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.

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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 umol/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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drugs even in acute cases.\textsuperscript{[1]} Homoeopathic treatment for uric acid, on the other hand, has no side effects and is safe to use.\textsuperscript{[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 \textit{Lycopodium clavatum}. This study concluded that homoeopathic treatment is very effective in reducing clinical symptoms and serum uric acid levels in subjects having gout.\textsuperscript{[3]} 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 \textit{Lycopodium clavatum} on monosodium urate crystals. There are several reports that this plant contains vanillic, coumaric, ferulic acids and syringic acids (glycoalkaloids).\textsuperscript{[9]} Further study yielded alkaloids lycopodine as major alkaloid, clavatine and clavatoxine; polyphenolic acids, including dihydrocaffeic and triterpenes (\textit{Lycopodium potential}).\textsuperscript{[7]}

\section*{Materials and Methods}

\subsection*{Drugs and chemicals}

Homoeopathic preparations of \textit{Lycopodium clavatum}\textsuperscript{\(d\)} 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).\textsuperscript{[9]}

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

\subsection*{Spectrophotometric crystallisation assay}

\textbf{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 \(\mu\)L 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.\textsuperscript{[10]}

It was then filtered through a 0.22 pm 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 \(\mu\)L of \textit{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:

\[
\text{Percentage inhibition} = \left(1 - \frac{Sm}{Sc}\right) \times 100
\]

where, Sm is the slope in the presence of the protein and Sc the slope of the control.\textsuperscript{[5]} This basic research followed reporting experiments in homoeopathic basic research (REHBaR) guidelines.\textsuperscript{[12]}

\section*{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 \textit{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 \textit{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 \textit{Lycopodium clavatum} calculated using the percentage inhibition formula are represented in a graph.

Here, the graph shows that all the potencies of homoeopathic medicine \textit{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

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Values of optical density of different potencies and control compared to the standard}
\end{figure}
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 the 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.

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**Conflicts of interest**

None declared.

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Perveen, et al.: Lycopodium as an inhibitor of monosodium urate crystallisation in gout

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Lycopodium clavatum as an inhibitor of monosodium urate crystallisation in gout

Contexte: L'hyperuricémie joue un rôle important dans le développement et la pathogénèse 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 sérique 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 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.