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Original Article

Lipid-lowering, hepatoprotective, and atheroprotective effects of the mixture Hong-Qu and gypenosides in hyperlipidemia with NAFLD rats

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Abstract

Background: Hyperlipidemia and its complications are among the most harmful of diseases with a worldwide impact, which creates an urgent imperative to find safe and effective drugs for treatment. HG is mainly composed of two kinds of traditional Chinese medicines (TCM), Hong-Qu and gypenosides. Previously, the ingredients of the mixture mainly composed by Hong-Qu and gypenosides (HG) were widely used for purposes of lipid-lowering, antiatherosclerosis effects, and maintaining cardiovascular health in China. The purpose of this study was to determine whether HG provides any benefit to patients with hyperlipidemia.

Methods: Forty-eight adult male Sprague-Dawley rats with fatty liver disease were randomly divided into six groups: normal, model, two positive controls, and two doses of HG-treated groups. The normal rats were fed a basal diet, and the other rats were fed a high-fat diet. Thereafter, the serum lipid profiles, hepatic steatosis, cytokines, enzymes, and relevant mRNA of rats were analyzed in serum, aorta tissue or hepatic tissues, respectively.

Results: After 65 days of feeding the high-fat diet to rats, there were significantly disordered serum lipid profiles, elevated oxidative stress biomarkers, and decreased antiinflammatory cytokines in the serum levels. Additionally, aortic foam cell formation was increased. The gene expression levels including hydroxymethylglutaryl-CoA reductase (HMGR), peroxisome proliferator-activated receptor alpha (PPAR-α), sterol response element-binding protein-1c (SREBP-1c), fatty acid synthase (FAS), acetyl-CoA carboxylase-1 (ACC-1) and carnitine palmitoyl transferase-1(CPT-1) in hepatic tissue were also altered by a high-fat diet fed to Sprague-Dawley rats, and HG treatment significantly resolved and normalized these alterations. Moreover, HG not only caused a significant decrease in the lipid drops on the hepatic tissues, but also restored the antioxidant components.

Conclusion: HG is beneficial for regulating the stability of blood lipids, has atheroprotective characteristics and may prevent nonalcoholic fatty liver disease (NAFLD), providing more than just a theoretical basis for drug research of cardiovascular disease (CVD) treatment.

Keywords: *Fermentum Rubrum*; *Gynostemma pentaphyllum*; gypenosides; Hong-Qu; hyperlipidemia; NAFLD

1. Introduction

Hyperlipidemia is a common metabolic syndrome characterized by diverse lipid profiles, such as hypercholesterolemia and hypertriglyceridemia, and it may induce significant adverse effects in humans.[1] The prevalence of hyperlipidemia has been correlated with different dietary habits and levels of physical activity, as well as genetic background.[2]

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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Hyperlipidemia is often accompanied by the occurrence of cardiovascular events, and active prevention and treatment of hyperlipidemia will reduce the risk of cardiovascular events.[3] Nonalcoholic fatty liver disease (NAFLD) is the hepatic counterpart of metabolic syndrome. As a risk factor for cardiovascular disease (CVD), it is the presence of excessive fat accumulation in the liver which frequently starts as a simple benign steatosis due to insulin resistance, obesity, high-fat food, chronic overnutrition, or metabolic abnormalities, becoming type 2 diabetes mellitus and hyperlipidemia without the alcohol abuse.[4–6] Some patients will even develop liver cell injury and hepatic fibrosis, which can progress to cirrhosis.[7,8]

Currently, there are several varieties of fast acting and effective lipid-lowering drugs that regulate serum lipid profiles. However, their use is limited by the differences between individual patients, poor tolerance, potential adverse effects, and drug dependence.[9–11] In addition, most NAFLD patients with hyperlipidemia require long-term medication, have poor tolerance and can be subject to obvious side effects. Presently, it is important to seek out safe and effective drugs to address hyperlipidemia and its associated health challenges. One possible solution may lie within the field of traditional Chinese medicine (TCM), where remedies are often natural and plant-derived, and have minimal adverse effects, presenting multiple targets for the prevention and cure of hyperlipidemia with NAFLD.[12]

One such well-known Chinese remedy, Hong-Qu, or red yeast rice (RYR, *Fermentum Rubrum*, Hong-Qu in Chinese), is produced and obtained conforming to an ancient Chinese method which is by fermenting the moist and sterile rice which was inoculated with the fungal strain *Monascus purpureus*.[13] Hong-Qu is used for treating hyperlipidemia, treatment or prevention of osteoporosis, as an antitumor, and assisting in the treatment of other CVDs caused by hyperlipidemia and atherosclerosis.[14–16] In China, Zhibituo tablet (ZT) is a Chinese patent medicine in which the main ingredient is Hong-Qu and is used for treating hyperlipidemia, CVD, and improving digestion.[17] Gypenosides, which are the extraction of total saponins of *Gynostemma pentaphyllum* (Jiaogulan in Chinese), are often applied to cure hyperlipidemia, atherosclerosis, hypoglycemia, and have been shown to be neuroprotective and immunomodulatory. Jiaogulan total saponins tablet (JT) is a Chinese patent medicine that is mainly composed of gypenosides and is used for treating hyperlipidemia and atherosclerosis.[18] Additionally, Hong-Qu is warm and gypenosides are cold by nature, according to TCM theory. What is more, both Hong-Qu and gypenosides can be used for nourishing the heart and spleen, eliminating phlegm and dampness, and activating blood circulation to dissipating blood stasis in the theory of TCM.[19,20] In this research, we combined Hong-Qu with gypenosides and named it HG. The purpose of the present study was to evaluate the cardiovascular, liver, and arterial protective effects of the mixture mainly composed by Hong-Qu and gypenosides (HG) as compared with the individual effect of two Chinese patent medicines (ZT and JT) using hyperlipidemia with NAFLD rats models.

2. Methods

2.1. Sources of drugs and kits

Hong-Qu was purchased from Zhejiang Sanhe Bio-tech Co., Ltd (Jiangshan, China) (batch number: 2014012301), and gypenosides was purchased from Ankang Chia Tai Pharmaceutical Co., Ltd. (Ankang, China) (drug approval number: Z61020872). The first control drug, ZT, was purchased from Chengdu Di'aojiuhong Pharmaceutical Factory (Chengdu, China) (drug approval number: Z20025688; batch number: 1310005). The other control drug, JT, was purchased from Hutchison Whampoa Guangzhou Baiyunshan Chinese Medicine Co., Ltd. (Guangzhou, China), (drug approval number: Z44021756; batch number: G3A001). Additionally, lovastatin (batch number: 130320) and gypenoside XLIX (batch number: 130320) were purchased from the Chengdu Pufei De Biotech Co., Ltd. (Chengdu, China).

The biochemical autoanalyzer assay kits for total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) were purchased from Sichuan Maker Bitotechnology Co., Ltd. (Chengdu, China). Protein quantification, TC, and TG in livers, reactive oxygen species (ROS), NO, methane dicarboxylic aldehyde (MDA), total antioxidant capacity (TAC), reduced glutathione (R-GSH), superoxide dismutase (SOD), and catalase activity kits were purchased from the Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China).

2.2. Quality control and preparation of drug

The composition of the HG is Hong-Qu, gypenosides, and excipients with a weight ratio of 3.6:1:0.4. The quality control confirmations of Hong-Qu, ZT, and HG were performed on a Waters 2998 HPLC system equipped with a Waters 1525 binary pump, photodiode array detector system, and a thermostatically controlled column compartment. Lovastatin was used as a standard substance. The sample was separated on 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: H_2O = 0.1:100, v/v) and phase B (ACN). An isocratic elution was constituted by mobile phase A and B as a ratio of volume of 35:65. The flow rate was 1 mL/min and the injection volume was 20 μL. Also, total saponins of gypenosides, JT, and HG determined by visible light spectrophotometry (UV-2550, Shimadzu, Japan), and gypenoside XLIX was used as the reference substance.

2.3. Animals and experimental design

Forty-eight healthy adult male Sprague-Dawley rats, weighing 200 ± 10 g, were purchased from the Lanzhou University School of Medicine, Gansu Province Key Laboratory of Drug Preclinical Research (laboratory animal certificate: scxk2013-0002). All rats were cared for and utilized according to the Guide for the Care and Use of Laboratory

Animals published by the US National Institutes of Health (NIH Publication Number 85−23, revised 1996). Rats were housed in an environmentally controlled room at 23 ± 1°C, 55 ± 5% relative humidity, and a 12-hour light/dark cycle. All rats were fed a commercial basal diet and tap water *ad libitum* for 1 week and were divided randomly into six groups: (1) the normal control (NC) group (n = 8, basal diet, Beijing Keaoxieli Feed. Co., Ltd); (2) the model control (MC) group (n = 8, high-fat diet consisting of 75% basal diet, 9% lard, 5.5% yolk powder, 7.5% sugar, 2.5% cholesterol, 0.3% sodium cholate, and 0.2% propylthiouracil by weight, Beijing Keaoxieli Feed Co., Ltd); (3) the ZT-treated group [n = 8, high-fat diet with ZT 188 mg/kg, intragastric (i.g.) administration]; (4) the JT-treated group (n = 8, high-fat diet with JT 157 mg/kg, i.g.); (5) the high dose HG (HGH)-treated group (n = 8, high-fat diet with HG 102 mg/kg, i.g.); and (6) the low dose HG (HGL)-treated group (n = 8, high-fat diet with HG 51 mg/kg, i.g.). The content of the Monacolin K in 188 mg of ZT is equal to 102 mg of HG and the content of the total saponins of Jiaogulan in 157 mg of JT is equal to 102 mg of HG. After rats in the ZT, JT, HGH, and HGL groups were given a high fat diet for 30 days and their serum lipids were measured, we continued to give them the corresponding drug and feed high-fat diet for 5 weeks.

2.4. Preparations of serum and tissue samples

The serum samples were prepared following blood clotting for purposes of measuring the biochemical parameters. Blood samples were allowed to clot at 4°C and centrifuged at 5000g for 10 minutes before harvesting the serum, and serum samples were then stored at −20°C until assayed. For the histopathological evaluation, and determination of mRNA and related enzymes and factors, the heart and liver were stripped and fixed in 10% formalin at 4°C until assayed. Another part of the aorta and liver was immediately placed in the 10 mL dorf tube which was soaked in 0.1% diethyl pyrocarbonate water and then autoclave sterilized, and thereafter stored at −80°C until assayed.

2.5. Serum lipid profiles

The serum levels of TC, LDL-C, HDL-C, and TG were determined according to the manufacturer's instructions using a biochemical autoanalyzer (Hitachi, Tokyo, Japan). Then, the arteriosclerosis index (AI) was calculated as follows: AI = (TC-HDL-C)/HDL-C.

2.6. Determination of total ROS in the serum

Determination of ROS in the serum was measured spectrofluorometrically according to the manufacturer's protocol, and 1 mmol/L 2′,7′-Dichlorofluorescin diacetate (DCFH-DA) work stock solution was prepared by 0.1 mL of DCFH-DA (10 mM) and 0.9 mL of Phosphate Buffered Saline (PBS) (0.1 M, pH = 7.4), 190 μL of serum was mixed with 10 μL of 1 mmol/L DCFH-DA work stock solution, and 10 μL of PBS was used as control. After 30 minutes of incubation at 37°C, the fluorescence was measured on a spectrofluorometer at 502 nm excitation and 525 nm emission wavelengths.

2.7. Determination of TC, TG, NO, oxidative stress biomarkers, and antioxidant components in the serum and liver tissue

Determination of NO and MDA in the serum and MDA, TC, TG, TAC, R-GSH, SOD, and catalase activity in liver tissues was measured according to the manufacturer's protocol.

2.8. Histopathological analysis of liver and artery

Paraffin sections of aortic arch and livers (4−6 μm) were collected and stained with hematoxylin-eosin (H&E) staining. Liver images were captured with a CX21 microscope (Olympus, Takachiho, Japan). The quantities of fat vacuoles in every random field of liver section and the percentage of foam cell area and the intima area of the aortic arch in every random field were quantified and calculated by computer image analysis using an i-solution image analyzer. We made a classification for liver disease based on the grade of inflammation, the stage of fibrosis, lobular inflammation, ballooning, NAFLD activity score, and the grade and location of steatosis regarding histopathological analysis of liver.

2.9. Real-time quantitative reverse transcription polymerase chain reaction

Total RNA was extracted from liver using RNAiso Plus reagent (Takara Bio-Technology Co., Ltd, Dalian, China) according to the manufacturer's protocol. Reverse transcriptions were performed using a PrimeScript RT Master Mix cDNA synthesis kit (Takara Bio. Inc., Otsu, Shiga, Japan). The reaction volume of 10 μL contained 500 ng total RNA. Real-time polymerase chain reaction (PCR) was performed using SYBR Premix Ex Taq II (Takara Bio. Inc., Otsu, Shiga, Japan) against gene expression for hydroxymethylglutaryl-CoA reductase (HMGR), peroxisome proliferator-activated receptor alpha (PPAR-α), fatty acid synthase (FAS), acetyl-CoA carboxylase-1 (ACC-1), carnitine palmitoyltransferase-1 (CPT-1), sterol response element-binding protein-1c (SREBP-1c), and β-actin with the Corbett Research Rotor-Gene RG 3000 Real Time PCR System (Corbett Research, Hilly Street, Mortlake NSW 2137, Australia). The primers that were used are described in Table 1. Reactions were performed with 10 μL of SYBR Premix Ex Taq II, 1.6 μL of 10 μM primer pair, 6 μL of distilled water, 0.4 μL of Rox Reference Dye (50×) and 2 μL of cDNA. Each PCR run was performed under the following conditions: initial denaturation at 95°C for 30 seconds, and 40 cycles at 95°C for 15 seconds and 60°C for 30 seconds. The gene expression levels were compared with those of β-actin as a reference gene.

Table 1
Target genes and their primer sequences (5'→3', forward and reverse).

Target genes	GenBank accession number	Gene sequences	
		Forward	Reverse
β-actin	NM_031144.3	GGAGATTACTGCCCTGGCTCCTA	GACTCATCGTACTCCTGCTTGCTG
HMGR	NM_013134.2	TGGCAGGACGCAACCTCTAC	AATAGTTACCACTGACCGCCAGAA
PPAR-α	NM_013196.1	GGCAATGCACTGAACATCGAG	GCCGAATAGTTCGCCGAAAG
FAS	NM_017332.1	GCTGCTACAAACAGGACCATCAC	TCTTGCTGGCCTCCACTGAC
ACC-1	NM_022193.1	CAATCCTCGGCACATGGAGA	GCTCAGCCAAGCGGATGTAGA
CPT-1	NM_031559.2	AGGTCGGAAGCCCATGTTGTA	GCTGTCATGCGCTGGAAGTC
SREBP-1c	NM_001276707.1	CCCTGCGAAGTGCTCACAA	GCGTTTCTACCACTTCAGGTTTCA

ACC-1 = acetyl-CoA carboxylase-1; CPT-1 = carnitine palmitoyl transferase-1; FAS = fatty acid synthase; HMGR = 3-hydroxy-3-methylglutaryl coenzyme A reductase; PPAR-α = peroxisome proliferator-activated receptor alpha; SREBP-1c = sterol response element-binding protein-1c.

2.10. Statistical analysis

The experimental data were expressed as the mean ± standard deviation (SD), and differences were considered significant when $p < 0.05$ or $p < 0.01$, as tested by one-way analysis of variance using SPSS 16.0 (IBM, Armonk, NY, USA).

3. Results

3.1. Analysis of the main ingredient of raw materials, positive control drug, and HG

On the basis of Chinese drug quality control standard, the content of Monacolin K in Hong-Qu detected by high performance liquid chromatography (HPLC) is no less than 4.00 mg/g, and the total saponins content in gypenosides determined by visible light spectrophotometry should be no lower than 700 mg/g against gypenoside XLIX. After applying detection and analysis techniques, the content of Monacolin K in Hong-Qu was 30.658 mg/g and analyzed by HPLC in our lab. The actual content of the total saponins in gypenosides was 820 mg/g and determined by visible light spectrophotometry. The main ingredients of raw materials are in line with the standard. As Table 2 shows, the Monacolin K content of ZT is 4.207 mg/0.35 g (each tablet), and the HPLC chromatogram of lovastatin, Hong-Qu, ZT, and HG is shown in Fig. 1; Monacolin K (i.e., lovastatin) is their common ingredient. The content of the total saponins in JT is 20.803 mg/0.195 g (each tablet). Their contents are consistent with provisions of their manufacturer's protocols. The only medicinal ingredient of ZT is Hong-Qu, and the main active substance in Hong-Qu is lovastatin, the content of which is very abundant in Hong-Qu. Therefore, lovastatin was set as a quality control index of Hong-Qu, ZT, and HG.

3.2. Effects on the serum lipid profile

In order to evaluate the success of the hyperlipidemia model of MC, ZT, and JT, the HGH and HGL group rats were fed a high-fat diet for 30 days. Thereafter, it was observed that TG, TC, LDL-C, and AI (> 4) were significantly higher and HDL-C was lower than in the NC group ($p < 0.01$), which has been noted in Table 3; this indicates that the model was successful in inducing hyperlipidemia in rats. As shown in Table 4, after 5 weeks of treatment with ZT, JT, HGH, and HGL groups, significant decreases in TG, TC, LDL-C, and AI (< 4) and increases in HDL comparable with the MC group were observed ($p < 0.01$). Additionally, the effect of HGH group therapy is more obvious compared with ZT, JT group ($p < 0.05$ or $p < 0.01$).

3.3. Effects on the oxidative stress biomarkers and antiinflammatory cytokines in the serum

The high-fat diet fed to the model group drastically increased total ROS and MDA in the serum levels approximately 2.1- and 1.6-fold more than the normal group, respectively. However, administration with HG significantly decreased serum levels of total ROS as compared with the

Table 2
Contents of the main ingredient of raw materials, positive control drug and the mixture composed by Hong-Qu, gypenosides and excipients (HG) and the corresponding contents method for the determination.

Drugs	Contents of the main ingredient		Method for determination	Compliance with quality standards or not
	Monaconlin K	Total saponins		
Hong-Qu	30.658 mg/g	–	HPLC	Compliance
Gypenosides	–	820 mg/g	Visible light spectrophotometry	Compliance
ZT	12.02 mg/g	–	HPLC	Compliance
JT	–	106.68 mg/g	Visible light spectrophotometry	Compliance
HG	22.07 mg/g	200 mg/g	HPLC and visible light spectrophotometry	Compliance

HG = the mixture composed by Hong-Qu, Gypenosides and excipients; HPLC = high performance liquid chromatography; JT = Jiaogulan total saponins tablets; ZT = Zhibituo tablets.

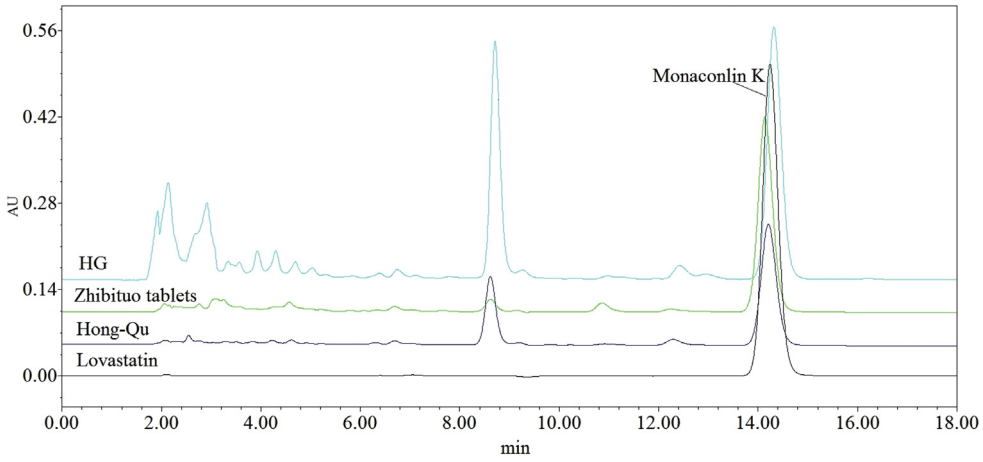

Fig. 1. High performance liquid chromatography (HPLC) chromatogram of lovastatin, Hong-Qu, Zhibituo tablet and Hong-Qu and gypenosides (HG). Monacolin K (lovastatin) is their common ingredient.

model group ($p < 0.01$ for 51 and 102 mg/kg, Fig. 2A), and decreased serum levels of MDA as compared with the model group ($p < 0.05$ for 51 mg/kg and $p < 0.01$ for 102 mg/kg, Fig. 2C). The effect of decreased serum levels of ROS of HG is more significant than the effect of two positive drugs ($p < 0.05$).

A high-fat diet fed in the model group had remarkably lower serum NO levels, approximately 0.77-folds less than those of the normal group, whereas administration with HG significantly decreased the alterations compared with the model group ($p < 0.05$ for 51 and 102 mg/kg, Fig. 2B), and serum NO levels were higher than the normal group. The positive drugs similarly promoted release of NO, but the effect of Jiaogulan total saponins is more significant than Zhibituo.

3.4. Effects on the TC and TG in liver tissue

The high-fat diet fed to the MC group caused considerably higher TC and TG levels in liver tissue which were approximately 1.8- and 3.1-fold than those of the normal group, respectively. ZT (188 mg/kg) and JT (157 mg/kg), which were used as drugs for positive control groups, markedly decreased the TC and TGs in liver tissue, respectively. Administration with HG significantly attenuated the elevated levels of TC and TGs in liver tissue as compared with model group ($p < 0.05$ for 51 mg/kg and $p < 0.01$ for 100 mg/kg, respectively, Fig. 3).The effect of reducing the TC and TG in liver tissue with HGH is separately better than use of JT ($p < 0.05$) and ZT ($p < 0.05$).

3.5. Effects on the oxidative stress biomarkers and antioxidant components in liver tissue

In hepatic tissues, the oxidative stress biomarkers of the model group, such as MDA levels, were approximately 1.9-fold higher than those of the normal group, respectively. However, administration of HG significantly attenuated those alterations ($p < 0.05$ for 51 mg/kg and $p < 0.01$ for 102 mg/kg in MDA, Fig. 4A). Administration of JT showed a similar effect on MDA levels, but ZT failed to produce a comparable result.

A high-fat diet provided in the model group caused considerable decreases of TAC levels in liver tissue by 1.5-fold compared to the normal group, while administration with HG (mainly 51 mg/kg and 102 mg/kg) significantly increased TAC levels compared with the model group ($p < 0.05$, Fig. 4B). Reduced GSH content in the hepatic tissue was depleted about 1.5-fold by a high-fat diet fed in the model group compared with the normal group, whereas administration with HG significantly restored R-GSH to its normal level compared

Table 3
Serum lipid levels of experimental rats induced by high-fat diet for 30 days. Data were expressed as mean ± standard deviation (SD).

Group	Serum lipid profiles before treatment (mmol/L)									
	TG	*p*	LDL-C	*p*	HDL-C	*p*	TC	*p*	AI	*p*
NC	0.679 ± 0.077	–	0.329 ± 0.057	–	0.970 ± 0.072	–	2.098 ± 0.177	–	1.181 ± 0.299	–
MC	1.565 ± 0.184**	< 0.0001	2.583 ± 0.279**	< 0.0001	0.734 ± 0.068**	< 0.0001	6.378 ± 0.534**	< 0.0001	7.751 ± 0.996**	< 0.0001
ZT	1.603 ± 0.076**	< 0.0001	2.689 ± 0.568**	< 0.0001	0.782 ± 0.038**	< 0.0001	6.658 ± 0.433**	< 0.0001	7.505 ± 0.285**	< 0.0001
JT	1.616 ± 0.176**	< 0.0001	2.650 ± 0.286**	< 0.0001	0.736 ± 0.066**	< 0.0001	6.139 ± 0.645**	< 0.0001	7.419 ± 1.298**	< 0.0001
HGH	1.659 ± 0.108**	< 0.0001	2.615 ± 0.257**	< 0.0001	0.729 ± 0.046**	< 0.0001	6.018 ± 0.679**	< 0.0001	7.319 ± 1.315**	< 0.0001
HGL	1.634 ± 0.148**	< 0.0001	2.603 ± 0.179**	< 0.0001	0.746 ± 0.062**	< 0.0001	5.968 ± 0.373**	< 0.0001	7.066 ± 0.946**	< 0.0001

**$p < 0.01$ for MC, ZT, JT, HGH and HGL vs. NC.
HGH = high dose HG-treated; HGL = low dose HG-treated; JT = Jiaogulan total saponins tablets-treated; MC = model control; NC = normal control; ZT = Zhibituo tablets-treated.

Table 4
Serum lipid levels of the six groups of rats after high-fat diet for 65 days and treatment for 5 weeks (data are expressed as mean ± standard deviation).

Group	Serum lipid profiles after treatment (mmol/L)									
	TG	*p*	LDL-C	*p*	HDL-C	*p*	TC	*p*	AI	*p*
NC	0.730 ± 0.059	–	0.335 ± 0.041	–	0.920 ± 0.048	–	2.149 ± 0.133	–	1.336 ± 0.107	–
MC	1.661 ± 0.076	–	2.700 ± 0.127	–	0.700 ± 0.027	–	6.690 ± 0.283	–	8.564 ± 0.404	–
ZT	1.408 ± 0.075$^{\#\#\triangle\triangle}$	$^{\#\#}$< 0.0001, $\triangle\triangle$< 0.0001	0.546 ± 0.079$^{\#\#\triangle\triangle}$	$^{\#\#}$< 0.0001, $\triangle\triangle$ 0.0002	0.879 ± 0.025$^{\#\#\triangle}$	$^{\#\#}$< 0.0001, $\triangle$ 0.0365	3.038 ± 0.232$^{\#\#}$	$^{\#\#}$< 0.0001, 0.0667	2.460 ± 0.296$^{\#\#\triangle}$	$^{\#\#}$< 0.0001, $\triangle$ 0.0220
JT	1.124 ± 0.071$^{\#\#\triangle\triangle}$	$^{\#\#}$< 0.0001, $\triangle\triangle$< 0.0001	0.924 ± 0.116$^{\#\#\triangle\triangle}$	$^{\#\#}$< 0.0001, $\triangle\triangle$< 0.0001	0.776 ± 0.031$^{\#\#\triangle\triangle}$	$^{\#\#}$ 0.0020, $\triangle\triangle$< 0.0001	3.154 ± 0.302$^{\#\#\triangle\triangle}$	$^{\#\#}$< 0.0001, $\triangle\triangle$ 0.0091	3.075 ± 0.473$^{\#\#\triangle}$	$^{\#\#}$< 0.0001, $\triangle$ 0.0452
HGL	1.115 ± 0.107$^{\#\#\triangle\triangle}$	$^{\#\#}$< 0.0001, $\triangle\triangle$< 0.0001	0.964 ± 0.106$^{\#\#\triangle\triangle}$	$^{\#\#}$< 0.0001, $\triangle\triangle$< 0.0001	0.756 ± 0.054$^{\#\#}$	$^{\#\#}$< 0.0001, $\triangle\triangle$< 0.0001	2.971 ± 0.133$^{\#\#}$	$^{\#\#}$< 0.0001, 0.0962	3.139 ± 0.133$^{\#\#\triangle\triangle}$	$^{\#\#}$< 0.0001, $\triangle\triangle$ < 0.0001
HGH	0.865 ± 0.084$^{\#\#}$	$^{\#\#}$< 0.0001,	0.339 ± 0.082$^{\#\#}$	$^{\#\#}$< 0.0001,	0.919 ± 0.0352$^{\#\#}$	$^{\#\#}$< 0.0001,	2.784 ± 0.268$^{\#\#}$	$^{\#\#}$< 0.0001,	2.130 ± 0.276$^{\#\#}$	$^{\#\#}$<0.0001,

$\#p < 0.05$, $\#\#p < 0.01$, ZT, JT, HGH and HGL vs. NC; $\triangle p < 0.05$, $\triangle\triangle p < 0.01$, ZT, JT and HGL vs. HGH.
HGH = high dose HG-treated; HGL = low dose HG-treated; JT = Jiaogulan total saponins tablets-treated; MC = model control; NC = normal control; ZT = Zhibituo tablets-treated.

with the model group ($p < 0.01$ for 102 mg/kg, Fig. 4C). Use of a high-fat diet administered to the model group also exhibited depletion of SOD and catalase activity in liver tissue compared with the normal group. Administration with HG significantly blocked the depletion of R-GSH, SOD, and catalase activity in liver tissue compared with control group ($p < 0.05$ for 102 mg/kg in catalase, where $p < 0.01$ for 102 mg/kg both in GSH and SOD, respectively, (Fig. 4C−4E). Administration with ZT increased R-GSH and catalase, but not TAC levels, GSH content, or SOD activities.

3.6. HG caused significant decreases of the lipid drops on the hepatic tissues

The high-fat diet fed to the MC group exhibited a typical feature of fatty liver showing the accumulation of many lipid droplets (dense small fat vacuoles) through histopathological examination compared with the normal group. Compared with the MC group, four treatment groups had reduced fat accumulation in the liver tissue. The liver steatosis of HGH group rats was more significantly reduced than the ZT and JT groups, and almost became normal (shown in Fig. 5). The fat vacuoles in liver induced by a high-fat diet were almost eliminated completely by HG, ZT, and JT treatment (shown in Fig. 5G), but the effect of HG treatment was more obvious ($p < 0.05$, HG vs. ZT or JT). In the results, the grade of inflammation, the stage of fibrosis, lobular inflammation, ballooning, NAFLD activity score, and the grade and location of steatosis differed among these groups of rats. We found that the liver inflammation, fibrosis, and steatosis of rats induced by a high fat diet become quite serious. The lesions in the HG group were mild, and close to normal (shown in Table 5). This suggested that hepatic steatosis induced by high fat diet can be reversed by HG.

3.7. Artery protection effects of HG

An extended duration high-fat diet induces a weakening or break in rat arterial walls; when ZT, JT, HGL, and HGH groups were treated to such a diet for 5 weeks, MC rat arterial intima thickened, revealing a small amount of foam cells, and a swelled and unsmooth vascular smooth muscle. This may be due to long-term dyslipidemia, which can cause inflammation and arterial wall immunity edema. This result, and the AI of MC being 8.564 (> 4), are consistent. AI is an internationally accepted indicator of atherosclerosis. If AI > 4, atherosclerosis will occur in rats.[21] However, in this study, MC rats were still in the initial stage of atherosclerosis. Arterial endothelia of HGH group rats have a slight deformation compared with rats in the NC group, a situation which is still superior to the MC group. Arterial endothelia of ZT, JT, and HGL group rats are similar to those found in the HGH group. However, the foam cell, immunity edema, and the disorder-related smooth muscle cells, and elastic fibers in the intima of the aortic arch induced by a high-fat diet were eliminated completely by HG, ZT, and JT treatment as compared with the model group ($p < 0.01$, Fig. 6G).

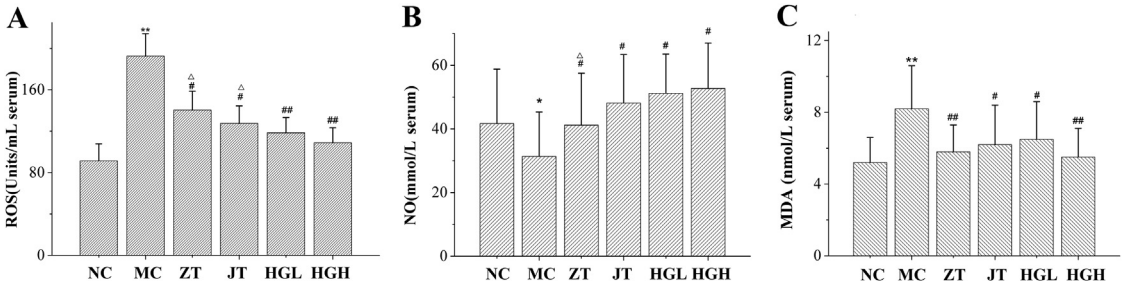

Fig. 2. Serum oxidative stress products and antiinflammatory cytokines (data are expressed as mean ± standard deviation). $^{**}p < 0.01$ and $^{*}p < 0.05$, MC vs. NC; $^{\#\#}p < 0.01$ and $^{\#}p < 0.05$, HGH, HGL, ZT and JT vs. MC; $^{\triangle}p < 0.05$, HGH vs. ZT and JT ($n = 8$). HGH = high dose HG-treated group; HGL = low dose HG-treated group; JT = Jiaogulan total saponins tablet-treated group; MC = model control group; MDA = methane dicarboxylic aldehyde; NC = normal control group; NO = nitric oxide; ROS = reactive oxygen species; ZT = Zhibituo tablets-treated group.

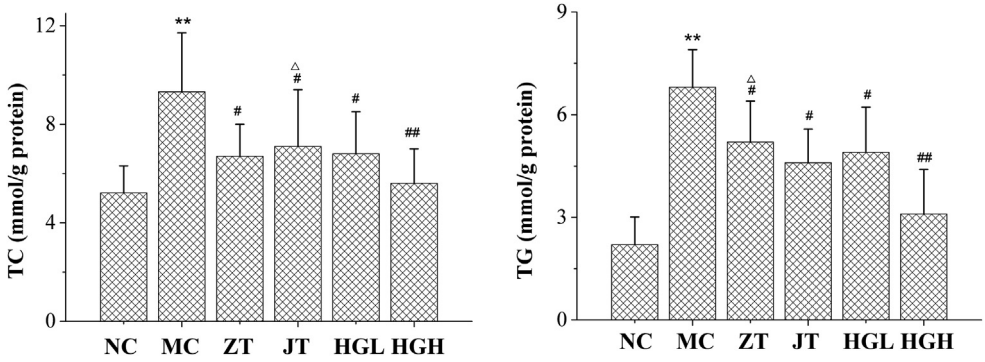

Fig. 3. Total cholesterol (TC) and triglyceride (TG) in the liver tissue (data are expressed as mean ± standard deviation). $^{**}p < 0.01$ and $^{*}p < 0.05$, MC vs. NC; $^{\#\#}p < 0.01$ and $^{\#}p < 0.05$, HGH, HGL, ZT and JT vs. MC; $^{\triangle}p < 0.05$, HGH vs. ZT and JT ($n = 8$). HGH = high dose HG-treated group; HGL = low dose HG-treated group; JT = Jiaogulan total saponins tablet-treated group; MC = model control group; NC = normal control group; ZT = Zhibituo tablets-treated group.

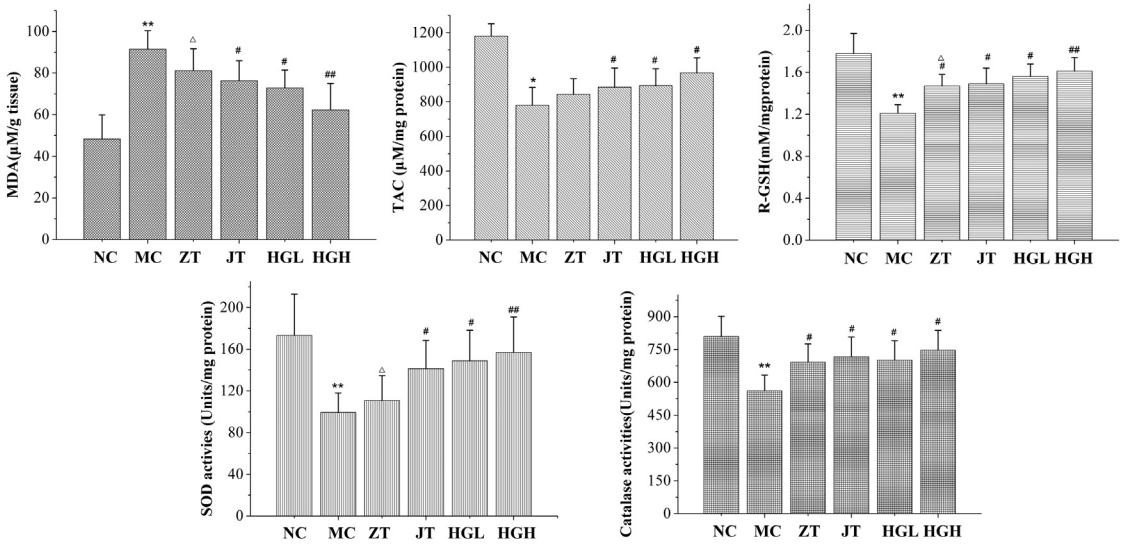

Fig. 4. Lipid peroxidation products and antioxidant compounds in the liver (data are expressed as mean ± standard deviation). $^{**}p < 0.01$ and $^{*}p < 0.05$, MC vs. NC; $^{\#\#}p < 0.01$ and $^{\#}p < 0.05$, HGH, HGL, ZT and JT vs. MC; $^{\triangle}p < 0.05$, HGH vs. ZT and JT ($n = 8$). HGH = high dose HG-treated group; HGL = low dose HG-treated group; JT = Jiaogulan total saponins tablet-treated group; MC = model control group; MDA = methane dicarboxylic aldehyde; NC = normal control group; R-GSH = reduced glutathione; SOD = superoxide dismutase; TAC = total antioxidant capacity; ZT = Zhibituo tablets-treated group.

3.8. Hepatic lipid metabolic gene expression

As shown in Fig. 7, after the rats of the MC group were fed a high-fat diet, which was then continued for 65 days, expression of HMGR, SREBP-1c, ACC-1, and FAS mRNA were significantly upregulated, and CPT-1 and PPAR-α were significantly downregulated compared with the NC group ($p < 0.01$). Comparison with the rats of MC group, the rats of

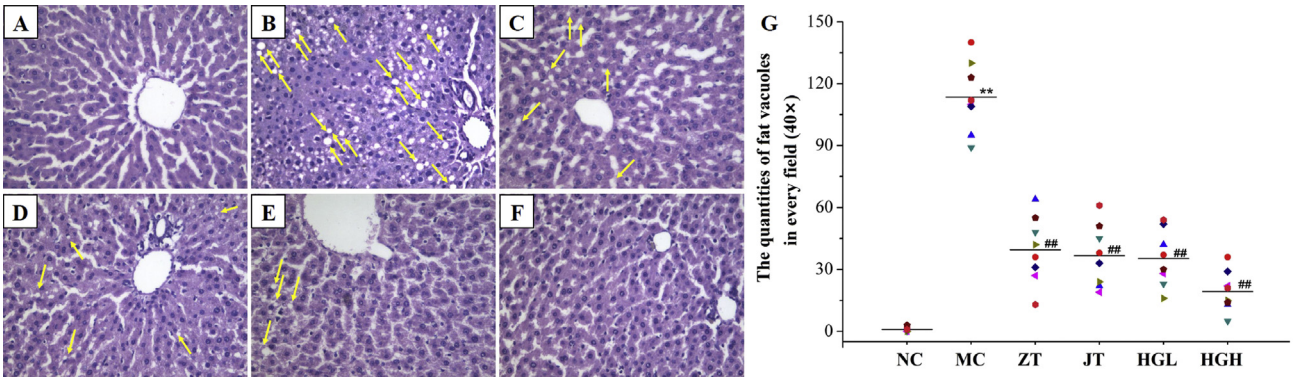

Fig. 5. Photomicrographs of liver sections from: (A) normal control (NC) group, (B) model control (MC) group, (C) Zhibituo tablet (ZT)-treated group, (D) Jiaogulan total saponins tablet (JT)-treated group, (E) low dose HG-treated group (HGL) group and (F) high dose HG-treated group (HGH) group. Where G is the statistical chart of the quantities of fat vacuoles in every field (40×). Liver sections were stained with hematoxylin & eosin staining (40×). Lipid droplets are indicated by yellow one-way arrows.

the ZT, JT, HGL and HGH groups, hyperlipidemia with NAFLD rats, were continuously administered drugs intervention 5 weeks, then the gene expression of HMGR, SREBP-1c, ACC-1 and FAS mRNA were obviously reduced and CPT-1, PPAR-α were increased ($p < 0.01$). The changes of ACC-1 and CPT-1 mRNA expression were more significant with HGH treatment as compared to ZT and JT ($p < 0.01$).

4. Discussion

Both dietary habits and genetic background are responsible for the pathogenesis and development of hyperlipidemia with NAFLD. To date, the commonly used models in rodents are genetic and diets or drug-induced models, such as with C57BL6 mice, and were induced by high-fat diets (18% lard, 12% egg yolk, 8% sugar, and 62% basic diet) for 12 weeks,[22] or mice with innate immune cell-deficiency received standard chow diet for 12 weeks,[23, 24] or Wistar rats were induced by an injection of a single dose of streptozotocin.[25] The present study successfully established a rat model of hyperlipidemia with NAFLD by providing nourishment with a high-fat diet for 65 days. The main components of the high-fat diet included: (1) lard; (2) bile acid salt to improve the absorption of fatty acid in the intestine; (3) propylthiouracil, which is an inhibitor of thyroid hormones synthesis, reducing the consumption of lipids as well as accelerating the lipid accumulation *in vivo*; and (4) cholesterol, suggesting that mature hyperlipidemia with NAFLD can develop in rodents in the absence of a relevant gene background. Overall, the time duration is shorter and the expense of such an experiment is lower when this method is utilized for modeling.

HG consists of Hong-Qu and gypenosides, according to the theory of TCM. As the principal agent, Hong-Qu contains a large number of Monaconlin compounds, especially Monaconlin K, which is an ingredient in natural medicines and health products and also inhibits HMGR activity. Therefore, there is an improved regulatory effect of blood lipids without similar side effects of statins.[26] Although here playing the role of an assisting agent, the majority of gypenosides have the same structure as ginsenosides. Indeed, gypenosides III, IV, VIII, XII, and malonyl gypenosides III and VIII are identical to ginsenosides Rb_1, Rb_3, Rd, F_2, and malonyl ginsenosides Rb_1 and Rd.[27] Gypenoside XLIX is a naturally occurring PPAR-α activator, and has antiinflammation and lipid-regulating characteristics.[28] Therefore, HG, when combined, not only can reduce lipogenesis, but also increase fatty acid oxidation, and the body's antiinflammatory and antioxidant capability.

Hyperlipidemia is a condition typically spared from steatosis, and cardiovascular risk is increased in NAFLD.[29] Cardiovascular disease is the major cause of mortality worldwide and accounts for approximately 40% of all deaths.[30] LDL-C is

Table 5
The grade of inflammation, the stage of fibrosis, lobular inflammation, ballooning, nonalcoholic fatty liver disease (NAFLD) activity score and the grade and location of steatosis different among these groups of rats.

Group	G [a]	S [b]	LI [c]	Ballooning	NAFLD activity score	G and L of steatosis [d]
NC	G0	S0	0	No	No	No
MC	G2	S3	2	Rare	Moderate fatty liver (10–25%)	Severe (> 66%), azonal
ZT	G1	S1	1	No	Mild fatty liver (< 10%)	Mild (5–33%), pan-acinar
JT	G1	S1	1	No	Mild fatty liver (< 10%)	Mild (5–33%), pan-acinar
HGH	G0	S0	0	No	No	No
HGL	G1	S0	1	No	Mild fatty liver (< 10%)	Mild (5–33%), pan-acinar

[a, b, c] According to the Chinese "Chronic hepatitis B Prevention Guide". G = grade of inflammation; S = stage of fibrosis; LI = lobular inflammation. Lobular inflammation: 0 = no inflammation; 1 = degeneration and a little of point or nidus necrosis lesions; 2 = degeneration and point or nidus necrosis lesions, or eosinophilic bodies.

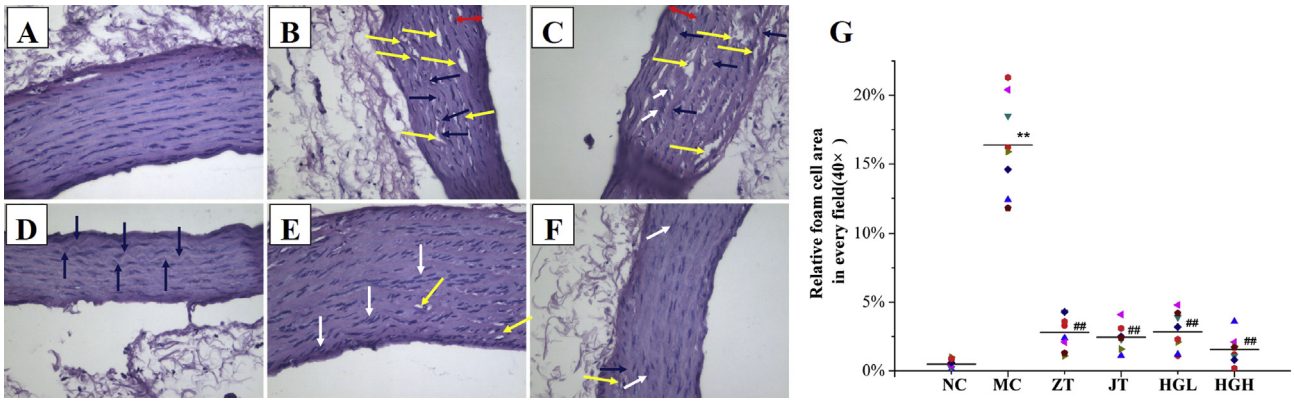

Fig. 6. Photomicrographs of artery sections from: (A) normal control (NC) group aortic arch, (B and C) model control (MC) group, (D) Zhibituo tablet (ZT)-treated group, (E) Jiaogulan total (JT) saponins tablet-treated group and (F) high dose HG-treated group (HGH) group aortic arch. A, B, C, D, E, and F were photographed under 40× micro objective and 10× ocular. Where G is the statistical chart of the relative foam cell area in every field (40×). Thickening areas of the aortic arch wall are indicated by red two-way arrows, elastic fibers are indicated by blue one-way arrows, foam cells are indicated by yellow one-way arrows, and the disordered arrangement of nucleus are indicated by white one-way arrows.

associated significantly with the risk of coronary heart disease (CHD), and high levels of TGs and low levels of HDL-C are particularly strong risk factors for CHD; the HMG-CoA reductase inhibitors can reduce LDL cholesterol levels as well as CHD events and total mortality.[31] After 35 days of treatment with HG, compared with the MC group, serum LDL-C, TG, TC, and AI were significantly reduced at a dose of 51 mg/kg and 102 mg/kg. The most likely cause of this change is that Hong-Qu is an inhibitor of HMG-CoA reductase, leading to decreased serum TC and HDL-C via suppressing the biosynthesis of endogenous cholesterol. Furthermore, gypenosides are agonists of PPAR-α, leading to decreased serum TG by inhibition of fatty acid synthesis and promoting β-oxidation of fatty acids. HDL particles have the ability to promote cholesterol efflux from macrophages in the artery wall, reduce oxidation, vascular inflammation and thrombosis, promote endothelial repair, improve endothelial function, promote insulin secretion, and enhance insulin sensitivity by pancreatic beta islet cells.[32] In the current study, when hyperlipidemia was induced by a high-fat diet in rats, although their serum HDL-C levels increased significantly compared with the NC group, the HG treatment significantly resolved and normalized the abnormal serum HDL-C. Hepatic lipid accumulation results from an imbalance between lipid availability and lipid disposal, which eventually triggers lipoperoxidative stress and hepatic injury. Identifying the origin of the accumulated TG and nonesterified fatty acids in the livers of patients with NAFLD may guide medical professionals in the prevention and treatment of this condition.[33] In the present study, according to the pathology examination, lipid droplets, TC, and TG in liver tissue were also significantly reduced by treatment with HG, suggesting that HG had a potent lipid-lowering effect and possibly could prevent the occurrence of cardiovascular events in hyperlipidemia rats.

In vivo, if there has been free radical involvement, including ROS and reactive nitrogen species, lipids will be peroxidated and the final product of the oxidation is malondialdehyde (MDA). Therefore, MDA is very often used as an index of oxidative status.[34] In the present study, lipid peroxidation and the oxidative stress levels of hyperlipidemia rats were obviously reduced by treatment with HG by eliminating ROS in serum and MDA in serum and liver tissue. The

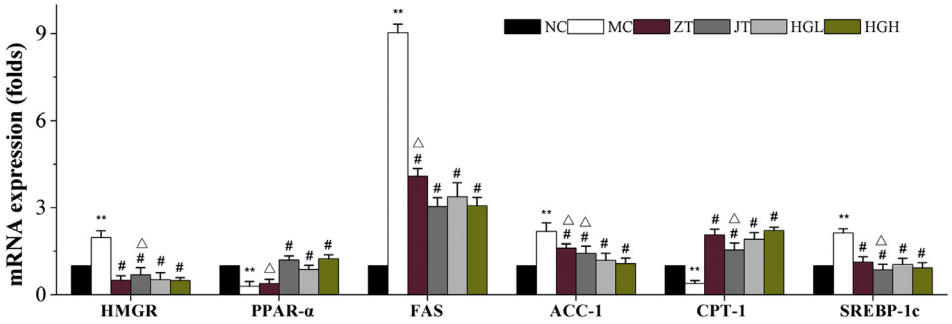

Fig. 7. The mRNA expression levels of liver. After acclimation for 1 week, the normal control (NC) group was given a basal diet for 65 days, the model control (MC) group was given a high-fat diet for 65 days and the high dose HG (HGH), low dose HG (HGL), Zhibituo tablet (ZT), and Jiaogulan total (JT) groups were given a high-fat diet for 65 days with corresponding drugs for 5 weeks. Data were expressed as mean ± standard deviation (SD). $^{**}p < 0.01$, MC vs. NC, $^{\#}p < 0.05$, HGH, HGL, ZT and JT vs. model control (MC) group; $^{\triangle}p < 0.01$, HGH vs. Zhibituo tablet-treated (ZT) group and Jiaogulan total saponins tablet-treated (JT) group ($n = 8$).

reduction of ROS also prevents oxidation of LDL to ox-LDL, thereby preventing the formation of foam cells in the artery. The histopathological examination also well supported the proposition that the foam cells in the artery were significantly decreased by treatment with HG. By contrast, the antioxidant components played a vital role in prevention of lipid peroxidation and relief of oxidative stress. GSH can scavenge free radicals, and the superoxide anion free radical was converted into hydrogen peroxide and catalyzed by SOD, and then hydrogen peroxide was converted into water by catalase (CAT). The antioxidant components were notably depleted in the MC group, while the HG treatment significantly restored them in hepatic tissue levels.

A small amount of nitric oxide is a kind of antiinflammatory substance. It has been reported that gypenosides not only suppressed NO synthesis in murine macrophages via attenuating NF-κB-mediated iNOS protein expression and inhibiting iNOS enzymatic activity, but also elicited a concentration-dependent increase in NO production from aortic endothelial cells.[35,36] HG contains 20% gypenosides, which suppressed inflammation in the arterial wall by inhibiting macrophage release of large amounts of NO and relaxed vascular smooth muscle by increasing nitric oxide production from aortic endothelial cells. Thus, those rats which manifested arterial inflammation after HG therapy increased the arterial endothelium NO release and normalized the serum NO levels.

HMGR is a rate-limiting enzyme in the process of cholesterol synthesis, disruption of which is a major cause of human morbidity and mortality.[37] Our results showed that administration of HG markedly lowered serum TC and decreased the gene expression of HMGR in high-fat diet-fed rats, and that the decrease in serum TC caused by HG may be related to reduced adiposity and subsequent decreases in adipose tissue mass and serum lipid levels.

The SREBP family has been established as a group of transcription factors regulating the transcription of genes involved in cholesterol, TG, and fatty acid synthesis, and SREBP-1c plays a pivotal role in the dietary regulation of most hepatic lipogenic genes including HMGR and ACC-1.[38] SREBP-1c levels are high in both alcoholic or nonalcoholic fatty liver disease.[39] In the current study, HG treatment reduced the expression of SREBP-1c, thus reducing the activation of HMGR and ACC-1, thereby inhibiting the synthesis of TC, TG, and fatty acids in liver. Furthermore, ACC catalyzes the carboxylation of acetyl-CoA to form malonyl-CoA, which is a key molecule in the control of intracellular fatty acid metabolism.[40] The FAS is a key enzyme in the metabolism of fatty acids and catalyzes the formation of saturated fatty acids.[41] ACC-1 and FAS lead to an increased synthesis of saturated fatty acids and their further conversion into monounsaturated fatty acids by stearoyl-CoA desaturase-1 (SCD).[42] HG significantly decreased the expression of ACC-1, and FAS consequently reduced the fatty acid and TG synthase. The results lead to a decrease in the accumulation of liver fat droplets, and blood lipid levels are normalized via inhibiting the ACC-1-FAS-SCD synthesis pathway of fatty acid.

Recent studies have proposed that upregulation of PPAR-α can prevent hepatic steatosis and decrease the progression of atherosclerosis.[43,44] PPAR-α is a major regulator of energy homeostasis, and regulates the expression of genes involved in fatty acid beta-oxidation; activation of PPAR-α could enhance the activity of CPT-1 and simultaneously inhibit activity of FAS.[45] The expression of PPAR-α was significantly reduced in rats induced by a high-fat diet, and serum TG levels were elevated. HG upregulated the expression of PPAR-α, and also activated it. CPT-1, the key enzyme for the transport of long-chain acyl-coenzymeA (acyl-CoA) compounds into mitochondria, promotes the metabolism of fatty acids.[46] PPAR-α is also the upstream regulatory element of CPT-1 in the cells,[47] which coincides with our result that PPAR-α and CPT-1 gene expression were synchronously increased after HG treatment. After high expression of PPAR-α was activated, β-oxidation of fatty acids was accelerated via activating CPT-1, which was activated by PPAR-α.

Taken altogether, it would appear that HG has a preeminent cardiovascular protective role, which promoted blood circulation, was good for the liver, and protected the artery due to adjusting the disturbance of lipoprotein metabolism, antioxidation, antiinflammatory effect, reducing liver fat lesions, and regulating enzymes association with lipid generated and metabolic. This evidence suggests that HG may have a synergistic effect in the treatment of hyperlipidemia with NAFLD. Therefore, our results indicate that the mixture HG may be developed as a hypolipidemic agent for the prevention of hyperlipidemia and a new drug to incorporate into therapy for treatment of NAFLD.

Acknowledgments

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References

1. Mahamuni SP, Khose RD, Menaa F, Badole SL. Therapeutic approaches to drug targets in hyperlipidemia. *BioMedicine* 2012;**2**:137−46.
2. Ruixing Y, Yuming C, Shangling P, Fengping H, Tangwei L, Dezhai Y, et al. Comparison of lipid levels, hyperlipidemia prevalence and its risk factors between Guangxi Hei Yi Zhuang and Han populations. *Arch Med Res* 2006;**37**:787−93.
3. Nelson RH. Hyperlipidemia as a risk factor for cardiovascular disease. *Prim Care* 2013;**40**:195−211.
4. Nascimbeni F, Pais R, Bellentani S, Day CP, Ratziu V, Loria P, et al. From NAFLD in clinical practice to answers from guidelines. *J Hepatol* 2013;**59**:859−71.
5. Lee JH, Friso S, Choi SW. Epigenetic mechanisms underlying the link between non-alcoholic fatty liver diseases and nutrition. *Nutrients* 2014;**6**:3303−25.
6. Anstee QM, Targher G, Day CP. Progression of NAFLD to diabetes mellitus, cardiovascular disease or cirrhosis. *Nat Rev Gastroenterol Hepatol* 2013;**10**:330−44.
7. Koppe SWP. Obesity and the liver: nonalcoholic fatty liver disease. *Transl Res* 2014;**164**:312−22.
8. Basaranoglu M, Ormeci N. Nonalcoholic fatty liver disease: diagnosis, pathogenesis, and management. *Turk J Gastroenterol* 2014;**25**:127−32.

9. Hou R, Goldberg AC. Lowering low-density lipoprotein cholesterol: statins, ezetimibe, bile acid sequestrants, and combinations: comparative efficacy and safety. *Endocrin Metab Clin* 2009;**38**:79−97.
10. Musso G, Cassader M, Gambino R. Cholesterol-lowering therapy for the treatment of nonalcoholic fatty liver disease: an update. *Curr Opin Lipidol* 2011;**22**:489−96.
11. Nseir W, Mahamid M. Statins in nonalcoholic fattyl iver disease and steatohepatitis: updated review. *Curr Atheroscler Rep* 2013;**15**:1−6.
12. Sham TT, Chan CO, Wang YH, Yang JM, Mok DKW, Chan SW. A review on the traditional Chinese medicinal herbs and formulae with hypolipidemic effect. *Biomed Res Int* 2014;**2014**:1153−66.
13. Ma JY, Li YG, Ye Q, Li J, Hua YJ, Ju DJ, et al. Constituents of red yeast rice, a traditional Chinese food and medicine. *J Agric Food Chem* 2000;**48**:5220−5.
14. Li XM, Shen XH, Duan ZW, Guo SR. Advances on the pharmacological effects of red yeast rice. *Chin J Nat Med* 2011;**9**:161−6.
15. Xie X, Wang Y, Zhang S, Zhang G, Xu Y, Bi H, et al. Chinese red yeast rice attenuates the development of angiotensin II-induced abdominal aortic aneurysm and atherosclerosis. *J Nutr Biochem* 2012;**23**:549−56.
16. Yang CW, Mousa SA. The effect of red yeast rice (Monascus purpureus) in dyslipidemia and other disorders. *Complement Ther Med* 2012;**20**:466−74.
17. Childress L, Gay A, Zargar A, Ito MK. Review of red yeast rice content and current Food and Drug Administration oversight. *J Clin Lipidol* 2013;**7**:117−22.
18. Yuan ZK, Wang DS, Huang XP, Guo ZH, Lu FG, Xie MZ, et al. Experimental study of tablet Yangxintongmai on treatment for atherosclerosis. In: xia J, editor. *Symposium of China Postdoctors and Academicians on Life Science*. Peoples Rep. of China: Science Press Beijing; 2003. p. 392−9.
19. Xie WD, Zhao YA, Du LJ. Emerging approaches of traditional Chinese medicine formulas for the treatment of hyperlipidemia. *J Ethnopharmacol* 2012;**140**:345−67.
20. Guo M, Liu Y, Gao ZY, Shi DZ. Chinese herbal medicine on dyslipidemia: progress and perspective. *Evid-Based Complement Altern Med* 2014;**2014**:489−99.
21. Chen J, Deng J, Zhang Y, Yang J, He Y, Fu W, et al. Lipid-lowering effects of Danhong injection on hyperlipidemia rats. *J Ethnopharmacol* 2014;**154**:437−42.
22. Wang YG, Fang WL, Wei J, Wang T, Wang N, Ma JL, et al. The involvement of NLRX1 and NLRP3 in the development of nonalcoholic steatohepatitis in mice. *J Chin Med Assoc* 2013;**76**:686−92.
23. Bhattacharjee J, Kumar JM, Arindkar S, Das B, Pramod U, Juyal RC, et al. Role of immunodeficient animal models in the development of fructose induced NAFLD. *J Nutr Biochem* 2014;**25**:219−26.
24. Iyer S, Upadhyay PK, Majumdar SS, Nagarajan P. Animal models correlating immune cells for the development of NAFLD/NASH. *J Clin Exp Hepatol* 2015;**5**:239−45.
25. Ziamajidi N, Khaghani S, Hassanzadeh G, Vardasbi S, Ahmadian S, Nowrouzi A, et al. Amelioration by chicory seed extract of diabetes- and oleic acid-induced non-alcoholic fatty liver disease (NAFLD)/non-alcoholic steatohepatitis (NASH) via modulation of PPARα and SREBP-1. *Food Chem Toxicol* 2013;**58**:198−209.
26. Patakova P. Monascus secondary metabolites: production and biological activity. *J Ind Microbiol Biotechnol* 2013;**40**:169−81.
27. Cui JF, Eneroth P, Bruhn JG. Gynostemma pentaphyllum: identification of major sapogenins and differentiation from Panax species. *Eur J Pharm Sci* 1999;**8**:187−91.
28. Huang THW, Li YH, Razmovski-Naumovski V, Tran VH, Li GQ, Duke CC, Roufogalis BD. Gypenoside XLIX isolated from Gynostemma pentaphyllum inhibits nuclear factor-kappaB activation via a PPAR-alpha-dependent pathway. *J Biomed Sci* 2006;**13**:535−48.
29. Loria P, Marchesini G, Nascimbeni F, Ballestri S, Maurantonio M, Carubbi F, et al. Cardiovascular risk, lipidemic phenotype and steatosis. A comparative analysis of cirrhotic and non-cirrhotic liver disease due to varying etiology. *Atherosclerosis* 2014;**232**:99−109.
30. Tziomalos K, Athyros VG. Fenofibrate: a novel formulation (Triglide (TM)) in the treatment of lipid disorders: a review. *Int J Nanomed* 2006;**1**:129−47.
31. Isles CG, Paterson JR. Identifying patients at risk for coronary heart disease: implications from trials of lipid-lowering drug therapy. *QJM* 2000;**93**:567−74.
32. Barter P. HDL-C: role as a risk modifier. *Atheroscler Suppl* 2011;**12**:267−70.
33. Musso G, Gambino R, Cassader M. Recent insights into hepatic lipid metabolism in non-alcoholic fatty liver disease (NAFLD). *Prog Lipid Res* 2009;**48**:1−26.
34. Janero DR. Malondialdehyde and thiobarbituric acid-reactivity as diagnostic indices of lipid peroxidation and peroxidative tissue injury. *Free Radic Biol Med* 1990;**9**:515−40.
35. Aktan F, Henness S, Roufogalis BD, Ammit AJ. Gypenosides derived from Gynostemma pentaphyllum suppress NO synthesis in murine macrophages by inhibiting iNOS enzymatic activity and attenuating NF-κB-mediated iNOS protein expression. *Nitric Oxide* 2003;**8**:235−42.
36. Tanner MA, Bu X, Steimle JA, Myers PR. The direct release of nitric oxide by gypenosides derived from the herb Gynostemma pentaphyllum. *Nitric Oxide* 1999;**3**:359−65.
37. Burg JS, Espenshade PJ. Regulation of HMG-CoA reductase in mammals and yeast. *Prog Lipid Res* 2011;**50**:403−10.
38. Dentin R, Girard J, Postic C. Carbohydrate responsive element binding protein (ChREBP) and sterol regulatory element binding protein-1c (SREBP-1c): two key regulators of glucose metabolism and lipid synthesis in liver. *Biochimie* 2005;**87**:81−6.
39. Kim YW, Kim YM, Yang YM, Kim TH, Hwang SJ, Lee JR, et al. Inhibition of SREBP-1c-mediated hepatic steatosis and oxidative stress by sauchinone, an AMPK-activating lignan in Saururus chinensis. *Free Radic Biol Med* 2010;**48**:567−78.
40. Cintra DE, Ropelle ER, Vitto MF, Luciano TF, Souza DR, Engelmann J, et al. Reversion of hepatic steatosis by exercise training in obese mice: the role of sterol regulatory element-binding protein-1c. *Life Sci* 2012;**91**:395−401.
41. Pappenberger G, Benz J, Gsell B, Hennig M, Ruf A, Stihle M, et al. Structure of the human fatty acid synthase KS−MAT didomain as a framework for inhibitor design. *J Mol Biol* 2010;**397**:508−19.
42. Igal RA. Stearoyl-CoA desaturase-1: a novel key player in the mechanisms of cell proliferation, programmed cell death and transformation to cancer. *Carcinogenesis* 2010;**31**:1509−15.
43. Giby VG, Ajith TA. Role of adipokines and peroxisome proliferator-activated receptors in nonalcoholic fatty liver disease. *World J Hepatol* 2014;**6**:570−9.
44. Van Raalte DH, Li M, Pritchard PH, Wasan KM. Peroxisome proliferator-activated receptor (PPAR)-alpha: a pharmacological target with a promising future. *Pharm Res* 2004;**21**:1531−8.
45. Jia Y, Bhuiyan MJH, H-j Jun, Lee JH, Hoang MH, Lee HJ, et al. Ursolic acid is a PPAR-α agonist that regulates hepatic lipid metabolism. *Bioorg Med Chem Lett* 2011;**21**:5876−80.
46. Zarain-Herzberg A, Rupp H. Therapeutic potential of CPT I inhibitors: cardiac gene, transcription as a target. *Expert Opin Investig Drugs* 2002;**11**:345−56.
47. Napal L, Marrero PF, Haro D. an intronic peroxisome proliferator-activated receptor-binding sequence mediates fatty acid induction of the human carnitine palmitoyltransferase 1A. *J Mol Biol* 2005;**354**:751−9.