Anti-atherosclerotic effects of *Camellia chrysantha* and *Gynostemma pentaphyllum* extracts mixture

Hong Hanh Nguyen 1, Ngan Hoang Nguyen 2, Tu Thi Thanh Nguyen 3*

1Department of Geriatric, E Hospital, 87-89 Tran Cung Street, Cau Giay District, Hanoi, Vietnam
2Department of Pharmacology, Vietnam Military Medical University (VMMU), 160 Phung Hung Street, Ha Dong District, Hanoi, Vietnam
3Faculty of Traditional Medicine, Hanoi Medical University, 01 Ton That Tung Street, Dong Da District, Hanoi, Vietnam

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**Abstract**

**Introduction:** The combination *Camellia chrysantha* and *Gynostemma pentaphyllum* is used in Vietnam against hyperlipidemia. This study aims to evaluate the effects of the extract mixture of *C. chrysantha* and *G. pentaphyllum* in the atherosclerosis-induced rat model.

**Methods:** Rats were administered with the extract mixture of *C. chrysantha* and *G. pentaphyllum* daily (7 and 14 g/kg/day) for 8 weeks upon the start of the study while they were simultaneously put on an atherosclerosis-induced diet. Blood samples were taken to examine the blood lipid indicators of the rats in the beginning, after 4 and 8 weeks of the study, respectively. After 8 weeks of treatment, the rats’ livers were removed to assess their overall health and the atherosclerosis patterns of their abdominal arteries.

**Results:** The mixture of leaf extracts of *C. chrysantha* and *G. pentaphyllum* in the doses of 7 and 14 g/kg/day reduced blood lipid indices, including triglycerides (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C) levels and the atherogenic index (AI). Furthermore, this mixture also increased the levels of high-density lipoprotein cholesterol (HDL-C) in the blood, decreased the incidence of liver fat, and prevented the development of atherosclerotic lesions in the abdominal aorta in the rats.

**Conclusion:** These results indicate that a mixture of *C. chrysantha* and *G. pentaphyllum* has a potential in preventing and treating atherosclerosis.

**Implication for health policy/practice/research/medical education:**
Our findings revealed that an extract mixture of *C. chrysantha* and *G. pentaphyllum* could reduce blood lipid indices and increase HDL-C levels in the rat atherosclerotic blood, indicating that this mixture is a potential source of natural agents for atherosclerosis treatment.


**Introduction**
Atherosclerosis is a condition where most of the arteries in the body, including the heart, brain, arms, legs, pelvis, and kidneys, become degenerated with the buildup of plaques (1). Atherosclerosis is the major cause of myocardial and cerebral infarction and the leading cause of death in developed countries in various groups of ages (2,3). About one-half of Americans between 45 and 84 years of age have atherosclerosis (1). Nowadays, the incidence of atherosclerosis is increasing among younger individuals (4). There is about a 50% risk of developing severe atherosclerosis for people over 40, and most people over 60 have atherosclerosis (5). Among people aged 30-79 years in 2020, 1066.70 million cases were affected with carotid intima-media thickness, a percentage change of 57.46% from 2000 (6). Meanwhile, atherosclerosis caused 49% of coronary heart disease of patients aged 15-64 years in Vietnam’s working population (7). The number of hospitalizations and deaths related to atherosclerosis has been increasing, making the use of drugs to prevent atherosclerosis a real need. Advancements in the pharmaceutical industry have made it possible to improve healthcare for atherosclerosis. Fibrates and statins are useful in preventing arteriosclerosis and thromboembolic...
events (8-10). However, long-term use of these medicines can have side effects, especially when these medicines are used together (8-13). Therefore, there has been an increasing need to develop medicines derived from herbs with both high effectiveness and safety. In Vietnam, many medicinal herbs, including C. chrysantha (family: Theaceae) and G. pentaphyllum (family: Cucurbitaceae), are used to cure atherosclerosis. C. chrysantha is a famous tea, rich in saponins, polyphenols, polysaccharides, flavonoids, and elements (14). C. chrysantha possesses several pharmaceutical effects such as anti-diabetic, antioxidant, anti-cancer, and diuretic properties. It was also used for treating certain nerves and brain disorders to control the heart rhythm and stimulating appetite (15). Gynostemma pentaphyllum contains saponins and flavonoids (16). Most of saponins in G. pentaphyllum belong to gypenosides skeleton (17). G. pentaphyllum was also found as a rich natural source of vitamins and minerals. Several applications of G. pentaphyllum in pharmaceutical science include reducing blood lipids and blood sugar levels, protecting the cardiovascular and central nervous systems, enhancing immune function, and killing cancer cells. The antioxidant, antipyrretic, sedative, analgesic, and anti-peptic ulcer effects were also found (18-20). Studies have shown that these two medicinal herbs contain saponins and flavonoids that have the effects of lowering blood lipids and preventing and treating atherosclerosis (19,21-24). Therefore, our study was conducted to evaluate the prevention of atherosclerosis with a mixture of C. chrysantha and G. pentaphyllum extracts in the atherosclerosis-induced rat model. This combination is used in Vietnam for these purposes.

Materials and Methods

Equipment
The automatic biochemical testing machine was a Biochemical Systems International model 3000 Evolution (Biochemical Systems International, Italy). The analytical balance 10-4, model CP224S was purchased from Sartorius (Germany) and blunt-tip needles for rats to drink were purchased from Natsume Seisakusho Co., Ltd (Japan).

Chemicals
Fried fat food included 42.7% cornmeal, 7% soybean oil, 20% casein, 10% sucrose, 5% cellulose, 3% gelatin, 10% lard (heated at 190 °C for 24 hours), 0.3% cholic acid, and 2% cholesterol. The ingredients for making fried fat food for the rats included pure cholesterol (Merck, Germany), cholic acid (Sigma, Singapore), cornstarch (Maizan, Vietnam), soybean oil (Simply, Vietnam), micellar casein (NZ, New Zealand), sucrose (CAS 57-50-1, China) and carboxymethyl cellulose (CAS: 9000-11-7, China). The reference drug, AtorVPC 10 (atorvastatin 10 mg) was purchased from Cuu Long Pharmaceutical Joint Stock Company (Vietnam).

Plant material
The leaves (6.0 kg) of C. chrysantha were collected from Vinh Phuc province, North of Vietnam (voucher specimen: NHN-0021) and the leaves (8.0 kg) of G. pentaphyllum were collected from Ha Giang province, north of Vietnam (voucher specimen: NHN-0022) in October 2020. The samples were taxonomically authenticated by Prof. Nguyen Hoang Ngan and the voucher specimens were deposited in the Pharmacology Laboratory, Pharmacy Training Institute, Vietnam Military Medical University, Vietnam.

Extract preparation for animal testing
The extraction procedure was described in the Vietnamese Pharmacopoeia V (25). Dried powdered leaves of C. chrysantha were extracted with hot water with a ratio of 1:10 (1 g of leaves into 10 mL of hot water) at 100 °C for 90 minutes, filtered (2 times) and then concentrated under decreased pressure in the ratio of 1:1 (1 g of herb to extract into 1 mL). The process of extracting G. pentaphyllum was also carried out the same way as that of C. chrysantha. After that, the two extracts were mixed in a ratio of 1:3 (1 mL of C. chrysantha and 3 mL of G. pentaphyllum) (26). This resulting mixture then was concentrated under a decreased pressure to produce a concentrated form in a ratio of 2:1 (2 g of the mixture to concentrate into 1 mL). The final extract was preserved in a cool compartment of the fridge. It has been reported in folk experience and previous studies that the recommended dose of C. chrysantha leaves for one person was 20 g/day, while the dose of G. pentaphyllum was 60 g/day (26). As a result, two medicinal herbs were combined at a ratio of 1:3 of C. chrysantha and G. Pentaphyllum, respectively. Various folk experiences and data on the preclinical safety of the mixture of these 2 herbs with a dose of 50 g/day (1 g/kg/ day) on some volunteer patients have shown the initial results with lipid-lowering effects. Thus, 50 grams per day for one person was considered suitable for human use, providing a basis for dose conversion in rats (27-29). As a typical guide to converting the human dosage into the animal dosage, rats should receive seven times the human dose (calculated based on dry herb weight), equivalent to their dose of 7 g/kg/day (30,31).

Experimental animals
Fifty healthy mature Wistar rats of both sexes weighing 160–180 g were supplied by Laboratory Animal, Vietnam Military Medical University (Hanoi, Vietnam). The animals had free access to food and water and were under the standard laboratory conditions for one week prior to the study. The lab temperature was 18-23 °C with 40-60% humidity and the light cycle/dark cycle was 12/12 hours. The experimental protocol was approved by Vietnam Military Medical University, Hanoi, Vietnam (Ethical Permission number IACUC-1610/20).
**Methods**

The rats were randomly divided into five groups of 10 (32) as described below:

- Physiological control group 1 (G1): the animals only drank distilled water and had a normal diet.
- Pathological control group 2 (G2): the animals drank distilled water; had an atherosclerosis-induced diet.
- Treatment group 3 (G3): the animals had an atherosclerosis-induced diet and oral administration of the extract with a dose of 7 g/kg/day.
- Treatment group 4 (G4): the animals had an atherosclerosis-induced diet and oral administration of the extract with a dose of 14 g/kg/day.
- Reference drug group 5 (G5): the animals had an atherosclerosis-induced diet with a dose of atorvastatin 10 mg/kg/day.

The atherosclerosis-induced method was described by Yurina et al with a slight modification (33). Rats in all groups except for G1 were fed fried fat food for 8 weeks. Rats in G3, G4 and G5 were administered daily for 8 weeks upon the start of the study while they were simultaneously put on an atherosclerotically-induced diet. At different times, including the start of the study, 4 weeks after and 8 weeks after the study, blood samples were taken through the retroorbital sinus. The animals were anesthetized with isoflurane. To determine the level of anesthesia, the right reflex was checked to determine whether or not the rat lost consciousness. The rat was restrained, the skin around the eye of the non-dominant hand was pulled taut, and the thumb and forefinger of the dominant hand were gently scuffed on the neck with the thumb and forefinger. With the dominant hand, a capillary of 2 to 2.5 cm was inserted into the medial canthus of the eye (30-degree angle to the nose) using a needle. In the case of the sinus or plexus, a slight pressure from the thumb was sufficient to puncture the tissue and enter the sinus. Whenever the sinus or plexus was pricked or punctured, blood would flow through the capillary tube as soon as the area was pricked or punctured. To prevent further bleeding, the capillary tube was gently removed from the plexus after the required volume of blood had been collected, and then wiped with sterile cotton to prevent further loss of blood. The bleeding was stopped by gently applying a little finger pressure for approximately 30 seconds to the area that was bleeding. Thirty minutes after blood collection, the animal was checked for postoperative and periorbital lesions.

Blood samples were taken from the rats to examine the blood lipid indicators of the rats via triglycerides (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C). The atherogenic index (AI) was calculated according to the following formula:

$$AI = \frac{TC - HDL-C}{HDL-C}$$

After 8 weeks of treatment, the rats were sacrificed with a high dose of anesthesia (thiopental sodium 50 mg/kg). Their livers were removed to assess their overall health; their abdominal arteries were examined to identify patterns and to determine the level of atherosclerosis they had (30,31,33).

**Statistical analysis**

Data were presented as mean ± standard error (SE). The data were evaluated using the independent samples t test and SPSS software (version 22, IBM Corp., USA). The differences were statistically significant at \( P < 0.05 \).

**Results**

Changes in the levels of TC, TG, HDL-C, LDL-C and AI in the rats’ blood

The results of TC, TG, HDL-C, LDL-C levels and AI in the rats’ blood are shown in Figure 1. The TC, TG, LDL-C levels and AI in G1 unchanged after 4 and 8 weeks (\( P > 0.05 \)). Also, the HDL-C levels in G1 and G2 unchanged after 4 and 8 weeks (\( P > 0.05 \)). The TC, TG, LDL-C levels in rats’ blood in G2, G3, G4 and G5 significantly increased compared with G1 after 4 and 8 weeks of treatment (\( p < 0.05 \)). The HDL-C levels in G3, G4, and G5 increased significantly compared with those in G1 and G2 after 4 and 8 weeks of treatment (\( P < 0.05 \)). The AI of G2 (after 4 weeks) and G3, G4, and G5 (after 8 weeks), which were compared with those in G1, increased significantly (\( P < 0.05 \)).

The results of the TC, TG, HDL-C, LDL-C levels and AI in the rats’ blood are shown in Figure 2. The TC, TG, and LDL-C levels and AI in the rats’ blood of G3, G4, and G5 were all significantly lower than that in G2 after 4 and 8 weeks of treatment (\( P < 0.05 \)). The HDL-C levels in G3, G4, and G5 were increased significantly, compared with those in G2 after 4 and 8 weeks of treatment (\( P < 0.05 \)). The TC, TG, HDL-C, and LDL-C levels and AI in G3, G4, and G5 compared with themselves were not significantly different after 4 and 8 weeks (\( P > 0.05 \)).

Macroscopic images of rats’ livers

Macroscopic images of the rat livers were presented in Figure 3. Macroscopic observation with the naked eye and under 25× magnification showed that the liver had a dark color in G1 (Figure 3A), and the liver was discolored to bright yellow, indicating a fat liver in G2 (Figure 3B). Nevertheless, the liver colors of G3, G4, and G5 were darker than the liver color of G2. As a matter of fact, the liver colors of G3 and G4 were close to that of G5 (Figures 3C, 3D, and 3E).

Assessment of abdominal aortic atherosclerosis

Figure 4 shows histopathological images of the abdominal aortas of the rats. In G1, the vessel walls were thin and smooth; the muscle cells were oriented transversely, with a patent ductus arteriosus (Figure 4A). There was a roughness in the vessel walls of G2 (Figure 4B). Foam
cells (black arrows), found in the submucosa layer, were the basis for the growth of atheroma (red arrows). The histopathological images of the rats’ abdominal aortas in G3, G4, and G5 were similar to that of G1. The vessel walls in G3, G4, and G5 were also rough but not significantly rougher than G1. There appeared no foam cells and atheromas in the above 3 groups (Figures 4C, 4D, and 4E).

**Discussion**

In this experiment, an atherosclerosis-induced model using a fried fat diet according to the method of Yurina et al was implemented (33). This was a simple, easy-to-implement and affordable model, suitable for studies over a reasonable period of time (2–3 months) (33). The results showed that the lipid indices in G1 rats did not change as

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**Figure 1.** Levels of (A) total cholesterol, (B) triglyceride levels, (C) HDL-C, (D) LDL-C, and (E) AI in the rats’ blood of G2, G3, G4, and G5 compared to G1 at T4 and T8. Data are mean ± SD. The asterisks above columns indicate a significant different compared to G1 ($P < 0.05$). HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.

**Figure 2.** The levels of (A) total cholesterol, (B) triglyceride levels, (C) HDL-C, (D) LDL-C, and (E) AI in the rats’ blood of G3, G4, and G5 compared to G2 at T4 and T8. Data are mean ± SD. The asterisks above columns indicate a significant different compared to G2 ($P < 0.05$). HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol.
the rats had no fried fat food. On the contrary, the rats in G2, G3, G4, and G5, which were all fed-fired fat food, had increased lipid indices. This confirmed the successful implementation of the atherosclerosis-induced model. Furthermore, after 4 and 8 weeks of treatment, the rats in G3, G4, and G5, which were orally administered with the extract mixtures and the reference drug, all showed decreased lipid indices including TC, TG, LDL-C, and AI, as well as an increased HDL-C, as compared to G2.

Another observation was that in the macroscopic images of the rats’ livers in G1, there appeared no fat livers while in G2 there appeared fat livers. In G3, G4, and G5, the livers’ colors did change to a brighter color. However, there appeared no fat livers, almost no different from G1.

From the microscopic images of the rats’ abdominal aortas, G1 rats showed thin vessel walls while G2 rats showed rough vessel walls, as well as foamy cells. This was the foundation to form atherosclerosis. Given treatment with the extract mixtures and the reference drug, the rats in G3, G4, and G5 showed no evidence of foamy cells and atherosclerosis, with almost similar images of G1.

Thus, based on the results of the lipid indices and the macroscopic images of the rats’ livers, and microscopic images of the rats’ abdominal aortas, the extract mixture of the *C. chrysantha* and *G. pentaphyllum* with the dosage of 7 g/kg/day and 14 g/kg/day had the potential to prevent atherosclerosis, similar to the benchmarked reference drug of atorvastatin with the dosage of 10 mg/kg/day.

The results of a study were different from ours in which the mixture of these two herbs was conducted in a rat model where the rats were fed fried fat food for 8 weeks, followed by the oral administration with two doses of 16.8 and 33.6 g/kg/day over a period of 4 weeks. The indices of TG, TC, HDL-C, LDL-C, and AI were evaluated on days 0,
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had the effect of lowering blood lipids. Forty-eight patients were divided into two groups, the original dose group and the increased dose group. All were treated with G. pentaphyllum capsules. The original dose group remained the same, 3 times a day, 1 tablet each time. The increased dose group increased the oral dose gradually, 3 times a day, 4 tablets each time. The results showed that the increased dose group after treatment had a marked improvement in TC, TG, and LDL-C levels compared to the initial dose group (45). Although there were many studies that had demonstrated the lipid-lowering effects, as well as the blood sugar-lowering effects of C. chrysantha and G. pentaphyllum separately, in clinical practice, in treating such diseases as diabetes mellitus, dyslipidemia, and hypertension, these two herbs were often combined (46).

In addition, to date, many dammarane-type saponins have been isolated from G. pentaphyllum. Its crude extracts, saponin-rich fractions (gypenosides), and purified compounds also have been reported to have a wide range of pharmacological activities in both in vitro and in vivo experiments. The most notable pharmacological effects were anticancer, cardioprotective, hepatoprotective, neuroprotective, anti-inflammatory, antidiabetic, and obesity prevention. The mechanism of saponins in reducing blood fat was explained by the bile acids that were absorbed by saponins. This reduced the excretion of bile acids in the stool, which was then replaced by an increase in the conversion of cholesterol into bile acids by the liver. Some saponins also interacted directly with cholesterol to form cholesterol-saponin complexes, thereby inhibiting the absorption of cholesterol from the small intestine. In addition to saponins, flavonoids were found in C. chrysantha and G. pentaphyllum. It had antioxidant effects and prevented and treated atherosclerosis by inhibiting the oxidation of LDL, inhibiting cytokine secretion, preventing the formation of atherosclerotic plaques and antiplatelet aggregation (47,48).

Conclusion
In a rat model of atherosclerosis induced with fed fried fat food, a mixture of leaf extract of C. chrysantha and G. pentaphyllum with the doses of 7 and 14 g/kg/day exhibited atherosclerosis-preventing effects by reducing blood lipids, including TC, TG, and LDL-C levels, as well as AI. In addition, the mixture of extracts increased HDL-C levels in the blood, reduced the fat content of the liver, and reduced atherosclerotic lesions of the abdominal aorta in the rats. The dose of 14 g/kg/day showed better improvement in atherosclerotic indices than the dose of 7 g/kg/day during 4 and 8-week periods. These results suggested that a mixture of leaf extract of C. chrysantha and G. pentaphyllum could be used to prevent and treat atherosclerosis.
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Authors’ contributions
Conceptualization: Hong Hanh Nguyen.
Data curation: Ngan Hoang Nguyen and Tu Thi Thanh Nguyen.
Formal analysis: Hong Hanh Nguyen and Ngan Hoang Nguyen.
Investigation: Hong Hanh Nguyen and Ngan Hoang Nguyen and Tu Thi Thanh Nguyen.
Methodology: Ngan Hoang Nguyen.
Resources: Hong Hanh Nguyen and Ngan Hoang Nguyen.
Supervision: Tu Thi Thanh Nguyen.
Validation: Ngan Hoang Nguyen and Tu Thi Thanh Nguyen.
Writing—original draft: Hong Hanh Nguyen and Tu Thi Thanh Nguyen.
Writing—review & editing: Tu Thi Thanh Nguyen.

Conflict of interests
The authors declare there is no conflict of interest.

Ethics considerations
The study protocol was approved by the Vietnam Military Medical University, Hanoi, Vietnam (Permission number IACUC-0302/21 issued on February 03, 2021).

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References
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