

INHIBITION OF CALCIUM OXALATE CRYSTALLIZATION BY AN EXTRACT OF *OCIMUM BASILICUM* SEEDS: AN *IN-VITRO* STUDYPATEL RAVINDRAKUMAR K^{1*}, PATEL SANDIP B²¹Department of Pharmacology, Indukaka Ipcowala College of Pharmacy, Anand, Gujarat, India. ²Department of Pharmacology, Ramanbhai College of Pharmacy, Anand, Gujarat, India. Email: ravi23iicp@gmail.com

Received: 15 April 2020, Revised and Accepted: 22 June 2020

ABSTRACT

Objective: *Ocimum basilicum* (OB) has been used to treat diverse illnesses which include urinary stone disorder for a reason that historical time in India. We investigated OB seeds for antiurolithic activity.

Methods: Calcium oxalate crystallization becomes triggered by the addition of 0.01 M sodium oxalate answers in normal human urine and nucleation was done.

Results: OB seeds were discovered to be robust and promising antiurolithic agents which are in accordance with its use in traditional medication.

Conclusion: An extract of the traditional herb OB has super inhibitory activity on crystalluria and therefore might be useful in dissolving urinary stone; however, in addition, a study in animal fashions of urolithiasis is needed to assess its capability antiurolithic interest.

Keywords: *Ocimum basilicum*, Urolithiasis, Calcium oxalate crystallization, Nucleation.

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INTRODUCTION

Urolithiasis (from Greek *ouron*, "urine" and *lithos*, "stone") is the situation in which urinary calculi are fashioned or located anywhere in the urinary system or the manner of formation of stones within the kidney, bladder, and ureters (urinary tract). Kidney stones are a commonplace purpose of blood in the urine and pain within the belly, flank, or groin [1]. Urinary stone formation influences 10–12% of the population inside the international. From epidemiological information, calcium oxalate is the maximum commonplace factor of the calculi [2,3]. Various etiologic factors, consisting of heredity, diet, metabolic abnormalities, and contamination, have been implicated in the pathobiology of kidney stone formation [4]. Kidney stones are specifically composed of calcium salts, uric acid, cysteine, and struvite. Among those sorts, more than 85% of the stones in human are created from calcium oxalate and calcium phosphate [5]. Development of plant-primarily based medicine as an opportunity or complementary to the conventional system of medicine has drawn great attention and serves as an immense supply of recent drug entities [6]. Based on those grounds, *Ocimum basilicum* (OB) was selected for the existing look at. Basil is well-known for its folk's medicinal value and is customary officially in a number of international locations [7]. The plant is effective in the treatment of belly troubles, fever, cough, and gout and given internally to treat cystitis, nephritis, and in internal piles [8]. No screening has been finished to this point to establish the antiurolithic efficacy of OB. Therefore, modern research turned into carried out for the screening of the antilithic capacity of OB in opposition to calcium oxalate crystallization in an *in vitro* putting.

METHODS

Plant collection

The plant material was collected in October 2018 and was taxonomically identified and authenticated as seeds of OB by the Department of Biosciences, Sardar Patel University, Vallabh Vidyanagar-388120, Gujarat, India. The vouchered specimens NO. SMSHAH-01 was deposited in the herbarium for future reference.

Processing and extraction

The plant material was thoroughly cleaned, dried below coloration, coarsely powdered, and used for the extract instruction 20 g of the powder is brought into the cellulosic cartridge after which placed inside the Soxhlet assembly. A general of 150 mL of hydroalcoholic (ethanol:water [70:30, v/v]) mixture was introduced into the flask and heated for 4 h at 65°C. Hence, the solvent when heated condenses and accumulates in the siphon tank, which increases the touch time between the solvent and the product to be extracted. While the solvent reaches a sure degree, it initiates the siphon and returns to the flask, inflicting the dissolved substance. At the stop of the of the extraction, the solvent contained inside the distillation flask with the extract is eliminated with the aid of a rotary vacuum evaporator [9,10]. Then dried extract became saved in a hermetic box at 4°C. The dried extract changed into dissolved in double distilled water and used for further take a look at.

Crystallization assay in complete urine

Urine samples (accrued over 24 h) from a healthful challenge had been collected in a polypropylene bottle containing an antibacterial agent; the urine sample turned into refrigerated throughout collection. Aliquots (2 ml) of urine were transferred to the tubes and allowed to heat to 37°C; for similarly examine the procedure as per [11].

Micro photographic research

Calcium oxalate turned into prompted within the absence and presence of hydroalcoholic extract of *Ocimum basilicum* (HOB). Following experiments were executed: With HOB at different concentrations (1 ml) 10 ml CaCl₂ (0.05 M) turned into admixed, delivered 1 ml (0.05 M) sodium oxalate solution became delivered and the tubes incubated at 37°C for 6 h, take on the identical temperature 1 ml of the suspension became unfold over the microslide and allowed to crystallize. Microphotographs had been fascinated about light microscopes (100×) fitted with the digital camera, the procedure as per reference [12]. The results are proven in Fig. 1.

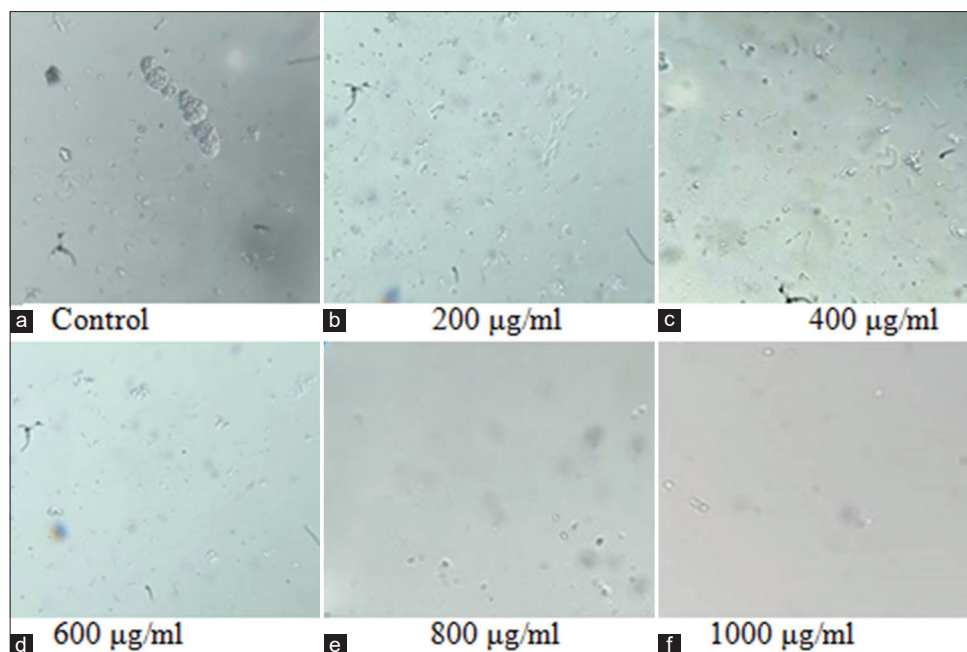


Fig. 1: Pictures of (a-f) calcium oxalate crystals as found below mild microscope ($\times 100$)

Experimental protocol

The effects of extracts on calcium oxalate crystallization become decided by the point path measurement of turbidity modifications because of the crystallization in urine samples (amassed over 24 h) from a healthful challenge after the addition of 0.01 M sodium oxalate answer. The precipitation of calcium oxalate at 37°C and pH 6.8 has been studied with the aid of the dimension of absorbance at 620 nm, the usage of ultraviolet/visible spectrophotometer.

Study without an inhibitor

A quantity of 1.0 ml of human urine turned into transferred into the mobile and 0.5 ml of distilled water brought to it and clean analyzing was taken. The 0.5 ml of 0.01 M sodium oxalate was added to the previous extent, and the size is without delay commenced for a duration of 10 min.

Study with an inhibitor

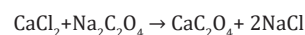
The HOB changed into dissolved in double distilled water, filtered through membrane filter, and the attention of 200, 400, 600, 800, and 1000 $\mu\text{g/ml}$ changed into prepared. A mixture of 1 ml of human urine and 0.5 ml of plant extract solution is versed inside the cellular. A clean studying turned into taken and then 0.5 ml of 0.01 M sodium oxalate answer turned into [13]. Added and at once the absorbance becomes measured for a period of 10 min at 620 nm the share of inhibition of calcium oxalate crystal formation become calculated the usage of the following formula:

$$\% \text{ Inhibition} = \frac{(\text{Absorbance of control} - \text{absorbance of test})}{\text{Absorbance of test}} \times 100$$

Nucleation assay

Nucleation assay changed into completed using the approach pronounced [14]. Solution of calcium chloride and sodium oxalate had been prepared at the final concentration of 5 mol/L and 7.5 mmol/L, respectively, in buffer containing Tris HCl 0.05 mol/L and NaCl 0.15 mol/L at pH 6.5. Nine hundred fifty microliter of calcium chloride answer was mixed with 100 μl of extract at exceptional concentration (200 $\mu\text{g/ml}$ –1000 $\mu\text{g/ml}$). Crystallization became commenced through including 950 μl of sodium oxalate answer. The temperature was maintained at 37°C. The absorbance of the solution became monitored at 620 nm. The charge of nucleation becomes anticipated by means of evaluating the induction time in the presence

of the extract with that of manipulate [15]. The growth of the crystal became predicted due to the following reaction.



The percent of inhibition produced using the herb extract was calculated the use of the components said within the above segment.

RESULTS

Nephrolithiasis is commonplace, affecting up to 10% of the populace at some point for the duration of their lifetime [16]. The supersaturation of urine with calcium oxalate, the maximum common thing of kidney stones, attributes to calcium oxalate debris crystallization in the urinary tract with later elements being nucleation, boom, and aggregation. Thus, if fantastic saturation or later steps in crystallization may be averted, then lithiasis ought to be avoided [17]. Calcium-containing stones are the most normally going on to a quantity of 75–90%, followed by magnesium ammonium phosphate (Struvite) to a quantity of 10–15%, uric acid 3–10%, and cystine 0.5–1% [18]. Calcium oxalate stones are discovered in two unique varieties, calcium oxalate monohydrate (COM) and calcium oxalate dihydrate (COD). COM, the thermodynamically most solid shape, is observed more often in scientific stones than COD and it has a greater affinity for renal tubular cells, thus answerable for the formation of stones within the kidney [19].

The *in vitro* inhibitory impact of extracts of OB on diverse phases of calcium oxalate crystallization changed into determined by the time route of absorbance measured in human urine at extract concentrations of 200, 400, 600, 800, and 1000 $\mu\text{g/ml}$. The mild micrographs at 6 h within the manipulate system confirmed the formation of each kinds calcium oxalate crystals with full-size aggregations (Fig. 1). Fig. 1a demonstrated calcium oxalate crystals while OB extract at the highest concentration (1000 $\mu\text{g/ml}$, Fig. 1f inhibited crystal formation).

Representative pictures of calcium oxalate crystals as discovered underneath a mild microscope ($\times 100$) within the absence A (control) and inside the presence of OB 200 $\mu\text{g/ml}$, 400 $\mu\text{g/ml}$, 600 $\mu\text{g/ml}$, 800 $\mu\text{g/ml}$, and 1000 $\mu\text{g/ml}$ attention.

At exclusive concentration, the formation and growth of the COM crystals from human urine changed into studied. Urinary salts along with

calcium oxalate stone formation are the end result of amazing saturation of urine. The increases of COM become observed to be most on top of things. To estimate the inhibiting capability of plant extract for oxalate crystallization special percentages of plant extract became examined.

The outcomes indicate that OB showed % inhibition for calcium oxalate crystal with a maximum inhibition of at 800 µg/ml, respectively (Fig. 2).

While in nucleation assay % inhibition for calcium oxalate formation become directly proportional to be growing attention of the plant extract. Maximum inhibition 90.45% became discovered with plant extract 1000 µg/ml and standard drug cystone gave inhibition of 84.75% as proven in Fig. 3.

The dose-dependent boom in percentage inhibition of nucleation by way of the extract and cystone had a coefficient of regression (R²) of 0.876 and 0.888. (Fig. 4).

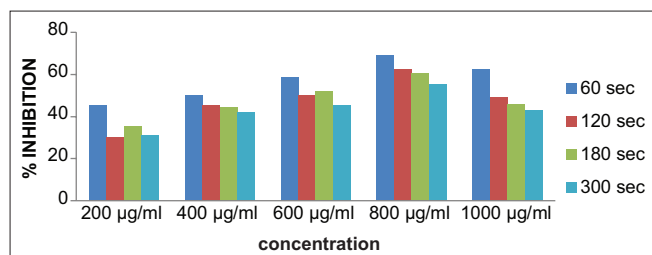


Fig. 2: The effect of *Ocimum basilicum* plant extract on calcium oxalate crystallization

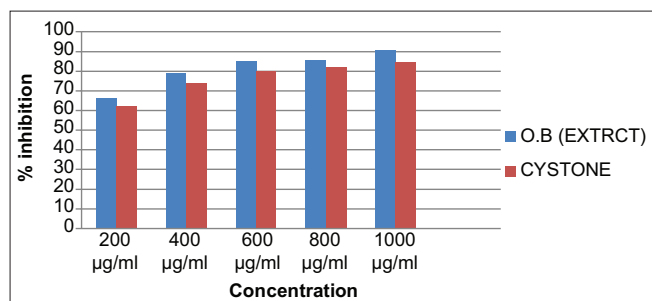


Fig. 3: The effect of plant extracts and standard on the nucleation of calcium oxalate

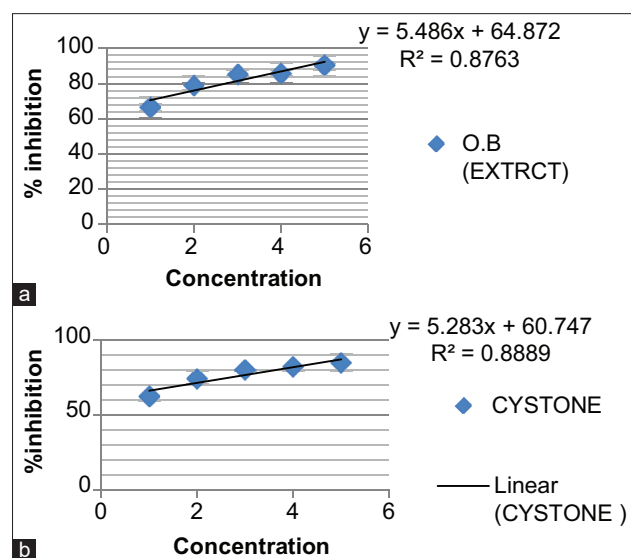


Fig. 4 The impact of (a) *Ocimum basilicum* seeds extract and (b) cystone at the nucleation assay

The extract may include some photochemical that inhibits the growth of calcium oxalate crystals [20]. Comparable inhibition of calcium oxalate stones became also mentioned for *Tamarindus indica* pulp, *Ocimum sanctum* leaves, and *Cucumis melo* seeds [21-23]. The *Balantidium ciliate* extract also incorporates a few materials that inhibit the aggregation of COM crystals. Crystal aggregation is the maximum crucial step, as it occurs very rapidly and has an extensive effect on particle length, and aggregated crystals are normally discovered in urine and renal stones [24]. Polyphenols compounds had been also contributed to the calcium oxalate calculi prevention [25]. Naturally going on, pentacyclic triterpenes of plant beginning have been diagnosed as owning an extensive variety of pharmacological outcomes. Lupeol (Lupa-21, 20 (29) dien, 3b-ol) has been observed to be green in decreasing the chance of stone formation in animals by way of preventing crystal-brought on tissue damage and dilution of urinary stone-forming elements. Two structurally associated triterpenes, lupeol and betulin (Lupa-20 (29) ene-3, 28 diol) had been assessed for his or her antilithiatic impact [26]. The phytochemical screening of OB discovered the presence of glycoside, gums, mucilage, proteins, amino acids, tannins, phenolic compound, triterpenoids steroids, sterols, saponins, flavones, and flavonoids in it [27]. Hydroalcoholic extract includes each polar and non-polar phytochemical compound and for this reason included predominant polyphenolic compounds such as phenolic compound, triterpenoids steroids, and flavonoids. Microscopy confirmed that the extract decreased the range of crystals in exclusive concentrations.

DISCUSSION

The supersaturation of urine with calcium oxalate, the most widely recognized segment of kidney stones, credits to calcium oxalate particles crystallization inside the urinary tract with later factors being nucleation, development, and conglomeration. Subsequently, in the event that supersaturation or later strides in crystallization can be forestalled, at that point, lithiasis ought to be dodged. Although a few measures are typically taken to decrease supersaturation, for example, expanding liquid admission and clinical treatment, it is commonly acknowledged that better techniques for the prevention of kidney stones should be created [28,29]. Nucleation is a significant initial step for the commencement of precious stones, which at that point, develops and structure totals. Calcium oxalate stone start develops, total with different stones, and held in the kidney.

In this *in vitro* examination, the disintegration strategies for calcium oxalate and calcium phosphate were utilized to know the job of OB separates in dissolving the effectively shaped stones core in renal framework. As *in vitro* crystallization study was performed since the outcomes obviously show that the precious stone nucleation was found to communicate a fixation subordinate restraint.

OB is a common herb that is known for its ornamental and therapeutic importance. The chemical constituents which have been isolated from the plant include terpenoids, alkaloids, flavonoids, tannins, saponin glycosides, and ascorbic acid. Conventionally, basil has been used as a medicinal plant in the treatment of headaches, coughs, diarrhea, constipation, warts, worms, and kidney malfunctions [30]. The study of the urinary chemistry with respect to the stone-forming minerals will provide a good indication of the risk of stone formation. From the study results, it is observed that hydroalcoholic extracts show the highest dissolution of calcium oxalate.

In the present study, the extract from seeds OB has proven good-sized antilithiatic interest. The inhibitory efficiency of the OB has become examined at the nucleation and boom of the most often taking vicinity kidney stones, COM. An awareness dependent inhibition turned into determined the usage of OB. Microscopy confirmed that the extract reduced the form of crystals. This suggests that phytochemicals from the flowers exert their motion right now on the crystals [31].

CONCLUSION

The HOB has established huge antilithiatic interest. However, those *in-vitro* results want to be confirmed *in vivo* for you to expand an

effective antilithic agent from this plant, as this assets of the extract are brilliant in preventing urinary stone formation by way of inducing the excretion of small particles from the kidney and lowering the risk of their retention within the urinary tract.

AUTHORS' CONTRIBUTIONS

Patel Ravindrakumar K: Principal investigator, completed practice and evaluation on all samples and write manuscript. Patel Sandip B: Guide, supervised improvement of work, helped in statistics interpretation, and manuscript assessment.

CONFLICTS OF INTEREST

The authors do not have the interests of conflicts.

FUNDING

This research not received any specific grant from any funding agencies.

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