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Original Article

# Anti-atherosclerotic effect of Fermentum Rubrum and *Gynostemma pentaphyllum* mixture in high-fat emulsion- and vitamin $D_3$-induced atherosclerotic rats

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**Abstract**

*Background*: The mixture of Hongqu and gypenosides (HG) is composed of Fermentum Rubrum (Hongqu, in Chinese) and total saponins of *Gynostemma pentaphyllum* (Thunb.) Makino (Jiaogulan, in Chinese) in a 3.6:1 weight ratio. Both Hongqu and Jiaogulan are considered valuable traditional Chinese medicines (TCMs); they have been commonly used in China for the treatment of hyperlipidemia and related diseases for centuries. The aim of the current study was assess the anti-atherosclerotic effect of HG.

*Methods*: Sixty-four Wistar rats were randomly divided into eight groups: normal, model, positive control (simvastatin, 1 mg/kg), Hongqu-treated (72 mg/kg), gypenoside (total saponin)-treated (20 mg/kg), and three doses HG-treated (50, 100, and 200 mg/kg). All of the rats were fed a basal diet. Additionally, the model group rats were intragastrically administered a high-fat emulsion and intraperitoneally injected with vitamin $D_3$. The serum lipid profiles, oxidative stress, inflammatory cytokine, and hepatic antioxidant levels were then determined. Furthermore, the liver histopathology and arterial tissue were analyzed, and the expression of hyperlipidemia- and atherosclerosis (AS)-related genes was measured using reverse transcription-polymerase chain reaction.

*Results*: The AS rat model was established after 80 days. Compared to the model group, the HG-treated groups showed an obvious improvement in the serum lipid profiles, oxidative stress, and inflammatory cytokine levels, and showed markedly increased hepatic total antioxidant capacity. Moreover, the expression of genes related to lipid synthesis and inflammation reduced and that of the genes related to lipid oxidation increased in the liver and arterial tissue, which also reflected an improved health condition.

*Conclusion*: the anti-atherosclerotic effects of HG were superior to those of simvastatin, Hongqu, and the gypenosides. Therefore, HG may be a useful anti-atherosclerotic TCM preparation.



*Keywords:* Atherosclerosis; Fermentum Rubrum; *Gynostemma pentaphyllum*; Gypenosides; Hyperlipidemia; Red yeast rice

*Abbreviations:* AS, atherosclerosis; ACC-1, acetyl-CoA carboxylase-1; CAT, catalase; CCL-5, chemokine (C−C motif) ligand 5; COX-2, cyclooxygenase-2; CPT-1, carnitine palmitoyl transferase-1; CRP, C-reactive protein; CVD, cardiovascular disease; FAS, fatty acid synthase; GSH, glutathione; HDL-C, high-density lipoprotein cholesterol; H&E, hematoxylin and eosin; HG, Hongqu and gypenosides; HMGR, 3-hydroxy-3-methylglutaryl coenzyme A reductase; ICAM-1, intercellular adhesion molecule-1; IL-6, interleukin-6; LDL-C, low-density lipoprotein cholesterol; MCP-1, monocyte chemotactic protein-1; MDA, malondialdehyde; NAFLD, non-fatty liver disease; NF-$\kappa$B, nuclear factor-$\kappa$B; NIH, National Institutes of Health; NO, nitric oxide; $NOS_1$, neuronal nitric oxide synthase; $NOS_2$, inducible nitric oxide synthase; $NOS_3$, endothelial nitric oxide synthase; PPAR-$\alpha$, peroxisome proliferator-activated receptor $\alpha$; PTU, propylthiouracil; ROS, reactive oxygen species; RT-PCR, reverse transcription-polymerase chain reaction; SOD, superoxide dismutase; SREBP-1c, sterol response element-binding protein-1c; TAC, total antioxidant capacity; TC, total cholesterol; TCM, traditional Chinese medicine; TG, triglyceride; TNF-$\alpha$, tumor necrosis factor-$\alpha$; VCAM-1, vascular cell adhesion molecule; $VD_3$, vitamin $D_3$.

Conflicts of interest: The authors declare that they have no conflicts of interest related to the subject matter or materials discussed in this article.

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## 1. Introduction

Atherosclerosis (AS) is characterized by chronic arterial inflammation caused by numerous factors such as dyslipidemia, neurovascular dysfunction, and oxidative stress.[1] Furthermore, it is the principal cause of cardiovascular disease (CVD), stroke, and peripheral necrosis, accounting for up to 50% of mortalities in Western countries.[2] Changing lifestyle habits of the modern society and environmental conditions have led to the development of AS at younger ages, with mortality rates due to its complications increasing yearly.[3−5]

Lipid lowering is widely recognized as an anti-AS treatment strategy. The risk of AS is greatly reduced by lowering the serum total cholesterol (TC) and triglyceride (TG) levels.[6] Therefore, excessive serum lipids, as well as high levels of oxidative stress in vivo, are also therapeutic targets of AS.[7,8] Moreover, the equal importance of anti-inflammatory and lipid-lowering therapies for AS cannot be ignored.[9,10] Consequently, the regulation of serum lipids, oxidative stress, and inflammation is an integral therapeutic regimen in the treatment of AS.

Hongqu, which is also known as red yeast rice, koji, and anka, is produced by fermenting moist, sterile rice with the yeast, *Monascus purpureus*. It is used as a food coloring agent and for brewing red yeast wine in China.[11] Modern research has shown that Hongqu contains numerous statin-like substances, and it has been used as an alternative to statins for the treatment of hyperlipidemia.[12−15] The commercial drugs, xuezhikang and zhibituo, which are red yeast rice preparations, have been clinically used to treat hyperlipidemia.[14] Gypenosides are extracts of the total saponins of Jiaogulan. The leaves of Jiaogulan are prepared from valuable and healthy teas in China.[16] Modern chemical and medical research studies have shown that Jiaogulan contains numerous dammarane-type glycosides, which are similar to ginsenosides,[17−19] and gypenosides that have shown potential in reducing the risk of cardiovascular disease, cancer, and Alzheimer's disease, and have anti-hyperlipidemic, hypoglycemic, anti-inflammatory, and antioxidant properties.[20−22] There are reports that gypenoside XLIX is an agonist of peroxisome proliferator-activated receptor (PPAR)-$\alpha$.[23] This suggests that similarly to fibrates, gypenosides can reduce blood triacylglycerol levels. Both Hongqu and gypenosides potently lower cholesterol and exert antioxidant and anti-inflammatory effects. Additionally, Hongqu is “warm” while “Jiaogulan” is cold by nature according to TCM theory; the combination of “warm” and “cold” leads to “neutral,” which is more acceptable to a human body that is under the “neutral” condition, thus reducing the side effects of the medication.

In a previous study, we investigated the lipid-lowering, hepatoprotective, and atheroprotective effects of HG in hyperlipidemia by using a non-fatty liver disease (NAFLD) rat model established by administering a high-fat diet.[24] We found that HG adequately regulated blood lipids, and exhibited anti-inflammatory and anti-oxidative effects on protein molecules and enzymes, and at the genetic level.[24] In this study aimed at investigating the anti-atherosclerotic effects of HG, an atherosclerotic rat model was established by intragastric administration of a high-fat emulsion and intraperitoneal injection of vitamin $D_3$ ($VD_3$).

The anti-atherosclerotic effects of HG were evaluated mainly based on four therapeutic strategies: regulating blood lipids, anti-inflammation, antioxidant effects, and histopathological outcomes. In addition, the possible mechanisms of action were elucidated by determining the expression of genes associated with lipid synthesis and oxidation in liver tissue, as well as those associated with the development of AS in arterial tissue, by reverse transcription-polymerase chain reaction (RT-PCR).

## 2. Methods

### 2.1. Primary reagents and drugs

Hongqu (batch number: 2014012301) was purchased from Zhejiang Sanhe Bio-tech Co., Ltd., (Jiangshan, China) and the gypenosides (approval number: Z61020872) were purchased from Ankang Chia Tai Pharmaceutical Co., Ltd., (Ankang, China). $VD_3$ injection was produced by Shanghai General Pharmaceutical Co., Ltd., (lot: 130126), while anhydrous sodium acetate (lot: L480P65), N'N-diethyl-p-phenylene diamine sulfate (DEPPD, lot: LG90P53), and analytical-grade iron sulfate heptahydrate (lot: L5B0P40) were purchased from J&K Scientific Ltd., (Beijing, China).

### 2.2. Preparation and quality control of HG

HG is composed of Hongqu, gypenosides, and excipients at a weight ratio of 3.6:1:0.4. The quality control analyses of Hongqu and HG were performed using a Waters 2998 high-performance liquid chromatography (HPLC) system equipped with a Waters 1525 binary pump and photodiode array detector system. Lovastatin was used as a standard substance. The sample was separated using a Lichrospher $C_{18}$ column (250 mm × 4.6 mm, 7-μm), and the temperature was maintained at 36 °C. The mobile phase consisted of phase A (phosphate: water = 0.1:100, v/v) and phase B (acetonitrile [ACN]). An isocratic elution was conducted with mobile phases A and B at a ratio of 35:65 (v/v). The total saponins of the gypenoside raw material and HG were determined using the vanillin-glacial acetic acid-perchloric acid method by ultraviolet visible light spectrophotometry (UV-2550, Shimadzu, Japan), and gypenoside XLIX was used as the reference substance.

### 2.3. Animal management and model establishment of AS

Sixty-four healthy 8-week-old adult male Wistar rats weighing 300 ± 5 g were purchased from the Lanzhou University School of Medicine, Gansu Province Key Laboratory of Drug Preclinical Research (laboratory animal certificate: scxk2013-0002). All experimental procedures were performed in accordance with the National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals (NIH Publications No. 8023, revised 1978). The rats were housed in

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