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Effect of mixed *Camellia chrysantha, Gynostemma pentaphyllum* and *Celastrus hindsii* Extract on Atherosclerotic Rat Models

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ABSTRACT

Atherosclerotic disease occurs due to lifestyle choices, such as being sedentary, eating fast foods, and a lack of physical exercise. Atherosclerosis has gradually become the leading cause of death worldwide. Currently, the trend of using natural herbal products to supplement and replace *synthetic drugs* is being considered in Vietnam. This study aims to evaluate the *in vivo* anti-atherosclerotic effect of a mixture extract of *C. chrysantha*, *G. pentaphyllum*, and *C. hindsii* in a Wistar rat model. Wistar rats were randomly assigned to 5 groups with 10 animals in each group and blood samples were obtained from rats at 0, 14, and 28 days after treatment began to examine blood lipid indicators: triglyceride (TG), total cholesterol (TC), high-density lipoproteins (HDL-C), and low-density lipoproteins (LDL-C). The results showed that a mixture of *C. chrysantha*, *G. pentaphyllum*, and *C. hindsii* extracts at doses of 16.8 and 33.6 g/kg/day reduced blood lipid test indexes, including triglyceride levels, total cholesterol, LDL-cholesterol, and atherogenic index. At the same time, this mixture increased the levels of HDL-cholesterol in the blood, reduced the incidence of fatty liver, and protected against atherosclerotic lesions of the abdominal aorta in the studied rats. These initial findings contribute to the search for new sources of raw materials and the development of natural products to prevent and treat atherosclerosis.

Keywords: Atherosclerosis, *Camellia chrysantha*, *Gynostemma pentaphyllum*, *Celastrus hindsii*, Wistar rat.

Introduction

Atherosclerosis has become more prevalent nowadays as a result of lifestyle issues such as sedentary behavior and eating bad food.[1] Atherosclerosis has become the main cause of death in the world. It is a gradual condition that begins at a young age. The exact cause of this disease remains unknown, but many factors are indicated to contribute to the formation of atherosclerotic plaque. Atherosclerosis is a combination of phenomena that change the structure of the intima of large and medium-sized arteries, including the local accumulation of lipids, glucide complexes, blood and blood products, fibrous tissue, and acid deposits, altering the arterial media. In industrialized countries, up to 50% of deaths are caused by cardiovascular disease, of which atherosclerotic causes accounted for 50%. In the United States, up to 88% of adults over the age of 60 have atherosclerosis and it is universal in individuals over the age of 80.[2] In Vietnam, there are no data for the entire population; however, the rate of people suffering from atherosclerosis-related cardiovascular problems is increasing and the average age of onset is decreasing.[3] Atherosclerosis can occur in many vascular entities, such as the carotid artery, coronary artery, lower extremity arteries, etc., and it can lead to a variety of related diseases. When atherosclerotic plaques rupture, they generate blood clots that fill the vessel-which is already narrowed by atherosclerotic plaques.[4]

These almost always play a major role in cardiovascular events: myocardial infarction, cerebral infarction, limb embolism, etc.[5,6] The risk factors for atherosclerosis include traditional risk factors such as dyslipidemia or high bad cholesterol in the blood, which are the main risk factors for atherosclerosis. Other risk factors include hypertension, smoking, diabetes, obesity, physical inactivity, stress, and taking birth control pills.[4-6]

The current treatment trend is to use active compounds extracted from natural herbal sources to supplement and replace synthetic drugs because of their effectiveness and limited side effects. Medicinal plants are still commonly utilized today, despite advances in modern medicine, and are the original sources for many medications and functional foods.[7-9] In Vietnam, natural medicinal plants have been widely used in traditional medicine for the treatment of cardiovascular diseases, including atherosclerosis. Among the significant medicinal herbs, *Camellia chrysantha*, *Gynostemma pentaphyllum*, and *Celastrus hindsii* have been used since ancient times to decrease weight, blood lipids, and blood glucose and to treat atherosclerosis.[7,8]

Camellia chrysantha belongs to the family Theaceae, genus *Camellia L.*, and species *hakodae* Ninh. A total of 310 species of camellia are widely distributed throughout East and Southeast Asia. The *C. chrysantha* tree is known to many people for its high economic value.[10] The leaves of some species are used to produce tea while the seeds of other species are used to produce cooking oils or cosmetics.[11,12] Some species are also grown as ornamental plants. Previous studies have identified more than 400 substances in *C. chrysantha*, with the main components being saponins, polyphenols, polysaccharides, flavonoids, and elements including germanium (Ge), zinc (Zn), selenium (Se), vanadium (V), molybdenum (Mo), manganese (Mn), kalium (K) and vitamins B1, B2, C. According to "The medicinal plants and medicinal animals in Vietnam" (Institute of Medicinal Plants [2004], Science and Technology Publishing House),

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Camellia chrysantha is anti-diabetic, anti-oxidant, and anti-cancer, diuretic, consumes energy, stimulates the nerves and brain, enhances brain function, regulates the heart rate, and stimulates the appetite, among other properties. In particular, there are active compounds in tea leaves that decrease the total lipid levels in blood serum.[13-17]

Gynostemma pentaphyllum (Cucurbitaceae) is also an important natural herb in Vietnam. This plant is rich in flavonoids and saponins; the saponin content of *G. pentaphyllum* was found to be 3–4 times higher than that of ginseng and some of them have chemical structures similar to those found in ginseng (ginsenoside).[18] Saponins in *G. pentaphyllum* are defined as gynosaponins or gypenoside-type saponins. The total gypenoside compounds accounted for about 2.4% of the dry mass of *G. pantaphyllum* leaves. Furthermore, more than 100 types of gypenoside have been isolated.[19] In addition, *G. pentaphyllum* contains vitamins and minerals such as selenium, zinc, iron, manganese, phosphorus, etc. The pharmaceutical effects of *G. pentaphyllum* are believed to be mediated primarily by saponins along with flavonoids. *G. pentaphyllum* has proven effects of reducing blood lipids and blood sugar, the cardiovascular system, the central nervous system, immune function, and cancer cells. Some other protective effects have been studied, such as antioxidative, antipyretic, sedative, analgesic, and anti-peptic ulcer effects.[20-22]

Celastrus hindsii Benth et Hook belongs to the Celastraceae family.[23]This plant contains polyphenol compounds including rutin, kaempferol 3-rutinoside, rosmarinic acid, lithospermic acid and lithospermic acid B, and three novel oligomers of rosmarinic acid (one dimer and two trimers).[24] From the trunk of *C. hindsii*, Huang et al. identified sesquiterpene and triterpenes. The biological evaluation showed that maytenfolone A can resist hepatocellular carcinoma and nasopharyngeal carcinoma cells. Celasdin B has been found able to inhibit active HIV replication in lymphocytes. The ongoing study of *C. hindsii* samples from Vietnam led to the isolation and clarification of the structure of glucosyringic acid, lup-20-ene-3β, 11β-diol, lup-20-ene-3-one (lupenone), and lup-5,20 -diene-3-one.[25] According to Lou et al., triterpenoids isolated from the trunk of *C. hindsii* were evaluated for their *in vitro* antiviral activities against the respiratory syncytial virus (RSV) with cytopathic pathogene effect (CPE) assays. Hindsiilactone A and hindsiiquinoflavan B were shown to have cytotoxic activity against four human tumor cell lines (NCI-H187, HCT116, BC-1, and HuH7).[26,27]

Previously, a preclinical assessment of the effects of the above three individual extracts was performed. However, no experiments have mixed these three plants. Therefore, in this study, the *in vivo* anti-atherosclerotic effects of a mixture of the extracts of *C. chrysantha*, *G. pentaphyllum*, and *C. hindsii* were evaluated.

Materials and Methods

Chemicals and research equipment

Research equipment: TG, TC, HDL-C, and LDL-C indicators were measured on a 3000 Evolution analyzer (Biochemical Systems International Srl; Italy). Analytical scale (10^{-4}) was obtained from a CP224S analytical balance (Sartorius; Germany). A curved needle was used to administer medications to the rats (Japan). Small animal dissection kits and other experimental tools were purchased from Roeyu Enterprises Inc. (Vancouver, Canada).

Chemicals: Pure cholesterol (Merck; Germany), cholic acid (Sigma; Singapore), and other ingredients to process fatty food for rats were purchased from Biochemical testing chemicals of MEDIA (Italy).

Plant extractions

The leaves of *Camellia chrysantha* (voucher specimen: NHN-0021), arial part of *G. pentaphyllum* (voucher specimen: NHN-0022), and stem bark of *Celastrus hindsii* Benth et Hook, (voucher specimen: NHN-0023) were purchased from The Ba Ba Tea company (Bac Ninh city, Vietnam) in October 2020. These plant materials met the standards of the Vietnamese pharmacopeia V 2020. Plants identification was done by Associate Professor Nguyen Hoang Ngan, plant sample were deposited at the Department of Pharmacology, Pharmacy Training Institute, Vietnam Military Medical University. The medicinal herbs were extracted separately by automatic extracting machine (KTP-EP-25, Korea Techno Pack, Bucheon-si, Korea) at the Department of Pharmacology, Pharmacy Training Institute, Vietnam Military Medical University. The parameters of the extraction process of medicinal herbs are in Table 1.

The extracts were filtered through several layers of thick cloth to remove insoluble impurities and then the extracts were evaporated over a water bath to obtain a 1:1 extract (from 1g of medicinal herb material, 1 ml of extract was obtained). According to folk experience, as well as some studies, the dose of *C. chrysantha* leaves is 20g/person/day while the doses of *Gynostemma pentaphyllum* and *Celastrus hindsii* are 60g/person/day. Therefore, we combined the three medicinal herbs at a 1:3:3 ratios of *Camellia chrysantha* leaves, *Gynostemma pentaphyllum*, and *Celastrus hindsii*, respectively. When all three herbs are combined, the initial dose is 140 g/person/day. The dose may be reduced when the ingredients are mixed. In this study, a dose of 70g/person/day (1.4g/kg/day) was considered the expected dose for human use, providing the basis for dose conversion in rats. The dose in rats is 12 times higher than the dose in humans, 16.8 g/kg/day (calculated according to dry herb weight).[6,7]

Animals

In vivo experiments were performed with 50 standardized mature Wistar rats. The rats were not selected by sex and each animal weighed 160–180g at the beginning of the experiment. Animals were provided by the Laboratory Animal Supply Board of the Military Medical University and raised in the laboratory animal room for one week before the experiment. Animals were fed according to research animal feeding guidelines and given free access to cooled boiled water. The experimental results were monitored and recorded daily. The study was conducted at the Department of Pharmacology, Pharmacy Training Institute of the Military Medical University from November 2020 to May 2021.

Ethical consideration

Experimental animals were always cared for in hygienic conditions and provided with adequate food and water. The procedures and manipulations were performed according to general regulations for laboratory animals. The experimental protocol was approved by the Vietnam Military Medical University, Hanoi, Vietnam (Permission number IACUC-1210/20 issued on October 12, 2020).

Animal grouping and administration of extract

Wistar rats, eligible for the experiment, were randomly assigned to 5 groups with 10 animals in each group.

+ Group 1 (G1, physiological control): no atherosclerosis + distilled water.
+ Group 2 (G2, pathological control): induced atherosclerosis + distilled water.
+ Group 3 (G3, dose 1): induced atherosclerosis + experimental extract at 16.8g/kg/24 h.
+ Group 4 (G4, dose 2): induced atherosclerosis + experimental extract at 33.6g/kg/24 h.
+ Group 5 (G5, reference drug): induced atherosclerosis + oral administration of atorvastatin at 10 mg/kg/24 hours.

Table 1: Extraction condition

No	Method and reagents	Parameters
1	Extraction method	Hot reflection
2	Solvent	Water
3	Ratio: solvent/plants	10/1
4	Extraction times	2 times
5	Temperature	100^{o}C
6	Extraction time	90 mins/times

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Atherosclerosis was induced according to the method described by Lee et al.[6] The rats were fed a high concentration of fried fats for 8 weeks. Fatty food for rats was prepared according to the following formula: cornmeal 42.7%, soybean oil 7%, casein 20%, sucrose 10%, cellulose 5%, gelatin 3% (heated at 1900C for 24 hours) 10%, cholic acid 0.3%, and cholesterol 2%. The treatment was given daily for 28 days at 8 am after the completion of 8 weeks of feeding a diet high in fried fats. Blood samples were obtained from rats at 0, 14, and 28 days after treatment began to examine blood lipid indicators: TG, TC, HDL-C, and LDL-C.

Atherogenic index (A.I) was calculated according to the formula below:

$$A.I = (TC - HDL\text{-}C) / HDL\text{-}C$$

After 28 days of treatment, the rats were sacrificed. Their livers were removed to evaluate their overall condition. In addition, their abdominal arteries were assessed to identify patterns and determine the level of atherosclerosis present.[6,7]

Statistical analysis

The data in this experiment were processed with the biomedical statistics method using SPSS 22.0 software. Figures are shown as $\overline{X} \pm$ SD. The differences were considered significant at $p < 0.05$.

Results and Discussion

Changes in total cholesterol

Changes in the total cholesterol in the rats' blood were investigated and the results are presented in Table 2.

After 8 weeks of feeding the high-fat diet, G2–5 had significantly high levels of total cholesterol compared with the physiological control group ($p < 0.01$). In G1, after 8 weeks of the high-fat diet, the total blood cholesterol levels had decreased slightly ($p > 0.05$). After 14 days of treatment, G4 (treated with the experimental extract at 33.6g/kg/24h) and G5 (treated with the reference drug atorvastatin) exhibited significantly decreased total cholesterol compared to the model group ($p < 0.01$) and G3 (treated with the experimental extract at 16.8g/kg/24 h) also showed a significant decrease ($p < 0.05$). After 28 days of treatment, G3, G4, and G5 had significantly decreased total cholesterol compared to G2 ($p < 0.01$).

Changes in TG levels

The results are presented in Table 3. At 0 days of treatment, G2–5 had blood TG levels that were significantly higher than those in the physiological control group (G1; $p < 0.01$). At 14 days of treatment, G4 and G5 showed significantly decreased blood TG levels compared with G2 ($p < 0.01$) and G3 showed a significant decrease with $p < 0.05$. At 28 days of treatment, G3–5 showed a significant decrease in TG levels in blood compared with G2 ($p < 0.01$).

Changes in HDL-cholesterol levels

The results are presented in Table 4. At 0 days of treatment, G2–5 showed a slight decrease in blood HDL-cholesterol compared with the physiological control group (G1; $p > 0.05$). At 14 days of treatment, G4 and G5 showed significantly increased blood HDL-cholesterol levels compared to G2 ($p < 0.05$), while G3 showed a slight increase ($p > 0.05$). At 28 days of treatment, G3–5 showed significantly increased blood HDL-cholesterol levels compared to G2 ($p < 0.05$ and $p < 0.01$).

Changes in LDL-cholesterol levels

The results are presented in Table 5. At 0 days of treatment, the blood LDL-cholesterol level in G2–5 had increased compared to the physiological control group (G1; $p < 0.01$). G4 and G5 had a significant decrease in blood LDL-cholesterol levels compared to the model group ($p < 0.01$), while G3 showed a significant decrease with $p < 0.05$. In addition, at 28 days, G3–5 had significantly decreased blood LDL-cholesterol levels in the blood compared to G2 ($p < 0.01$).

Table 2: Total cholesterol (mmol/l) in rat blood ($n = 10, \overline{X} \pm SD$)

Experiment groups	Total cholesterol (mmol/L)			
	Before treatment (a)	**After 14 days** (b)	**After 28 days** (c)	*p*
G1	1.76 ± 0.35	1.81 ± 0.22	1,79 ± 0,37	$p > 0.05$
G2	4.24 ± 0.72	4.15 ± 0.51	4,09 ± 0,64	$p > 0.05$
G3	4.21 ± 0.66	3.25 ± 0.38*	2,61 ± 0,46**	$p_{b\text{-}a} < 0.05$
G4	4.26 ± 0.83	2.93 ± 0.44**	2,47 ± 0,42**	$p_{c\text{-}b} < 0.05$
G5	4.18 ± 0.68	2.97 ± 0.56**	2,52 ± 0,35**	$p_{c\text{-}a} < 0.01$
p	$p_1 < 0.01$	$p_1 < 0.01$; * $p_2 < 0.05$; ** $p_2 < 0.01$		-

Values are represented as Mean ± standard deviation. Values with different figures as superscripts in a row differ significantly ($p \leq 0.05$). Values with different alphabets as superscripts for a parameter in a column differ significantly ($p \leq 0.05$).

Table 3: TG levels (mmol/l) in rat blood ($n = 10, \overline{X} \pm SD$)

Experiment groups	TG levels (mmol/L)			
	Before treatment (a)	**After 14 days** (b)	**After 28 days** (c)	*p*
G1	0.89 ± 0.29	0.93 ± 0.25	0.91 ± 0.21	$p > 0.05$
G2	1.73 ± 0.36	1.65 ± 0.62	1.58 ± 0.53	$p > 0.05$
G3	1.71 ± 0.42	1.48 ± 0.51*	1.37 ± 0.54**	$p_{b\text{-}a} < 0.05$
G4	1.68 ± 0.31	1.38 ± 0.42**	1.23 ± 0.46**	$p_{c\text{-}b} < 0.05$
G5	1.70 ± 0.34	1.42± 0.48**	1.26 ± 0.49**	$p_{c\text{-}a} < 0.01$
p	$p_1 < 0.01$	$p_1 < 0.01$; * $p_2 < 0.05$; ** $p_2 < 0.01$		-

Values are represented as Mean ± standard deviation. Values with different figures as superscripts in a row differ significantly ($p \leq 0.05$). Values with different alphabets as superscripts for a parameter in a column differ significantly ($p \leq 0.05$).

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The results from Table 1 to Table 5 show that, after 8 weeks of feeding rats with a high concentration of fried fats, there was a significant increase in total cholesterol, TG, LDL-cholesterol, and a decrease in HDL-cholesterol. This represents dyslipidemia, increasing the undesirable cholesterols and decreasing the concentration of HDL-cholesterol. Along with the increase in blood lipids, especially bad fat components, lipid peroxidation is accumulated greatly. The high consumption of unhealthy fats leads to the pathogenesis of dyslipidemia, a risk factor for atherosclerosis and cardiovascular disease.[28] A diet high in fried fats raises the bad cholesterol level in the blood and makes it more vulnerable to oxidation. Lipid oxidation acts as an initiation for epithelial dysfunction, which leads to the development of atherosclerotic plaque and atherosclerosis.[29] After inducing atherosclerosis in rats, the mixture of three medicinal plants was given at doses of 16.8 and 33.6 g/kg/24h to evaluate their therapeutic effect in repairing the atheroma on the rats' vessel walls. The results show that the mixture of plants reduced blood lipids and dyslipidemia. Thus, liver fat was reduced and atherosclerosis in the rat models fed a diet high in bad fats was also reduced (Figure 1 and Figure 2). The effect of these extracts was evaluated compared to the reference drug atorvastatin, at a dose of 10 mg/kg/24 hours. Atorvastatin belongs to the statin group and effectively regulates blood lipid disorders and is anti-atherosclerotic. The research results clearly show that a mixture of *C. chrysantha*, *G. pentaphyllum*, and *C. hindsii* at doses of 16.8 or 33.6 g/kg/day effectively treated atherosclerosis and were equivalent to the reference drug. This offers a foundation for the development of preparations to help treat dyslipidemia and prevent atherosclerosis.

Changes in atherogenic index (A.I)

The results are presented in Table 6. At 0 days, the G2–5 A.I had increased significantly compared to the physiological control group (G1; $p < 0.01$). After 14 days, G4–5 exhibited a significant decrease in A.I ($p < 0.01$) and G3 showed a significant decrease with $p < 0.05$. After 28 days, G3–5 showed a significant decrease in A.I compared to G2 ($p < 0.01$).

Appearance analysis of the rats' livers

The general appearance of the rats' livers, representing each rat group, is presented in Figure 1.

Observation shows that, in the physiological control group (G1), the liver was dark, while in G2, the liver is discolored and bright yellow, demonstrating signs of fatty liver. Interestingly, in G3–5, the color of the liver was darker than the liver in the model group, close to the colour of the liver in the physiological control group (G1) (Figure 1C, D, and E).

The prevalence of non-alcoholic fatty liver disease (NAFLD) is strongly increasing and may put patients at increased risk for atherosclerotic cardiovascular disease (CVD). Both disease phenotypes often co-occur, in the case of obesity, insulin resistance, diabetes mellitus type 2, and the metabolic syndrome.[4,30] On atherosclerotic rat models, the effects of the study drug were often evaluated for both atherosclerosis and hepatic steatosis, suggesting a strong association of drug efficacy in improving these two symptoms.[31,32]

A*ssessment of abdominal aortic atherosclerosis*

Histopathology specimens of the rats' abdominal aortas were interpreted at the Department of Pathology, Hospital 103. Histopathological images of the rats' abdominal aortas, representing each rat group, are presented in Figure 2.

The results of the microscopic image analysis of the rats' abdominal aortas showed that the histopathology of the rat abdominal aorta of the G1 is thin and smooth and the muscle cells are oriented horizontally. However, in G2, the vessel wall is rough and foam cells (black arrow) appear in the submucosal layer, which is the basis of the atheroma growth (red arrow) forming above. Interestingly, histopathological images of the abdominal aorta in G3–5 were similar to those of the physiological control group, G1. The vessel walls of these groups were slightly rougher than those of G1, with no expression of foam cells or atheroma.

Atherosclerosis is a chronic inflammatory disease with lipid deposition, oxidative stress, foam cell formation. The persistence of cholesterol-engorged macrophages (foam cells) in the artery wall fuels the development of atherosclerosis. Atherosclerosis can occur in many vascular entities, such as the carotid artery, coronary artery, lower extremity arteries, etc., and it can lead to a variety of related diseases. When atherosclerotic plaques rupture, they generate blood clots that fill the vessel-which is already narrowed by atherosclerotic plaques.[33] These almost always play a major role in cardiovascular events: myocardial infarction, cerebral infarction, limb embolism, etc.[5,6] Many studies have been focused on the treatment of cardiovascular diseases, including atherosclerosis by using natural medicinal products.[7,8,34,35] *C. chrysantha* regulated the heart rate and decreased the total lipid levels in blood serum.[13-17] *G. pentaphyllum* had effects of reducing blood lipids, blood sugar, and the cardiovascular system.[20-22] and *C. hindsii* had effects on cardiovascular disorders.[36] In our experiment, a mixture of *C. chrysantha*, *G. pentaphyllum*, and *C. hindsii* extracts reduced the lipid test indexes in the blood, including TG levels, total cholesterol, LDL-cholesterol, increased the blood HDL-cholesterol, and inhibited foam cell or plaque formation. Furthermore, at doses of 16.8 and 33.6 g/kg/day, the extracts exhibited good effects in treating atherosclerosis, equivalent to the reference drug atorvastatin at a dose of 10mg/kg/day after inducing atherosclerosis in white rats with a diet high in fried fats. This might be a potential product to treat atherosclerotic.

Table 4: HDL-Cholesterol levels in the blood (mmol/l) of rats ($n = 10, \overline{X} \pm SD$)

Experiment groups	HDL-Cholesterol levels (mmol/L)			
	Before treatment (a)	**After 14 days** (b)	**After 28 days** (c)	*p*
G1	0.81 ± 0.23	0.83 ± 0.16	0.79 ± 0.25	$p > 0.05$
G2	0.75 ± 0.19	0.77 ± 0.24	0.80 ± 0.28	$p > 0.05$
G3	0.77 ± 0.22	0.98 ± 0.27	1.05 ± 0.31**	$p_{b-a} < 0.05$
G4	0.73 ± 0.26	1.08 ± 0.30*	1.12 ± 0.33**	$p_{c-b} < 0.05$
G5	0.79 ± 0.32	1.06 ± 0.23*	1.09 ± 0.24**	$p_{c-a} < 0.01$
p	$p_{-1} > 0.05$	$p_{3,4,5-1} < 0.01$; *$p_{2} < 0.05$; **$p_{2} < 0.01$		

Values are represented as Mean ± standard deviation. Values with different figures as superscripts in a row differ significantly ($p \leq 0.05$). Values with different alphabets as superscripts for a parameter in a column differ significantly ($p \leq 0.05$).

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Table 5: LDL-Cholesterol levels (mmol/l) in rat blood (n = 10, $\overline{X}$ ± SD)

Experiment groups	LDL-Cholesterol levels (mmol/L)			
	Before treatment (a)	**After 14 days** (b)	**After 28 days** (c)	*p*
G1	0.62 ± 0.26	0.59 ± 0.23	0.64 ± 0.20	p > 0.05
G2	2.71 ± 0.48	2.59 ± 0.59	2.48 ± 0.63	p > 0.05
G3	2.64 ± 0.51	1.62 ± 0.50*	1.03 ± 0.47**	$p_{b-a} < 0.05$
G4	2.68 ± 0.56	1.25 ± 0.46**	0.81 ± 0.45**	$p_{c-b} < 0.05$
G5	2.72 ± 0.43	1.28 ± 0.53**	0.84 ± 0.44**	$p_{c-a} < 0.01$
p	$p_1 < 0.01$	$p_1 < 0.01$; * $p_2 < 0.05$; ** $p_2 < 0.01$		-

Values are represented as Mean ± standard deviation. Values with different figures as superscripts in a row differ significantly ($p \leq 0.05$). Values with different alphabets as superscripts for a parameter in a column differ significantly ($p \leq 0.05$).

Table 6: Atherogenic index (A.I) (n = 10, $\overline{X}$ ± SD)

Experiment groups	Atherogenic index			
	Before experiment (a)	**After 14 days** (b)	**After 28 days** (c)	*p*
G1	1.25 ± 0.41	1.22 ± 0.36	1.19 ± 0.32	p > 0.05
G2	4.51 ± 0.55	4.26 ± 0.48	4.03 ± 0.59	p > 0.05
G3	4.62 ± 0.52	2.41 ± 0.65*	1.56 ± 0.47**	$p_{b-a} < 0.05$
G4	4.60 ± 0.49	1.85 ± 0.42**	1.32 ± 0.34**	$p_{c-b} < 0.05$
G5	4.48 ± 0.63	1.88 ± 0.36**	1.36 ± 0.45**	$p_{c-a} < 0.01$
p	$p_1 < 0.01$	$p_1 < 0.01$; * $p_2 < 0.05$; ** $p_2 < 0.01$		-

Values are represented as Mean ± standard deviation. Values with different figures as superscripts in a row differ significantly ($p \leq 0.05$). Values with different alphabets as superscripts for a parameter in a column differ significantly ($p \leq 0.05$).

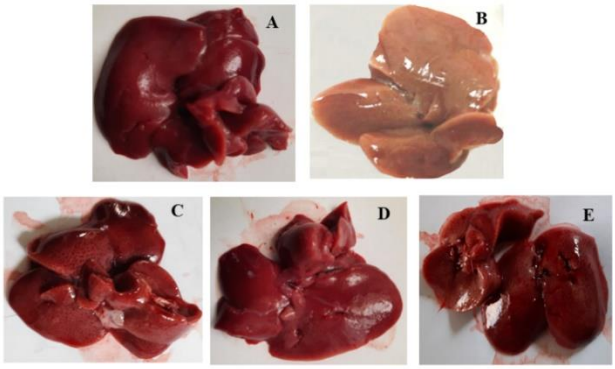

Figure 1: The general appearance of the rats' livers. (A) physiological control group, (B) model group, (C) experimental extract dose 1 group, (D) experimental extract dose 2 group, (E) atorvastatin reference group.

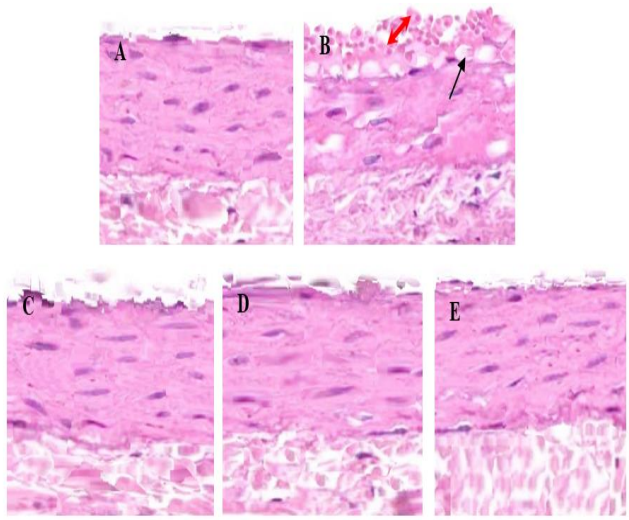

Figure 2: Microscopic image of rats' abdominal aortas. (A) physiological control group; (B) model group: the vessel wall is rough and foam cells (black arrow) appear in the submucosal layer, which is the basis of the atheroma growth (red arrow) forming above; (C) experimental extract dose 1 group; (D) experimental extract dose 2 group; and (E) atorvastatin reference group (HE x 400). (C), (D), (E): Vessel wall thickness was normal. Intimal endothelium structure and submucosal layer were no difference compared with physiological control group.

Conclusion

A mixture extracts of *C. chrysantha*, *G. pentaphyllum*, and *C. hindsii* at doses of 16.8 and 33.6 g/kg/day exhibited good effects in treating atherosclerosis, equivalent to the reference drug atorvastatin at a dose of 10mg/kg/day. The experimental extract reduced the lipid test indexes in the blood, including TG levels, total cholesterol, LDL-cholesterol, and the atherogenic index, increased the blood HDL-cholesterol, and inhibited foam cell or plaque formation. The extract also seemed to reduce hepatic steatosis in white rats with atheroma. These results suggest that mixture extracts of *C. chrysantha*, *G. pentaphyllum*, and *C. hindsii* can provide anti-atherosclerotic properties, as well as being an important promising source of natural anti-atherosclerotic.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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