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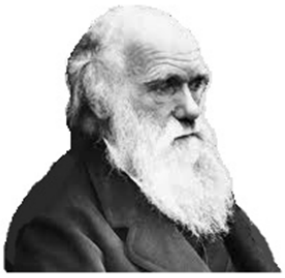

World News of Natural Sciences

An International Scientific Journal

WNOFNS 55 (2024) 16-24 EISSN 2543-5426

Study on preparation and lipid-lowering activity of a compoud based on "*Cymbopogon flexuosus*, *Salvia miltiorrhiza*, *Panax ginseng* and *Pueraria lobata*" active components

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ABSTRACT

Objective: to prepare an active compound from the extracts of four traditional Chinese medicines that can reduce blood lipids. Methods: In this study, the active components were respectively extracted from four Chinese herbal medicines, namely, "*Cymbopogon flexuosus*", "*Salvia miltiorrhiza*", "*Panax ginseng*" and "*Pueraria lobata*", and then the active components were mixed in different proportions to prepare a compound. Subsequently, the *in vivo* lipid-lowering effects were investigated, and the results showed that the compound prepared in this study has the efficacy of lowering blood lipids. Conclusion: The "compound" prepared in this study can be developed as a functional food or beverage with auxiliary lipid-lowering efficacy.

***Keywords*:** *Cyanococcus aurantium, Salvia miltiorrhiza, Panax ginseng, Pueraria lobata,* active components, extractions, compound, hypolipidemic

1. INTRODUCTION

Dyslipidemia, also known as hyperlipidemia, is a prevalent chronic metabolic disease in developed and highly urbanized areas, with increasing incidence rates [1]. Recent decades have

(Received 25 April 2024; Accepted 21 May 2024; Date of Publication 23 May 2024)

seen a significant rise in blood lipid levels and dyslipidemia prevalence among the Chinese population, particularly in hypercholesterolemia. National survey data from 2018 indicates that 35.6% of adults aged ≥18 years in China have dyslipidemia, affecting nearly 4 out of 10 individuals [2]. The "Report on Nutrition and Chronic Disease Status of Chinese Residents 2020" reports 200 million cases of dyslipidemia and 100 million cases of hyperlipidemia in the country.Patients with dyslipidemia are not only adults, as the prevalence of hypercholesterolemia is also high among children and adolescents, with blood lipid levels on the rise. Data indicates that the expected increase in serum cholesterol levels in the Chinese population may result in approximately 9.2 million cardiovascular events in the country from 2010 to 2030 [3].

Currently, clinically used drugs for regulating blood lipids mainly consist of active lipid regulators like fibrates (e.g. pravastatin, atorvastatin, lovastatin) and non-fibrates (e.g. ezetimibe); insulin sensitizers such as glitazones (e.g. rosiglitazone, daglitazone) and metformin; insulin secretion promoters, and drugs that block absorption like bile acid chelating resins (e.g. cholestyramine, cholinine) and cholesterol absorption inhibitors (e.g. ezetimibe). Long-term use of lipid-modifying drugs can lead to side effects such as abnormal liver function, muscle pain, cramps, and digestive issues [4, 5]. Traditional Chinese medicines are known for their precise curative effects and minimal side effects, disocovering new blood-lipid-lowering drugs from Traditional Chinese medicines become the mainstream [6].

As common Chinese medicinal materials, *Salvia miltiorrhiza*, *Panax notoginseng*, and *Pueraria lobata* have been proven to have certain blood lipid regulating effects [7-10]. The polysaccharides and flavonoids in *Cyclocarya paliurus* can lower blood lipids [7, 11, 12]; salvianolic acid and other components in *Salvia miltiorrhiza* can inhibit platelet aggregation and lower cholesterol [13, 14]; *Panax notoginseng* has the effects of lowering blood lipids and anti-inflammatory properties. It has medicinal effects such as inflammation, antioxidant, and immune regulation [15]; *Pueraria lobata* can improve cholesterol metabolism and has obvious blood-lipid-lowering effects [16]. To investigate the synergism of blood lipid-lowering properties of *Cyclocarya paliurus*, *Salvia miltiorrhiza*, *Panax notoginseng*, and *Pueraria lobata*, in this study, we extracted the active ingredients using water from above four Chinese plant medicines, and then prepared a compound with the four extracts in different proportions. Subsequently, we tested the *in vivo* hyperlipidemic. The aim of this work is to present novel possibilities for the development of anti-hyperlipidemic drugs.

2. EXPERIMENTAL SECTION

2. 1. Main instruments and reagents

The Dirui CS-480 fully automatic biochemical analyzer was provided by Dirui Medical Technology Co., Ltd. The triglyceride (TG) detection kit (A110-1-1), total cholesterol (TC) detection kit (F002-1-1), low-density lipoprotein cholesterol (LDL-C) detection kit (Cat. No.: A113-2-1), and serum high-density lipoprotein cholesterol (HDL-C) detection kit (A114-1-1) were sourced from Nanjing Jiancheng Bioengineering Research Institute Co., Ltd. Ethanol (95%) was obtained from Sinopharm Chemical Reagent Co., Ltd. Green Qianliu was purchased from Jianshui County Clover Biotechnology Co., Ltd. Salvia miltiorrhiza, Panax notoginseng, and *Pueraria lobata* were purchased from Anguo Kangtai Jiaye Traditional Chinese Medicine Co., Ltd. Other chemical reagents used were of commercially available analytical grade.

2. 2. Experimental animals

SPF grade SD male rats [Certificate: SCXK (Beijing) 2019-0008, provided by Beijing Huafukang Biotechnology Co., Ltd.] weighing between 180 g and 220 g were used in the study. The rats were housed at the Tianjin Center for Disease Control and Prevention and fed a maintenance diet (certified by SCXK (Beijing) 2019-0008, provided by Beijing Huafukang Biotechnology Co., Ltd.) supplemented with 20.0 % sucrose, 15 % lard, 1.2 % cholesterol, and 0.2 % sodium cholate to create the model feed. The diet also included appropriate amounts of casein, calcium hydrogen phosphate, stone powder, among others. The rats were kept in a barrier-level environmental animal laboratory with a temperature range of 20 to 25 °C and a relative humidity of 40 to 70 % RH. The laboratory held a license for animal use with the number SYXK (Tianjin) 2019-0005.

2. EXPERIMENT PROCEDURE

2. 1. Preparation of active ingredients of *Cyclocarya paliurus* leaves (CPAE)

1.5 kg of powdered *Cyclocarya paliurus* leaves (CPAE) was placed into a 30L extraction tank. It was extracted using water for twice: for the first time, adding 12 times the amount of water, extracting at 90 °C for 1.5 hours, and filter using a 200 mesh filter; for the second extraction, adding 10 times the amount of water, and extracting at 90 °C for 1.5 hours, and filter using a 200 mesh filter. The two filtrates were combined and concentrated under reduced pressure (80 °C, -0.08 Mpa) to obtain a solution with the relative density of 1.10. Then, the solution was placed in an open pot (30L spherical concentrator), and steamed through the interlayer to concentrate at 100 °C for 4 hours to obtain a paste with the specific gravity of d=1.18. Subsequently, the paste was dried under reduced pressure (80 °C, -0.08 Mpa) until the moisture content is less than 5 % to obtain the water extract. Crushing the extract and passing it through a 60-mesh sieve to obtain dry paste powder (marked as **A**). The extraction rate of *Cyclocarya paliurus* leaves (CPAE) is 9%.

The other three extracts of "*Salvia miltiorrhiza*, *Panax notoginseng*, and *Pueraria lobata*" were obtained according to the processes of "*Cyclocarya paliurus* leaves", and which were marked as " **B**, **C**, and **D**" respectively.

2. 2. The process for preparation of the compound

A (150.0 g), B (187.5 g) , C (53.3 g), D (75.0 g), microcrystalline cellulose (34.2 g), and magnesium stearate (5.0 g) were added in a mixer and mixed for 30 minutes to create the mixture (the title compound). The mixture was added in the capsules with a filling capacity of 0.5 g per pellet, containing A (0.15 g), B (0.1875 g), C (0.0533 g), and D (0.075 g).

2. 3. Lipid lowering activity test

The prepared compound is intended for human dosage at 4.0g/60kg·BW·day. The specific dosage design includes 0.335g·BW, 0.67g·kg·BW, and 1.34g·kg·BW for low, medium, and high dose groups, respectively. These doses are equivalent to 5 times, 10 times, and 20 times as the intended human dose. Animals are individually housed in cages.

Eighty rats were fed a specific diet for 15 days and then randomly divided into two groups based on body weight, ensuring that the weight difference within each group did not exceed

10% of the average body weight. Fourteen rats were assigned to the blank control group, which received regular maintenance feed, while sixty-six rats were assigned to the model group, which received the experimental model feed. The rats were weighed weekly. After two weeks of feeding the model group, 2 mL of blood was collected from both the blank control and model groups without fasting.

The blood samples were centrifuged at high speed (3000 rpm, 10 min) to separate the serum, and serum total cholesterol (TC) and triglyceride levels were measured using a kit. Additionally, lipid (TG), high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein cholesterol (LDL-C) levels were assessed. The model group was then divided into four groups based on TC levels, with the highest and lowest values being excluded. Each group consisted of 10 animals, with three groups serving as experimental groups and one group serving as the model control group. Similarly, the highest TC value was excluded in the blank control group, and 10 animals were selected, with one rat assigned to the blank control group.

The experimental group received low, medium, and high doses of the active component complex once a day, while the model control group and blank group were given the same amount of distilled water daily. The experimental and model groups were fed model feed, and the blank group was fed maintenance feed. The weight of each mouse was measured weekly. After 30 days, 2 mL of blood was collected from each group of mice, and the serum was separated by high-speed centrifugation (3000 rpm, 10 min). The levels of TC, TG, HDL-C, and LDL-C in the serum samples were then measured.

3. RESULTS AND DISCUSSION

3. 1. Validation of animal models

A mixed hyperlipidemia animal model was utilized in this study. The model group received model feed, while the blank control group received maintenance feed for a duration of 2 weeks. Following this period, serum levels of total cholesterol (TC), triglycerides (TG), and low-density lipoprotein cholesterol (LDL-C) were assessed in the experimental mice. The results (shown in **Table 1**) indicate that the model group exhibited significantly higher levels of TC, TG, and LDL-C compared to the blank control group ($P<0.05$), confirming the successful establishment of the mixed hyperlipidemia animal model.

Table 1. Comparison results of TC, TG, HDL-C and LDL-C in animals after modeling and blank control (mmol/L, X±SD).

Dose (g/kg·BW)	Number of animals (only)	TC	TG	HDL-C	LDL-C
Blank control group	10	2.08±0.25	1.80±0.58	1.13±0.13	0.26±0.039
model group	40	3.01±0.37$^{\#}$	4.48±1.31$^{\#}$	1.08±0.22	0.75±0.16$^{\#}$

3. 2. Effects of the compound prepared in this study on serum total cholesterol (TC) in experimental rats

The experimental process described in 2.4 was used to test the impact of the prepared compound on rat serum TC levels at various concentrations.

The results were shown in **Table 2**. From **Table 2**, we can know that both the control and model groups before and after the experiment demonstrated the success and stability of the model.

In the experimental group, the results before and after the experiment revealed that the compound prepared in this study effectively reduces serum total cholesterol (TC) in experimental mice, with the most optimal effect observed at lower doses.

Table 2. Effect of compound prepared in this study on serum TC levels in rats.

Dose (g/kg·BW)	Number of animals (only)	Before experiment	After experiment
Blank control group	10	2.03±0.12	2.12±0.07
model group	10	3.03±0.11*	3.07±0.06*
0.335	10	3.05±0.12	2.54±0.09#
0.67	10	3.03±0.10	2.91±0.06#
1.34	10	3.02±0.08	3.01±0.11

*Compared with the blank group, the difference is statistically significant(P<0.05).
#Compared with the model group, the difference is statistically significantt (P<0.05).

3. 3. Effects of the complex of active components of four traditional Chinese medicines on serum triglycerides (TG) in experimental rats

In order to evaluate the impact of this compound on serum triglyceride (TG) levels in experimental mice, we followed the experimental process outlined in section 2.4. The results (shown in **Table 3**) indicated that this compound effectively reduced TG levels in the mice. Prior to and post-experiment, both the normal and model groups displayed successful and stable results.

The experimental group data further supported the efficacy of this compound prepared in this study in reducing TG levels. Moreover, as the duration of medication increased, this compound exhibited significant improvements in TG levels in rats induced by a high-fat diet, with smaller doses yielding more favorable results.

Table 3. Effect of this compound prepared in this study on serum TC levels in rats

Dose (g/kg·BW)	Number of animals (only)	Before experiment	After experiment
Blank control group	10	1.79±0.07	2.3±0.07
model group	10	4.45±0.15*	4.48±0.07*
0.335	10	4.54±0.09	3.22±0.07#
0.67	10	4.58±0.13#	3.6±0.12#
1.34	10	4.43±0.06	3.74±0.05#

*Compared with the blank group, the difference is statistically significant (P<0.05).
#Compared with the model group, the difference is statistically significantt (P<0.05).

3. 4. Effect of this compound preapared in this study on serum low-density lipoprotein cholesterol (LDI-C) in experimental mice

The impact of this compound on serum low-density lipoprotein cholesterol (LDL-C) in experimental mice was evaluated according to the experimental procedure described in section 2.4. The results were listed in **Table 4**. Comparison with the serum LDL-C levels before and after experiment among the experimental group, model group, and normal control group revealed a significant improvement in LDL-C levels in the experimental mice treated with this compound, particularly at higher concentrations.

Table 4. Effect of active ingredient complex on serum LDI-C levels in rats

Dose (g/kg·BW)	Number of animals (only)	Before experiment	After experiment
Blank control group	10	0.26±0.04	0.22±0.01
model group	10	0.7±0.09*	0.67±0.02*
0.335	10	0.76±0.08	0.54±0.01#
0.67	10	0.77±0.05	0.49±0.02#
1.34	10	0.79±0.05#	0.42±0.02#

*Compared with the blank group, the difference is statistically significant (P<0.05).
#Compared with the model group, the difference is statistically significantt (P<0.05).

3. 5. Effect of this compound prepared in this study on serum high-density lipoprotein cholesterol (HDI-C) in experimental mice

The impact of this compound on serum high-density lipoprotein cholesterol (HDI-C) in experimental mice was investigated according to the experimental procedure described in section 2.4. The results were presented in **Table 5**.

The results reveals that, in comparison to the control group, the serum HDL-C levels of the model group rats (which were fed with a high-fat diet) exhibited a slight upward trend throughout the testing period, although this increment was not deemed statistically significant (P>0.05).

Furthermore, it was observed that this compound did not have a notable effect on the serum HDL-C levels in experimental mice. These findings suggest that this compound prepared in this study does not directly impact high-density lipoprotein cholesterol.

Table 5. Effect of active ingredient complex on rat serum HDI-C levels

Dose (g/kg·BW)	Number of animals (only)	Before experiment	After experiment
Blank control group	10	1.13±0.05	1.18±0.09
model group	10	1.13±0.04	1.18±0.06
0.335	10	1.13±0.10	1.15±0.03
0.67	10	1.11±0.04	1.16±0.06
1.34	10	1.12±0.08	1.20±0.08

4. CONCLUSION

The compound developed in this study, utilizing "*Cyclocarya paliurus*, *Salvia miltiorrhiza, Panax notoginseng*, and *Pueraria lobata*" as raw materials, has been demonstrated to has the ability to lower blood lipids. This compound shows potentials for the development of functional foods or beverages with reducing blood lipids activity.

Acknowledgment

This work was financially supported by STS project in Xiamen, Fujian Province (2023T3017); the Cooperation Program of Xiamen Institute of Rare-earth Materials and Academician workstation, Xiamen Tasman Bio-Tech Research Institute (Nos: 2022350204002401:2022350204004248) and Xiamen Science and Technology Proiects(Nos: 20210706:2021FCX012502040011); Xiamen Science and Technology Bureau Project (20210706).

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