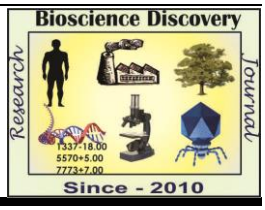
Bioscience Discovery, 8(2):153-157, April - 2017

Print & Online, Open Access, Research Journal Available on http://jbsd.in
ISSN: 2229-3469 (Print); ISSN: 2231-024X (Online)
Research Article



In vitro study of aqueous leaf extract of *Raphanus Sativus var.* for inhibition of calcium oxalate crystallization

Syed Salman Akhtar, S.M. Mular, Nazia D. Khan*, Z.H Khan, Syed Sohail

Department of Biochemistry, Shri Shivaji College of Arts, Commerce, & Science Akola
onlynazia247@gmail.com

Article Info

Received: 10-02-2017,
Revised: 16-03-2017,
Accepted: 25-03-2017

Keywords:
Raphanus Sativus, Calcium oxalate crystallization, Nucleation,Aggregation Cystone**.**

Abstract

The leaves of *Raphanus Sativus* var. Longipinnatus are traditionally used for treatment of kidney diseases and urinary stones. The present work investigated the effect of aqueous extract of leaves of *R.Sativus* (RSAE) on *in-vitro* crystallization of CaOx crystals. Crystallization was studied by using nucleation and aggregation assay of calcium oxalate (CaOx) crystals and growth assay of calcium oxalate monohydrate .The effects of RSAE and cystone on slope of nucleation and aggregation as well as growth of calcium oxalate crystallization was evaluated spectrophotometrically. The densities of the formed crystals were compared under microscope. RSAE significantly inhibited the slope of nucleation and aggregation of CaOx crystallization,and decreased the crystal density. It also inhibited the growth and caused the dissolution of Calcium oxalate crystals. The standard drug cystone also exhibited similar effects. The study reveals that the leaves of *Raphanus Sativus* were found effective in the prevention of theexperimentally induced urinary stones and substantiate the traditional claim. It is concluded that the leaves of *R.sativus* have beneficial inhibitory effect on *in-vitro* crystallization of CaOx crystals.

INTRODUCTION

Urolithiasis is defined as the presence of one or more calculiin any location within the urinary tract. It is a common disorder estimated to occur in approximately 12% of the population, with a recurrence rate of 70–80% in male and47–60% in female. Majority of the stones are calcium containing stones, especially calcium oxalate (80%) and others are 20% (Thomas and Hall, 2005).

The medical management of urolithiasis involves drug treatment and extracorporeal shock wave lithotripsy (ESWL). The various therapies including thiazide as diuretic and alkali citrate are used to prevent the recurrence of hypercalciuria and hyperoxaluria, which induce calculi formation, but evidencefor their efficacy is less (Smith *et al*., 1992). The surgical endoscopic stoneremoval and extracorporeal shock wave lithotripsy have revolutionizedthe treatment of urolithiasis but does not preventthe likelihood of new stone formation. Besides imposing the high cost, shock waves in therapeutic doses may cause acute renal injury, decrease in renal function and an increasein stone recurrence. In addition, persistent residual stone fragments and the possibility of infection after ESWL represent a serious problem in the treatment of stones.Thus, medical management of urolithiasis is either costly or poses serious side effects (Moe, 2006) .

The crystallization of the stone begins with increased urinary super saturation, with the subsequent formation of the solid crystalline particles within the urinary tract. This is followedby nucleation, by which stone-forming salts in

supersaturated urinary solution coalesce into clusters that then increasein size by the addition of new constituents. These crystalsthen grow and aggregate with other crystals in solution, andare ultimately retained and accumulated in the kidney. Therefore, if this progression of crystallization can be prevented, then lithiasis can also be prevented.There is growing interest of public in herbal medicine, particularly in the treatment of urolithiasis partly because of limited choice in the pharmacotherapy (Pak, 1989). Data from *in-vitro*, *in-vivo* and clinical trials reveal that phytotherapeutic agents could be useful as either an alternative or an adjunctivetherapy in the management of urolithiasis. Many Indian plants are useful as antilithiatic agents. Hence, the Indianmedicinal plants are constantly being evaluated for possible antilithiatic effects (Basavaraj *et al*., 2007).

The radish or muli is a root vegetable belongs to the Brassicaceae family, found and consumed all over the world. It is found in many varieties, sizes, shapes (round, long, oval) and colors (white, purple, red, pink, gray-black, green, yellow and etc) according to the season and duration of cultivation.

The medicinal property of this plant is mainly present in leaves and root. The leaves of *R, sativus* are used in ethno-medicinal practices for treatment of kidney diseases and urinary stones. Ethno botanical studies of Vidharba region of Maharashtra (India) report the folk medicinal uses of leaves of *R, sativus* in kidney stones and urinary tract troubles. Leaves are also used traditionally for controlling painful urination (Karadi *et al.*, 2006). *R.sativus* is an important medicinal plant of Marathwada useful in the treatment of urinary retention and kidney diseases.

In view of traditional and ethno-medicinal use of leaves of *R,sativus* in the treatment of kidney stones, the present work demonstrated the effect of aqueous extract of the leaves of *R,sativus* on *in-vitro* crystallization of CaOx crystals. As this plant is consumed as food substance by human beings and as weed fodder by cattle, its antilithiatic property would be good preventive option available (Kok *et al*., 1990). Therefore the present study was carried out to investigate *in vitro* potential of aqueous leaf extract of *Raphanus Sativus var*. for inhibition of calcium oxalate crystallization.

MATERIALS AND METHODS

Plant material

The leaves of *R.sativus* were collected from the local market of Akola. All the other chemicals and reagents were of pure analytical grade and obtained from local supplier.

Drugs and chemicals

Cystone from Himalaya Drug Company India Ltd., Mumbai were purchased from the local market. All remaining chemicals used in the experiment were of the highest grade commercially available.

Preparation of aqueous extract of the leaves of R. Sativus (RSAE)

The leaves were separated from other extraneous matter and subjected to shade drying. The dried leaves were subjected to a coarse powder by using dry grinder. The powder (10g) was soaked (maceration) in 100 ml purified water and kept in dark and dry place for 48 h at a temperature range of 20–26°C. Chloroform was added in quantity of 1% total mixture to avoid microbial growth. After 48 h, solutions were filtered by Whatman Filter Paper No. 1. The filtered extracts were dried in a rotary evaporator to obtain a dark brown powdery extract (1.34 % w/w) (Garimella *et al*., 2001).

Effect of RSAE on in-vitro crystallization

In-vitro crystallization of calcium oxalate Nucleation and aggregation assay.

Nucleation and aggregation assay were performed as per method previously described by (Hess *et al*., 2000) with minor modifications. Briefly, freshly prepared solution of 10 mM calcium chloride dihydrate and 1.0 mM sodium oxalate, containing 200 mM NaCl and 10 mM sodium acetate trihydrate, was adjusted to pH 5.7. All experiments were performed at 37 °C, using a circulating water bath. For crystallization experiments, 25 ml of sodium oxalate solution was transferred into a beaker and placed in the hot plate magnetic stirrer (Model 2MLH, REMI), which was maintained at 37°C and constantly stirred at 800 rpm. An additional 1 ml of distilled water/standard (cystone)/extract were added and finally calcium chloride solution (25 ml) was added. The optical density was measured at 620 nm in spectrophotometer (UV 1800, Shimadzu Corporation, Japan) after addition of calcium containing solution, on every 15 s over 5 min and then every 1 min over 10 min. All the experiments were performed in triplicate. The final solutions were seen under a light microscope to analyze the density of formed crystals in the solution (Olympus, USA). Percent inhibition in the presence of cystone

or RSAE was compared with the control by the following formula.

The percentage inhibition was calculated as:

$$[1 - (Tsi / Tsc)] \times 100$$

Where Tsc, the turbidity slope of the control; and Tsi, the turbidity Slope in the presence of the inhibitor.

In vitro calcium oxalate crystal growth assay. Inhibitory activity of RSAE against CaOx crystal growth was measured using previously described methods. Briefly, 20 ml each of 4mMcalcium chloride and 4mMsodium oxalate were added to a 30 ml of solution, containing NaCl (90 mM) buffered with Tris HCl (10 mM) Ph 7.2.To this 600 µl of calcium oxalate monohydrate (COM) crystal slurry (1.5 mg/ml acetate buffer) was added. Consumption of oxalate begins immediately after COM slurry addition and was monitored for 600 s by disappearance of absorbance at 214 nm. One ml of RSAE (500) and RSAE (1000 µg/ml) was added separately into this solution.The depletion of free oxalate ions will decrease if RSAE inhibits calcium oxalate crystal growth. Rate of reduction of free oxalate was calculated using the baseline value and the value after 30 s incubation with or without the extract. The relative inhibitory activity was calculated as follows:

% Relative inhibitory activity (CS/C) x 100

Where C is the rate of reduction of free oxalate without any extract and S is the rate of reduction of free oxalate with RSAE (Kalyan *et al.*, 2009 and Joshi *et al.*, 2005a).

RESULTS AND DISCUSSION

The changes in the turbidity or optical density of different solutions, *viz* control, cystone (1000 µg/ml), and RSAE (500 µg/ml and 1000 µg/ml),were plotted at different time intervals. The turbidity increased linearly up to 5 minutes, which indicated the nucleation process and then decreased linearly up to 15 minutes indicating the aggregation process. RSAE (500 µg/ml and 1000 µg/ml) and cystone (1000 µg/ml) inhibited both the rate of nucleation and the rate of aggregation. The maximum optical density of the solutions, viz control, cystone (1000 µg/ml) and RSAE (500 µg/ml) and RSAE (1000 µg/ml), recorded was 0.150, 0.062, 0.110 and 0.090. The percent inhibition rates of nucleation of CaOx by cystone (1000 µg/ml), RSAE (500 µg/ml) and RSAE (1000 µg/ ml) were found to be 57.33, 26.66 and 40.00 percent, respectively (Fig. 1). The percent inhibition rates of aggregation of CaOx by cystone (1000 µg/ml), RSAE (500 µg/ml) and RSAE (1000 µg/ ml) were found to be 66.66, 40.66 and 33.33 respectively (Fig. 1). The photomicrographs of the CaOx crystals in solutions of control, cystone (1000 µg/ml) and RSAE (500 µg/ml and 1000 µg/ ml) showed that CaOx crystals were less denser in cystone (1000 µg/ml), RSAE (500 µg/ml) and RSAE (1000 µg/ml) as compared to control (Fig. 2).

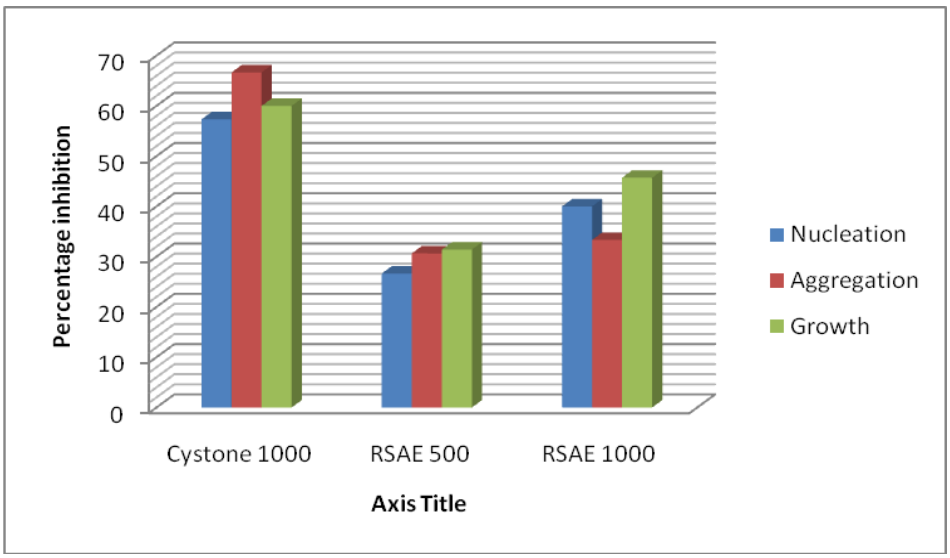


Fig. 1: Effect of RSAE on In-vitro crystallization of calcium oxalate

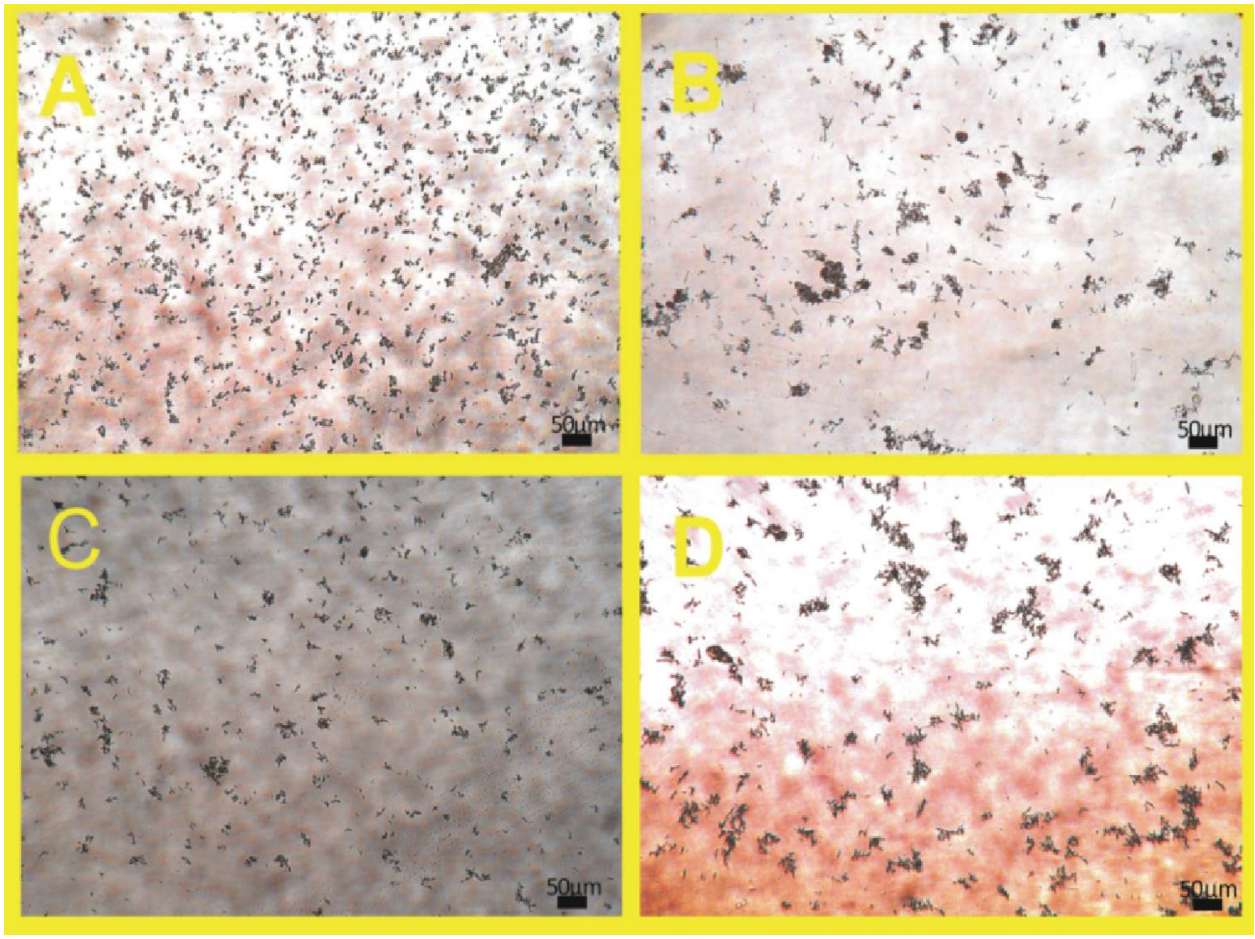


Figure 2 :– Photomicrographs of CaOx crystal density in different solutions, viz control, cystone (1000 µg/ml), and RSAE (500 µg/ml and 1000 µg/ml)
[A = control, B = cystone, C = RSAE $_{500}$, D = RSAE$_{1000}$], magnification 100×.

***In vitro* calcium oxalate crystal growth assay.**

In calcium oxalate growth assay, RSAE inhibited calcium oxalate monohydrate (COM) growth. The percentage inhibition of cystone (1000 µg/ml), RSAE (500 µg/ml) and RSAE (1000 µg/ml) were 60.00, 31.42 and 45.71 respectively (Fig. 1).

The recurrence of stone is a very serious concern in the medical management of urolithiasis. Drug treatments like thiazide as diuretic and alkali-citrate, used to prevent the recurrence of hypercalciuria and hyperoxaluria, are considered to be less efficacious.Although the surgical endoscopic stone removal and extracorporeal shock wave lithotripsy have revolutionized the treatment of urolithiasis, they increase the chances of new stone formation and the shock waves in therapeutic doses may cause acute renal injury, decrease in renal function and an increase in stone recurrence (50–80%).

In view of these, the effect of leaves of *R.Sativus*, having traditional use in the treatment of kidney stones and urinary tract troubles, was studied in in-vitro models of urolithiasis. In invitro calcium oxalate crystallization study, the process of nucleation and aggregation was studied in sodium acetate buffer of pH 5.7 to simulate the conditions of urine so as to favor the above processes. In the crystallization study, the turbidity increased linearly up to 5 min and then decreased linearly up to 15 min after the addition of calcium chloride dihydrate. Earlier increase in the turbidity was suggestive of the nucleation phenomenon, while the decrease in the later part indicated the aggregation. These two phenomena represented the complete process of in-vitro crystallization. Simultaneous addition of RSAE (500 and 1000 µg/ml) and cystone (1000 µg/ml) along with calcium chloride dihydrate inhibited the nucleation as well as aggregation process of CaOx crystallization as indicated by dose-dependent decrease in turbidity of the solution in both phenomena.

The inhibition of in-vitro crystallization of CaOx suggests that RSAE has influence on the formation of crystals from sodium oxalate and calcium chloride and/or their aggregation. Most of the previous papers stated that the test drug or extract inhibited the crystallization by favoring the formation of calcium oxalate dehydrate (COD) crystals instead of calcium oxalate monohydrate (COM). The study needs a use of polarized light to differentiate between COM and COD crystals, which is a limitation of the present study and thus could not state whether the RSAE favored the formation of COD and less of COM, but the possibility of this cannot be ignored. The non-significant decrease in turbidity as observed in the process of aggregation may be due to the possibility of more amounts of COD crystals in the solution (Barros *et al*., 2003). Moreover, growth assay showed that RSAE also inhibited the growth of calcium oxalate monohydrate crystals with maximum inhibition at 1000 µg/ml concentration. This indicates that RSAE has inhibitory influence on nucleation/aggregation and growth of calcium oxalate crystals. The results indicated that RSAE has significant influence on the formation, growth and dissolution of crystals, and further suggest that the extract has beneficial effect in preventing the formation of crystals and their growth. Literature of the previous studies is silent on the exact mechanism involved in the inhibition of in-vitro crystallization and stated that the extracts contained some chemical components that inhibited the crystallization. There are reports that flavonoids inhibit calcium oxalate crystallization in human urine as well as in animal models and crystal deposition. Saponins showed anti-crystallization properties by disaggregating the suspension of mucoproteins, the promoters of crystallization (Joshi *et al.,* 2005b). Finally, the results of the present investigations suggest that the leaves of the *R.Sativus* have in-vitro anti-crystallization effect on CaOx crystals. These findings substantiate the traditional use of the leaves in the treatment of urinary stones and kidney problems. In order to substantiate its in-vitro effect, the in-vivo studies need to be carried out in experimental animals.

The leaves of *R.Sativus* have beneficial inhibitory effect on *in vitro* Crystallization of CaOx crystals.

REFERENCES

Barros ME, Schor N and Boim MA, 2003. Effects of an aqueous extract from Phyllanthus niruri on calcium oxalate crystallization *in vitro. Urol Res*;**30**:374–9.

Basavaraj DR, Biyani CS, Browning AJ and Cartledge JJ, 2007. The role of urinary kidney stone inhibitors and promoters in the pathogenesis of calcium containing renal stones. *EAU-EBU Update Ser*;**5**:126–36.

Garimella TS, Jolly CI and Narayanan S, 2001. *In-vitro* studies on antilithiatic activity of seeds of *Dolichos biflorus Linn*. And rhizomes of Bergenia ligulataWall. *Phytother Res* ;**15**:351– 5.

Joshi VS, Parekh BB, Joshi MJ and Vaidya AB, 2005a. Herbal extracts of Tribulus *terrestris* and *Bergenia ligulata* inhibit growth of calcium oxalate monohydrate crystals *in-vitro. J Cryst Growth;***275**:e1403–8.

Joshi VS, Parekh BB, Joshi MJ and Vaidya ADB, 2005b. Inhibition of the growth of urinary calcium hydrogen phosphate dehydrate crystals with aqueous extracts of *Tribulus terrestris and Bergenia ligulata. Urol Res*;**33**:80–6.

Kalyan SB, Christina AJM, Syama SB, Selvakumar S and Sundara SK, 2009. Urolithiatic activity of *Hibiscus sabdariffa Linn.* on ethylene glycol induced lithiasis in rats. *Nat Prod Rad* ;**8**:43–7.

Karadi RV, Gadge NB, Alagawadi KR and Savadi RV, 2006. Effect of *Moringa oleifera* Lam. root-wood on ethylene glycol induced urolithiasis in rats. *J Ethnopharmacol*;**105**:306–11.

Kok DJ, Papapolous SE and Bijovet OL, 1990. Crystal agglomeration is a major element in calcium oxalate urinary stone formation. *Kidney Int;* **37**:51–6.

Moe OW, 2006. Kidney stones: pathophysiology and medical management. *Lancet*; **367:** 333–44.

Pak CY, 1989. Prevention and treatment of kidney stones. Role of medical prevention. *J Urol*; **141**(3):798–801.

Smith CL and Guay DRP. 1992. Pharmacotherapy and pathophysiologic approach, *Elsevier* **2**: 720–36.

Thomas B and Hall J, 2005. An overview on Urolithiasis. Urolithiasis Surgery (*Oxford*); **23**:129–33.

How to Cite this Article:

Syed Salman Akhtar, S.M. Mular, Nazia D. Khan, Z.H Khan, Syed Sohail, 2017. *In vitro* study of aqueous leaf extract of *Raphanus Sativus var*. for inhibition of calcium oxalate crystallization. *Bioscience Discovery,* **8**(2):153-157.