

Lycopodium clavatum as an inhibitor of monosodium urate crystallisation in gout: An *in vitro* study

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Abstract

Background: Hyperuricaemia plays a significant role in the development and pathogenesis of several metabolic and systemic disorders including metabolic syndrome, hypertension, stroke and atherosclerosis. *Lycopodium clavatum* is the most widely used drug in homoeopathy for treating hyperuricaemia and gout. However, its mechanism of action in reducing serum uric acid remains uncertain. **Objective:** The objective of the study was to study the potential role of homoeopathic preparation of *Lycopodium clavatum* in different potencies on monosodium urate crystallisation *in vitro*. **Methods:** Spectrophotometric crystallisation assay was carried out on a stock solution of 5 ml of uric acid after its preparation. The time course of the optical density was measured in a standard solution and values were measured every 5 min after agitation with cyclo-vortex mixer. The optical density values were also measured in the homoeopathic preparation of *Lycopodium clavatum* in different potencies with same method. Inhibiting effect of this medicine was found from graphs by measuring slope of nucleation and aggregation from optical density value and the percentage inhibition exerted by the proteins was calculated. **Results:** Spectrophotometric analysis showed all potencies of *Lycopodium clavatum* inhibited aggregation of monosodium urate crystals with a maximum inhibition at 200C and 1M potency. **Conclusion:** We found that the homoeopathic medicine *Lycopodium clavatum* could be effective in inhibiting the formation of monosodium urate crystals *in vitro* and also causes the dissolution of crystals, especially in high potencies. Further studies are required to understand the exact mechanism of action.

Keywords: Gout, Hyperuricaemia, *In vitro*, *Lycopodium clavatum*, Monosodium urate crystal

INTRODUCTION

Hyperuricaemia is a condition where plasma (or serum) urate concentration is >420 $\mu\text{mol/L}$ (7.0 mg/dL).^[1] It can result from increased production or decreased excretion of uric acid. This increased production is mainly due to altered purine metabolism. Uric acid is a weak acid, so it gets ionised easily to form urates. These urates predominate in plasma extracellular fluid and synovial fluid. At a pH of 7.4, 98% of this urate exists as monosodium urate.^[1] When the concentration of uric acid in plasma increases, it gets supersaturated, forming monosodium urate crystals.^[1]

Deposition of these crystals results in complications such as gouty arthritis, nephrolithiasis, urate nephropathy and uric acid nephropathy. Hyperuricaemia has also been reported as an independent risk factor for metabolic syndrome, obesity, diabetes, stroke and atherosclerotic disease.^[1]

The incidence of hyperuricaemia has increased dramatically in recent decades. From 2006 to 2014, the prevalence of

hyperuricaemia increased from 19.7% to 25.0% among men and from 20.5% to 24.1% among women.^[2]

In the stage of asymptomatic hyperuricaemia, people may not experience any symptoms, but the crystals get deposited in and around the joints slowly. Asymptomatic hyperuricaemia is also a valuable biomarker for predicting the development of cardiometabolic and renal diseases.^[3]

Pharmacologic treatment for asymptomatic hyperuricaemia carries some risks and is not considered beneficial or cost effective and generally is not recommended. Also, there are adverse effects and contraindications for the urate-lowering

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Received: 27 August 2021; **Accepted:** 25 November 2022

Access this article online

Quick Response Code:

Available in print version only

Website:
www.ijrh.org

DOI:
10.53945/2320-7094.1055

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How to cite this article: Perveen PS, Ali KRM, Veluthoor S, Jijith US, Nithya T, Afiya Alloor, Shijil VK. *Lycopodium clavatum* as an inhibitor of monosodium urate crystallisation in gout: An *in vitro* study. Indian J Res Homoeopathy 2022;16(4):263-268.

drugs even in acute cases.^[3] Homoeopathic treatment for uric acid, on the other hand, has no side effects and is safe to use.^[4] Febuxostat is a urate-lowering drug used frequently and this is used as our positive control for comparing with our medicines.

An observational study was conducted on 150 diagnosed cases of gout, where 14.67% of patients were treated with *Lycopodium clavatum*. This study concluded that homoeopathic treatment is very effective in reducing clinical symptoms and serum uric acid levels in subjects having gout.^[5] Homoeopathic treatment is used by various homoeopathic practitioners all over the world, but there are few studies to show its efficiency in hyperuricaemia.

This study is an effort to prove the action of *Lycopodium clavatum* on monosodium urate crystals. There are several reports that this plant contains vanillic, coumaric, ferulic acids and syringic acids (glycoalkaloids).^[6] Further study yielded alkaloids lycopodine as major alkaloid, clavatine and clavatoxine; polyphenolic acids, including dihydrocaffeic and triterpenes (*Lycopodium* potential).^[7]

MATERIALS AND METHODS

Drugs and chemicals

Homoeopathic preparations of *Lycopodium clavatum*^[8] mother tincture (Q), 6C, 12C, 30C, 200C, 1M and 10M) were procured from the pharmaceutical manufacturers, SBL India Pvt. Ltd., New Delhi, India. All other chemicals and reagents used were of analytical grade (Merck company, India).^[9]

Three controls were taken, one of distilled water, second of vehicle that is, rectified spirit and third of febuxostat as a positive control.

Spectrophotometric crystallisation assay

Preparation of a 100 mg/L stock solution of uric acid

A uric acid standard solution was prepared by mixing 100 mg uric acid in a volumetric flask. About 100 µL of 0.6 N NaOH was added to help dissolve the uric acid. Then, pH was adjusted to 7.4 and the solution was then made up to 1 L.^[10]

It was then filtered through a 0.22 µm pore Millipore membrane. A 5 mL of sample of uric acid stock solution was placed into 10 mm path length cuvette holder and standard optical density was measured using a spectrophotometer with wavelength 300 nm. Then, values were measured every 5 min after agitation with cyclo-vortex mixer. Spectrophotometric crystallisation assay was also carried out for the homoeopathic medicines using the same technique.

The time course of the optical density at 300 nm was measured automatically using a UVIKON 930 Spectrophotometer and values were also measured in the 100 µL of *Lycopodium* (Q, 6C, 12C, 30C and 200C, 1M, 10M). Independent experiments were conducted on each potency at least 3 times. OD620 (optical density) increases initially during the nucleation phase and decreases during the aggregation phase. 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 proteins was calculated using the formula:

Percentage inhibition = $(1 - S_m/S_c) \times 100$ where, S_m is the slope in the presence of the protein and S_c the slope of the control.^[11] This basic research followed reporting experiments in homoeopathic basic research (REHBaR) guidelines.^[12]

RESULTS

The mean ± SE was calculated of each optical density of different potencies after conducting independent experiments at least 3 times.

Figure 1 shows a standard graph from the values of optical density (O.D) obtained from spectrophotometer by keeping solution of monosodium urate crystals without additives. An increase in the slope of O.D indicates crystal formation and increase in particle number and thus indicates nucleation. After nucleation, when the solution gets saturated, equilibrium is reached. Then, there is a decrease in O.D with time which indicates a decrease in particle number due to crystal aggregation. Thus, the slope of O.D with time indicates crystal aggregations and can be used as a standard for all comparisons.

Figure 1 graph also shows the crystal inhibiting effect of *Lycopodium clavatum* 6C, 12C, 30C, 200C, 1M, 10M and control on nucleation and aggregation of crystals compared with standard graph.

Figure 2 shows the effect of homoeopathic medicine *Lycopodium clavatum* tincture and ethanol (negative control), positive control, 200 and 1M on nucleation and aggregation of crystals comparing with the standard graph.

Figure 3 shows that nucleation and aggregation of different potencies of *Lycopodium clavatum* calculated using the percentage inhibition formula are represented in a graph.

Here, the graph shows that all the potencies of homoeopathic medicine *Lycopodium clavatum* 6C, 12C, 30C, 200C, 1M, 10M and tincture prevented the aggregation, while mother tincture showed comparatively lesser aggregation. Maximum inhibition was observed in 200C and 1M potencies that

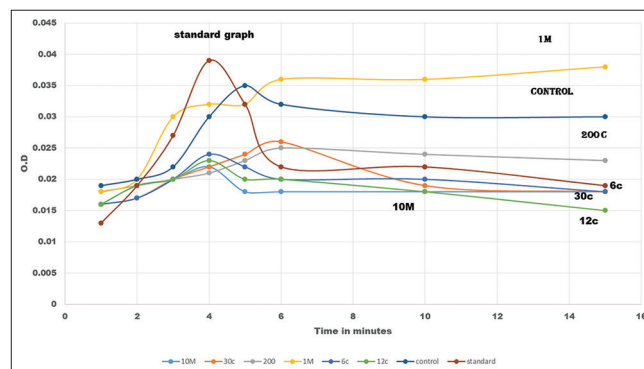


Figure 1: Values of optical density of different potencies and control compared to the standard

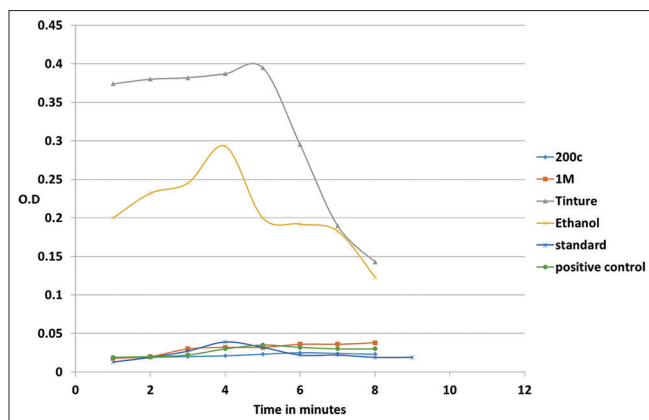


Figure 2: Values of optical density of the tincture, different potencies and controls compared to the standard

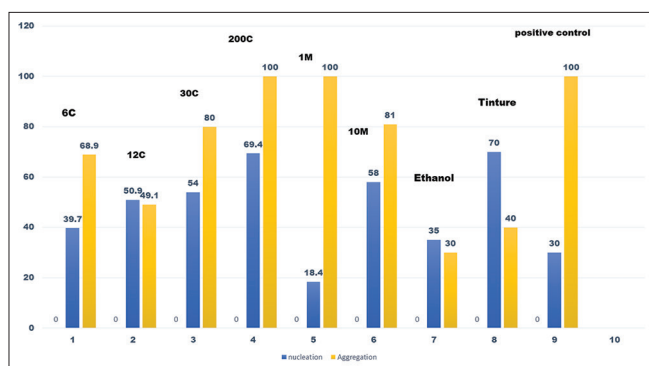


Figure 3: Nucleation and aggregation of *Lycopodium clavatum* potencies and controls

is, they showed aggregation more than 100 similar to the positive control. *Lycopodium* 1M showed very little nucleation. This indicates that homoeopathic medicine *Lycopodium clavatum*, especially 1M potency, prevents the formation of crystals.

DISCUSSION

Earlier, *in vitro* studies conducted on crystals showed the efficiency of homoeopathic medicine, *Berberis vulgaris* on calcium oxalate crystallisation and results showed that it could prevent the early phase of crystallisation.^[13] In homoeopathy, there are many medicines known to reduce uric acid levels, but *Lycopodium clavatum* is most frequently used. A case report has shown the effect of *Lycopodium clavatum*, the treatment of joint arthritis from hyperuricaemia syndrome in birds.^[14] In the present study, we found that *Lycopodium clavatum*, in various potencies, is effective in inhibiting the nucleation, which suggests that it prevents the formation of monosodium urate crystals, and also inhibits aggregation of crystals, thus promoting the dissolution of crystals.

In addition, the results indicate that high potencies were more effective than low potencies, especially 200C and 1M potencies, which were verified by spectrophotometry. Further

studies are required to understand the exact mechanism of action.

This *in vitro* study was time-bound and we followed the outcomes only for a few days, the results could thus be restricted to an extent. However, since the duration of action of *Lycopodium clavatum* is mentioned as 40–50 days in the homoeopathic literature, better results can be expected on clinical front, especially if the medicine is prescribed on the basis of individualisation, and future studies on this front may be considered. Further studies must be conducted on the basis of light or electron microscopy to understand the structure of crystals to generalise the findings that *Lycopodium clavatum* can cause dissolution of crystals, thus helping alleviating the symptoms of gout and preventing formation of tophi.

CONCLUSION

Thus, in the present study, we found that the homoeopathic medicine *Lycopodium clavatum* could be effective in inhibiting the formation of monosodium urate crystals *in vitro* and also causes the dissolution of crystals, especially in high potencies. Further studies are required to understand the exact mechanism of action.

ACKNOWLEDGEMENT

Authors would like to express thanks to biochemical laboratory core valleys herbal technologies Pvt. Ltd., for providing biochemist and necessary laboratory facilities.

Financial support and sponsorship

Nil.

Conflicts of interest

None declared.

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***Lycopodium clavatum* comme inhibiteur de la cristallisation de l'urate monosodique dans la goutte: Une étude in vitro**

Contexte: L'hyperuricémie joue un rôle important dans le développement et la pathogenèse de plusieurs troubles métaboliques et systémiques, notamment le syndrome métabolique, l'hypertension, les accidents vasculaires cérébraux et l'athérosclérose. *Lycopodium clavatum* est le médicament le plus largement utilisé en homéopathie pour traiter l'hyperuricémie et la goutte. Cependant, son mécanisme d'action dans la réduction de l'acide urique reste incertain. **Objectif:** L'objectif de l'étude était d'étudier le rôle potentiel de la préparation homéopathique de *Lycopodium clavatum* à différentes puissances sur la cristallisation de l'urate monosodique in vitro. **Méthodes:** Un test de cristallisation spectrophotométrique a été réalisé sur une solution mère de 5 ml d'acide urique après sa préparation. L'évolution dans le temps de la densité optique a été mesurée dans une solution standard et les valeurs ont été mesurées toutes les 5 minutes après agitation avec un mélangeur cyclo-vortex. Les valeurs de densité optique ont également été mesurées dans la préparation homéopathique de *Lycopodium clavatum* à différentes puissances avec la même méthode. L'effet inhibiteur de ce médicament a été déterminé à partir de graphiques en mesurant la pente de la nucléation et de l'agrégation à partir de la valeur de la densité optique et le pourcentage d'inhibition exercé par les protéines a été calculé. **Résultats:** L'analyse spectrophotométrique a montré que toutes les puissances de *Lycopodium clavatum* ont inhibé l'agrégation des cristaux d'urate monosodique avec une inhibition maximale à 200C et des puissances de 1M. **Conclusion:** Nous avons constaté que le médicament homéopathique *Lycopodium clavatum* pouvait être efficace pour inhiber la formation de cristaux d'urate monosodique in vitro et également provoquer la dissolution des cristaux, en particulier à forte puissance. Des études supplémentaires sont nécessaires pour comprendre le mécanisme d'action exact.

***Lycopodium clavatum* als Hemmstoff der Kristallisation von Mononatriumurat bei Gicht: Eine in vitro Studie**

Hintergrund: Hyperurikämie spielt eine wichtige Rolle bei der Entwicklung und Pathogenese verschiedener Stoffwechsel- und Systemerkrankungen wie metabolisches Syndrom, Bluthochdruck, Schlaganfall und Atherosklerose. *Lycopodium clavatum* ist das in der Homöopathie am häufigsten verwendete Arzneimittel zur Behandlung von Hyperurikämie und Gicht. Sein Wirkmechanismus bei der Senkung der Serumharnsäure ist jedoch noch unklar. **Zielsetzung:** Ziel der Studie war es, die potenzielle Rolle einer homöopathischen Zubereitung von *Lycopodium clavatum* in verschiedenen Potenzen auf die Kristallisation von Mononatriumurat in vitro zu untersuchen. **Methoden:** Der spektrophotometrische Kristallisationstest wurde mit einer Stammlösung von 5 ml Harnsäure nach deren Herstellung durchgeführt. Der zeitliche Verlauf der optischen Dichte wurde in einer Standardlösung gemessen, und die Werte wurden alle 5 Minuten nach dem Schütteln mit einem Cyclo-Vortex-Mixer gemessen. Die Werte der optischen Dichte wurden auch in der homöopathischen Zubereitung von *Lycopodium clavatum* in verschiedenen Potenzen mit derselben Methode gemessen. Die hemmende Wirkung dieses Arzneimittels wurde anhand von Diagrammen ermittelt, indem die Steigung der Keimbildung und Aggregation anhand der optischen Dichte gemessen und die prozentuale Hemmung durch die Proteine berechnet wurde. **Ergebnisse:** Die spektrophotometrische Analyse zeigte, dass alle Potenzen von *Lycopodium clavatum* die Aggregation von Mononatriumuratkristallen hemmten, mit einer maximalen Hemmung bei 200C und 1M Potenzen. **Schlussfolgerung:** Wir haben festgestellt, dass das homöopathische Arzneimittel *Lycopodium clavatum* die Bildung von Mononatriumurat-Kristallen in vitro wirksam hemmen und auch die Auflösung der Kristalle bewirken kann, insbesondere in hohen Potenzen. Weitere Studien sind erforderlich, um den genauen Wirkmechanismus zu verstehen.

गठिया में मोनोसोडियम यूरेट क्रिस्टलीकरण के अवरोधक के रूप में लाइकोपोडियम क्लैवेटम: एक इन विट्रो अध्ययन

पृष्ठभूमि: हाइपरयूरिसीमिया चयापचय सिंड्रोम, उच्च रक्तचाप, हृदयाघात और एथेरोस्क्लेरोसिस सहित कई चयापचय और दैहिक विकारों के विकास और रोगजनन में महत्वपूर्ण भूमिका निभाता है। होम्योपैथी में हाइपरयूरिसीमिया और गठिया के इलाज के लिए लाइकोपोडियम क्लैवेटम सबसे व्यापक रूप से इस्तेमाल की जाने वाली दवाई है। हालांकि, सीरम यूरिक एसिड को कम करने में इसकी कार्यप्रणाली पर अनिश्चिता बनी हुई है। **उद्देश्य:** अध्ययन का उद्देश्य कृत्रिम परिवेश में होम्योपैथिक औषधि लाइकोपोडियम क्लैवेटम की विभिन्न पोटेंसियों में मोनोसोडियम यूरेट क्रिस्टलीकरण पर संभावित भूमिका का अध्ययन करना था। **विधि:** इसकी तैयारी के बाद 5 मिलीलीटर यूरिक एसिड के मूल विलयन पर स्पेक्ट्रोफोटोमेट्रिक क्रिस्टलीकरण की परख की गई। यूरिक अम्ल के बनाने के पश्चात 5 मिली. मूल विलयन पर स्पेक्ट्रोफोटोमीट्रिक क्रिस्टलीकरण जांच विधि द्वारा अध्ययन किया गया। मूल विलयन को हिलाने के बाद साइक्लो-भंवर मिक्सर द्वारा 5 मिनट के अंतराल पर प्रकाशीय घनत्व को मापा गया। लाइकोपोडियम क्लैवेटम की होम्योपैथिक औषधियों की विभिन्न पोटेंसियों में प्रकाशीय घनत्व को भी एक ही विधि के साथ विभिन्न शक्तियों में मापा गया था। न्यूक्लियेशन के ढलान को मापने और ऑप्टिकल घनत्व मूल्य से एकत्रीकरण द्वारा ग्राफ से इस दवा का अवरोधक प्रभाव पाया गया और प्रोटीन द्वारा लगाए गए प्रतिशत अवरोध की गणना की गई। **परिणाम:** स्पेक्ट्रोफोटोमेट्रिक विश्लेषण ने लाइकोपोडियम क्लैवेटम की सभी पोटेंसियों को मोनोसोडियम यूरेट क्रिस्टल के एकत्रीकरण को 200C और 1M पोटेंसी पर अधिकतम अवरोध के साथ दिखाया। **निष्कर्ष:** हमने पाया कि होम्योपैथिक दवा लाइकोपोडियम क्लैवेटम इन विट्रो में मोनोसोडियम यूरेट क्रिस्टल के गठन को रोकने में प्रभावी हो सकती है और विशेष रूप से उच्च शक्ति में क्रिस्टल के विघटन का कारण भी बन सकती है। कार्रवाई के सटीक तंत्र को समझने के लिए आगे के अध्ययन की आवश्यकता है।

***Lycopodium clavatum* como inhibidor de la cristalización del urato monosódico en la gota: Estudio in vitro**

Fundamento: La hiperuricemia desempeña un papel importante en el desarrollo y la patogénesis de varios trastornos metabólicos y sistémicos, como el síndrome metabólico, la hipertensión, el accidente cerebrovascular y la aterosclerosis. *Lycopodium clavatum* es el fármaco más utilizado en la homeopatía para tratar la hiperuricemia y la gota. Sin embargo, su mecanismo de acción para reducir el ácido úrico en suero sigue siendo incierto. **Objetivo:** El objetivo del estudio fue estudiar el papel potencial de la preparación homeopática de *Lycopodium clavatum* en diferentes potencias sobre la cristalización in vitro del urato monosódico. **Métodos:** El ensayo espectrofotométrico de cristalización se realizó sobre una solución madre de 5 ml de ácido úrico tras su preparación. El curso temporal de la densidad óptica se midió en una solución estándar y los valores se midieron cada 5 minutos después de la agitación con un mezclador cyclo-vórtex. Los valores de densidad óptica también se midieron en la preparación homeopática de *Lycopodium clavatum* en diferentes potencias con el mismo método. El efecto inhibidor de este medicamento se encontró a partir de gráficos midiendo la pendiente de nucleación y agregación a partir del valor de la densidad óptica y se calculó el porcentaje de inhibición ejercido por las proteínas. **Resultados:** El análisis espectrofotométrico mostró todas las potencias de *Lycopodium clavatum* inhibían la agregación de cristales de urato monosódico con una inhibición máxima a 200C y 1M potencias. **Conclusión:** Encontramos que la medicina homeopática *Lycopodium clavatum* podría ser eficaz en inhibir la formación in vitro de cristales de urato monosódico y también causar la disolución de cristales, especialmente en altas potencias. Se requieren más estudios para comprender el mecanismo exacto de acción.

狼牙棒作为痛风患者尿酸单钠结晶的抑制剂。一项体外研究

背景介绍:高尿酸血症在一些代谢性和系统性疾病的发展和发病机制中起着重要作用,包括代谢综合征、高血压、中风和动脉硬化。· 枸杞子 克拉瓦特是顺势疗法中最广泛使用的药物,用于治疗高尿酸血症和痛风。然而,其降低血清尿酸的作用机制仍不确定。· 目标:该研究的目的是研究不同药效的狼牙棒对体外尿酸单钠结晶的潜在作用。· 方法:分光光度法结晶测定是在制备5毫升尿酸原液后进行的。在标准溶液中测量光密度的时间过程,用旋涡混合器搅拌后每5分钟测量一次数值。· 用同样的方法也测量了不同药效的枸杞子克拉瓦特的同种制剂的光密度值。通过测量光密度值的成核和聚集的斜率,从图表中发现这种药物的抑制作用,并计算出蛋白质的抑制百分比。· 结果:分光光度法分析显示,狼牙棒的所有效力都能抑制单钠尿酸盐晶体的聚集,在200C和1M的效力下,抑制作用最大。· 总结:我们发现,顺势疗法药物枸杞子 克拉瓦特可以有效地抑制体外尿酸单钠晶体的形成,也可以引起晶体的溶解,特别是在高药效下。· 需要进一步研究以了解确切的作用机制。