for 10 min and ana-
lyzed 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 extra-
neous 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 archi-
tecture and their photomicrographs were taken.

Statistical analysis [21]

The data obtained by the various parameters was sta-
tistically evaluated by one way analysis of variance (ANO-
VA) 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 calcu-
lated for each parameter. The differences in biochemical parameters between the calcu li 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 calcu li induced group were ana-
lyzed accordingly. Level of significance was kept at P<0.05.

RESULTS AND DISCUSSION

Ethylene glycol induced urolithiasis resulted in signif-
icant elevation of urine and kidney calcium, oxalate, inor-
ganic phosphate and serum blood urea nitrogen, creatinine, uric acid. compared to normal control group. Treatment with cystine (750 mg/kg) and Momordica charantia linn. (AqE-200 mg/kg; AlcE- 250 mg/kg) prevented the bio-
chemical changes induced by ethylene glycol. (Table No. 1,2,3)

In order to probe the possible mechanism by which M. charantia linn. prevents renal damage caused by ethylene glycol, investigation on levels of various stone inhibitors like total protein, magnesium and citrate. There was significan-
cially rise on total protein, magnesium and citrate after treatment with cystine and M. charantia linn. (Table No. 4).

Administration of ethylene glycol significantly re-
duced the body weight, urine volume and pH of urine as compared to normal group. Rats treated with cystone and M. charantia linn. showed significant decreased in body weight, urine volume and pH of urine as compared to control group. (Table No. 5).

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 AlcE-PR of fruits treated groups showed normal histology of the kidney, and shows normal glomeruli, slight oedema of the tubular cells. The AqE of fruits treated animals also showed the recovery; however, the renal tubular epithelial recovery was less significant compared to standard drug treated animals. (Table No. 6)

| Table No. 1: Changes in urinary excretion of stone forming constituents in control and experimental animals |
| --- |
| **Group** | **Dose (mg/kg)** | **Urine parameters (mean ± SEM)** |
| Normal | Vehicle | 0.45 ± 0.65 | 1.18 ± 0.25 | 3.80 ± 0.10 |
| Calculi induced | Vehicle | 3.20 ± 0.42b | 3.94 ± 0.053b | 7.315 ± 0.114b |
| Cystone treated | 750 | 1.22 ± 0.22b | 1.53 ± 0.14b | 4.21 ± 0.21b |
| AqE fruits (CR) | 250 | 2.22 ± 0.31b | 3.28 ± 0.18b | 5.68 ± 0.09b |
| AlcE fruits (CR) | 200 | 1.91 ± 0.14b | 3.31 ± 0.27b | 5.011 ± 0.10b |
| AqE fruits (PR) | 250 | 2.00 ± 0.15b | 3.20 ± 0.16b | 5.02 ± 0.08b |
| AlcE fruits (PR) | 250 | 1.80 ± 0.38b | 3.11 ± 0.16b | 5.19 ± 0.05b |

Each value represents Mean±SEM., N=6; a compared to Normal (P<0.001); b compared to calculi induced (P<0.001)
Table No. 4: Changes in various inhibitors in control and experimental animals

| Group                  | Dose (mg/kg) | Total protein (mean ± SEM) | Magnesium (mean ± SEM) | Citrate (mean ± SEM) |
|------------------------|--------------|----------------------------|------------------------|-----------------------|
| Normal                 | Vehicle      | 2.96 ± 0.02                | 3.005 ± 0.05           | 51.86 ± 1.98          |
| Calculi induced        | Vehicle      | 1.39 ± 0.079<sup>a</sup>  | 0.625± 0.049<sup>a</sup> | 48.69 ± 6.86<sup>a</sup> |
| Cystone treated        | 750          | 2.79 ± 0.060<sup>b</sup>  | 2.003 ± 0.131<sup>b</sup> | 82.9 ± 13.29<sup>b</sup> |
| AqE fruits (CR)        | 250          | 2.28 ± 0.12<sup>b</sup>   | 1.47 ± 0.159<sup>b</sup> | 37.47 ± 2.83<sup>b</sup> |
| AlcE fruits (CR)       | 200          | 2.09 ± 0.274<sup>b</sup>  | 1.37 ± 0.13<sup>b</sup>  | 53.42 ± 2.23<sup>b</sup> |
| AqE fruits (PR)        | 250          | 2.50 ± 0.15<sup>b</sup>   | 1.50 ± 0.13<sup>b</sup>  | 39.72 ± 4.72<sup>b</sup> |
| AlcE fruits (PR)       | 250          | 2.27 ± 0.07<sup>b</sup>   | 1.37 ± 0.15<sup>b</sup>  | 45.2 ± 1.28<sup>b</sup> |

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

Table No. 5: Change in physical parameters in ethylene glycol induced urolithiasis.

| Group                  | Dose (mg/kg) | % change in Body weight | Urine volume | pH       |
|------------------------|--------------|-------------------------|--------------|----------|
| Normal                 | Vehicle      | 7.82 ± 0.38             | 14.03 ± 0.36 | 6.65 ± 0.18 |
| Calculi induced        | Vehicle      | -7.66 ± 0.61<sup>a</sup> | 10.45 ± 0.25<sup>a</sup> | 5.88 ± 0.32<sup>a</sup> |
| Cystone treated        | 750          | 3.85 ± 0.42<sup>b</sup>  | 19.48 ± 1.03<sup>b</sup>  | 6.66 ± 0.21<sup>b</sup>| |
| AqE fruits (CR)        | 250          | -4.63 ± 0.34<sup>b</sup> | 13.78 ± 0.2<sup>b</sup>      | 6.03 ± 0.3<sup>b</sup>  |
| AlcE fruits (CR)       | 200          | -4.11 ± 0.23<sup>b</sup> | 17.03 ± 0.19<sup>b</sup>     | 6.45 ± 0.17<sup>b</sup> |
| AqE fruits (PR)        | 250          | -4.94 ± 0.56<sup>b</sup> | 14.80 ± 0.14<sup>b</sup>     | 6.0 ± 0.1<sup>b</sup>   |
| AlcE fruits (PR)       | 250          | -1.79 ±0.79<sup>b</sup>  | 19.06 ± 0.29<sup>b</sup>     | 6.21 ± 0.17<sup>b</sup>| |

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

Table No. 6: histological features found from L.S of kidneys of different groups

| Histological feature                  | Normal | Calculus induced | Cystone treated | AqE-CR | AlcE-CR | AqE-PR | AlcE-PR |
|---------------------------------------|--------|-----------------|-----------------|--------|---------|--------|---------|
| Tubular congestion                    | -      | +++             | ++              | +      | ++      | +      | +       |
| Tubular cast                          | -      | +++             | +               | -      | +       | -      | -       |
| Epithelial disquamation                | -      | +++             | +               | -      | +       | +      | +       |
| Glomerular congestion                 | -      | +               | -               | +      | -       | -      | -       |
| Blood vessel congestion                | -      | +               | -               | -      | +       | +      | +       |
| Inflammatory cells                     | -      | +               | -               | +      | -       | +      | -       |

+++ ; Presence of histological abnormality; - ; Absence of histological abnormality
A. Normal Group

B. Calculi induced (untreated)

C. Cystone treated group

D. AqE of fruit of *M. charantia* Linn. treated Group (CR)

E. AlcE of fruit of *M. charantia* Linn. treated Group (CR)

F. AqE of fruit of *M. charantia* Linn. treated group (PR)
CONCLUSION

The presented data indicate that administration of the AqE and AlcE of *M. charantia* Linn. fruits to rats with ethylene glycol induced lithiasis reduced and prevented the formation of urinary stones, supporting folk information regarding antiurolithiatic activity of the plant part. 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. These effects could conclude the antiurolithiatic property of *M. charantia* Linn.