Research Article

Inhibition of Calcium Oxalate Crystallization in Vitro by Extract of *Momordica Charantia Linn*

JN. Vyawahare*, PA. Shelke, PD. Aragade and DG. Baheti

Department of Pharmaceutics, Sitabai Thite College of Pharmacy, Shirur, Pune, Maharashtra, India.

ABSTRACT

Present study designed to evaluate the antilithiatic potential of *Momordica charantia Linn* under in vitro condition. In the present study the fruits as well as leaves of *Momordica charantia Linn* have been selected for their antiurolithiatic activity. The extract was subjected to phytochemical screening and found to contain alkaloid glycosides, reducing sugar, saponin, terpenoids, and steroids. The physical constant /physicochemical parameters were also studied. The given research work was studied under in vitro conditions using two critical assays such as crystal nucleation and aggregation Assay. For evaluation purpose extraction were carried out by using different solvent of different polarity. The inhibitory potential of the *Momordica charantia Linn* extract were tested for the COM crystal formation, which are predominantly present in most of the kidney stones, under in vitro conditions. The nucleation and aggregation of calcium oxalate crystals were measured separately using Spectro-photometric methods.

Keywords: Lithiasis, Kidney stones, *Momordica charantia Linn*, Calcium oxalate, Nucleation Assay.

INTRODUCTION

Urolithiasis (urinary calculi) is one among the three prevalent disorders in the urinary system. Approximately 80% of these calculi are composed of calcium oxalate and calcium phosphate, followed by cystine, struvite and ammonium acid urate stones. A kidney stone, also known as a renal calculus is a solid concretion or crystal aggregation formed in the kidneys from dietary minerals in the urine. Urolithiasis is a complex process that occurs from series of several physicochemical event including super-saturation, nucleation, growth, aggregation and retention within the kidneys. Calcium- containing stones, especially calcium oxalate monohydrate, calcium oxalatedihydrate and basic calcium phosphate are the most commonly occurring ones to an extent of75-90% followed by magnesium ammonium phosphate (Struvite) to an extent of 10- 15%, uric acid 3-10% and cystine 0.5-1%[1] Patients suffering from diseases like hyperparathyroid-ism, renal tubular acidosis, cystinuria, hypercalciuria, 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. It was also found that there is strong correlation of genetic defect with stone formation. Decreased in urine volume, increased amount of solute in urine, change in urinary pH and infec-tion may also lead to stone formation[4]

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[2]. The market for Ayurvedic medicine is estimated to be expanding at rate of 20% annually in India. The drugs of herbal origin have been used in traditional system of medicines such as Unani and Ayurveda since ancient time. The ayurvedic system of medicine uses about 700 species, Unani 700 species, Siddha 600 species, Amchi 600 species and modern medicine uses around 30 species[3].

Momordica charantia Linn. is a monoecious 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 [4].It is cultivated in warm season i.e during April to July by using 2-3 seeds in pit. The pits are prepared at a distance of half a meter and provided with manures. Only one plant is retained and seedlings are watered once or twice a week. Plants pegin to flower 30-35

days after sowing and the fruits are ready for harvesting 15-20 days after flowering. Bitter melon contains an array of biologically active plant chemicals including triterpenes, proteins, and steroids. In addition, a protein found in bitter melon, momordin, has clinically demonstrated anticancerus activity. Other proteins in the plant, alpha- and beta-momorcharin and cucurbitacin B, have been tested for possible anticancerous effects.The chemicals that lower blood sugar include a mixture of steroidal saponins known as charantins, insulin-like peptides, and alkaloids. The study was designed with an objective to carry out in vivo anti-urolithiatic activity of aqueous and alcoholic extract of fruits of Momordica charantia Linn.[5]

MATERIALS AND METHODS

Plant sample collection and preparation of extract

The fresh fruits as well as leaves of Momordica charantia Linn were collected from local areas of Shirur, Dist-pune, India. Taxonomical Identification of the plant was confirmed by Mr. Acharya sir, head of dept of botany, C T. Bora College, Shirur, Dist: Pune. The fresh Fruits and leaves were harvested, rinsed with tap water and air dried under shade for 14 days and reduced to coarse powder using pestle and mortar and then grinded to fine powder using the Kenwood electric blender. The powder was stored in an airtight bottle until needed for use. The aqueous extract of fruits was prepared using chloroform water, I.P., by maceration method for 7 days at room temperature and the alcoholic extract was prepared using 70% (v/v) alcohol by soxhlet method at a temperature of 60–70 °C. All the extracts were evaporated in a water bath at 60°C. The residue was stored in an airtight container and refrigerated, which was utilized for the *in vitro* assays.

Experimental Work

In vitro assays[3, 9, 11]

Formation of kidney stone involves three critical stages which include nucleation of calcium oxalate crystals, growth and aggregation. These three stages can be analyzed under in vitro conditions both in presence and absence of the *Momordica charantia Linn* extract. In order to determine the maximum efficacy of the extracts, a varying concentration of all the extracts ranging from 100µg/mlto 500µg/ml were utilized for these assays.

1) Nucleation assay

The stone formation begins with the occurrence of nuclei, therefore we chose the classical model for the study of oxalate crystallization described by Hennequin et al. (1993)[9] with some minor modifications. Solutions of calcium chloride and sodium oxalate were prepared separately at a final concentration of 3mM/L and 0.5mM/L respectively in a buffer containing Tris 0.5mM/L and NaCl 0.15mM/L of pH 6.5. Both the solutions were filtered thrice. For the assay, 950µl of calcium chloride and varying concentration of corm extracts (final volume of 100µl) were pipetted out against a reagent blank (without extract). To this added 950µl of sodium oxalate and shook well. The absorbance was measured at 620nm. The percentage inhibition produced by the herb extract was calculated as:

$$\% \text{ inhibition} = \frac{(\text{Absorbance of Control} - \text{Absorbance of Test})}{\text{Absorbance of Control}} \times 100$$

Where; Ab Test: Absorbance in the presence of inhibitor (Extract), Ab Control: Absorbance of graph without inhibitor (Control

2) Aggregation assay

The method used was similar to that described by Hess et al. with some modifications ‘Seed’ CaOx monohydrate (COM) crystals were prepared by mixing calcium chloride and sodium oxalate at 50 mmol/L, Both solutions were equilibrated to 600c in a water bath for 1 h and then cooled to 370c overnight. The Crystals were harvested by centrifugation and then evaporated at 370c.COM crystals were used at a final concentration of 0.8 mg/ml, buffered with Tris 0.05 mol/L and Nacl 0.15mol/L at pH 6.5 .then experiments were conducted at 370c in the presence or absence of plant extract after stopping the stirring. The rate of aggregation was estimated by comparing the slopes of turbidity in presence of extract with that obtain in control.

IR = (Turbidity of sample/ Turbidity of control) × 100

RESULTS AND DISCUSSION

Phytochemical evaluation of *Momordica charantia Linn.*

The plant extract were subjected to preliminary phytochemical screening for the detection of various plant constituents present. The active ingredients, after isolation, can be incorporated into the modern medicine system for the development of newer formulation for therapeutic ailments Preliminary qualitative phytocemical screening of ethanolic extract of leaves of euphorbia hirta linn showed the presence of alkaloid, glycoside, tannin, Saponin, flavanoids [table no.2].the physical constant /physicochemical parameters were also studied [table no.1]

Table 1: result of physicochemical parameters

S.No	Parameters	Fruits	Leaves
1	Loss on drying	4.37%	10.76%
2	Total Ash value	7.1%	7%
3	Water soluble ash value	2.33%	2.16 %
4	Acid insoluble ash value	1.66%	1.16%
5	Water soluble extractive value	9.26 %	9.3 %
6	Alcohol Soluble extractive value	7.04 %	7.06 %
7	Swelling index(ml)	1.3ml	1.1ml

Table 2: Preliminary Phytochemical Screening of the Entire plant Powder of *Momordica charantia linn.*

S. No	Test	Leaves (Alcohol Extract)	Leaves (Aqueous extract)	Fruit (Alcohol Extract)
1	Alkaloid	+	+	+
2	Glycoside	+	-	+
3	Saponine	+	+	+
4	Flavonoid	-	-	-
6	Fixed oil	+	+	+
7	Carbohydrate	+	+	+
8	Protein	+	+	+
9	Steroids	-	-	+

In Vitro Assays

Figure 1 displays the effect of the different concentration of the aqueous extract and Ethanol Extract of *Momordica charantia linn* leaves as well as Fruits on the nucleation of calcium oxalate crystals. The increase in the concentration of *Momordica charantia linn* extract showed increase in the inhibition of nucleation. Maximum inhibition of nucleation 88 % observed at concentration of 500 µg/ml. as we observe in the figure 1 the Ethanol Extract of fruits has good potential decrease the nucleation process, as compare to aqueous extract of fruits and leaves.

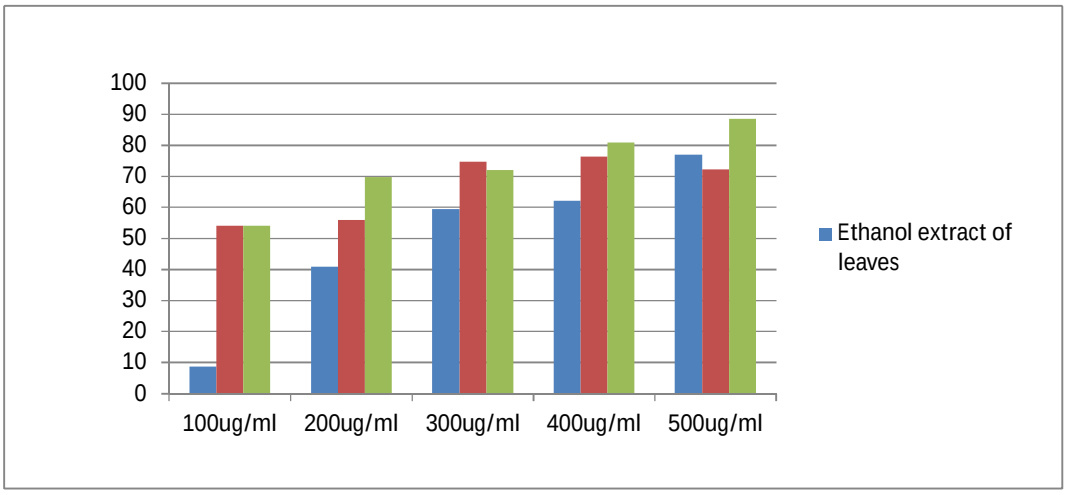

Fig. 1: Effect of Momordica charantia Linn. Extracts on nucleation of CaOx

The % inhibition of turbidity (aggregation) in the presence of herb extracts was lower than in the control, showing that crystals were less aggregated. The inhibited aggregation associated with the extract increased with concentration. This inhibition was found with alcoholic and aqueous extract. (Figure2). In this study also the Ethanol extract of fruits shows high inhibition of aggregation as compare to aqueous extract of leaves and fruits.

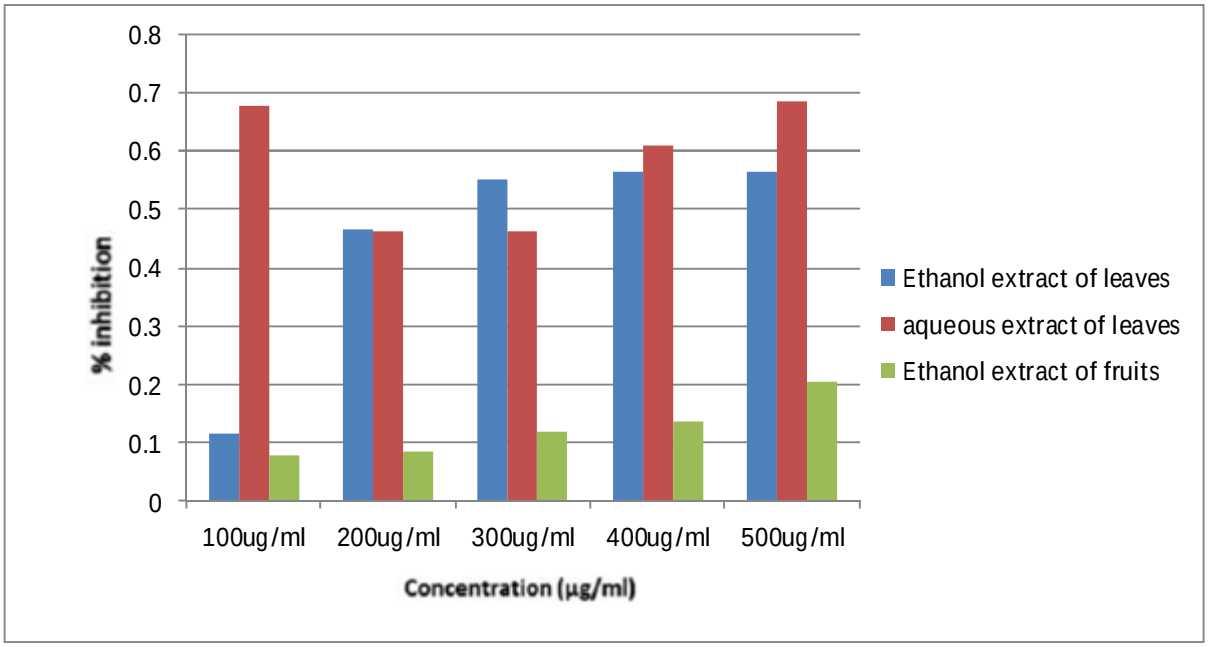

Fig. 2: Effect of *Momordica charantia linn* extracts on CaOx crystal aggregation

The main findings of the present study were that extracts from plants inhibited the nucleation and aggregation process of kidney stone formation. The herb extracts may contain substances that inhibit the growth of CaOX crystals. The extract may also contain substances that inhibit CaOX crystal aggregation; the agglomeration of particles is a critical step in urinary stone formation. If the extract keeps CaOX particles dispersed in solution they are more easily eliminated.

CONCLUSION

From the above results and study we can conclude that, *Momordica charantia linn.* Is having a significant antiurolithic activity. The results clearly indicate that under in vitro conditions, the crystal nucleation, and

aggregation, was found to express a concentration dependent inhibition. From phytochemical and in-vitro crystal inhibition study of plant *Momordica charantia linn.* It was found that the leaves of plant may contain chemical constituent like, Pentacyclic triterpenoid, Saponin glycoside, Cardic glycoside. The plant shows in-vitro crystal inhibition action. It might be because of presence of saponin. So this experiment will be helpful in future for scientific evaluation constituent which show for urolithasis activity in-vivo.

ACKNOWLEDGEMENTS

Special thanks to Dr.D.V.Derle Department of Pharmaceutics, MVP Samaj's College of Pharmacy, Nashik and Baheti D.G. from Sitabai Thite College of pharmacy, Shirur College, India.

REFERENCES

1. Atodariya U, Barad R, Upadhyay S and Upadhyay U. Anti-Urolithiatic Activity of Dolichos Biflorus Seeds. Journal of Pharmacognosy and Phytochemistry. 2013;2(2):209-213.
2. Patel RK, Patel SB and Shah JG. Anti-Urolithiatic Activity of Ethanolic Extract of Seeds of Benincasa Hispid (Thumb). Pharmacologyonline. 2011; 3:586-591.
3. Kalpana S, Nirmaladevi R, Shrinidhi Rai T and Karthika P. Inhibition Of Calcium Oxalate Crystallization In Vitro By Extract Of Banana Cultivar Monthan. International Journal of Pharmacy and Pharmaceutical Sciences. 2014;5(4):
4. Shah BN, Raiyani KD and Modi DC. Antiurolithiatic Activity Studies of Momordica charantia Linn. Fruits. International Journal of Pharmacy Research and Technology. 2011:1(1):
5. Kokate CK, Purohit AP and Gokhale SB. Pharmacognosy, Nirali Prakashan, fourty third edition: 8.58-8.59.
6. Rangari VD. A Pharmacognosy and Phytochemistry. Part I second editionCareer publication, Page no. 105(TLC) 366.
7. Mohammad Ali. A book of Pharmacognosy and Phytochemistry. Volum1, CBS publishers. 445-446
8. Khandelwal KR. Practical Pharmacgnosy. 12th ed., Nirali Prakashan, Pune.P.N. 149-156 (2004).
9. Pulok Mukherjee. quality control herbal drugs and approach to evaluation of botanical, published by business horizwns, first edition 2002;P.N 214-217.
10. Atmani F and Khan SR. Effect of an extract from herniaria hirsuta on calcium oxalate crystallization in vitro, BJU International. 2000;85:621-625.
11. Ashok Kumar Bagepalli Srinivasa, Kuruba L, Khan S and Saran GS. Antiurolithiatic Activity of Gokhsuradi Churan, an Ayurvedic Formulation by In Vitro Method. Advanced Pharmaceutical Bulletin. 2013;3(2):477-479
12. Patel MA, Patel PK and Seth AK. Effect of Seed Extracts Of Elettaria Cardamomum On Calcium Oxalate Crystallization. International Journal of Pharmacy Research and Technology. 2011;1(1):21-25.
13. Sekkoum K, Cheriti A, Taleb S, Belboukhari N and Djellouli HM. Inhibition Effect of Some Algerian Sahara Medicinal Plants on Calcium Oxalate Crystallization. Asian Journal of Chemistry. 2010;22:2891-2897.
14. Chandaka Madhu, Harish L, Narsimha Reddy K, Sagar S, Soumya D and Pavan Kumar G. Antiurolithic Activity of Aqueous Extract on Roots and Seeds of Plectranthus tomentora on Ethylene Glycol Induced kidney Stones in Male Albino Rats. Asian J Res Pharm Sci. 2012;2(4):129-133.