

Cholesterol lowering activity of homoeopathic preparations of *Colchicum autumnale* – An *in vitro* study

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Abstract

Background: Total cholesterol content of erythrocyte membranes plays a critical role in atherosclerotic plaque progression and instability. Several homoeopathic medicines are known to have a significant role in the control of hyperlipidaemia. *Colchicum autumnale* is a plant remedy used mainly for gout, but also for hyperlipidaemia. **Objectives:** This is a study to assess the *in vitro* anti-cholesterol action of the *Colchicum autumnale* (*Colch. at.*) on the erythrocyte membrane. **Materials and Methods:** The study was conducted using a hyperlipidaemic blood sample which was divided into eight parts. Six different potencies of *Colch. at.* were tested, and two controls, one negative and another positive were used. Cholesterol was estimated using Liberman Burchard reagent and spectrophotometry. **Results:** In a hyperlipidemic blood sample, the range of reduction of cholesterol concentration was from 18.20% to 19.16%. The 6C potency removed 18.24% of cholesterol, 12C potency removed 18.50% cholesterol, 30C potency removed 19.02% cholesterol, 200C potency removed 19.68% cholesterol and 10M potency removed 19.16% cholesterol. The maximum effect was seen in 1M potency where the reduction was 19.68%. **Conclusion:** This study suggests that the *Colchicum autumnale* might be useful for the management of hypercholesterolemia.

Keywords: Cholesterol, *Colchicum autumnale*, Hyperlipidaemia, *In vitro*

INTRODUCTION

Hyperlipidaemia is estimated to cause 2.6 million deaths (4.5% of total) and 29.7 million disability-adjusted life years (DALYs), or 2.0% of total DALYS. It may be the major explanation for disease burden in both the developed and developing countries as a risk factor for ischemic heart condition and stroke.^[1]

India is undergoing a rapid epidemiological transition with increasing population, economic prosperity, urbanization and ageing with associated risk factor transition.^[2] About 79% of the population had abnormalities in one of the lipid parameters. According to the Indian Council of Medical Research-INDIA diabetes study, hyperlipidaemia is more common in rural and urban areas. Indians are known to have a unique pattern of dyslipidaemia with lower high-density lipoprotein cholesterol, increased triglyceride levels and higher proportion of low-density lipoprotein cholesterol, and there have been no large-scale representative studies on dyslipidaemia to assess the magnitude of the problem.^[3]

Many studies suggest that the total cholesterol content of erythrocyte membranes also might play a critical role in atherosclerotic plaque progression and instability.^[2]

Total cholesterol level is the widely used measurement for diagnosing hypocholesterolemia.^[3]

The main aim of treatment in patients with hyperlipidaemia is to scale back the danger of developing ischemic heart disease or the occurrence of further cardiovascular or cerebrovascular disease. In conventional treatment, statins and fibrates are common lipid-modulating agents, and a more modern lipid-lowering agent, alirocumab (Praluent), has recently been approved for the treatment of dyslipidaemia.^[4]

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Received: 27 August 2021; **Accepted:** 26 August 2022

Access this article online

Quick Response Code:

Available in print version only

Website:
www.ijrh.org

DOI:
10.53945/2320-7094.1053

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How to cite this article: Nithya T, Ali KRM, Veluthoor S, Jijith US, Perveen PS, Alloor A, Shijil VK. Cholesterol lowering activity of homoeopathic preparations of *Colchicum autumnale* – An *in vitro* study. Indian J Res Homoeopathy 2022;16(3):211-215.

At present, available hypolipidaemic drugs are related to a variety of side effects. The consumption of synthetic drugs results in hyperuricemia, diarrhoea, nausea, myositis, gastric irritation, flushing, dry skin and abnormal liver function.^[5] Moreover, several herbal drugs are also advocated for hypolipidaemic actions.^[3] Due to these concerns, it is necessary to develop alternative natural therapies against hyperlipidaemia.

Plant products are frequently considered to be less toxic and free from side effects than synthetic ones. Plants play a serious role in the introduction of new therapeutic agents and have received much attention as sources of biologically active substances including antioxidants, hypoglycaemics and hypolipidaemics.^[3]

Homoeopathic medicines are prepared from traces of animal, plant, mineral and other natural substances, by standard methods called dynamisation or potentization, which comprises of successive dilutions and succussions.^[6]

Colchicum autumnale is a biennial plant under the lileacea family. The active principle, colchicine, is a bitter poisonous alkaloid. It is frequently used as an ingredient in many medicines especially used for gout and rheumatic complaints.^[7] It also contains some bioactive substances like polyphenols, the foremost abundant (poly)-phenolic compounds are lignans, flavonoids, phenolic acids and tannins. This reveals that it has significant antioxidant activity.^[8]

The first proving of *Colch. at.* was carried out by Anton Storck before Hahnemann and several symptoms were produced in a healthy man. Staff and Von Reil followed a systematic proving. The experiment was carried out on 17 provers, of whom ten were men and seven women, using the potencies: 6X, 6C and 30C. Dr. Othon Andre Julian from materia medica of new homoeopathic medicine has also mentioned about its effect on hyperlipidaemia.^[9] *Colch. at.* is also added by Dr. Frederick Schroyens in the generalities chapter of synthesis repertory under the rubric hyperlipidaemia.^[10]

There have been various *in vitro* experiments performed on the plant, animal and human cellular models to plausibly explaining the action of these ultra-high diluted medicines.^[11] There are only a few *in vitro* and clinical verification studies conducted in homoeopathy on hyperlipidaemia.^[12] Therefore, this study is an attempt to evaluate the action of *Colch.at.* in different potencies in hyperlipidaemic samples through an *in vitro* assay.

The amount of cholesterol can be determined using the colour reagent method measuring the optical density or absorbance of the coloured complex.^[13]

MATERIALS AND METHODS

Study setting

The study was conducted at Core Valleys Herbal Technologies Pvt. Ltd., laboratory. Nallalam, Kozhikode, Kerala, India.

Study design

An *in vitro* study was conducted with six potencies of a homoeopathic medicine, *Colch. at* in hyperlipidaemic blood

sample. The sample was divided into eight equal parts. One was taken as positive control using atorvastatin and another as negative control using ethanol, while the rest were tested using 6C, 12C, 30C, 200C, 1M and 10M potencies of *Colch.at.*

Drugs and chemicals

Homoeopathic preparations of *Colch.at.* with different potencies (6C, 12C, 30C, 200C, 1M and 10M) were obtained from standard GMP-certified manufacturer of homoeopathic medicines (SBL, GMP and ISO 9001 certified Company). Liebermann Burchard reagent was standardised using 0.5ml acetic anhydride and 0.5 mL H₂SO₄. All other chemicals and reagents were of analytical grade. A hyperlipidaemic blood sample was collected from the medical laboratory of Government Homoeopathic Medical College.

Methods

The study was conducted using a hyperlipidaemic blood sample of a patient diagnosed with hyperlipidaemia with an initial concentration of total cholesterol of 210 mg/dL. The duration of the study was 2 days. Experiments were standardized in the laboratory with the help of a biochemist. As an anticoagulant agent, EDTA was added. Blood was centrifuged using a centrifuging tube at 1000 rpm for 5 min to separate plasma and red blood cells. After washing with distilled water, the glass wares were dried using an oven. 100 mg of red blood cell was weighed and taken in a dried test tube and diluted using 20 mL of water and converted to 500 mL/dL red blood cell. 2 mL of this solution was taken in eight dried test tubes, respectively. Among these one test tube was taken negative control using ethanol, and another was taken as a positive control using atorvastatin (5 mg). Moreover, the remaining test tubes were added with 0.5 ml of various six potencies of *Colch. at.*^[14]

Cholesterol estimation using Liebermann Burchard reagent

In each test tube, 1 mL of Liebermann Burchard reagent was added. A greenish-brown colour appeared, and there was a slight change in the intensity of colour based on the medicinal reaction [Figure 1]. The reading was taken using ultraviolet Vis spectrophotometry. Optical density was measured by setting 420 nm wavelength.

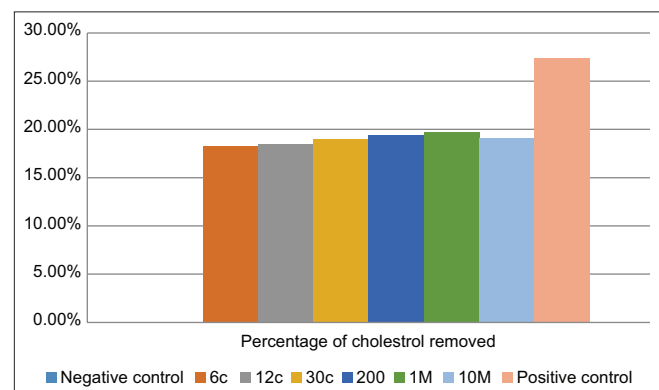


Figure 1: Cholesterol reduction with different potencies of *Colchicum autumnale* in blood sample

RESULTS

After adding *Colch. at.* potencies in hyperlipidaemic blood, the spectrophotometric reading was 0.623 for 6C potency, 0.621 for 12C potency, 0.617 for 30C potency, 0.614 for 200c potency, 0.612 for 1M potency and 0.616 for 10 M potency. The negative control showed the value same as that of the hyperlipidaemic sample and reagent, that is, 0.762 and the positive control showed 0.553.

The percentage of cholesterol removed from each sample was calculated using the following formula:

$$\frac{(\text{Absorbant reading of control} - \text{Absorbant reading of sample})}{(\text{Absorbant reading of control})} * 100$$

In a hyperlipidaemic blood sample, the range of reduction of cholesterol concentration was from 18.20% to 19.16%. 6C potency removed 18.24% of cholesterol, 12C potency removed 18.50% cholesterol, 30C potency removed 19.02% cholesterol, 200C potency removed 19.68% cholesterol and 10M potency removed 19.16% of fats. The maximum effect was seen in 1M potency, where the reduction was 19.68%. Figure 1 shows different potencies of *Colch.at.* and percentage of cholesterol reduction in the hyperlipidaemic sample.

DISCUSSION

A review published in the year 2015 has positively highlighted the role of homoeopathy in dyslipidaemia, suggesting future research on pragmatic and qualitative designs.^[12] Another *in vitro* study conducted on the effect of polyphenols extracts from *Brassica* vegetables on erythrocyte membranes revealed that the cholesterol concentrations in membranes of hypercholesterolemic erythrocytes were lowered after incubation with the extract.^[13]

As mentioned earlier, *Colch. at.* is represented as one mark medicine in the Synthesis repertory.^[9] The present study suggests that *Colch.at.* can reduce cholesterol, most effectively with 1M potency.

In this study, the reduction of cholesterol concentration was from 18.20% to 19.16%. Further studies are needed to identify the hypolipidaemic or hypercholesterolemic activity of *Colch.at.*

CONCLUSION

The study suggests that *Colch. at.* might be useful in the treatment of hypercholesterolemia. Further studies are needed to identify the mode of action, as well as effect, of *colchicum autumnale* in hyperlipidaemic samples. Further, as this *in vitro* study was for only 2 days, and the duration of action

of this medicine is 14–20 days, more reliable results are expected in clinical trials with follow-ups of longer duration.

ACKNOWLEDGEMENT

Authors are thankful to the research laboratory of the Government Homeopathic Medical College, and Core Valleys Herbal Technologies Pvt Ltd., Kozhikode, Kerala for providing biochemistry and laboratory facilities.

Financial support and sponsorship

Nil.

Conflicts of interest

None declared.

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Activité hypocholestérolémiant des préparations homéopathiques de *Colchicum autumnale* - une étude in vitro

Le contexte: Le contenu en cholestérol total des membranes érythrocytaires (CEM) joue un rôle critique dans la progression et l'instabilité des plaques d'athérosclérose. Plusieurs médicaments homéopathiques sont connus pour leur rôle important dans le contrôle de l'hyperlipidémie. *Colchicum autumnale* est un remède végétal utilisé principalement pour la goutte, mais aussi pour l'hyperlipidémie. **Objectif:** Il s'agit d'une étude visant à évaluer l'activité hypocholestérolémiant in vitro du *Colchicum autumnale*. (Colch. at.) sur la membrane érythrocytaire. **Matériaux et méthodes:** L'étude a été menée en utilisant un échantillon de sang hyperlipidémique qui a été divisé en huit parties. Six puissances différentes de Colch. at. ont été testées, et deux contrôles, un contrôle négatif et un autre positif, ont été utilisés. Le cholestérol a été estimé en utilisant le réactif Liberman Burchard et la spectrophotométrie. **Résultat:** Dans un échantillon de sang hyperlipidémique, l'intervalle de réduction de la concentration de cholestérol était de 18,20 % à 19,16 %. La puissance 6C a éliminé 18,24 % du cholestérol, la puissance 12C a éliminé 18,50 % du cholestérol, la puissance 30C a éliminé 19,02 % du cholestérol, la puissance 200C a éliminé 19,68 % du cholestérol et la puissance 10M a éliminé 19,16 % du cholestérol. L'effet maximal a été observé dans la puissance 1M où la réduction était de 19,68 %. **Conclusion:** Cette étude suggère que le *Colchicum autumnale* pourrait être utile dans la gestion de l'hypercholestérolémie.

Cholesterinsenkende Wirkung von homöopathischen Zubereitungen aus *Colchicum autumnale* - eine In-vitro-Studie

Hintergrund: Der Gesamtcholesteringehalt der Erythrozytenmembranen (CEM) spielt eine entscheidende Rolle bei der Entstehung und Instabilität atherosklerotischer Plaques. Mehrere homöopathische Arzneimittel sind dafür bekannt, dass sie eine wichtige Rolle bei der Kontrolle der Hyperlipidämie spielen. *Colchicum autumnale* ist ein pflanzliches Mittel, das hauptsächlich bei Gicht, aber auch bei Hyperlipidämie eingesetzt wird. **Zielsetzung:** Dies ist eine Studie zur Bewertung der cholesterinsenkenden Aktivität von *Colchicum autumnale*. (Colch. at.) auf die Erythrozytenmembran. **Materialien und Methoden:** Für die Studie wurde eine Blutprobe von Hyperlipidämikern verwendet, die in acht Teile aufgeteilt war. Es wurden sechs verschiedene Potenzen von Colch. at. getestet und zwei Kontrollen, eine Negativkontrolle und eine Positivkontrolle, verwendet. Cholesterin wurde mit dem Liberman-Burchard-Reagenz und Spektralphotometrie bestimmt. **Ergebnis:** In einer hyperlipidämischen Blutprobe lag die Spanne der Cholesterinreduzierung zwischen 18,20% und 19,16%. Die 6C-Potenz entfernte 18,24% Cholesterin, die 12C-Potenz 18,50% Cholesterin, die 30C-Potenz 19,02% Cholesterin, die 200C-Potenz 19,68% Cholesterin und die 10M-Potenz 19,16% Cholesterin. Die maximale Wirkung wurde in der 1M-Potenz beobachtet, wo die Reduzierung 19,68 % betrug. **Schlussfolgerung:** Diese Studie legt nahe, dass *Colchicum autumnale* für die Behandlung von Hypercholesterinämie nützlich sein könnte.

कोलचीकम ऑटोमनेल को होम्योपैथिक तरीके से तैयार करने के दौरान कॉलेस्ट्रॉल घटाना - एक इन विट्रो अध्ययन

परिचय: इराथरोसाइट मेंबरेस (सीईएम) की कुल कॉलेस्ट्रॉल मात्रा एथिरोस्केलोरोटिक प्लाक को बढ़ने देने एवं अस्थिर करने में महत्वपूर्ण भूमिका निभाती है। हाइपरलिपिडेमिया की रोकथाम में कई होम्योपैथिक दवाइयाँ महत्वपूर्ण भूमिका अदा करती हैं। कोलचीकम ऑटोमनेल एक पौध औषधि है जिसका इस्तेमाल मुख्यतः गठिया के साथ-साथ, हाइपरलिपिडेमिया के उपचार में भी होता है। **विषय सारांश:** यह वह अध्ययन है जिसके माध्यम से इराथरोसाइट मेंबरेस पर कोलचीकम ऑटोमनेल (कोलच. एट.) की इन विट्रो कॉलेस्ट्रॉल घटाने वाले काम का मूल्यांकन किया जाता है। **सामग्रियाँ एवं विधि:** यह अध्ययन हाइपरलिपिडेमिक खून के नमूने का इस्तेमाल करके संचालित किया गया था जिसे आठ भागों में बांटा गया था। कोलच.एट. की छह अलग-अलग पोटेंसी को जांचा गया था, तथा दो नियंत्रण, एक नकारात्मक एवं दूसरा सकारात्मक नियंत्रण इस्तेमाल किया गया था। लिबरमेन बुरकार्ड अभिकर्मक तथा स्पेक्ट्रोफोटोमेट्री का इस्तेमाल करके कॉलेस्ट्रॉल की मात्रा का आंकलन किया गया था। **परिणाम:** एक हाइपरलिपिडेमिक खून के नमूने में, कॉलेस्ट्रॉल घटाने की मात्रा सीमा 18.20 प्रतिशत से 19.16 प्रतिशत थी। 6सी पोटेंसी ने 18.24 प्रतिशत कॉलेस्ट्रॉल, 12सी पोटेंसी ने 18.50 प्रतिशत कॉलेस्ट्रॉल, 30 सी पोटेंसी ने 19.02 प्रतिशत कॉलेस्ट्रॉल, 200 सी पोटेंसी ने 19.68 प्रतिशत कॉलेस्ट्रॉल तथा 10 एम पोटेंसी ने 19.16 प्रतिशत कॉलेस्ट्रॉल खत्म किया था। सबसे ज्यादा प्रभाव 1 एम पोटेंसी में देखा गया था जहाँ गिरावट 19.68 प्रतिशत थी। **निष्कर्ष:** यह अध्ययन प्रस्तावित करता है कि हाइपरकॉलेस्ट्रॉलेमिया के लक्षणों को रोकने में कोलचीकम ऑटोमनेल उपयोगी साबित हो सकता है।

Actividad de Reducción del Colesterol de Preparaciones Homeopáticas de *Colchicum autumnale* – Un Estudio In Vitro

Antecedentes: El contenido total de colesterol de las membranas eritrocitas (CEM) juega un papel crítico en la progresión y la inestabilidad de la placa aterosclerótica. Se sabe que varios medicamentos homeopáticos tienen un papel significativo en el control de la hiperlipidemia. *Colchicum autumnale* es un remedio vegetal utilizado principalmente para la gota, pero también para la hiperlipidemia. **Objetivo:** Estudio para evaluar la actividad de reducción del colesterol in vitro del *Colchicum autumnale*. (Colch. At.) en la membrana eritrocítica. **Materiales y métodos:** El estudio se realizó utilizando una muestra de sangre hiperlipidémica que se dividió en ocho partes. Seis potencias diferentes de Colch. en. se probaron dos controles, uno negativo y otro positivo. El colesterol se estimó usando reactivo de Liberman Burchard y espectrofotometría. **Resultado:** En una muestra de sangre hiperlipidémica, el rango de reducción de la concentración de colesterol fue de 18,20% a 19,16%. La potencia 6C quitó el 18,24% del colesterol, 12C potencia quitó el 18,50% del colesterol, 30C potencia quitó el 19,02% del colesterol, 200C potencia

quitó el 19,68% del colesterol y 10M potencia quitó el 19,16% del colesterol. El efecto máximo se observó en la potencia de 1M, donde la reducción fue del 19,68 %. **Conclusión:** Este estudio sugiere que el *Colchicum autumnale* podría ser útil para el manejo de la hipercolesterolemia.

秋水仙同型制剂降胆固醇活性的体外研究

背景资料: 红细胞膜 (CEM) 的总胆固醇含量在动脉粥样硬化斑块进展和不稳定性中起关键作用. 已知几种顺势疗法药物在控制高脂血症中具有重要作用. 秋水仙是一种植物疗法, 主要用于痛风, 但也用于高脂血症. **目标:** 这是一项评估秋水仙的体外降胆固醇活性的研究. (教练. 在.) 红细胞膜上. **材料和方法:** 该研究是通过使用高脂血症血液样本进行的, 该血液样本分为八个部分. 科尔切斯特的六种不同效力. 在. 进行了测试, 并使用两个对照, 一个阴性对照和另一个阳性对照. 使用利伯曼*伯查德试剂和分光光度法估计胆固醇. **结果:** 在高脂血症血液样本中, 胆固醇浓度降低的范围为18.20%至19.16%. 6c效价去除了18.24%的胆固醇, 12c效价去除了18.50%的胆固醇, 30C效价去除了19.02%的胆固醇, 200c效价去除了19.68%的胆固醇, 10M效价去除了19.16%的胆固醇. 在1m效力中看到最大效果, 其中减少量为19.68 %. **结论:** 这项研究表明秋水仙可能是高胆固醇血症的管理有用.