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Original Research Article

EVALUATION OF IN-VITRO ANTI-UROLITHIATIC ACTIVITY OF *PORTULACA OLERACEA L.*

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ABSTRACT

The inhibition of in-vitro calcium-oxalate crystal formation by *Portulaca oleracea L.* extract was investigated by different methods i.e nucleation assay and synthetic urine assay. In nucleation assay, the aim was to evaluate the effectiveness of the extract on calcium oxalate crystallization in vitro while in synthetic urine method the percentage inhibition and growth of the COM crystals of oxalate from synthetic urine at different concentrations of extract was investigated. In nucleation assay % inhibition for CaOx crystal formation was found directly proportional to the increase in concentration of the plant extract with maximum inhibition of 71.07%, while in synthetic urine assay maximum inhibition was 79.31 %. Thus *Portulaca oleracea L.* was found to be a potent anti-urolithiatic agent.

Key words: Urolithiasis; calcium oxalate (CaOx); calcium oxalate monohydrate (COM); *Portulaca oleracea L.* (PO); in-vitro)

INTRODUCTION

According to the World Health Organization, approximately 75% of the global population, of the developing world, depends on botanical medicines for their basic healthcare needs.[1] Urolithiasis is derived from the Greek words 'ouron' (urine) and 'lithos' (stone). It is an ancient and common affliction whose clinical occurrence and presentation is described in Aphorisms of Hippocrates. Found in the tombs of Egyptian mummies dating back to 4000 BC and in the graves of North American Indians from 1500-1000BC. Sanskrit documents in India between 3000 and 2000 BC have reference of stone formation.[2] It is considered as the third most common affliction of the urinary tract.[1] The deposition or formation of stones in any part of the urinary system i.e the kidney, the ureters or the urinary bladder is called Urolithiasis. A stone is an aggregation of solute materials from urine into a solid form. Its constituents could be calcium, oxalate, phosphate or uric acid**.** Life time risk of urolithiasis varies from 1-5% in Asia, 5-9% in Europe, 10-15% in USA and 20-25% in middle-east. In India, calcium oxalate remains the most predominant constituent of urolithiasis. Stone formation is the culmination of a series of physiochemical events i.e. supersaturation and nucleation, growth of the crystal and aggregation that occurs as the glomerular filtrate traverses through the tubules of nephron. Urine remains supersaturated with most stone forming salt components[3] as well as chemicals that prevent or inhibit the crystals from urinary tract. These crystals remain tiny enough; they will travel through the urinary tract and pass out of the body in the urine without being noticed. [4] However, the presence of certain molecules raise the level of supersaturation of salts needed to initiate crystal nucleation or reduce the rate of crystal growth or aggregation and prevents stone formation. [3] Calcium oxalate stones represent upto 80% of analyzed stones.[5] Calcium phosphate account for 15-25%, while 10-15% is mixed stones. The others are struvite 15-30%, cystine 6-10%, and uric acid stones 2-10%.[4] Calcium oxalate stones are of primary two types, calcium oxalate monohydrate (COM), commonly known as whewellite and

calcium oxalate dihydrate, commonly known as weddellite. The occurrence frequency of whewellite is 78% while that of weddellite is 43%.[6] Urolithiasis has plagued human kind since antiquity but until about 40 years ago the progress in the pathophysiological understanding and management of urolithiasis was slow. Though technological advancements have made dramatic improvement, still some of the drawbacks of the methods exists which includes their being too costly and recurrence of stone formation along with number of other side effects.[7] Hence, search for new antilithiatic drugs from natural sources has assumed greater importance as herbal drugs are cost effective as well as confer least side effects. Ayurvedic prescriptions also suggest many phytomedicines for dissolving the stones, which are referred as pashanbheda.

Portulaca oleracea L. commonly known as purslane is a fast growing succulent weed belonging to the family Portulacaceae, cultivated in many areas of the world, used as vegetable and for medicinal purposes since ancient times. Archaeological data indicates purslane seeds dating back to the first millennium B.C. having been discovered at an archaeological site at Salts Cave, Kentucky. Known since the time of Hippocrates, purslane was used by Theophrastus and Dioscorides for its diuretic, anthelmintic, cathartic properties. Ancient Egyptians used purslane for heart failure and heart diseases. Lot of research work has been done on this plant for evaluating its pharmacological activities and phytochemical constituents and recently, a review has been published on the same. [8] Literature search has revealed its use in treatment of various ailments related to the kidney as well as urinary system. It is prescribed in the treatment of dysuria, haematuria, diuretic [9] and in urinary disorders[10] urinary tract infections, retention and cystitis.[11] Its leaf and shoot is used as vegetable for nephrolithiasis.[12;13] Various researches done on purslane for evaluating its use in urinary diseases include studies were, aqueous extract was found effective in dissolution of tri-calcium phosphate[14] as well as protective against cisplatin[15] and gentamicin[16] induced renal toxicity in rats while ethanolic extract showed protection against cisplatin [15] and

ethylene glycol and ammonium chloride induced urolithiasis in albino rats.[17] The aim of the present study is to evaluate the effectiveness of alcoholic extract of *Portulaca oleracea L.* for its anti-urolithiatic activity using two in-vitro methods; nucleation assay and synthetic urine assay. In nucleation assay the aim was to evaluate the effectiveness of the extract on calcium oxalate crystallization in vitro while in synthetic urine method the percentage inhibition and growth of the COM crystals of oxalate from synthetic urine at different concentrations of extract was the object of this investigation.

MATERIALS AND METHODS

Chemicals:

All chemicals used were of high purity grade and were purchased from Sumeet enterprises Pvt. Ltd, Bhopal. Ethanol was of GR Merck grade. Calcium chloride and sodium oxalate were obtained from Burgoyne reagents, while sodium chloride and calcium chloride dihydrate were procured from Sigma Aldrich.

Plant collection and Identification:

The whole plant of *Portulaca oleracea L.* was collected from Vitthal market, Bhopal, Madhya Pradesh, during the month of April 2012 and plant was identified with the help of regional Floras [18] and taxonomists and finally confirmed with the herbarium of Botanical Survey of India (BSI), Allahabad, voucher specimen No.1114-23.01-52.

Extraction:

Fresh plant, after collection was shade dried at room temperature then grinded and then 100 gm plant material was extracted by Soxhlet apparatus for 48 hours. Then the extract was concentrated in vacuo to dryness at 30-40°c temperature, obtaining 24% w/w of dried extract. The dried extract was stored in refrigerator until used for further analysis.

Experimental Work:

Nucleation assay

The classical model for the study of oxalate crystallization was chosen because of its simplicity and satisfactory reproducibility.[19] This model includes the study of crystallization without inhibitor and with it, in order to assess the inhibiting capacity of the plant extract used. Solution of calcium chloride and sodium oxalate were prepared at the final concentrations of 5mmol/L and 7.5mmol/L respectively in a buffer

containing Tris 0.05mol/L and NaCl0.15 mol/L at pH 6.5. 950 mL of calcium chloride solution mixed with 100 mL of herb extracts at different concentrations. Crystallization was started by adding 950 mL of sodium oxalate solution. The temperature was maintained at 37 °C. The OD of the solution was monitored at 620 nm using spectrophotometer (Systronics digital spectrophotometer 166) after 30 minutes. The rate of nucleation was estimated by comparing the induction time in the presence of the extract with that of control. Data was represented in percentage inhibition.

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

CaCl2 + Na2C2O4→ CaC2O4 + 2NaCl

Synthetic urine assay

The classical model for the study of oxalate crystallization was chosen because of its simplicity and satisfactory reproducibility.[20] This model includes the study of crystallization without inhibitor and with it, in order to assess the inhibiting capacity of the plant extract used. Two solutions of following composition were mixed: A: $Na_2C_2O_4$ (2 m mol/1) and B: Ca Cl_2 $2H_2O$ (10 mmol/1). The two solutions were prepared alongwith adding NaCl 9 g to obtain the ionic force like the indoor environments. Synthetic urine is prepared by mixing and stirring two equal volumes of 50 ml of solutions A and B at constant temperature (37°C) in capped vessels to give final synthetic urine prepared artificially. Mixture agitation was maintained to prevent sedimentation.

Simulation of the sedimentary crystal formation

The crystal size development was monitored in sample drops every five minutes by polarized microscope. A drop of sample was put on hemacytometer counting chamber and observed sample under microscope at time after 30 minutes. Then calculated the number of crystals and catches of sight with a camera. A series of experiments corresponding to the physiological concentrations of plant extract was used. The follow-up of the crystal size development by microscope was carried out at time after 30 minutes of formation of crystals and catches of sight with a camera. The percentage of inhibition (I %) was

calculated from the difference of the number of CaOx crystals without inhibitor, from that with it.

RESULTS

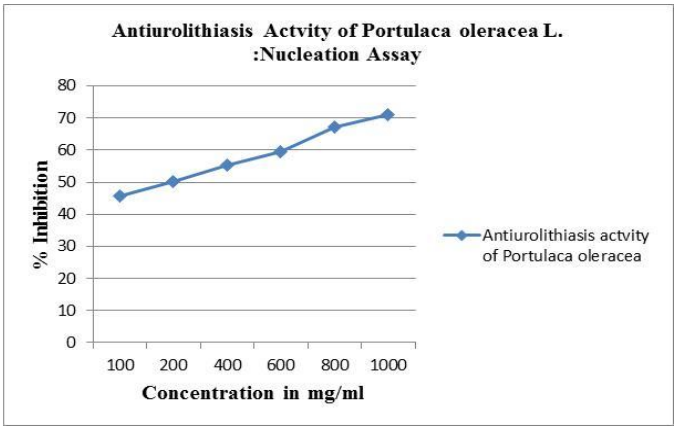

Fig.1.The effect of different concentrations of *Portulaca oleracea L.*(PO) extract on calcium oxalate crystal inhibition by nucleation assay

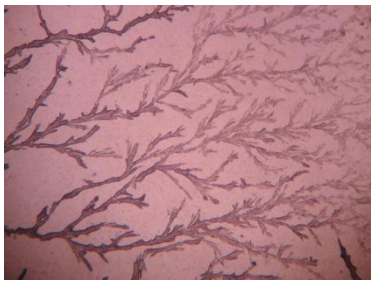

Fig.2.COM crystal development in control

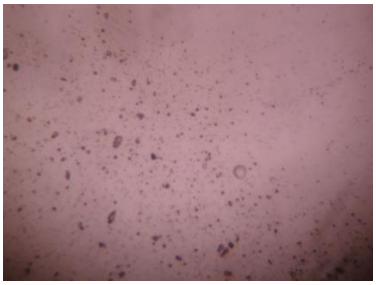

Fig.3. COM crystal development in 25% concentration of *Portulaca oleracea L.*(PO) extract, in synthetic urine assay

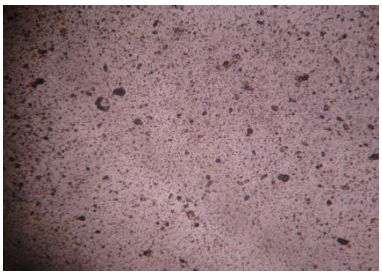

Fig.4. COM crystal development in 100% concentration of *Portulaca oleracea L.*(PO) extract, in synthetic urine assay

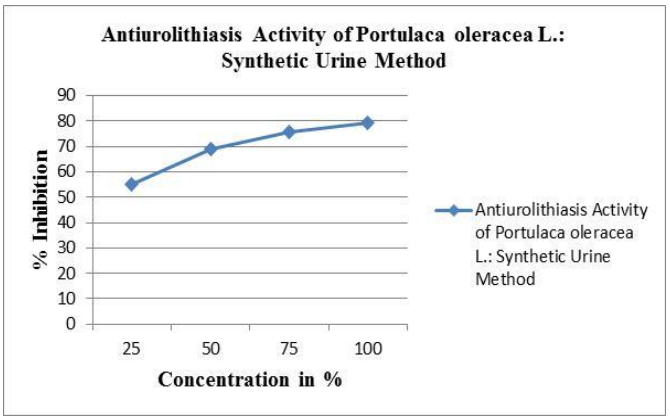

Fig.5. The effect of different percentages of *Portulaca oleracea L.*(PO) extract on the number of COM crystals formed (COM/ mm3) in synthetic urine assay

***Effect on nucleation assay*:**

Incubating the metastable solutions of calcium chloride and sodium oxalate resulted in the formation of CaOx crystals. The rate of nucleation was estimated by comparing the induction time in the presence of the extract with that of control. The O.D. was monitored at 620nm after 30 minutes. The turbidity of solution in the presence of herb extract was lower than in the control, showing that oxalate crystallization was less in the presence of extract with minimum inhibition of 45.67 % at 100 mg/ml while a maximum inhibition of 71.07% at 1000mg/ml extract

concentration. (Figure1). Data represents that % inhibition for CaOx crystal formation was directly proportional to the increase in concentration of the plant extract.

***Effect on synthetic urine assay*:**

The formation and growth of the COM crystals from synthetic urine at different extract concentration was studied. Stone formation is the result of supersaturation of urine with certain urinary salts such as calcium oxalate. Since crystallizable oxalate species are pH independent[20] the crystallization of oxalate in the absence of inhibitor, led to the formation of COM crystals monitored by polarized light microscopy. The number of COM crystals in control was found to be maximum (Figure.2). In order to assess the inhibiting potential of plant extract for oxalate crystallization, different percentages of plant extract were tested. In the presence of different percentages of plant extract, the length and the width of the crystals were reduced. The average length of the crystals grown in the presence of the inhibitors was less than that of the control sample. It was found that the plant used in this study inhibited potently the crystal development with maximum number of crystals (162.5/mm^3) at 25% (Figure 3.) extract concentration while minimum number of crystals (75/mm^3) were formed at 100% (Figure 4.) concentration. Results showed that the decrease in number of crystal as well as % inhibition of the formation of COM crystals was directly proportional to the increase in percentage of plant extract, with minimum inhibition 55.17 % at 25% extract while maximum inhibition of 79.31 % at 100 % extract concentration.(Figure 5.) The supersaturation of urine with CaOx, is an important factor in crystallization, with later factors being nucleation, growth and aggregation. Thus if supersaturation or initial stages in crystallization can be prevented, then lithiasis could be avoided. Indeed, several measures are usually taken to reduce supersaturation, e.g. increasing fluid intake and medical therapy.

DISCUSSION

In the present work the inhibition of in-vitro calcium-oxalate crystal formation by *Portulaca oleracea L.* alcoholic extract was studied by different methods. Interestingly in both the assays the calcium oxalate crystallization in vitro and the percentage

inhibition and growth of the COM crystals of oxalate was found directly proportional to the increase in concentration or percentage of the plant extract. The extract may contain phytochemicals that cause inhibition. This property of plant may be important in preventing stone formation. These in vitro results should be confirmed in vivo; while the preliminary studies showed that the extract of *Portulaca oleracea L.* has anti-lithogenic effects. The mechanism by which it exerts its effects remains unknown as well as chemicals responsible could be studied in future. To the best of our knowledge and in accordance with the literature survey, this is the first report on potent antiurolithiatic activity in methanolic extract of *Portulaca oleracea L.*

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