In vitro evaluation of Rotula aquatica Lour. for antiurolithiatic activity

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1. Introduction

Urolithiasis, formation of kidney stone presence of one or more calculi in any location within the urinary tract, is one of the oldest and widespread diseases known to man. It is a serious, debilitating problem in all societies throughout the world, affecting approximately 12% of the population and men are three times more prone than women. It is more prevalent between the ages of 20 and 40 in both sexes. Etiology is multifactorial and is strongly related to dietary lifestyle habits or practices. Increased rates of hypertension and obesity, also contribute to an increase in stone formation.

The most common (about 80%) renal stones are calculi of calcium oxalate (CaOx) crystals. CaOx crystals, primary constituent of human renal stones, exist in the form of CaOx Monohydrate (COM) and CaOx Dihydrate (COD). Calcium-containing stones, especially COM (Whewellite), COD (Weddellite) and basic calcium phosphate (Apatite) occurs to an extent of 75–90% followed by magnesium ammonium phosphate (Struvite) to an extent of 10–15%, uric acid 3–10% and cystine 0.5–1%. The stone formation requires

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http://dx.doi.org/10.1016/j.jopr.2013.02.026
supersaturated urine which depends on urinary pH, ionic strength, solute concentration and complexities. Various substances in the body have an effect on one or more of the above processes, thereby influencing a person’s ability to promote or prevent stone formation. Management of stone disease depends on the size and location of the stones. Stones larger than 5 mm or stones that fail to pass through should be treated by some interventional procedures such as extracorporeal shock wave lithotripsy (ESWL), ureteroscopy (URS), or percutaneous nephrolithotomy (PNL). Unfortunately, the propensity for stone recurrence is not altered by removal of stones with ESWL and stone recurrence is still about 50%. In addition, ESWL might show some significant side effects such as renal damage, ESWL induced hypertension or renal impairment. Although there are a few recent reports of beneficial effects of medical treatments in enhancing clearance of stones in the distal ureters, de facto there is still no satisfactory drug to use in clinical therapy, especially for the prevention of the recurrence of stones. Many remedies have been employed during the ages to treat urinary stones. In the traditional systems of medicine, most of the remedies found to be effective were having medicinal plants.

In the present manuscript, experimental evidences regarding antiurolithiatic activity of Rotula aquatica belongs to the family Boraginaceae, known as pashanbed in Ayurveda. It is commonly called as ceppunerinji, is a well known medicinal plant in ayurvedic system of medicines. It is represented by about 100 genera and 2000 species. It is a small branched shrub, 60–180 cm in height with numerous short lateral arrested branches often rooting. The plant is scattered throughout peninsular and Western Ghats of India in the sandy and rocky beds of streams and rivers. The plant is reported to contain baunerol, steroid and alkaloid. In Ayurveda, R. aquatica has been reported to be used as diabeties, antilithic effect, cardiotonic activity, and is used in stone bladder. The plant contains baunerol, steroid, alkaloids which showed antimitotic effect. Allantoin found in root which is responsible for diuretic activity. The aqueous extract of the root of R. aquatica showed antioxidant activity. It also contains sterol, rhabdol which is found to be active to induce diuresis. In light of the above study, R. aquatica has been selected for antiurolithiatic activity.

2. Materials and methods

2.1. Plant material and extraction

The fresh plant parts of R. aquatica Lour. were collected from Kuttiyadi (Malapuram District) in Kerala state. The Herbarium of Botanical Survey of India, Southern Circle, Coimbatore, Tamil Nadu and were authenticated as R. aquatica Lour. The dried samples were grounded to coarse powder. The drug was first defatted with petroleum ether (60–80 °C) and then chloroform, methanol and aqueous extract was prepared using Soxhlet apparatus. The different solvent was evaporated using a rotary vacuum-evaporator (Yamato RE300, Japan) at 50 °C and the remaining water was removed by lyophilization (VirTis Benchtop K, USA). The dried extracts were stored in airtight container and kept in a refrigerator.

2.2. Preliminary phytochemical screening

For preliminary phytochemical screening, the extracts was tested for the presence of alkaloids, flavonoids, phenols saponis, steroids, terpenoids, anthraquinones, proteins and aminocids following the standard procedures.

2.3. Experimental protocol

The effect of extracts on CaOx crystallization was determined by the time course measurement of turbidity changes due to the crystal nucleation and aggregation. The precipitation of calcium oxalate at 37 °C and pH 6.8 has been studied by the measurement of turbidity at 620 nm. A spectrophotometer UV/Vis (Shimadzu) was employed to measure the turbidity of the formation of calcium oxalate.

2.4. Nucleation assay

We chose the classical model for the study of oxalate crystallization because of its simplicity and satisfactory reproducibility. This model includes the study of crystallization without inhibitor and with it, in order to assess the inhibiting capacity of any chemical species used. Solution of calcium chloride and sodium oxalate were prepared at the final concentrations of 5 mmol/L and 7.5 mmol/L respectively in a buffer containing Tris 0.05 mol/L and NaCl 0.15 mol/L at pH 6.5. 950 μL of calcium chloride solution mixed with 100 μL of herb extracts at the different concentrations (100 μg/ml–1000 μg/ml). Crystallization was started by adding 950 μL of sodium oxalate solution. The temperature was maintained at 37 °C. The OD of the solution was monitored at 620 nm. The rate of nucleation was estimated by comparing the induction time in the presence of the extract with that of control.

The growth of crystals was expected due to the following reaction:

\[ \text{CaCl}_2 + \text{Na}_2\text{C}_2\text{O}_4 \rightarrow \text{CaC}_2\text{O}_4 + 2\text{NaCl} \]

2.5. Aggregation assay

The method used was similar to that described by Atmani and Khan with some minor modifications. ‘Seed’ CaOx monohydrate (COM) crystals were prepared by mixing calcium chloride and sodium oxalate at 50 mmol/L. Both solutions were equilibrated to 60 °C in a water bath for 1 h and then cooled to 37 °C overnight. The crystals were harvested by centrifugation and then evaporated at 37 °C. CaOx crystals were used at a final concentration of 0.8 mg/mL, buffered with Tris 0.05 mol/L and NaCl 0.15 mol/L at pH 6.5. Experiments were conducted at 37 °C in the absence or presence of the plant extract after stopping the stirring. The percentage aggregation inhibition rate (Ir) was then calculated by comparing the turbidity in the presence of the extract with that obtained in the control using following formula:

\[ \text{Ir} = \left(1 - \frac{\text{Turbidity}_{\text{sample}}}{\text{Turbidity}_{\text{control}}}\right) \times 100 \]
3. Results

Fig. 1 showed CaOx crystallization without the addition of extract (control) while Fig. 2 showed CaOx crystallization in the presence of extract in the concentration of 100, 200, 300, 400 and 500 µg/ml respectively. 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 greatest with aqueous extract of root when compared to petroleum ether, chloroform and methanol extracts of leaf and stem (Figs. 3–8).

4. Discussion

Kidney stone function is a complex process that results from a succession of several physico-chemical events including
supersaturation, nucleation, growth, aggregation and retention within renal tubules.31 Thus if supersaturation or later steps in crystallization can be prevented, then lithiasis should be avoided. Indeed, several measures are usually taken to reduce supersaturation, e.g. increasing fluid intake and medical therapy. In India, as in many less developed areas, phytotherapy is a common method of primary health care because pharmaceutical products are expensive and the ‘folk’ pharmacopoeia provides apparently effective remedies for many diseases. These results could be considered positives because the herb extracts inhibits crystallization and prevents stone formation.

The main findings of the present study were that extracts from plants inhibited the crystallization of CaOx in solution, there were less and smaller particles with increasing concentrations of extract as shown in various microphotographs i.e. Figs. 1 and 2. Fig. 1 showed maximum number and largest size of crystals as it was without plant extracts while Fig. 2 showed comparatively less number and smaller size of crystals. The increasing concentration of plant extracts (100, 200, 300, 400 and 500 µg/ml) had inhibited the CaOx crystal growth (Fig. 2). These results were also supported by the Figs. 3–8. The extract of plant causes fewer numbers of crystals in solution, thereby reduced supersaturation and the size of the particles. This property of the extract is therefore, advantageous in preventing urinary stone formation by inducing the excretion of small particles from the kidney and reducing the chance of retention in urinary tract. Further R. aquatica root also claimed to have diuretic effect24 and diuretic effects may also reduce stone development when total fluid intake and output increased, and such effects have been attributed to several herbal preparations.

Herbal extracts may contain substances that inhibit the growth of CaOx crystals. This property of plants may be
important in preventing kidney stone formation; CaOx crystals induced by urinary macromolecules was less tightly bound to epithelial cell surfaces, which are then excreted with urine. The extract may also contain substances that inhibit CaOx crystal aggregation; the agglomeration of particles is a critical step in urinary stone formation, as larger crystals are less likely to pass spontaneously in the urinary tract. If the extract keeps CaOx particles dispersed in solution they are more easily eliminated.

5. Conclusions

The aqueous extract of *R. aquatica* root have inhibitory effect on CaOx crystallization thus may be beneficial in the treatment of urolithiasis but there is a need of detailed investigation in elaborated preclinical experimentations and clinical trials to establish the use of plant as anti-urolithiatic agent.

Conflicts of interest

All authors have none to declare.

Acknowledgments

The authors are very grateful to the University Grants Commission New Delhi (UGC letter No: F.No.39-434/2010 (SR)) for financial support of this major research project work.

References