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RESEARCH ARTICLE

Phytochemical Analysis and Antiurolithiatic Activity of Aqueous Fruit Extract of *Momordica charantia* Linn in Ethylene Glycol-induced Urolithiasis Rats Model

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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. However, enough scientific evidences were not available about the effect of this plant as nephroprotective and antiurolithic. Hence, the aim of the present study was to evaluate qualitative phytochemical analysis and antiurolithiatic activity of an aqueous extract from the fruits of *M. charantia* collected from Sagar region of Madhya Pradesh. Qualitative analysis of various phytochemical constituents was determined by the well-known test protocol available in the literature. Ethylene glycol (0.75%) in drinking water was fed to all the groups (Groups II-V) except normal control (Group I) for 28 days to induce urolithiasis for curative and preventive regimen. Groups I, II, and III served as normal control, negative control (hyperurolithiatic), and standard (Allopurinol 120 mg/kg), Groups IV and V were treated with aqueous extracts of *M. charantia* fruits (100 mg/kg and 200 mg/kg), respectively. The urolithiasis-related biochemical parameters were evaluated in the rat's urine and serum. Phytochemical analysis revealed the presence of carbohydrates, alkaloids, saponins, and flavonoids. The treatment with aqueous extract (100, 200 mg/kg, p.o) of fruits of *M. charantia* significantly lowered ($P < 0.001$) the increased levels of serum creatinine, urine protein, and urine calcium. The treatment with aqueous extract fruits significantly ($P < 0.001$) increases levels of urine output, urine creatinine, and serum calcium. The results were comparable to that of negative control group. The presented data indicate that administration of *M. charantia* 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* could contribute to its antiurolithiatic property.

Keywords: Antiurolithiatic activity, biochemical parameters, ethylene glycol, *Momordica charantia* Linn, phytochemical analysis

INTRODUCTION

Urolithiasis commonly known as kidney stone or renal stone and has caused a major impact on public health in the past two decades. There are various types of calcareous (calcium oxalate monohydrate and calcium oxalate dihydrate and apatite) and non-calcareous (uric acid, struvite, cystine, uric acid, and others) stones, among which over 80% cases of calcium oxalate, where 5–10% cases of uric acid stone are found in population. As per the survey of National Health and Nutrition Examination, 7.1% of women and 10.6% of men were affected by renal stone disease.[1] In India, approximately 12% population is suffering with renal stone

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every year with the high incidence states such as Gujarat, Rajasthan, Punjab, Maharashtra, Delhi, and Haryana.[2] Renal stone formation is highly unpredictable with complex etiology. Various endogenous and exogenous factors and multivariate pathogenesis are involved in renal stone formation. Nitrogenous waste products like urea also contribute to renal stone formation. People's food habits, dehydration, hot climate, and hard water usages are involved in exogenous factors.[3] Supersaturation of urine with components likes calcium, oxalate, and phosphate which initiate renal stone formation which is followed by nucleation, crystal growth, and crystal aggregation process. Many stone inhibitors are available in urine such as magnesium and citrate which make the soluble complex with calcium ions and reduces the supersaturation level of CaOx ions, but its inhibition capacity varies person to person.[4] The treatment of renal stones depends on stone size and location. Many therapies such as diuretics, probiotics, citrate, and chelating agents are given but they have their own pharmacological limitations, side effects on long use and not removing stone. Hence, in majority cases, renal stones are removed by surgical treatment such as extra-corporeal shock wave lithotripsy, ureteroscopy, and percutaneous nephrolithotomy, but unfortunately stone recurrence rate was observed in about 50% cases after removal of stone by surgical treatment.[5] Surgical treatment causes side effects such as hypertension, tubular necrosis, hemorrhage, and fibrosis of the kidney.[6] Hence, in renal stone, the treatment needs preventive as well as curative therapy for better relief. However, there are not any proper effective drugs in current therapy which completely removes the stones. In Ayurveda, it has been mentioned that many herbal plants have been used in treatment of urolithiasis. Herbal plants have a complex spectrum of action, such as antioxidant, diuretic, antimicrobial, anti-inflammatory, analgesic, antispasmodic properties, litholytic, and anti-calcifying activities without any side effects.[7] *Momordica charantia* is a plant belonging to the *Cucurbitaceae* family and is widely distributed in tropical and subtropical areas around the world.[8,9] *M. charantia* a vegetable consumed in India and also considered to have traditional medicinal use, even in Ayurveda, the fruit is considered as tonic, stomachic, stimulant, emetic, antibilous, laxative, and alterative. *M. charantia* has been used in various Asian traditional medicine systems for a long time.[10] *M. charantia* contains a collection of biologically active plant chemicals including triterpens, proteins, steroids, alkaloids, saponins, flavonoids, and acids due to which plant possesses anti-fungal, anti-bacterial, anti-parasitic, anti-viral, anti-fertility, anti-tumorous, hypoglycemic, and anti-carcinogenic properties. Fruits are used as traditional medication to cure various diseases such as rheumatism, gout, worms, colic, illness of liver, and spleen. It is also found useful in the treatment of cancer and diabetes.[11] Hence, the study was designed with an objective to carry out *in vivo* anti-urolithiatic activity of aqueous extract of fruits of *M. charantia*.

MATERIALS AND METHODS

Plant Materials

Fresh fruits of *M. charantia* collected from the local area of Sagar, MP. Herbarium file of plant part was prepared and authenticated by Dr. Pradeep Tiwari (Professor), Department of Botany, Dr. HS Gour University Sagar, (M.P.) and the specimen voucher no. assigned was BOT/H/03/74/103. After that herbarium file was submitted in department. Plant material (fruits) selected for the study were washed thoroughly under running tap water and, then, were rinsed in distilled water; they were allowed to dry for some time at room temperature. Then, the plant material was shade dried without any contamination for about 3–4 weeks. Dried plant material was grinded using electronic grinder. Powdered plant material was observed for their colour, odour, taste, and texture. Dried plant material was packed in air tight container and stored for phytochemical and biological studies.

Chemical Reagents

Allopurinol was obtained from Himalaya Health Care Ltd. Ethylene glycol was obtained from Merck India Ltd. (Mumbai, India). Diagnostic kits for creatinine and protein were purchased from HiMedia Laboratories Pvt. Ltd. (Mumbai,

India). All other chemicals and reagents used were analytical grade and procured from approved chemical suppliers.

Extraction by Soxhletion Method

Using the soxhletion method, 1000 g of dried plant material were thoroughly extracted with water (1.5 L) at 60–70°C for 24 h. Over their boiling temperatures, the extract was evaporated. To determine the extractive yield, the dried crude concentrated extract was weighed. It was, then, transferred to glass vials (6 × 2 cm) and kept in a refrigerator (4°C) until it was needed for analysis.[12]

Phytochemical Screening of the Extract

Various phytoconstituents, including alkaloids, carbohydrates, glycosides, phytosterols, saponins, tannins, proteins, amino acids, and flavonoids were analyzed qualitatively in the *M. charantia* extract.[13,14]

Animals

Albino Wistar rats (either sex, 5–6 weeks, 200 ± 20 g) were selected randomly from animal house of Sagar Institute of Pharmaceutical Sciences, Sagar, India and, further, divided into various treatment groups randomly and kept in propylene cage with sterile husk as bedding. Relative humidity of 30.7% at 22 ± 2°C and 12:12 light and dark cycle was maintained in the animal house and fed with standard pellets (Golden Feeds, New Delhi, India) and water was available *ad libitum*. The Institutional Animal Ethics Committee of SIPS, Sagar has approved all animal experiments. The experimental ethics committee no. is SIPS/EC/2019/67.

Acute Oral Toxicity

Acute toxicity study of the prepared extracts of *M. charantia* was carried out according to the Organization for Economic Co-Operation and Development (OECD) Guidelines-423[15] that the animals were fasted for 4 h, but allowed free access to water throughout. As per the OECD recommendations, the starting dose level should be that which is most likely to produce mortality in some of the dosed animals, and when there is no information available on a substance to be tested in this regard; for animal welfare reasons, the dose level to be used as the starting dose is selected from one of three fixed levels 5, 300, and 2000 mg/kg body weight. Acute toxicity was determined as per reported method.[16]

Ethylene Glycol-Induced Urolithiasis Model in Albino Rats

Animals were divided in five groups containing six animals in each and kept in cages. All animals had free access to regular rat chow and drinking water *ad libitum* for 28 days. Renal calculi were induced in Group II to V by supplementing with 0.75%v/v ethylene glycol in drinking water *ad libitum*. Group IV to V were treated with plant extracts starting from 1st day to 28th day.[17]

Assessment of Antiurolithiatic Activity

Collection and analysis of urine

All animals were kept in individual metabolic cages and urine samples of 24 h were collected on 28th day. Animals had free access to drinking water during the urine collection period. After urine collection, urine volume and pH of urine were measured. A drop of concentrated hydrochloric acid was added to the urine before being stored at 4°C. Urine was analyzed for calcium, protein, and creatinine.

Serum analysis

After the experimental period, blood was collected by heart puncture under anesthetic condition. Serum was separated by centrifugation at 10,000 × *g* for 10 min and analyzed for creatinine and calcium.

Statistical Analysis

Data were calculated as Mean ± SD/SEM. One-way ANOVA was used to interpret the results followed by Dunnett test. $P < 0.001$ was considered as level of significance.

RESULTS AND DISCUSSION

The crude extracts so obtained after Soxhletion extraction process was concentrated on water bath by evaporation the solvents completely to obtain the actual yield of extraction. The yield of extracts obtained from the fruits of the plants was founds to be 4.85% w/w. Phytochemical analysis of aqueous extract of *M. charantia* fruits showed the presence of carbohydrates, alkaloids, saponins, and flavonoids, as shown in Table 1. The anti-urolithiatic activity of *M. charantia* (100 and 200 mg/kg) was assessed in this study by evaluating different biochemical parameters in ethylene glycol-induced urolithiasis in male rats. It is also evident from the previous studies that ethylene glycol-induced urolithiasis decreased urine volume. Stones with smaller size can easily travel through the urinary system followed by excretion, but large size stones may lead to obstruction in urine passage, so urine output decreases in urolithiasis. Since *M. charantia* increased urine output volume significantly compared to the negative control group (2.10 ± 0.22 mL/dL), this suggests that the *M. charantia* possesses significant antiurolithiatic activity and *M. charantia* 200 mg/kg (7.01 ± 0.32 mL/dL) shows significant activity than *M. charantia* 100 mg/kg (6.82 ± 0.20 mL/dL), as shown in Table 2. It was earlier evident that ethylene glycol induces urolithiasis increased serum creatinine level. Since *M. charantia* decreased the serum creatinine levels significantly compared to the urolithiatic group, this suggests that *M. charantia* possesses significant anti-urolithiatic activity compared with negative control group (2.78 ± 0.04 mg/dL). *M. charantia* 200 mg/kg (0.81 ± 0.02 mg/dL) shows significant activity than *M. charantia* 100 mg/kg (0.91 ± 0.13 mg/dL), as shown in Table 3. The previous study also demonstrated that in urolithiasis urine creatinine levels decrease as the glomerular filtration rate decrease due to obstruction of urine flow by the stones in the urinary system. This causes impairment of renal function resulting decreased excretion of waste products, such as creatinine. Since *M. charantia* increased the urine creatinine levels (2.95 ± 0.02 mg/dL) significantly compared to the ethylene glycol treated group (0.95 ± 0.04 mg/dL), it can be said that *M. charantia* possesses significant anti-urolithiatic activity and *M. charantia* 200 mg/kg (2.95 ±0.02 mg/dL) showed more significant activity than *M. charantia* 100 mg/kg (2.86 ± 0.13 mg/dL), as shown in Table 4. It was evident that ethylene glycol induced urolithiasis increases total protein content in urine. Due to hemodynamic stress, capillary integrity gets damaged, which predisposes to leakage of proteins into the urine cause and increased urine protein levels. *M. charantia* decreased the protein content (0.79 ± 0.005 mg/dL) in urine significantly compared to the ethylene glycol treated group (4.19 ± 0.018 mg/dL), this suggests

Table 1: Result of phytochemical screening of extracts of *M. charantia*

S. No.	Constituents	Aqueous extract
1	Alkaloids	
	Mayer's test	+Ve
	Dragendroff's test	+Ve
	Wagner's test	+Ve
	Hager's test	+Ve
2	Glycosides	
	Borntrager's test	-Ve
	Killer killiani's test	-Ve
	Leagal test	+Ve
3	Flavonoids	
	Lead acetate test:	+Ve
	Alkaline test:	+Ve
4	Phytosterol and triterpenoids	
	Lieberman test	-Ve
	Lieberman buchrard test	-Ve
	Shalkowaski test	-Ve
5	Phenol and tannin	
	Ferric chloride test	-Ve
	Lead acetate test	-Ve
6	Proteins and amino acid	
	Millon's test	-Ve
	Ninhydrin test	-Ve
7	Carbohydrate	
	Molisch's test	+Ve
	Fehling's test	+Ve
	Borfoed's test	-Ve
	Benedict's test	-Ve
8	Saponins	
	Foam test	+Ve

Momordica charantia: M. charantia

that the *M. charantia* 200 mg/kg possesses significant anti-urolithiatic activity, as shown in Table 5. Increased urine calcium levels were observed in animals developing urolithiasis. An increased urinary calcium concentration is a factor favoring nucleation and precipitation of calcium oxalate from urine and subsequent crystal growth. This fact, combined with the increased urinary calcium, leads to their super saturation in urine and finally stone formation. Since *M. charantia* decreased the urine calcium levels (5.71 ± 0.11 mg/dL) significantly compared to ethylene treated group (8.12 ± 0.09 mg/dL), this suggests that *M. charantia* possesses very significant anti-

Table 2: Effect of aqueous extract of *M. charantia* fruits on urine output in ethylene glycol-induced kidney stone in rat

S. No.	Groups	Estimation of urine output(ml/dl)					
		0 Days	7 Days	14 Days	21 Days	28 Days	35 Days
1	Control	7.32±0.25	7.41±0.26	7.38±0.28	7.42±0.13	7.33±0.28	7.34±0.37
2	Disease-induced	7.33±0.16	6.59±0.29	6.12±0.30	5.41±0.26	3.59±0.29	2.10±0.22
3	Allopurinol 120 mg/kg	7.41±0.26	7.05±0.41	5.55±0.24	6.26±0.11**	6.58±0.08***	7.23±0.15***
4	AEMC 100 mg/kg	7.37±0.32	7.01±0.39	5.48±0.33	6.24±0.21*	6.47±0.21**	6.82±0.20***
5	AEMC 200 mg/kg	7.34±0.15	6.58±0.21	5.53±0.16	6.28±0.14*	6.51±0.25***	7.01±0.32***

Value are expressed Mean±SEM (n=6) One-way analysis of variance (ANOVA) followed by multiple comparison Dunnett's test. *P<0.05, **P<0.01, and ***P<0.001, *Compare with negative control group, *Momordica charantia: M. charantia*

Table 3: Effect of aqueous extract of *M. charantia* fruits on serum creatinine in ethylene glycol-induced kidney stone in rat

S. No.	Groups	Estimation of serum creatinine (mg/dl)					
		0 Days	7 Days	14 Days	21 Days	28 Days	35 Days
1	Control	0.71±0.04	0.72±0.09	0.71±0.05	0.73±0.02	0.70±0.06	0.68±0.02
2	Disease-induced	0.72±0.06	1.62±0.01	1.88±0.09	2.04±0.05	2.45±0.05	2.78±0.04
3	Allopurinol 120 mg/kg	0.71±0.05	1.43±0.06	1.54±0.01	1.11±0.06*	0.92±0.06**	0.75±0.06***
4	AEMC 100 mg/kg	0.73±0.06	1.59±0.01	1.69±0.09	1.54±0.07*	1.11±0.09*	0.91±0.13**
5	AEMC 200 mg/kg	0.72±0.05	1.45±0.03	1.51±0.07	1.14±0.05*	1.08±0.03*	0.81±0.028***

Value are expressed Mean±SEM (n=6) One-way analysis of variance (ANOVA) followed by multiple comparison Dunnett's test. *P<0.05, **P<0.01, and ***P<0.001, *compare with negative control group, *Momordica charantia: M. charantia*

Table 4: Effect of aqueous extract of *M. charantia* fruits on urine creatinine in ethylene glycol-induced kidney stone in rat

S. No.	Groups	Estimation urinary creatinine (mg/dl)					
		0 Days	7 Days	14 Days	21 Days	28 Days	35 Days
1	Control	3.03±0.04	3.02±0.09	3.05±0.05	3.07±0.02	3.06±0.06	3.08±0.02
2	Disease-induced	3.05±0.06	2.82±0.01	2.54±0.09	1.84±0.05	1.21±0.05	0.95±0.04
3	Allopurinol 120 mg/kg	3.02±0.05	2.98±0.06	2.66±0.01	2.71±0.06*	2.86±0.07**	3.01±0.05***
4	AEMC 100 mg/kg	3.03±0.06	3.01±0.01	2.61±0.09	2.75±0.07*	2.82±0.09**	2.86±0.13***
5	AEMC 200 mg/kg	3.05±0.05	3.01±0.03	2.64±0.07	2.68±0.05*	2.87±0.03**	2.95±0.02***

Value are expressed Mean±SEM (n=6) One-way analysis of variance (ANOVA) followed by multiple comparison Dunnett's test. *P<0.05, **P<0.01, and ***P<0.001, *compare with negative control group, *Momordica charantia: M. charantia*

Table 5: Effect of aqueous extract of *M. charantia* fruits on urine protein in ethylene glycol-induced kidney stone in rat

S.No.	Groups	Estimation of urine protein (mg/dl)					
		0 Days	7 Days	14 Days	21 Days	28 Days	35 Days
1	Control	0.75±0.005	0.75±0.017	0.73±0.008	0.73±0.015	0.74±0.118	0.71±0.138
2	Disease-induced	0.74±0.009	1.11±0.014	1.47±0.024	2.19±0.008	3.20±0.17	4.19±0.018
3	Allopurinol 120 mg/kg	0.75±0.008	0.84±0.002	1.25±0.010	1.11±0.006**	0.91±0.004**	0.86±0.007***
4	AEMC 100 mg/kg	0.74±0.007	0.99±0.08	1.31±0.001	1.25±0.002*	1.09±0.004**	0.95±0.002**
5	AEMC 200 mg/kg	0.75±0.007	0.92±0.004	1.29±0.002	1.17±0.004**	1.01±0.004**	0.79±0.005***

Value are expressed Mean±SEM (n=6) One-way analysis of variance (ANOVA) followed by multiple comparison Dunnett's test. *P<0.05, **P<0.01, and ***P<0.001, *Compare with negative control group, *Momordica charantia: M. charantia*

Table 6: Effect of aqueous extract of *M. charantia* fruits on urine calcium in ethylene glycol-induced kidney stone in rat

S. No.	Groups	0 Days	7 Days	14 Days	21 Days	28 Days	35 Days
1	Control	5.53±0.21	5.53±0.16	5.49±0.25	5.54±0.12	5.57±0.12	5.58±0.18
2	Disease-induced	5.51±0.04	5.49±0.09	5.64±0.12	6.98±0.11	7.52±0.19	8.12±0.09
3	Allopurinol 120 mg/kg	5.46±0.21	5.71±0.11	6.24±0.17	5.97±0.16*	5.64±0.12**	5.68±0.18***
4	AEMC 100 mg/kg	5.48±0.16	5.59±0.21	6.13±0.13	6.01±0.14*	5.92±0.11**	5.84±0.09**
5	AEMC 200 mg/kg	5.42±0.09	5.52±0.05	6.21±0.11	5.84±0.16*	5.78±0.14**	5.71±0.11***

Value are expressed Mean±SEM (n=6) One-way analysis of variance (ANOVA) followed by multiple comparison Dunnett's test. *P<0.05, **P<0.01, and ***P<0.001, *Compare with negative control group, *Momordica charantia: M. charantia*

Table 7: Effect of aqueous extract of *M. charantia* fruits on serum calcium in ethylene glycol-induced kidney stone in rat

S. No.	Groups	Determination of serum calcium (mg/dl)					
		0 Days	7 Days	14 Days	21 Days	28 Days	35 Days
1	Control	5.53±0.03	5.53±0.09	5.49±0.07	5.54±0.11	5.57±0.09	5.42±0.12
2	Disease-induced	5.51±0.02	5.09±0.12	4.54±0.08	3.11±0.09	2.17±0.08	1.61±0.12
3	Allopurinol 120 mg/kg	5.46±0.11	5.41±0.16	4.18±0.08	4.87±0.05*	5.11±0.11**	5.31±0.17***
4	AEMC 100 mg/kg	5.48±0.16	5.39±0.07	4.07±0.05	4.64±0.09*	4.81±0.11**	5.01±0.06**
5	AEMC 200 mg/kg	5.42±0.08	5.32±0.06	4.15±0.03	4.75±0.12*	4.97±0.07**	5.22±0.05***

Value are expressed Mean±SEM (n=6) One-way analysis of variance (ANOVA) followed by multiple comparison Dunnett's test. *P<0.05, **P<0.01, and ***P<0.001, *Compare with negative control group, *Momordica charantia: M. charantia*

urolithiatic activity. From some earlier studies, it was evident that decreased urine calcium levels were observed in animals developing urolithiasis, as shown in Table 6. The previous studies showed that urolithiasis decreases serum calcium level. Since *M. charantia* increased serum calcium levels (5.22 ± 0.05 mg/dL) significantly compared to the negative control group (1.61 ± 0.12 mg/dL), this suggests that *M. charantia* possesses significant antiurolithiatic activity. Furthermore, from the previous studies, it was evident that urolithiasis increases serum potassium level. Since *M. charantia* decreased serum potassium levels significantly compared to the positive control group, this suggests that *M. charantia* possesses very good anti-urolithiatic activity, as shown in Table 7. Hence, it can be said that administration of *M. charantia*, in a dose of 100 and 200 mg/kg to urolithiatic rats significantly recovered the elevated levels of urine and serum parameters. It also recovered the decreased levels of urine and serum parameters compared to ethylene glycol treated group, this was nearly equally efficacious with the standard drug Allopurinol 120 mg/kg.

CONCLUSION

The presented data indicate that administration of the aqueous extract of *M. charantia* 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*.

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