



In Vitro Inhibition of Calcium Oxalate Crystallization by *Phagnalon rupestre* aqueous extract

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ABSTRACT

Kidney stone disease is a common urologic condition that affects around 10% of the general population at some point in their lives. Unfortunately, currently, available management aims to treat the result of an underlying metabolic abnormality, which results in further recurrence. The formation of urinary stones is related to decreased urine volume or increased excretion of stone-forming components. With its multifactor etiology and high rate of recurrences, kidney stone provides a medical challenge; therefore, there is a demanding need to prevent this disease and its recurrence. In Libya, using herbal medicines, especially *Phagnalon rupestre*, as a traditional therapy to treat kidney stones is not science-based, though it is widely used. Therefore, this study aims to evaluate the effect of *Phagnalon rupestre* extract on the dissolution of calcium oxalate stones. The aqueous plant extract was extracted from the dried aerial parts of *Phagnalon rupestre* using the reflux method. Using spectrophotometric methods, the nucleation of calcium oxalate crystals was measured. The inhibitory effect of herb extract on the nucleation growth of calcium oxalate formation was compared with that of the control.

Interestingly, the highest dissolution rate of calcium oxalate crystals was observed by the *Phagnalon rupestre* extract in comparison to the control. Our results demonstrate that an extract of *Phagnalon rupestre* inhibits the nucleation of calcium oxalate crystals. The extract of *Phagnalon rupestre* may contain substances that inhibit calcium oxalate crystal aggregation. Future work will focus on testing the extract in vivo and confirming our findings in an animal model.

التثبيط المخبري لتبلور أو كسالات الكالسيوم بواسطة مستخلص عشبة الارنب *Phagnalon rupestre*

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الكلمات المفتاحية:

حصوات الكلى
عشبة الارنب
نواة بلورات اكسالات الكالسيوم
أنوية الحصوات

الملخص

مرض حصوات الكلى هو حالة مسالك بولية شائعة تصيب حوالي 10% من سكان العالم في مرحلة ما من حياتهم، للاسف تهدف الطرق الحالية المتاحة إلى معالجة النتيجة النهائية لخلل الاستقلاب الأساسي، مما يؤدي إلى تكرارها، يرتبط تكوين حصوات المسالك البولية بنقص حجم البول أو زيادة إفراز العناصر المكونة للحصى، بسبب عواملها المتعددة وارتفاع معدل تكرارها تشكل حصوات الكلى تحديًا طبيًا، وبالتالي، هناك حاجة ملحة للوقاية من هذا المرض وتكرار حدوثه، لا يعتبر استخدام الأعشاب في ليبيا، وخاصةً عشبة الارنب، قائمًا على اساس علمي على الرغم من استخدامها على نطاق واسع، لذلك فإن الهدف من هذه الدراسة هو تقييم تأثير مستخلص عشبة الارنب على تثبيط تكوين حصوات أكسالات الكالسيوم، تم استخلاص النبات من الأجزاء الهوائية المجففة من عشبة الارنب باستخدام طريقة الاسترجاع، وباستخدام طرق القياس الضوئي الطيفية، تم قياس تثبيط نواة بلورات أكسالات الكالسيوم، وتمت مقارنة التأثير المثبط لمستخلص الأعشاب على نمو النواة المكونة لأكسالات الكالسيوم مع تأثير المجموعة الشاهدة، ومن المثير للاهتمام، لوحظ أعلى معدل انحلال لبلورات أكسالات الكالسيوم بواسطة مستخلص عشبة الارنب مقارنة بالمجموعة الشاهدة، اظهرت النتائج أن

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مستخلص عشبة الارنب يثبط تكوين بلورات أكسالات الكالسيوم، قد يحتوي مستخلص عشبة الارنب على مواد (مكونات) تمنع تراكم بلورات أكسالات الكالسيوم، سيركز العمل المستقبلي على اختبار المستخلص في الجسم الحي وتأكيد النتائج التي توصلنا إليها في نموذج حيواني.

Introduction

Kidney stone disease is a common urologic condition that affects around 10% of the general population at some point in their lives[1]. It is a major socio-economic problem of human beings. Unfortunately, currently, available management aims to treat the result of an underlying metabolic abnormality, which results in further recurrence. Many patients still undergo surgery to remove the stones[2]. The formation of urinary stones is related to decreased urine volume or increased excretion of stone-forming components (calcium, oxalate, urate, cystine, xanthine, phosphate, etc.)[3]. Approximately 75% of renal stones are primarily calcium oxalate CaOx, 10–20% are magnesium ammonium phosphate (struvite or triple phosphate), 5% are composed of urate, and 1-2% are cysteine[4]. With its multifactor etiology and high rate of recurrences, urinary tract stone disease provides a medical challenge. Therefore, there is a demanding need to prevent this disease and its recurrence. Using herbal medicines to treat various diseases has historical roots in ancient medicinal practices. In Libya, using herbal medicines, especially *Phagnalon rupestre*, as a traditional therapy to treat kidney stones is not science-based. However, it is widely practiced with no report or publication to emphasize its positive impact. Therefore, this study aims to evaluate the effect of *Phagnalon rupestre* extract used in traditional medicine on the dissolution of calcium oxalate stones.

Material and methods

Plant Sample Collection and preparation of extract

The aqueous extract was extracted from the dried aerial parts of *Phagnalon rupestre* supplied by the plant science department herbarium (science faculty). 50 g of dried aerial parts were subjected to 500 ml of sterile distilled water using the reflux method for 4 hours[5], and the extract was filtered with Whatman filter paper No.1.

Nucleation Assay

Calcium chloride (5 mmol/L) and sodium oxalate (7.5 mmol/L) solution were prepared in a buffer containing Tris 0.05 mol/L and NaCl 0.15 mol/L at pH 6.5. 950 µl calcium chloride solutions were added to 1ml aqueous extract (100mg/ml) in 3ml test tubes. Crystallization was started by adding 950 µl of sodium oxalate. The absorbance (optical density, OD) was recorded at 620 nm after 30 minutes and hours of incubation at room temperature[6]. The test was done in triplicate. The formation of the CaOx stone was observed using a microscope for comparison and conformation of dissolution.

Statistical analysis

The inhibitory effect of herb extract on the nucleation growth of calcium oxalate formation was compared with that of the control using an unpaired t-test and one-way ANOVA. P value > 0.05 was considered significant, and the test was done in Minitab 19 Ltd[7]

Result

The result presented in Fig (1)(2) represents the effect of *Phagnalon rupestre* aqueous extract in two different intervals. The mean result between samples was significant (P values < 0.002 and < 0.0043 after 30, 1Hours, respectively) compared to the control.

Light microscope fig(3) confirms the presence of calcium oxalate crystals of interest. The *Phagnalon rupestre* extract observed the highest calcium oxalate crystals dissolution rate compared to the control.



Fig. 1 Microscopic examination 100X of calcium oxalate dissoaltion (Arrow) (Left treated with *Phagnalon rupestre* extract 1Hrs showing crystal Deformation) (Right calcium oxalate without treatment in Normal Crystal)

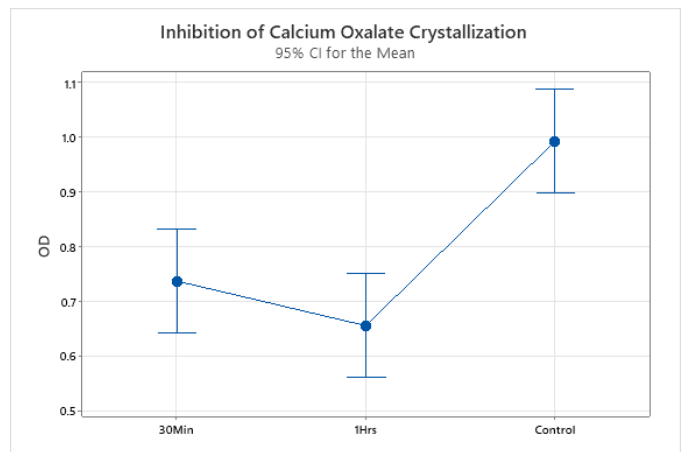


Fig.2 Means for inhibition of calcium Oxalate crystallization

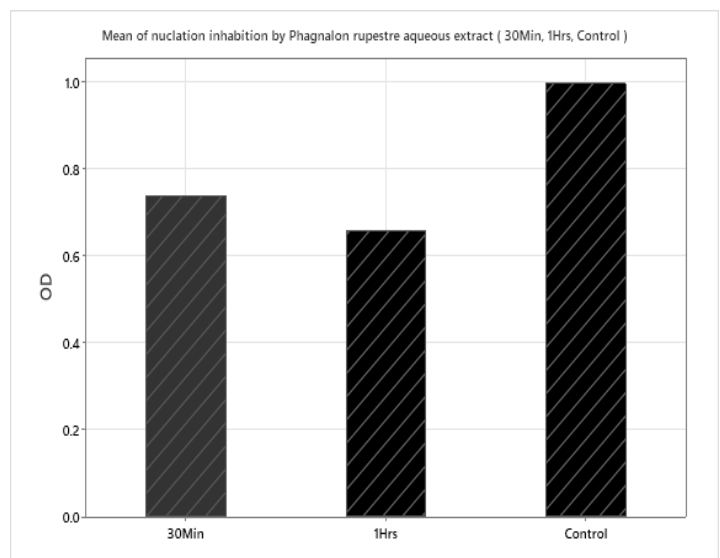


Fig.3 Inhibition of nucleation by *Phagnalon rupestre* at 30 min – 1Hrs in comparison to control

Discussion

To ascertain the development and crystallization of CaOx, we used a traditional model of synthetic urine supersaturated with calcium chloride and sodium oxalate. Human normal urine is not a static mixture since new solutes are continuously added to and removed. The formation of crystals in synthetic urine in a static environment can, to some extent, be beneficial for explaining the growth of urinary calculi in humans; however, it is challenging to duplicate the urinary system in vitro[8].

We use crystal aggregation because it is the most important process. It happens quickly, significantly affects particle size, and is frequently observed in urine and renal stones[9].

In the present result, the aqueous extract showed a significant dissolution of calcium oxalate crystal, especially at 1 hour. To our knowledge, no previous work has been done for the same Phagnalon spp. However, several studies[10, 11] indicate that Phagnalon spp contains a high level of polyphenolic constituents, e.g., alkaloids, flavonoids, saponins, terpenoids, and Saponins which are known[12, 13] to have anti-crystallization properties by disaggregating the suspension of mucoproteins, the promoters of crystallization. Moreover, these result comes In agreement with other reports despite the species differences. The results of the nucleation assay confirmed that the extract contained nucleation-preventing agents.

Controlling the crystallization process is the most important for the stone formation, i.e., nucleation is the best way to prevent and treat urolithiasis. This can be achieved by using plant extracts like *Phagnalon rupestre* since they have been widely used in Libyan traditional medicine[14] for many years to treat kidney stones. Generally, plant extracts consist of a unique mixture of numerous bioactive compounds that can be extracted with various techniques.

Conclusion

Our results demonstrate that an extract of *Phagnalon rupestre* inhibits the nucleation of calcium oxalate crystals. The extract of *Phagnalon rupestre* may contain substances that inhibit calcium oxalate crystal aggregation. Therefore, the extract might be beneficial in preventing and treating kidney stone formation. Future work will focus on testing the extract in vivo and confirming our findings in an animal model.

Conflict of interest

The authors declare no conflict of interest

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