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ORIGINAL ARTICLE

Access this article online

Website: www.ijrh.org

DOI: 10.4103/0974-7168.166374

Quick Response Code:

Homoeopathic preparation of *Berberis vulgaris* as an inhibitor of Calcium oxalate crystallization: An *in vitro* evidence

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Received: 09-10-2014
Accepted: 06-07-2015

ABSTRACT

Background: *Berberis vulgaris* is the most widely used drug in Homoeopathy for treating urolithiasis. However, its mechanism of action in alleviating its consequences remains uncertain.

Objective: To explicate the potential role of Homoeopathic preparation of *B. vulgaris* on *in vitro* Calcium oxalate (CaOx) crystallization.

Materials and Methods: Spectrophotometric crystallization assay was carried out, and the slopes of the nucleation (till the maximum) and aggregation (after the peak) phases were calculated using linear regression analysis, and the percentage inhibition exerted by the modifiers was calculated. Light microscopic observation of CaOx crystals formed in the presence or absence of modifiers was carried out to support the outcome with spectrophotometric crystallization assays and to ascertain the potential role of *B. vulgaris* in CaOx crystallization.

Results: The crystallization studies performed so far signifies *B. vulgaris* to be a potent drug against CaOx crystallization both at the level of nucleation and aggregation.

Conclusion: Our present findings add up to the experimental evidence to support the efficacy of the homeopathic preparation of the *B. vulgaris* in modulating the primary events of stone formation.

Keywords: *Berberis vulgaris*, Calcium oxalate crystals, Crystallization, Urolithiasis, Anti-urolithic agent

INTRODUCTION

Urolithiasis has been a menace to mankind since the distant past and continues to be on the rise worldwide besides being an imperative issue because of its incidence, recurrence, and vicious consequences.[1] In India, approximately 5–7 million patients suffer from kidney stone disease and at least

How to cite this article: Ganesan T, Ravi DB, Vasavan J, Khurana A, Nayak D, Periandavan K. Homoeopathic preparation of *Berberis vulgaris* as an inhibitor of Calcium oxalate crystallization: An *in vitro* evidence. Indian J Res Homoeopathy 2015;9:152-7.

1/1000 of Indian population need hospitalization due to kidney stone diseases.[2] About 70 to 80% of the calculi are composed primarily of Calcium oxalate (CaOx) mixed with varying amounts of Calcium phosphate.[3]

The major objective while treating renal stones is to accomplish utmost clearance of stone, while causing the quite lowest amount of morbidity to the patient. Various minimally invasive modalities are described for this, like shockwave lithotripsy, percutaneous nephrolithotomy, and retrograde intra renal surgery; nevertheless, the recurrence rates are estimated at 50% over a 10-year and 75% over 20-year period, with some people experiencing 10 or more episodes over the course of a lifetime besides exhibiting equally worse side effects.[4] As an alternative, other novel treatment options such as Homoeopathy can be considered as a replacement for the invasive treatment strategies. Several epidemiological data suggest and support the role of *Berberis vulgaris*, the most widely used drug in homoeopathic medicine for urolithiasis in alleviating the pain and stone formation.[5]

The earlier studies performed in our lab have assessed the efficacy of the Homoeopathic preparation of *B. vulgaris* in treating urolithiasis in a rat model, and the results have justified its prominent role in reducing oxalate deposition in the tissues, protecting renal cell membrane integrity and thereby might prove as a potential antilithiatic agent.[6]

Several observations that highlight the effectiveness of this homoeopathic preparation of *B. vulgaris* with a characteristic display of biochemical parameters exist. However, data analysis studies for its efficiency on modulating the CaOx crystal structure and morphology are scant so far.

Hence, the main objective of this study is to elucidate the potential role of homoeopathic preparation of *B. vulgaris* on *in vitro* CaOx crystallization.

MATERIALS AND METHODS

Drugs and Chemicals

Homoeopathic preparation of *B. vulgaris* (Φ, 6C, 30C, and 200C) was procured from Hahnemann Publishing Co., Pvt., Ltd., Kolkata, India. All other chemicals and reagents used were of analytical grade.

Spectrophotometric Crystallization Assay

Spectrophotometric crystallization assay was carried out by the method of Hess *et al*. (1995). In a quartz cuvette containing 1 ml of Potassium oxalate solution, 1 ml of Calcium chloride solution was added to give a final concentration of 4.25 mmol/L Calcium and 0.75 mmol/L oxalate. All the solutions were prepared in deionized water containing 200 mmol/L Sodium chloride and 10 mmol/L Sodium acetate (pH 5.7). The time course of the optical density at 620 nm was measured automatically using a UVIKON 930 Spectrophotometer (Kontron Instruments, Italy). The values were also measured in the 20, 50, and 100 µl of *B. vulgaris* (Φ, 6C, 30C, and 200C). OD620 increases initially during nucleation phase and decreases during the aggregation phase.

Slopes of the nucleation (till the maximum) and aggregation (after the peak) phases will be calculated using linear regression analysis, and the percentage inhibition exerted by the proteins will be calculated using the formula:

$$\text{Percentage inhibition} = (1 - \text{Sm/Sc}) \times 100$$

where, Sm is the slope in the presence of the protein and Sc the slope of the control.

Light Microscopic Studies

CaOx crystals for light microscopic studies were prepared according to the method of Nakai *et al*. (1996). CaOx crystals were formed by mixing 0.2 ml of 20 mM Calcium chloride with 0.1 ml of 20 mM potassium oxalate. All the solutions were prepared in 10 mM sodium acetate containing 200 mM sodium chloride. Each solution was adjusted to the pH 6.5. Crystallization was carried out by mixing appropriate ratio of the solutions, and the suspension formed was spread on a glass plate and a cover slip was placed on it and investigated under light microscope (Eclipse E400, NIKON Microscope, Japan) and was photographed at × 40 magnification. The same procedure was repeated by adding each concentration of *B. vulgaris* procured.

RESULTS AND DISCUSSION

The majority of urinary calculi found in patients with urolithiasis are predominantly of CaOx composition. Calcium and oxalate are the two urine substances responsible for CaOx crystallization. Hence, generally, the inhibitors of CaOx crystallization have been used as a prophylactic agent to prevent the recurrence.

The problem of calculating the supersaturation in urine (i.e., the driving force of this particular case of phase transformation) and finding out the possible

natural or pharmaceutical regulators of this driving force, in the process of investigating the kinetics of crystal nucleation and growth, as well as in the investigation of the kinetics of the dissolution of already existing stones, appears to be of utmost significance.[7] In this context, we set into assessing the CaOx crystallization kinetics in the presence of an established homoeopathic preparation of *B. vulgaris*. *In vitro* crystallization systems are widely used for different purposes in urolithiasis research so as to understand the pathology and thus to emerge with efficient treatment strategies.

Several *in vitro* and also *in vivo* studies on medicinal plants have proven them to play a crucial role in delaying and/or preventing the early phases of crystallization and thus conferring a remedy as antiurolithic agents.[8,9]

To analyze the effect of the homoeopathic preparation of *B. vulgaris* on CaOx crystal formation events, the crystallization studies were carried out.

In Figure 1, the time from addition of Calcium chloride until the first detectable increment of OD620, reflects the time required for CaOx crystal nuclei to appear and grow which allow for detection. The increase in slope of OD620 with time in turn mainly reflects an increase in particle number in function of time and thus crystal nucleation.[10,11]

In a while, an equilibrium is reached where the solution gets saturated, and the crystal mass has to remain stable. However, data support the view that the observed decrease in OD620 with time reflects a decline in particle number due to crystal aggregation, which indeed has been demonstrated by scanning electron microscope.[12] Thus, this slope of decrease of OD620 with time can be taken as a measure of crystal aggregation and can be used as the standard for all comparisons.

Figure 2 represents the *in vitro* CaOx crystallization that was carried out with 50% ethanol which served as a control in the present study. The alcohol was found to influence the CaOx crystallization process.

Figures 3-6 show the effect of *B. vulgaris* homoeopathic preparations of the mother tincture (Φ), 6C, 30C, and 200C, respectively, on the nucleation and aggregation of CaOx.

Table 1 represents the effect of different *B. vulgaris* homoeopathic formulations on *in vitro* CaOx nucleation and aggregation [Figure 7]. Surprisingly, the mother tincture is shown to favor aggregation

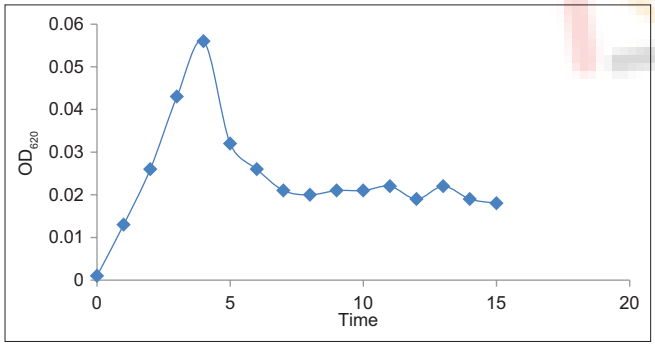

Figure 1: Standard graph for *in vitro* Calcium oxalate crystallization

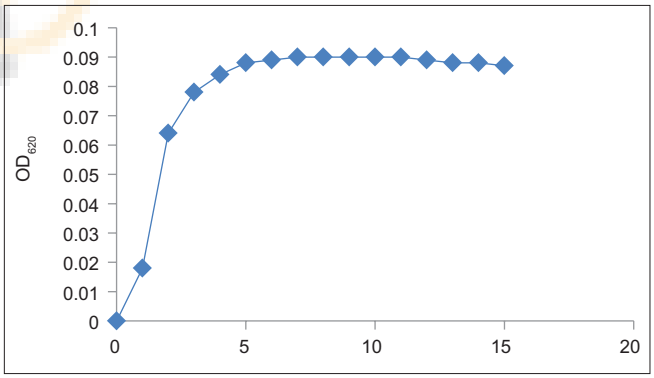

Figure 2: *In vitro* Calcium oxalate crystallization with 50% ethanol control

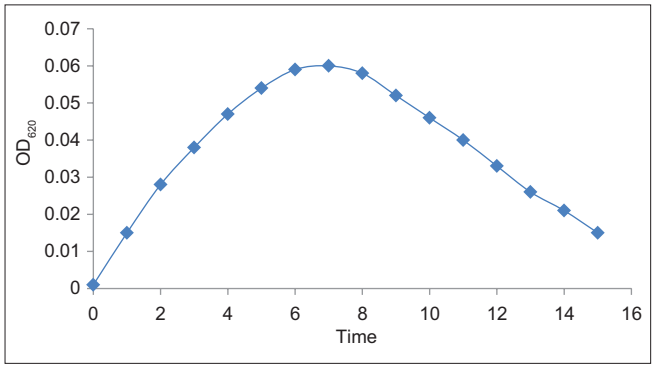

Figure 3: Effect of *Berberis vulgaris* (Ø-50µl) on Calcium oxalate crystallization

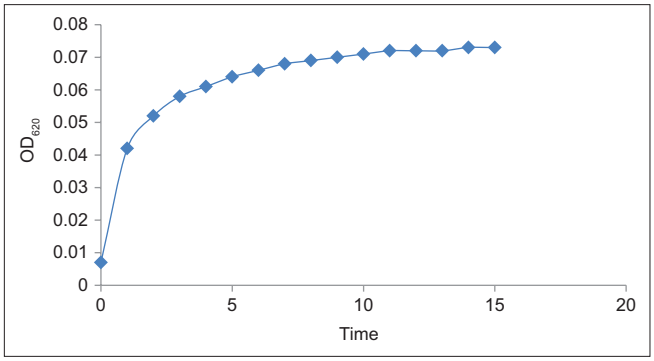

Figure 4: Effect of *Berberis vulgaris* (6C-50µl) on Calcium oxalate crystallization

of CaOx to a greater extent of about 229%. On the contrary, the 6C, 30C and 200C have been found to be inhibitory in nature, that too, they inhibited the aggregation by 100%.

These suggest that the maximum inhibition (>100%) was observed when 6C, 30C, and 200C preparation were tested for their efficacy to modulate the CaOx crystal aggregation.

Light microscopic observation studies were carried out to affirm our outcomes with spectrophotometric crystallization assays. The CaOx crystals appear as hemispherulitic clusters of crystals with the X-shaped habit in the control (without any additives) [Figure 8], which is generally observed in increased supersaturation.[13] The increase of crystal number is related to supersaturation, and hence, the observed crystallization pattern in the control dictates the prevalent high supersaturation. In the citrated one, only bipyramidal CaOx dihydrate (COD) crystals somewhat intertwining were observed and scanty in numbers. Similar but not the exact observations were made in the 30C preparation. However, these crystals were not thermodynamically stable; they tend to change to COM crystals. This reveals that it could relieve the supersaturation and prevent crystal

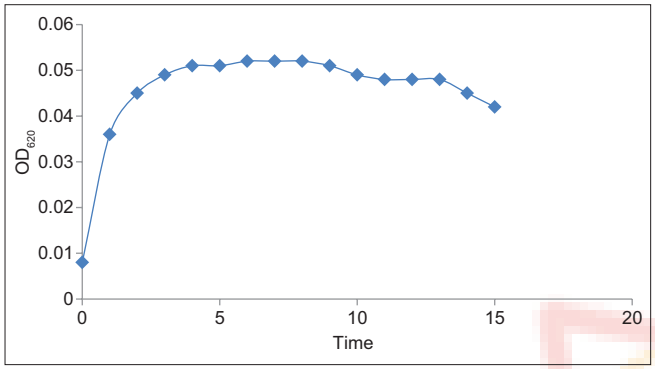

Figure 5: Effect of *Berberis vulgaris* (30C-50µl) on Calcium oxalate crystallization

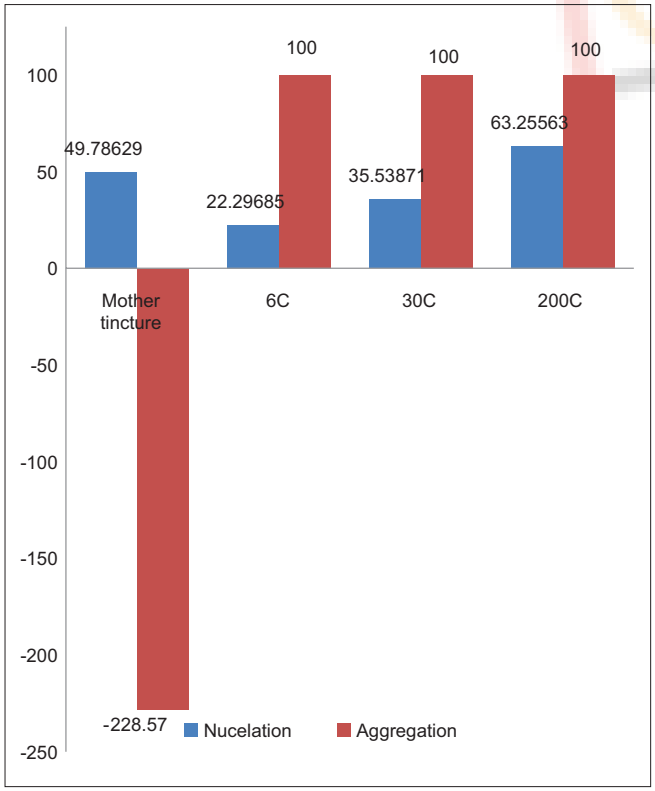

Figure 7: Percentage inhibition of nucleation and aggregation by *Berberis vulgaris*

Table 1: Effect of different *Berberis vulgaris* homoeopathic formulation on *in vitro* Calcium oxalate nucleation and aggregation

Particulars (100 µl each)	Nucleation	Aggregation
Mother tincture	49.78629	−228.7
6C	22.29685	>100
30C	35.53871	>100
200C	63.25563	>100

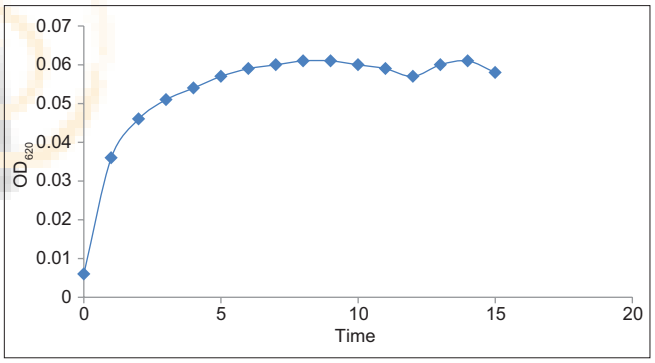

Figure 6: Effect of *Berberis vulgaris* (200C-50µl) on Calcium oxalate crystallization

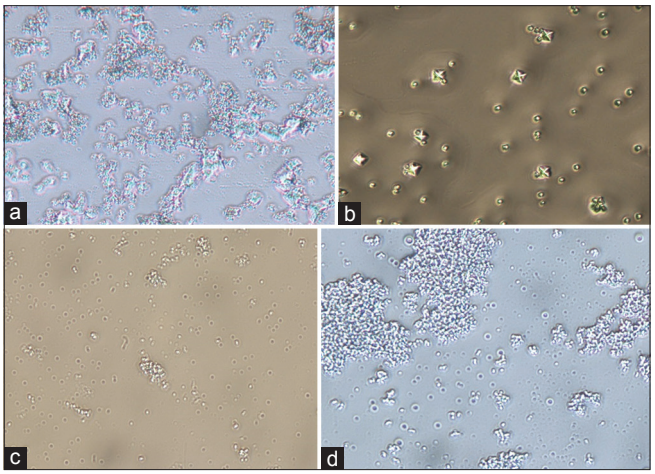

Figure 8: (a-d) Pattern of Calcium oxalate crystals. (a) Non-Citrated, (b) Citrated, (c) 30C, (d) Ø

aggregation. Thus, a negative supersaturation in respect to CaOx-precipitation can occur in the presence of the homoeopathic preparation, and even the direct dissolution of CaOx calculi situated in the kidney pelvis, or elsewhere in the urinary tract, could be expected.

Our present findings suggest that homoeopathic preparation of the *B. vulgaris* in appropriate concentrations proves to be effective against the early events of crystallization.

CaOx stones are found in two different varieties, CaOx monohydrate (COM) or Whewellite, and COD or weddellite. COM, the thermodynamically most stable form, is observed more frequently in clinical stones than COD, and it has a greater affinity for renal tubular cells, thus responsible for the formation of stones in the kidney (Jyothilakshmi *et al*., 2013). Hence, the formation of COD crystals forms a favorable environment of harmless excretion of CaOx. In this regard, *B. vulgaris* tends to form COD crystals.

CONCLUSION

Thus, our *in vitro* crystallization assays demonstrate even a possibility to dissolve CaOx stones in the human urine, thereby safeguarding it from crystalluria and its resultant injury. Although the relative effect of this homoeopathic preparation on the solubility of CaOx has been determined in simple salt solutions, the exact combination of the factors that is responsible for variations in CaOx solubility in urine is very yet insufficiently known. Hence, further studies are warranted to conclude its *modus operandi* in *in vivo* systems.

Acknowledgment

The authors are highly indebted to the Central Council for Research in Homoeopathy (CCRH), New Delhi for their financial assistance.

Financial Support and Sponsorship

Funded by CCRH.

Conflicts of Interest

There are no conflicts of interest.

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मूल लेख

कैल्शियम ऑक्ज़लेट क्रिस्टलीकरण के प्रावरोधक के रूप में *बेरबेरिस वल्गेरिस* का होम्योपैथिक योगः एक *पात्रे* प्रमाण

सार

पृष्ठभूमिः *बेरबेरिस वल्गेरिस* मूत्राश्मरता (यूरोलिथायसिस) के उपचार हेतु होम्योपैथिक चिकित्सा में सर्वाधिक व्यापक रूप से प्रयुक्त औषधि है। हालांकि, उसके परिणामों की गंभीरता कम करने की कार्य–क्रियाविधि अभी तक अनिश्चित बनी हुई है।

उद्देश्यः *पात्रे* कैल्शियम ऑक्ज़लेट (CaOx) क्रिस्टलीकरण में *बी.वल्गेरिस* के होम्योपैथिक योग की संभाव्य भूमिका की व्याख्या करना।

सामग्रियाँ एवं विधियांः स्पेक्ट्रमीप्रकाशमितीय क्रिस्टलीकरण आमापन किया गया एवं नाभिकन (अधिकतम तक) एवं समुच्चयन (शीर्ष के बाद) प्रावस्थाओं की प्रवणताओं की गणना रैखिक समाश्रयण विश्लेषण द्वारा की गई तथा संशोधकों द्वारा लगाए गए प्रतिशत प्रावरोध की गणना की गई। स्पेक्ट्रमीप्रकाशमितीय क्रिस्टलीकरण आमापनों के परिणामों का समर्थन करने एवं CaOx क्रिस्टलीकरण में *बी.वल्गेरिस* की संभाव्य भूमिका को निश्चित करने के लिए संशोधकों की उपस्थिति या अनुपस्थिति में निर्मित हुए CaOx क्रिस्टलों का प्रकाश सूक्ष्मदर्शीय प्रेक्षण किया गया।।

परिणामः अब तक किए गए क्रिस्टलीकरण अध्ययन दर्शाते हैं कि *बी.वल्गेरिस* नाभिकन और समुच्चयन, दोनों स्तरों पर CaOx क्रिस्टलीकरण के विरुद्ध एक प्रभावकारी औषधि है।

निष्कर्षः हमारे प्रस्तुत जांच–परिणाम अश्मरी निर्माण की प्राथमिक घटनाओं के मॉडुलन में *बी.वल्गेरिस* के होम्योपैथिक योग की प्रभाविकता का समर्थन करने में प्रयोगात्मक प्रमाण का गठन करते हैं।

मुख्य शब्दः *बेरबेरिस वल्गेरिस*, कैल्शियम ऑक्ज़लेट क्रिस्टल, क्रिस्टलीकरण, मूत्राश्मरता, मूत्राश्मर–रोधी अभिकर्ता

Preparado homeopático de *Berberis vulgaris* como inhibidor de la cristalización del oxalato de calcio: Evidencia *in vitro*

RESUMEN

Fundamentos: *Berberis vulgaris* es el medicamento más ampliamente utilizado en homeopatía para el tratamiento de la litiasis renal. Sin embargo, todavía no se ha esclarecido su mecanismo de acción en aliviar las secuelas.

Objetivo: Explicar el papel potencial del preparado homeopático de *B. vulgaris* en la cristalización *in vitro* del oxalato de calcio (CaOx).

Materiales y métodos: Se ha realizado un ensayo espectrofotométrico de la cristalización. ymediante un análisis de regresión lineal, se calcularon las pendientes de las fases de nucleación (hasta el máximo) y agregación (después del máximo). Asimismo, se calculó la inhibición porcentual ejercida por los modificadores. Se realizó una observación por microscopio óptico de los cristales de CaOx formados en presencia o ausencia de los modificadores para apoyar los resultados con los ensayos espectrofotométricos de cristalización y determinar la función potencial de *B. vulgaris* en la cristalización del CaOx.

Resultados: Los estudios de cristalización realizados hasta la fecha muestran que *B. vulgaris* es un medicamento potente contra la cristalización del CaOx tanto en la fase de nucleación como en la de agregación.

Conclusiones: Los resultados del presente estudio aportan evidencias experimentales adicionales que muestran la eficacia del preparado homeopático *B. vulgaris* en la modulación de los eventos primarios de la formación de cálculos.