



Effects of *Hypericum perforatum* on serum lipid vascular systems in mice

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Abstract

Herbal products are increasingly used against lipid to attenuate its negative impact on vasculature system. However, there are uncertainty regarding the best administration approaches and preparation of herbal product to be used due to variation in absorption rate and extent with subsequent impaction on the lipid levels. The aim of the present study was comparing the pharmacological and analytical aspects of soaked versus powder form of *Hypericum perforatum*; a well-known anti-hyperlipidemic herbal product, using atorvastatin drug as a reference drug for comparison in addition to controlling groups (on/off lipid diet) handled as an additional control group. To conduct this research, the hyperlipidemia mice models were created by exposing the mice to a fatty diet and using *H. perforatum* and atorvastatin for evaluation. The plant extracts were also analyzed for active constituents. The results indicate that the plant analysis detected the presence of different antihyperlipidemic agents and the plant has effectively reduced plasma lipid parameters in exposed mice compared to control group; especially when taken forms of the plant was used. The study concluded that; soaked *H. perforatum* extract shown an effective reduction of lipid parameters and analysis of which revealed the presence of herbal active constituent which might be utilized in industrial pharmacy for new drug innovation.

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Introduction

The vast majority of clinical research has been done aimed at discovering drug treatments to avoid the atherosclerosis formation cycle. The use of herbal and natural medicines increased commonly because of fewer side effects and easier availability (1). The Clusiaceae is a family of about 400 flowering plant species and *Hypericum perforatum* (*H. perforatum*) is one genus of them. Different species of *H. perforatum* occur worldwide with a diverse geographic range including North America, Europe, Asia, Russia, India, and China (2). Hyperforin, hypericin, pseudohypericin, flavonoids, and tannins are considered the main active constituents. *H. perforatum* has several pharmacological activities including antipyretic, analgesic, antispasmodic, antiviral, and antimicrobial activities and its role in the treatment of psychological and neurological disorders such as depression and migraine (3).

In the health insurance industry, efforts have long been made to find a solution for preventing or treating atherosclerosis by managing elevated lipid levels which play an important role in the formation of atheroma plaque (4). Unfortunately, none of these attempts have been fully effective, and the concerns about the safety of prescription drugs have generated a growing desire for patients to use natural cholesterol-lowering products. Also, the scientists are searching for cholesterol-lowering and antiatherogenic activities of various plant products and their active ingredients in order to prevent or regulate atherosclerosis (5,6). Which is a complex inflammatory disease (7,8). And significant risk factors for atherosclerosis are cholesterol and triglyceride; this plays an important role in initiating coronary artery disease and atheroma plaque formation (9). The etiology of these disorders is complicated by oxidative stress. It has been discovered that hyperlipidemia induces oxidative stress, which causes cellular damage (10). Since

atheroma plaque in diseased arteries usually contains inflammatory cells, many people now assume there is a connection between the inflammatory process and coronary atherosclerosis (11).

The effect of different forms of *H. perforatum* (powder and soaked) on cardiovascular disorders were explored in this research. And we have used atorvastatin, a chemical drug in the prevention and treatment of atherosclerosis, to equate its effect with that of HP. Also in this study, the lipid-lowering and antioxidative properties of *H. perforatum* were investigated in terms of lipid profiles and histologically in mice fed a high-fat diet (12).

Materials and methods

Preparation of extracts

The collected plant was dried at room temperature for about 14 days. Then, this was grinded and kept in a dark glass bottle before extraction. The natural products were extracted from the stalk of the *H. perforatum* plant using the continuous soxhlet apparatus method. It is a technique in which different polar solvents are used sequentially to extract the active ingredients from plants and obtaining pure ore free of impurities. Three types of different polarity solvents have been tested petroleum ether, chloroform and ethanol, the separation process was carried out at different temperatures according to the boiling point of each solvent provided that it does not exceed 80°C. The sample was soaked for 24 hours in continuous soxhlet apparatus, as 250 ml of solvent was used for every 20 gm of the ground dried sample, the extraction process lasted 24 hours for each type of solvent until the color of the solvent disappeared, after obtaining the crude extract, it was filtered by filter paper and then evaporated to half its volume by (Rotary Vacuum Evaporator) to obtain concentrated extracts it was placed in tightly closed dark bottles and kept in the refrigerator until the subsequent operations.

High performance liquid chromatography (HPLC)

A weighted quantity of extract was dissolved in HPLC grade methanol for HPLC analysis. The sample was analyzed by HPLC, Quaternary Gradient Pump, Auto sampler model: (S5200), Detector: UV S2340 and column oven. The mobile phase was A and B. In A phase Methanol : DW : Acetic acid at ratio 85:13:2, while B Methanol : DW : Acetic acid at ratio 25:70:5. The column is C18-ODS 25cm*4.6mm and detector UV 280-360 nm at flow rate 1 ml/min (13).

Gas chromatography (GC)

Sample was analyzed by using Gas chromatography device, model (Shimadzu 2010), Japan, using an ionized flame detector and using a capillary separation column in lengths 0.25mm*0.25um*30m where the temperature of the injection area and the detector were 330-280°C respectively. Whereas, the temperature of the separation

column was gradually starting from 120-280°C at a rate of 8 degrees/min). Inert nitrogen gas was used as a carrier gas at a rate of 100KPa (14).

Experimental groups

A healthy adult albino Swiss mice weighing 20-30 g of both sex was randomly divided into five experimental groups of five mice each. Group 1, negative control group, control diet + water ad libitum. Group 2, positive control group, fatty diet + water with 1% H₂O₂. Group 3, hyperlipidemia group, control diet 50% + powder form of *H. perforatum* plant 25% + forage wheat 25% with water ad libitum. Group 4, hyperlipidemia group, control diet + soaked of *H. perforatum* taken orally. Group 5, hyperlipidemia group, control diet + water ad libitum + Atorvastatin drug 7mg/kg orally. The dried plant was grinded to produce the powder, and the soaked solution was produced by soaking 30g of the powder of *H. perforatum* plant overnight in boiling water 2L then filtered. Normal saline as a solvent for preparation of drug suspensions, 20 mg of atorvastatin tablet dissolved in 10ml of normal saline. Then the mice were administered 0.2 ml of it by intra-gastric gavage once per day for 30 consecutive days.

Induction of hyperlipidemia

Hyperlipidemia was induced by feeding the mice a fatty diet containing: wheat flour 34%, barley flour 20%, corn flour 25%, fatty milk powder 10%, animal protein 10%, salt 1% and vegetable oil and water for kneading with 1% water with H₂O₂ for a period of three months (15). After 12 weeks, the triggered hyperlipidemia demonstrated itself in the mice fed a fatty diet as a significant rise in mean triglyceride (TG), total cholesterol (TC) and low density lipoprotein cholesterol (LDL-C) levels (P<0.05) when compared to those fed a control diet containing: animal protein 10%, soybean cake 20%, forage yellow corn 55%, forage wheat 14%, salt 0.5%, lime 0.5% and tap water.

Measuring the serum lipids

The lipid profile parameters were measured three times on separate days, first at baseline and then at the twelfth week and at the end of the sixteenth week. Blood samples after 12 h of fasting were collected from the vein of the eye of the mice. The serum was obtained by centrifuging the blood samples at 3000 rpm for 15 min then the lipid profile parameters were measured using special kits (Biolabo, Zenith Lab Co., Ltd). Atherosclerosis index (AI) was calculated according to the following formula: AI=TC/HDL-C (16).

Evaluation of aortic atherosclerosis

At the end of the experiment, the cervical dislocation was used to sacrifice all of the animals for histopathological research. After incising the chest wall, the aorta was cut and removed, then rinsed with ordinary saline solution before being stored in 10% formalin for the next stage. Harris's

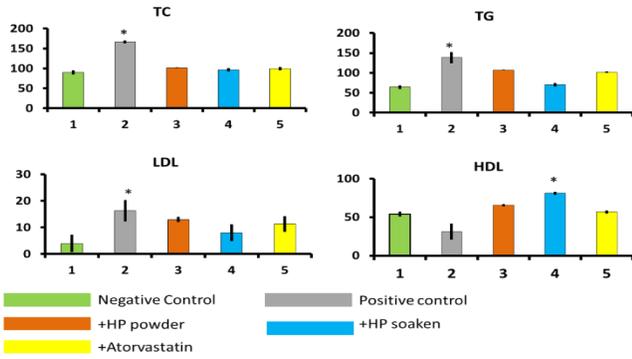


Figure 2: Plant extract reduced plasma lipid profile in experimental animal comparing soaked versus powder form versus atorvastatin in comparison to positive and negative control. Data displayed as mean \pm standard deviation, * mean significant at $P < 0.05$.

Histopathological study

A representative image of atherosclerotic alterations in the aortas from the five groups (Figure 3). The aortas of mice in group 1 on a regular diet had no atherosclerotic plaques. On a fatty diet and water with 1% H_2O_2 for 90 days, the grade of fatty streak formation in the aorta of group 2 was significantly raised. The amount of atherosclerosis was significantly reduced in the mice on powder form of *H. perforatum* plant with regular diet in group 3 and in group 4 mice on *H. perforatum* plant soaks taken orally with regular food for 30 days respectively, compared to the animals in group 2, and mice in group 5 treated with atorvastatin drug showed a lowering in the amount of atherosclerosis in contrast to group 2. The group has taken soaked form of *H. perforatum* plant shown a better reduction in the amount of atherosclerosis in contrast with the group taken powder form of *H. perforatum* plant with regular diet.

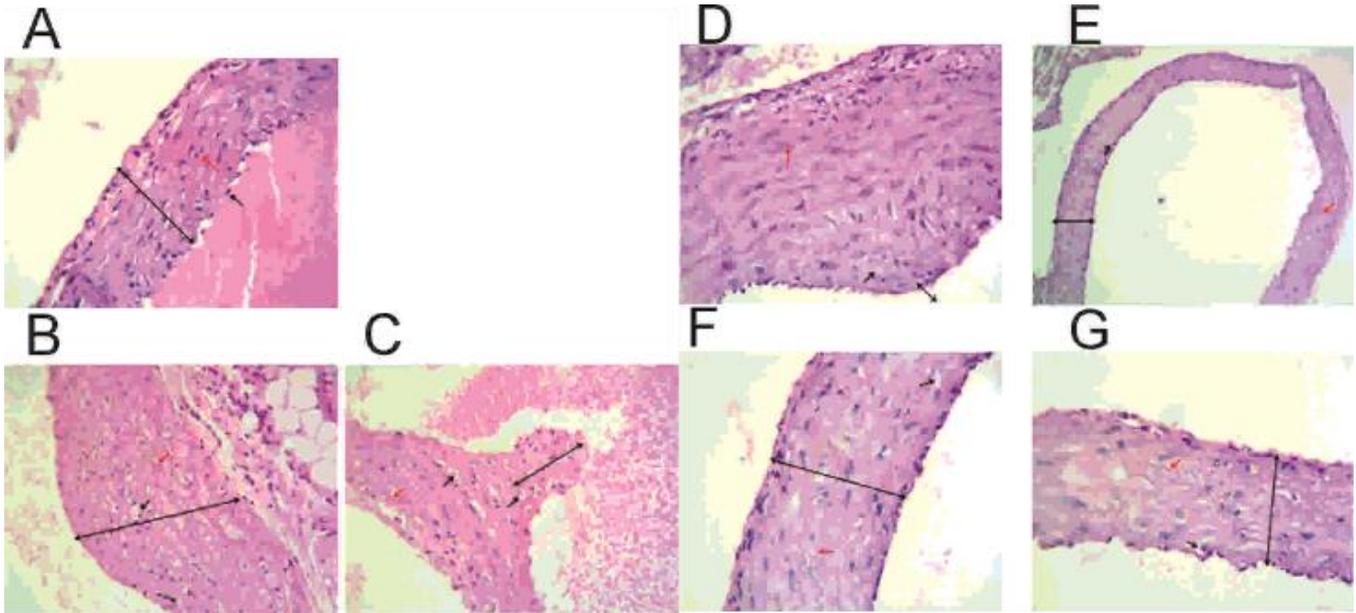


Figure 3: A representative image for mice aorta showing different histopathological changes using H&E stain, E (100X) others (400X). Arrows indicate thickness of layers (\leftrightarrow), endothelium (\rightarrow) and smooth muscle cells (\rightarrow). A: photomicrograph of mice aorta of negative control group shows normal architecture representing by the thickness of layers (\leftrightarrow), endothelium (\rightarrow) and smooth muscle cells (\rightarrow). B: photomicrograph of mice aorta of positive control group shows thickening of layers (\leftrightarrow), the present foam cells in intimal and medial layers (\rightarrow) and hypertrophy of smooth muscle fibers (\rightarrow). C: photomicrograph of mice aorta of positive control group shows a projection of intimal to the lumen as atherosclerotic plaque (\leftrightarrow), presence of foam cells in intimal and medial layers (\rightarrow) and hypertrophy of smooth muscle fibers (\rightarrow). D: photomicrograph of mice aorta of atorvastatin drug shows a projection of intima to the lumen as atherosclerotic plaque (\leftrightarrow), presence of foam cells in intimal and medial layers (\rightarrow) and hypertrophy of smooth muscle cells with the presence of inflammatory cells and necrosis (\rightarrow). E: photomicrograph of mice aorta of *H. perforatum* plant group shows normal architecture representing by the thickness of layers (\leftrightarrow) and smooth muscle cells (\rightarrow) with presence of few foam cells (\rightarrow). F: photomicrograph of mice aorta of the group treated with soaked form of the plant shows normal architecture representing by the thickness of layers (\leftrightarrow) and smooth muscle fibers (\rightarrow) with presence of few foam cells (\rightarrow). G: photomicrograph of mice aorta of the group treated with powdered form of the plant shows normal architecture representing by the thickness of layers (\leftrightarrow) and smooth muscle fibers (\rightarrow) with presence of few foam cells (\rightarrow).

Discussion

The present study confirmed that the dried plant of *H. perforatum* has a positive effect on overall measured parameters that reflect the etiology of cardiovascular diseases. This outcome has been confirmed by biochemical and histopathological studies compared to control groups. Moreover, analysis of the plant extract confirmed that the extract contains various biomolecules; some of which are well-known for their cardiovascular actions. Furthermore, the study also proved that the soaked form of the plant has induced a better effect than the dried powdered form. Nevertheless, in comparison to atorvastatin, the plant shown improved lipid profile whether dried or soaked. The positive effect of different *H.* species has been reported with different outcomes supporting our findings (3).

Also, the study mentioned that analysis of the extract of *H. perforatum* shown presence of various bioactive molecules. Analysis by HPLC revealed the presence of various extract constituents compared to standard and represented by various retention peaks (17). HPLC peaks matched the retention time of catechine, ferulic acid, quercetin, rutin, kaempferol, and apigenin while the retention peaks of GC matched only low molecular weight, volatile compounds: formic acid, acetic acid, propionic acid, butyric acid, and valeric acid (13).

This work reported that soaked form of the dried plant *H. perforatum* has induced a remarkable lipid parameters modulation represented by favorable reduction of LDL, TC and TG alongside elevation of HDL in comparison to the dried powdered form of the plant or atorvastatin group (18). Despite that, the dried powder is shown favorably better antihyperlipidemic effect than atorvastatin (19). In line with our study, the results of Zou *et al.* (20), investigation showed that *H. perforatum* has a strong hypolipidemic effect. It has the potential to lower blood TC, TG, and LDL-C levels while increasing blood HDL-C levels, Zou *et al.* (20), also confirmed that *H. perforatum* reduced malondialdehyde (MDA) levels in the blood and liver whereas dramatically increasing Superoxide dismutase (SOD) activity in both serum and liver, as well as catalase (CAT) activity in the liver (20). *H. perforatum* extract significantly decreased low density lipoprotein cholesterol, cholesterol, triglyceride, C-reactive protein (CRP), MDA as well as apolipoprotein B (apoB), atherosclerosis index, and increased apoA and high density lipoprotein in rabbits. This extract also confined the atherosclerotic lesions, according to histopathological findings to Asgary *et al.* (21). Moghaddam *et al.* (22), reported that LDL-C was dramatically lowered, and TC was also greatly reduced. The levels of TG and HDL-C did not alter significantly. However, MDA, aspartate aminotransferase, and alanine aminotransferase were all dramatically reduced (22). The histopathological studies are represented by tissue slices of mice aorta at the end of the study to further confirm the outcome (18,23). Fatty diet has induced atherosclerotic

changes compared to the control group on a regular diet. These atherosclerotic changes approximately absent in mice treated by plants in soaked or dried form with slightly better outcomes with soaked form (24). On the other hand, atorvastatin shown greatly reduced atherosclerotic changes compared to a positive non-treated control group with slightly better outcomes obtained with plant preparation compared to atorvastatin (11).

Conclusion

The study concluded that the plant *H. perforatum* in dried or soaked form reduced lipid parameters compared to control groups (with/without fatty diet) and the soaked form showed better hypolipidemic effects than dried form. Both dried and soaked form has shown a better or comparable effect to atorvastatin; a well-known hypolipidemic agents. The hypolipidemic effect of the plant *H. perforatum* was confirmed through histopathology study. Analysis of the extract of the plant revealed the presence of effective biomolecules; some of which could be utilized for biosynthesis of new antihyperlipidemic drugs.

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Conflict of interests

The authors declare no conflict of interest

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تأثيرات العرن المثقوب على دهون مصل الدم في الأوعية الدموية للفئران

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الخلاصة

تستخدم المنتجات العشبية على نحو متزايد ضد الدهون لتخفيض تأثيرها السلبي على نظام الأوعية الدموية. ومع ذلك، هناك عدم إثبات بالدليل القاطع بشأن أفضل طريقة لإعطاء المستخلصات العشبية وتحضير المنتجات العشبية لاستخدامها بسبب الاختلاف في معدل وكمية الامتصاص والتأثير اللاحق على مستويات الدهون. وكان الهدف من هذه الدراسة هو مقارنة الجوانب الدوائية والتحليلية للنقيع مقابل شكل مسحوق من العرن المثقوب ويعرف أيضا في الغرب باسم نبتة القديس يوحنا المثقبة؛ منتج عشبي معروف مضاد للفرط في الدهون، باستخدام عقار أتورفاستاتين كدواء مرجعي للمقارنة بالإضافة إلى مجموعات التحكم (يتناول / لا يتناول الطعام الدهني) التي يتم التعامل معها كمجموعات مراقبة إضافية. لإجراء هذا البحث، تم إنشاء نموذج فرط الدهون لدى الفئران المختبرية عن طريق تعريض الفئران لنظام غذائي دهني واستخدام عشبة القديس يوحنا وأتورفاستاتين للتقييم. كما تم تحليل مستخلص عشبة القديس يوحنا للتأكد من وجود المادة الفعالة. وتشير النتائج إلى أن تحليل النبات كشف وجود عوامل مختلفة مضادة لفرط الدهون والعشبة قد خفضت بشكل فعال مؤشرات الدهون في البلازما في الفئران المعرضة للعشبة بالمقارنة مع مجموعة التحكم؛ وخصوصا عندما تم استخدام شكل النقيع المائي من النبات. وخلصت الدراسة إلى أن استخدام عليقة عشبة القديس يوحنا أظهرت انخفاضا فعالا من المعلمات الدهنية والتحليل الذي كشف عن وجود مواد عشبية فعالة التي يمكن استخدامها في الصيدلة الصناعية لابتكار عقارات جديدة.