MEDICAL SCIENCE MONITOR

ANIMAL STUDY

e-ISSN 1643-3750
© Med Sci Monit, 2017; 23: 3412-3427
DOI: 10.12659/MSM.905544

Received: 2017.05.29
Accepted: 2017.06.19
Published: 2017.07.14

Effects of an Enriched Extract of Paeoniflorin, a Monoterpene Glycoside used in Chinese Herbal Medicine, on Cholesterol Metabolism in a Hyperlipidemic Rat Model

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
Literature Search F
Funds Collection G

ABCDEF 1,2,3 **Huiming Hu***
BCE 4 **Qiaoqiao Zhu***
AE 1 **Jie Su**
DE 1 **Yajun Wu**
CEF 2 **Yanchen Zhu**
BC 5 **Yin Wang**
DF 5 **Hui Fang**
DF 1 **Minxia Pang**
CE 1 **Bo Li**
ADEF 3,4 **Suhong Chen**
AEG 1 **Guiyuan Lv**

1 College of Pharmaceutical Science, Zhejiang Chinese Medical University, Hangzhou, Zhejiang, P.R. China
2 Department of Computer Science, Jiangxi University of Traditonal Chinese Medicine, Nanchang, Jiangxi, P.R. China
3 Collaborative Innovation Center of Yangtze River Delta Region Green Pharmaceuticals, Zhejiang University of Technology, Hangzhou, Zhejiang, P.R. China
4 College of Pharmaceutical Science, Wenzhou Medical University, Wenzhou, Zhejiang, P.R. China
5 Department of Pharmacy, The PLA 117 Hospital, Hangzhou, Zhejiang, P.R. China

* Huiming Hu and Qiaoqiao Zhu contributed equally to this work and should be considered co-first authors

Corresponding Authors: Guiyuan Lv, e-mail: zjtcmlgy@163.com, Suhong Chen, e-mail: chensuhong@aliyun.com

Source of support: The project supported by China National Natural Science Foundation (81274123 to Guiyuan Lv) and by China National Major Scientific and Technological Special Project for "Significant New Drugs Development" (2014ZX09301307-013 to Guiyuan Lv). The reported work was supported by China National Natural Science Foundation (81673638 to Suhong Chen)

Background: Paeoniflorin is a monoterpene glycoside extracted from the roots of *Paeonia lactiflora* and is used in Chinese herbal medicine to treat hyperlipidemia. The aim of this study was to evaluate the effects of an enriched extract of paeoniflorin on cholesterol levels, hemodynamics, and oxidative stress in a hyperlipidemic rat model.

Material/Methods: Male Sprague-Dawley rats were fed high-cholesterol diets and treated with three different doses of paeoniflorin for 12 weeks. The effects of paeoniflorin treatment were assessed on cholesterol levels, cholesterol metabolism, red blood cell vascular flow using hemorheology, antioxidant enzymes, and expression of the rate-limiting enzyme in the mevalonate pathway, 3-hydroxy-3-methylglutharyl-coenzyme A reductase (HMG-CoAR). Rat liver histology and immunohistochemical analysis were performed to evaluate the expression of nuclear factor erythroid 2–related factor 2 (Nrf2), cytochrome P450 7A1 (CYP7A1), and peroxisome proliferator-activated receptors (PPAR)-α. Protein expression HMG-CoAR, low-density lipoprotein receptor (LDLR), PPAR-α and CYP7A1 was measured by Western blotting. Antioxidant activity in rat liver was determined by measuring superoxide dismutase (SOD) and malondialdehyde (MDA).

Results: Serum and hepatic cholesterol, hepatic steatosis and the products of cholesterol metabolism were reduced by paeoniflorin treatment, which also reduced the activity of HMG-CoAR and upregulated the expression of LDLR, PPAR-α, and CYP7A1 expression, increased SOD, decreased MDA, and upregulated Nrf2 expression.

Conclusions: The findings of this study in a rat model of hyperlipidemia have shown that paeoniflorin regulates hepatic cholesterol synthesis and metabolism and may also protect the liver from oxidative stress.

MeSH Keywords: **Cholesterol • Diet, High-Fat • Hemorheology • Hyperlipidemias • Oxidative Stress**

Full-text PDF: http://www.medscimonit.com/abstract/index/idArt/905544

4561 — 11 59

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

Background

Hyperlipidemia, including hypercholesterolemia and hypertriglyceridemia, is associated with reduced levels of high-density lipoprotein (HDL) [1,2]. Due to changes in lifestyle and diet, hyperlipidemia is associated with obesity, ischemic heart disease, and diabetes mellitus [3]. Because hyperlipidemia is a risk factor for cardiovascular disease that can reduce life expectancy, control of hyperlipidemia is an important area of preventive medicine [4,5]. Currently, statin therapy is used in the medical treatment of hyperlipidemia, but there are several side effects of statins, including rhabdomyolysis, polyneuropathy, and hepatotoxicity [6–9]. In Chinese herbal medicine, alternative approaches are used for the treatment of hyperlipidemia.

Paeoniflorin is a monoterpene glycoside extracted from the roots of *Paeonia lactiflora* and is used in Chinese herbal medicine to treat hyperlipidemia [10]. Paeoniflorin also has vasodilator effects [11]. A previous study by our group showed that an enriched extract of paeoniflorin could reduce the level of blood lipids and protect hepatic function in hyperlipidemic mice mice on a diet containing egg yolk [12]. Paeoniflorin has also been shown to reduce cholesterol levels in the experimentally-induced hyperlipidemia in Wistar rats [13].

Circulating plasma cholesterol levels depend on the balance among intestinal absorption, hepatic synthesis, and hepatic conversion into bile acids [14]. The enzyme 3-hydroxy-3-methylglutharyl-coenzyme A reductase (HMG-CoAR) is one of the most important enzymes involved in cholesterol biosynthesis in organisms and is the rate-limiting enzyme that synthesizes cholesterol via mevalonic acid (MVA) [15]. When reduction of cholesterol synthesis occurs, cells begin to consume stored cholesterol, there is an increase in the number of low-density lipoprotein receptors (LDLR) resulting in a decrease in levels of total cholesterol (TC) and LDL-C, as well as influencing other lipid metabolic processes, with LDLR playing a vital role in the endocytosis of cholesterol [16].

Total cholesterol includes free cholesterol (FC) and cholesterol esters (CE). Although hyperlipidemia is one of the common causes of nonalcoholic fatty liver disease, the liver is a vital organ in lipid metabolism, with peroxisome proliferator-activated receptors (PPAR)-α playing a significant role in the pathogenesis of hepatic steatosis and enhancing hepatic elimination of excess cholesterol [17]. The liver plays an important role in the synthesis and net excretion of cholesterol either directly as free cholesterol in the bile or after its conversion into bile acid, initiated by cholesterol 7α-hydroxylase (CYP7A1) [18,19]. CYP7A1 plays an important role in cholesterol metabolism [20,21].

Hyperlipidemia is associated with increased blood viscosity, which can increase the risk of platelet aggregation and thrombosis [22]. An increase in levels of total cholesterol (TC) and triglyceride (TG) increases cell membrane cholesterol, which could decrease the fluidity of red cell membranes and the deformation of erythrocytes, resulting in microcirculation disturbance, hypoxia, blood stasis, lowing hemorheology, reduction of blood flow, and increased blood viscosity. Nitric oxide (NO) is maintains hemodynamic balance in the cardiovascular system and results in the relaxation of blood vessels, while nitric oxide synthase (NOS) catalyzes NO. Hyperlipidemia and atherosclerosis restrain the production and release of NO, which leads to increased endothelin and vasoconstriction, which damage endothelial cells [23].

Oxidative free radicals are associated with lipid peroxidation, which leads to vascular endothelial damage, and is associated with atherosclerosis [24]. In the process of production of oxidized lipids, malondialdehyde (MDA) is the product of polyunsaturated fatty acids and an active oxygen reaction [25]. Superoxide dismutase (SOD) is an antioxidant enzyme with a level that reflects the ability of free radicals to remove oxygen in the body [25]. Antioxidants such as nuclear factor erythroid 2–related factor 2 (Nrf2), which is a redox-sensitive transcription factor that is activated under conditions of oxidative stress and activates a range of antioxidant and cytoprotective genes such as the *SOD* gene [26,27].

At this time, there have been few studies to investigate the mechanisms of action of paeoniflorin in the reduction of hyperlipidemia. Furthermore, it is still unclear whether paeoniflorin also is effective in improving hemodynamic function and in protecting the liver from oxidative injury. Therefore, the aim of this study was to evaluate the effects of an enriched extract of paeoniflorin on cholesterol levels, hemodynamics, and oxidative stress in a hyperlipidemic rat model.

Material and Methods

Material and reagents

Paeoniflorin was obtained from Zelang Medical Technology Co. (Nanjing, Jiangsu, China) and the purity was 52% as detected by high-performance liquid chromatography (HPLC) analysis (Figure 1). Simvastatin was obtained from Zhejiang Jingxin Pharmaceutical Co., Ltd. (Zhejiang, China) (Lot No: 0908221) and was dissolved in distilled water at 0.2 mg/ml as a positive control. Commercial kits used for determination of serum and total fecal cholesterol (TC), were obtained from Shanghai Fosun Long March Medical Science Co. Ltd (Shanghai, China). The liver TC, malondialdehyde (MDA), superoxide dismutase (SOD), nitric oxide (NO), and nitric oxide synthase (NOS) were

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

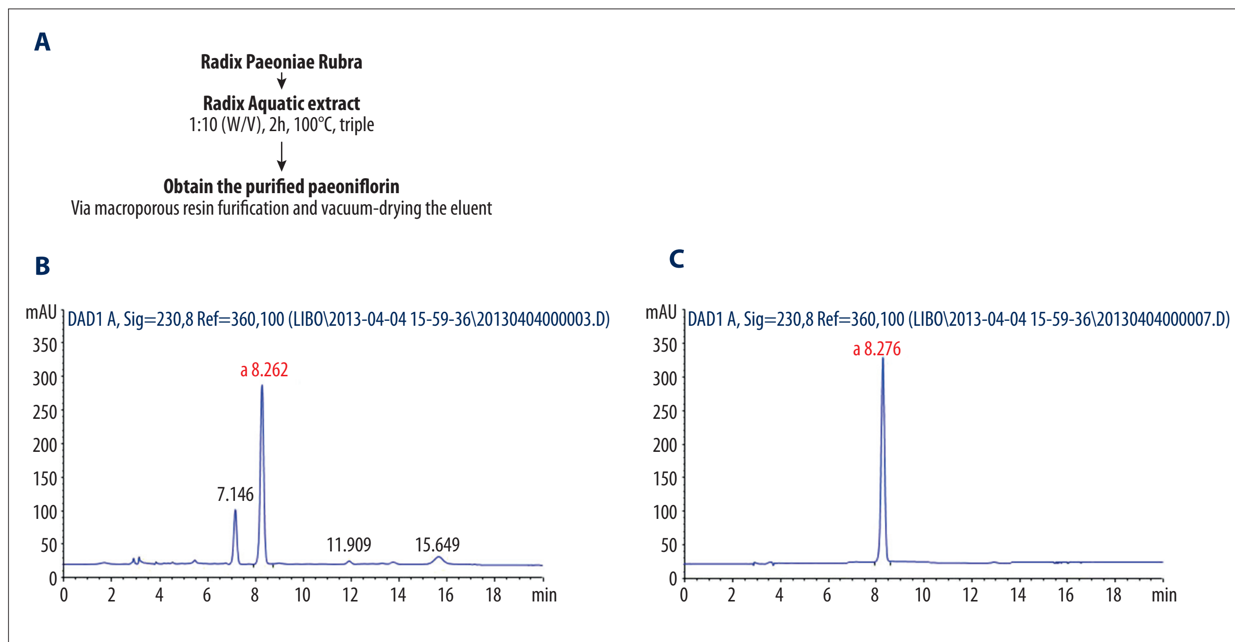

Figure 1. High-performance liquid chromatography (HPLC) diode array detection (DAD) analysis of paeoniflorin. (**A**) The route diagram of preparing paeoniflorin enriched extracts. (**B**) HPLC chromatogram of paeoniflorin extracts detected at 230 nm. 'Peak a' was identified to be paeoniflorin. (**C**) HPLC chromatogram of paeoniflorin standard substance detected at 230 nm. 'Peak a' was identified to be paeoniflorin.

obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Enzyme-linked immunosorbent assay (ELISA) kits for measuring hepatic 3-hydroxy-3-methylglutharyl-coenzyme A reductase (HMG-CoAR) activity, plasma free cholesterol (FC) and cholesterol ester (CE) were obtained from Beijing Rigorbio Science Development Co. Ltd (Beijing, China). Hematoxylin (Lot No: 20140919), eosin (Lot No: 20140919) and Masson trichrome reagent (Lot No: 20140919) were purchased from Nanjing Technology Co., Ltd. (Jiangsu, China). Antibodies against peroxisome proliferator-activated receptor (PPAR)-α (Lot No: sc-9000), low-density lipoprotein receptor (LDLR) (Lot No: sc-11824) and nuclear factor erythroid 2–related factor 2 (Nrf2) (Lot No: SC-722) were from Santa Cruz Biotechnology (CA, USA). Mouse and rabbit specific horseradish peroxidase (HRP)/diabenobenzidine (DAB), avidin-biotin complex (ABC) detection kit for immunohistochemistry (IHC) kit (Ab64264) were obtained from Abcam (Cambridge, MA, USA).

HPLC analysis of the purity of paeoniflorin

Paeoniflorin crude extract from *Radix paeoniae rubra* was analyzed with HPLC-diode array detection (DAD). Briefly, the paeoniflorin sample extract was diluted with methanol and filtered through a 0.22 mm membrane filter. The Agilent HPLC 1200 (Agilent Technologies Inc., Palo Alto, CA, USA) was used to determinate the content of paeoniflorin in extracts with the use of a C18 column (250×4.5 mm). The mobile phases consisted of acetic acid and phosphoric acid solutions (19: 81, v/v); the solvent flow rate was 1 ml/min with a column temperature of 25 °C. The injection volume was 5ml. The setting for the photodiode array detector was 230 nm with a total running time of 20 min. The HPLC chromatogram of the extracts is shown in Figure 1B.

Experimental animals and animal diets

Male Sprague–Dawley rats weighing 180–220 g were all purchased from Zhejiang Academy of Medical Sciences Laboratory Animal Center (Certificate No: SCXK2008-0033, Zhejiang, China) and acclimated for at least one week. All animals were supplied with fresh water and standard rodent laboratory chow was available *ad libitum*, as well as daily inspection in a controlled room, with a temperature of 25±1°C, humidity of 55±5°C, and a 12/12h light/dark cycle. All procedures were performed in strict compliance with the Peoples' Republic of China Legislation on the Use and Care of Laboratory Animals and with the Animal Management Rules of the Health Ministry of China (Document No: 55,2001).

After one week all rats were randomly divided into six groups of ten animals each. The normal control group (NC) was fed basic diet (8.1% water, 18.6% protein, 5.3% fiber, 3.6% fat, 1.54% calcium); the other groups were fed a high cholesterol diet (77.5% basic diet, 10% lard, 2% cholesterol, 0.5% sodium cholate, 10% dried egg yolk) [28]. The rats were randomly divided into five groups as follows: a high cholesterol

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

group (HC); a high cholesterol + simvastatin group (HC-S); a high cholesterol + high dose of paeoniflorin group (HC-PNH); a high cholesterol + medium dose of paeoniflorin group (HC-PNM); a high cholesterol + low dose of paeoniflorin group (HC-PNL), respectively.

Rats in the HC-PNH, HC-PNM, and HC-PNL groups were treated daily with paeoniflorin intragastrically (i.g.) at the doses of 500 mg/kg, 300 mg/kg, and 100 mg/kg for twelve weeks. The NC group and the HC group of experimental animals were given the same volume of physiological saline for several weeks by intragastric administration. All animals were given free access to food and water during the experimental period. Food intake and rat body weight were recorded daily.

During the experimental period, blood samples were drawn from the ophthalmic venous plexus at regular intervals and then centrifuged at 3,000 rpm for 10 min using a Thermo Scientific MicroCL 17 microcentrifuge (Thermo Ltd., Budapest, Hungary). At the end of the experiment, all animals had fecal collection to measure fecal cholesterol were then fasted for twelve hours before being sacrificed. Before death, blood samples were drawn from the ophthalmic venous plexus and divided into two. One sample was directly centrifuged at 3,000 rpm for 10 min for lipid analysis; the other sample was evaluated using hemorheology.

At post-mortem, the liver was excised from each rat, rinsed in ice-cold normal saline, gently blotted on filter paper, and fresh samples were taken of 0.5 gm in weight and stored in ice-cold normal saline; the remaining liver was fixed in 4% neutral-buffered formalin, processed, and sectioned for light microscopic evaluation.

Serum and hepatic lipid analysis

Concentrations of total cholesterol (TC) and low-density lipoprotein-cholesterol (LDLC) in serum were determined using commercially available enzyme kits and analyzed with a Selectra E Chemistry Analyzer (Vital, Netherlands). Concentrations of TC in the liver were determined using a PowerWave 340 absorbance microplate reader (Biotek Medical Device Co.Ltd., VT, USA).

Products of cholesterol metabolism: fecal TC and CE

The fecal samples of the rats were homogenized in cold saline (1g feces in 9ml of normal saline) and then centrifuged at 3,500 rpm for 10 min, and the supernatants were collected. The concentrations of fecal TC were determined using commercially available enzyme kits and analyzed with a Selectra E Chemistry Analyzer (Vital, Netherlands). The concentrations of fecal FC and CE were measured using commercial radioimmunoassay kits (Beijing Rigorbio Science Development Co., Ltd, Beijing, China) by Thermo LabSystems Multiskan MK3 (Thermo Fisher Scientific Inc. MA, USA).

Hemorheology analysis

Blood samples (5 ml) from the fundus vein plexus, were heparinized to anticoagulate the samples. Whole blood viscosity was determined and the whole blood reductive viscosity under high, middle and low shear force were analyzed from 1 ml anticoagulated blood, respectively using an LBY-N6B Automatic Hemorheological Tester (Beijing Precil Instrument Co., Ltd., Beijing, China), and the remaining anticoagulated blood was used to determine plasma viscosity.

Liver antioxidant profiles

The liver tissues were homogenized in cold saline (1 gm liver tissue in 9 ml of normal saline) and then centrifuged at 3,000 rpm for 20 min, and the supernatants were collected. The Coomassie blue protein binding method was used to determine the protein concentrations using bovine serum album as a standard [29]. The levels of MDA and the activity of SOD were measured according to the manufacturer's specification (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) [29,30].

Serum measurements of nitric oxide (NO) and activity of nitric oxide synthase (NOS)

Fresh blood (1 ml) was centrifuged at 3,000 rpm for 10 min at room temperature. The concentration of NO and the NOS activity were determined using a nitrate reductase kit and NOS kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China) and a PowerWave 340 absorbance microplate reader (Biotek Medical Device Co.Ltd., VT, USA).

Measurement of 3-hydroxy-3-methylglutharyl-coenzyme A reductase (HMG-CoAR) activity

The hepatic HMG-CoAR activities were measured using commercial radioimmunoassay kits (Beijing Rigorbio Science Development Co., Ltd. Beijing, China) and a Thermo LabSystems Multiskan MK3 (Thermo Fisher Scientific Inc. MA, USA).

Rat liver histopathology and immunohistochemistry (IHC)

Fresh rat liver was fixed in 4% neutral-buffered formalin, processed and embedded using an EC360 Tissue Embedder (Meiko, Germany) and cut into 4 mm sections using a LEICA ARM2245 tissue sectioning machine (Leica, Germany) and stained with hematoxylin and eosin (H&E) and Masson trichrome.

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

Immunohistochemistry (IHC) was performed using the mouse and rabbit specific HRP/DAB (ABC) detection kit were used for the development localization reaction to primary antibodies to PPARα, CYP7A1 and Nrf2 in hepatic tissues, which were then were counterstained with hematoxylin. Light microscopy was performed using a Leica B5-223IEP (Leica, Germany).

Western blots

Samples were collected and lysed in RAPI buffer (Solarbio, Beijing, China) with a protease/phosphatase inhibitor (Cell Signaling Technology, Canada). After treatment on ice for 30 min, lysates centrifuged at 12,000 rpm for 15 min at 4°C, and the protein content was measured using a BCA protein assay kit (Beyotime, Jiangsu, China).

Protein samples were mixed with 5×loading buffer (Beyotime, Jiangsu, China). The samples were separated by SDS-PAGE and electrotransferred onto polyvinylidene difluoride (PVDF) membranes (Pall Corporation, Mexico). The membranes were blocked with BSA blocking buffer for two hours at room temperature, incubated overnight at 4°C with the primary antibodies (Santa Cruz Biotechnology, USA or Cell Signaling Technology, Canada) in PBST. After washing, the membranes were incubated with the appropriate secondary antibody (Santa Cruz Biotechnology, USA) for 30 min. The membranes were incubated with streptavidin HRP (Thermo, USA) for 30 min after washing. The blotted protein bands were detected using a chemiluminescent substrate kit (Bio-Rad, USA). The signal intensity of blotting was normalized to the Western signal of the corresponding total protein. Relative intensities of protein bands were analyzed by Image J software.

Statistical analysis

All values are expressed as the mean ± standard deviation (SD) and subjected to one-way analysis of variance (ANOVA) by using SPSS 19.0 for Windows. The least significant difference (LSD) t-tests were applied when homogeneity of variance assumptions were satisfied. Otherwise, the Dunnett's t-test was used. The value of $P<0.05$ was considered to be statistically significant.

Results

Changes in rat body weight

As shown in Figure 2, the initial body weight of the six groups of rats showed no significant difference, while five groups fed high-cholesterol diet (HC, HC-S, HC-PNH, HC-PNM, HC-PNL) were heavier than the normal control (NC) group fed basic diet from the ninth day. The data suggested that a high-cholesterol diet in the longterm could cause weight gain.

Serum and hepatic lipids

As shown in Figure 3A, rats fed a high cholesterol diet from the fifth week, showed serum levels of total cholesterol (TC) in the HC group were greater than that of the NC group ($P<0.01$), indicating that the hypercholesterolemic model was successfully established. However, the serum levels of TC in all the three groups treated with paeoniflorin (HC-PNH, HC-PNM, HC-PNL) were significantly reduced by 18.67%, 31.92%, and 23%, respectively ($P<0.05$) after treatment with paeoniflorin for five weeks, when compared with the HC group. Furthermore, after treatment with paeoniflorin for eleven weeks, the serum levels of TC in these groups (HC-PNH, HC-PNM, HC-PNL) were significantly reduced by 31.99%, 34.64%, 17.38%, respectively ($P<0.05$), compared with the HC group.

As shown in Figure 3B, the hepatic levels of TC in the HC-PNH, HC-PNM, and HC-PNL groups were significantly reduced by 22.58%, 30.77%, 23.95%, respectively when compared with the HC group. These data suggested that paeoniflorin could reduce cholesterol levels.

As shown in Figure 3C, in rats fed high-cholesterol diets from the third week, the serum levels of LDLC in the HC group were significantly greater than that of the NC group ($P<0.05$), indicating that the hypercholesterolemic model was successfully established. However, the serum levels of LDLC in all the three groups treated with paeoniflorin (HC-PNH, HC-PNM, HC-PNL) were significantly reduced by 26.83%, 43.40%, and 30.63%, respectively ($P<0.05$) following treatment with paeoniflorin for five weeks, compared with the HC group. Furthermore, after administration paeoniflorin for eleven weeks, the serum levels of LDLC in the groups treated with paeoniflorin significantly declined by 36.43%, 44.08%, and 15.70%, respectively ($P<0.05$), compared with the HC group.

The products of cholesterol metabolism

The changes in free cholesterol (FC) and cholesterol ester (CE) in each group are shown in Figure 4A and Figure 4B. When compared with the NC group, the plasma FC and CE in the HC groups were significantly increased ($P<0.01$). However, the three different doses of paeoniflorin reduced plasma FC by 16.27%, 14.95%, and 22.88%, respectively ($P<0.01$), and also reduced the plasma CE by 13.45%, 14.84%, and 19.94%, respectively ($P<0.01$).

The concentrations of fecal TC in each group are shown in Figure 4C. When compared with the HC group, the concentrations of fecal TC in the group treated with paeoniflorin were increased, while the concentrations of plasma CA, as shown in Figure 4D, decreased significantly by 23.80%, 34.92%, and19.01%, respectively ($P<0.05$). These results showed that paeoniflorin could promote the metabolism of cholesterol.

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

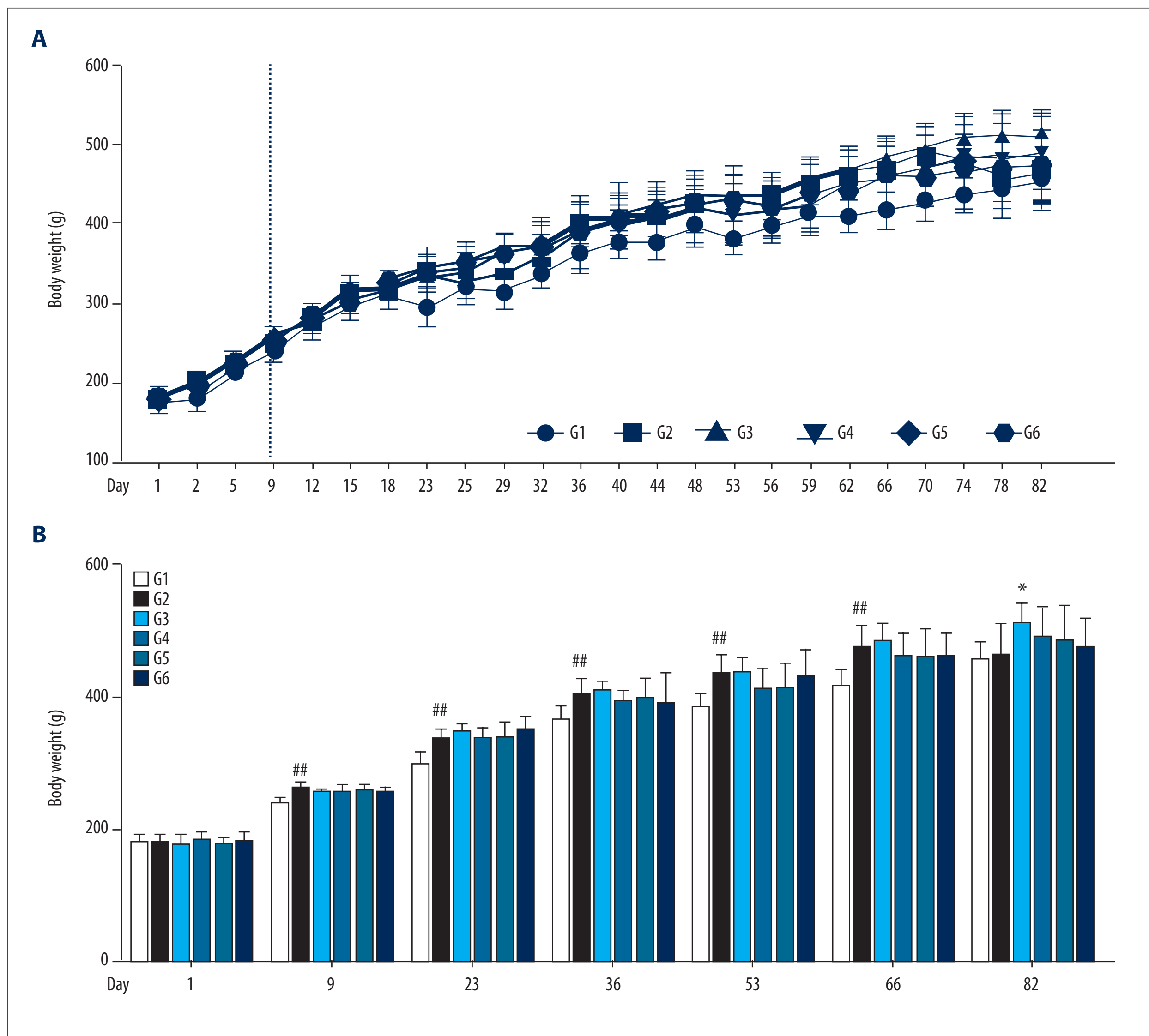

Figure 2. Effect on body weight of rats fed a basic diet or a high-cholesterol diet during the twelve-week feeding period. (**A**) Growth curves in body weight of rats. Each bar represents the mean from ten rats. (**B**) Changes in body weight of rats. The data are expressed as mean ±SD (n=10). G1=NC: normal control group; G2=HC: high cholesterol group; G3=HC-S: high cholesterol with simvastatin group; G4=HC-PNH: high cholesterol with high dose of paeoniflorin group (500 mg/kg body weight); G5=HC-PNM: high cholesterol with medium dose of paeoniflorin group (300 mg/kg body weight); G6=HC-PNL: high cholesterol with low dose of paeoniflorin group (100 mg/kg body weight).

Hemorheology

As shown in Figure 5A and Figure 5B, in rats fed a high-cholesterol diet for the twelve weeks, the whole blood viscosity (WBV) and the whole blood reductive viscosity (WBRV) in the HC group were significantly greater when compared with the NC group ($P<0.05$), while the WBV and WBRV in all the three groups treated with paeoniflorin (HC-PNH, HC-PNM, HC-PNL) were reduced.

As shown in Figure 5C, the plasma viscosity (PV) in the HC group was increased, compared with the NC group, but in the treated HC-PNM group PV was reduced by 4.53%. These data demonstrated that paeoniflorin could improve hyperviscosity induced by a high-cholesterol diet.

Superoxide dismutase (SOD) and malondialdehyde (MDA) in serum and liver

The changes in SOD and MDA in each group are shown in Figure 6A and Figure 6B. When compared with the NC group,

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

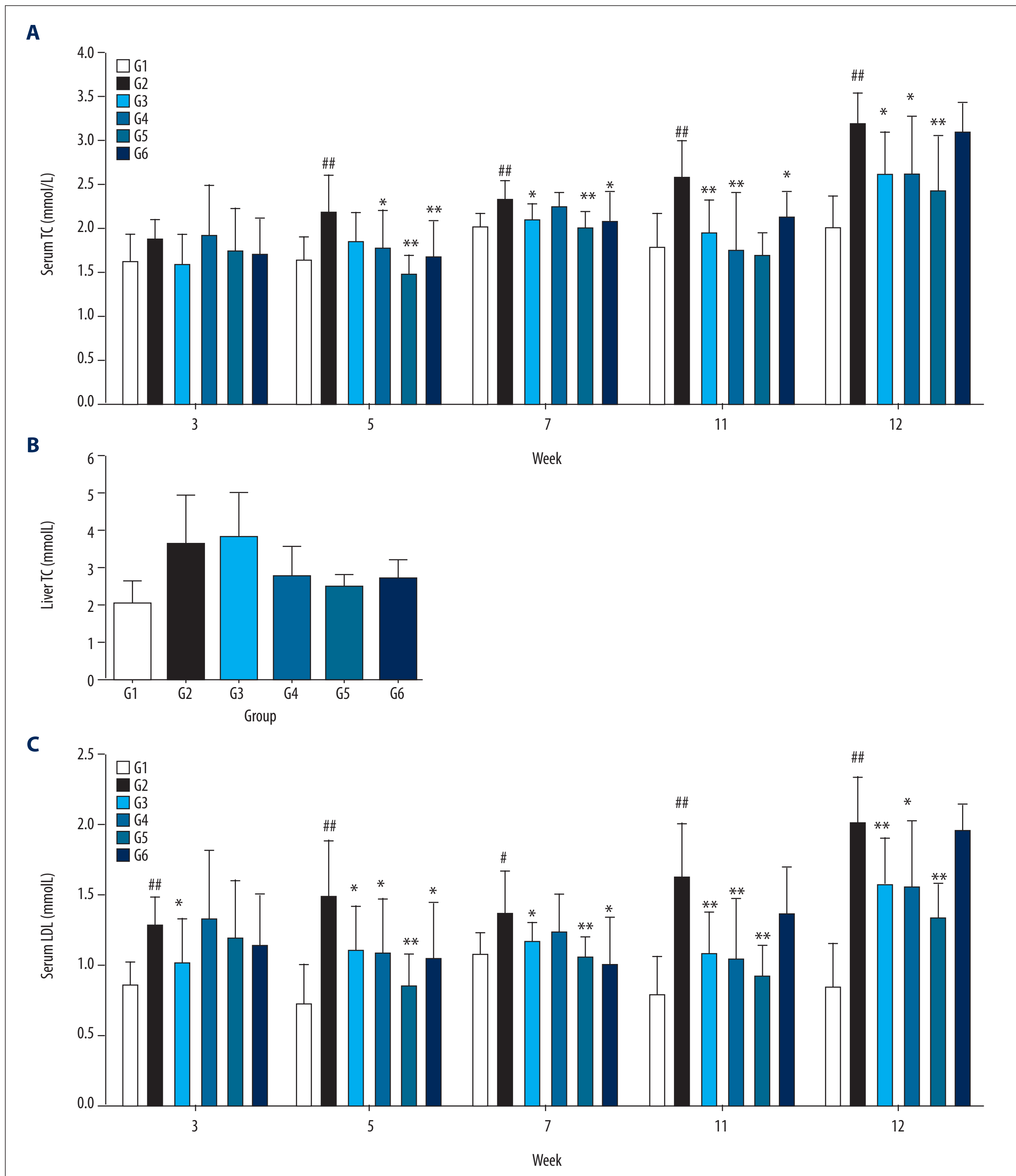

Figure 3. Hyperlipidemia lowering effects of paeoniflorin on total cholesterol (TC) and (low-density lipoprotein-cholesterol) LDL-C in rats fed a high cholesterol diet. (**A**) The effect on serum TC. The serum TC was measured at 3w, 5w, 7w, 11w, 12w after paeoniflorin treatment. The data are expressed as mean ±SD (n=9–10). (**B**) The effect on hepatic TC. The hepatic total cholesterol was measured after the last paeoniflorin treatment. The data are expressed as mean ±SD (n=9–10). (**C**) The effect on serum LDL-C. The serum LDL-C was measured at 3w, 5w, 7w, 11w, 12w after paeoniflorin treatment. The data are expressed as mean ±SD (n=9–10). G1=NC: normal control group; G2=HC: high cholesterol group; G3=HC-S: high cholesterol with simvastatin group; G4=HC-PNH: high cholesterol with 500 mg/kg paeoniflorin group; G5=HC-PNM: high cholesterol with 300 mg/kg paeoniflorin group; G6=HC-PNL: high cholesterol with 100 mg/kg paeoniflorin group. # *P*<0.05, ## *P*<0.01 *vs.* NC group, * *P*<0.05, ** *P*<0.01 *vs.* HC group.

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

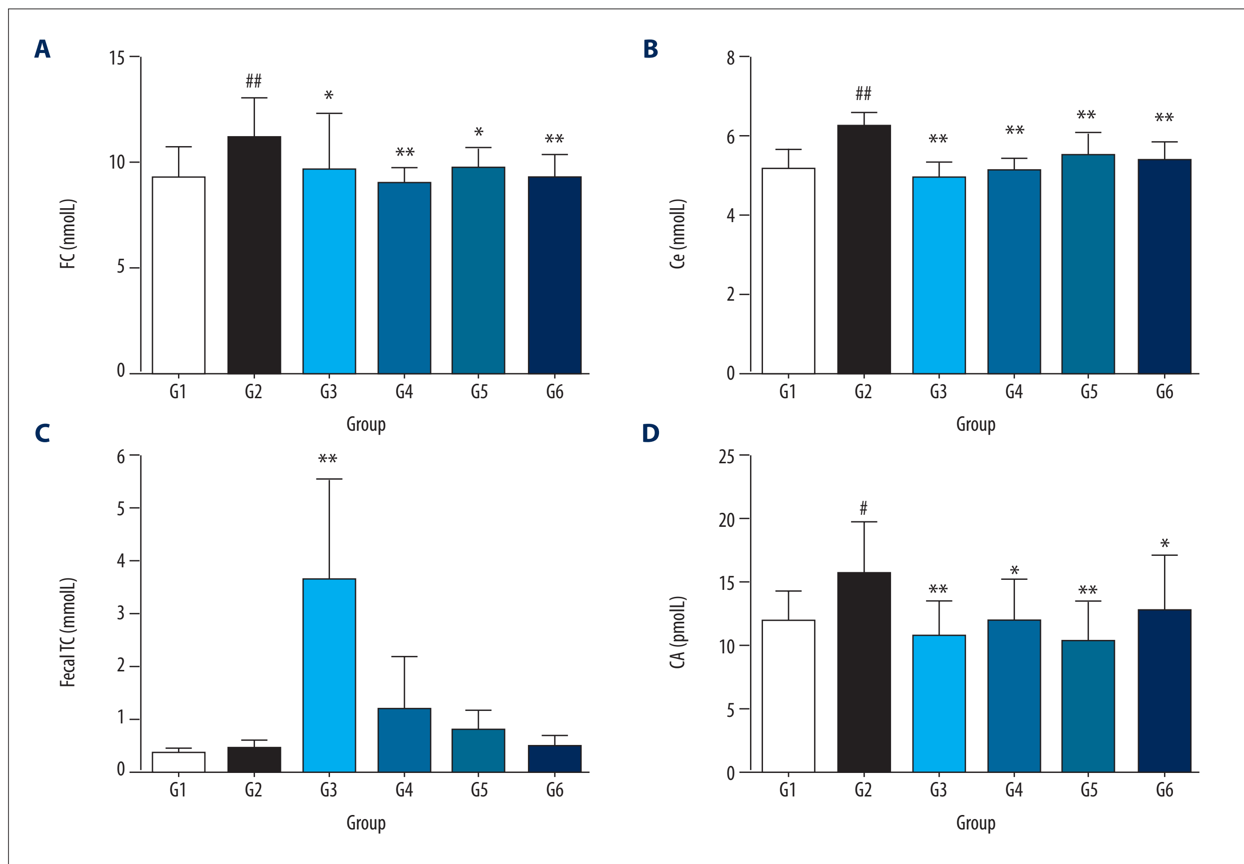

Figure 4. Effect of paeoniflorin treatment on cholesterol metabolism in rats. (**A**) The effect on plasma fecal cholesterol (FC). The data are expressed as mean ±SD (n=12). (**B**) The effect on plasma cholesterol ester (CE). The data are expressed as mean ±SD (n=12). (**C**) The effect on total fecal cholesterol (TC). The data are expressed as mean ±SD (n=12). (**D**) The effect on plasma CA. The data are expressed as mean ±SD (n=12). G1=NC: normal control group; G2=HC: high cholesterol group; G3=HC-S: high cholesterol with simvastatin group; G4=HC-PNH: high cholesterol with 500 mg/kg paeoniflorin group; G5=HC-PNM: high cholesterol with 300 mg/kg paeoniflorin group; G6=HC-PNL: high cholesterol with 100 mg/kg paeoniflorin group. # *P*<0.05, ## *P*<0.01 *vs.* NC group, * *P*<0.05, ** *P*<0.01 *vs.* HC group.

the activity of hepatic SOD in the HC group were significantly lower (*P*<0.01), while the levels of MDA were significantly greater (*P*<0.05). However, the three different doses of paeoniflorin in the rats fed high-cholesterol diet significantly reduced hepatic SOD by 10.94%, 22.98%, and 18.80%, respectively and also significantly reduced the hepatic MDA by 6.01%, 13.23%, and 8.77%, respectively. As shown in Figure 6C and Figure 6D, in the HC-PNM group, the concentration of serum NO and the activity of serum NOS were significantly increased, 60.85% and 14.73%, respectively, compared with the HC group.

Hepatic HMG-CoAR activity

As shown Figure 7, the hepatic HMG-CoAR activities were significantly greater in the HC group as compared with that of the NC group. However, in all the three groups, paeoniflorin treatment had a significant inhibitory effect on the hepatic HMG-CoAR activity of rats fed a high cholesterol diet (*P*<0.05). These results suggested that paeoniflorin could inhibit the biosynthesis of cholesterol.

Macroscopic hepatic morphology

The livers of the NC group rats, had a normal color, while the livers of the HC group rats were enlarged in size with a pale color. The livers of the three groups treated with paeoniflorin were more normal in appearance than the HC group.

Microscopic hepatic morphology

The livers of the NC group rats, showed normal hepatic histology, with normal hepatocytes, no cell enlargement, no inflammation, no fatty change, and no necrosis, as shown in Figure 8A. Liver histology of the rats fed high-cholesterol diets showed fatty change (steatosis) with cytoplasmic lipid vacuoles and an inflammatory cell infiltrate (steatohepatitis) as shown in Figure 8B.

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

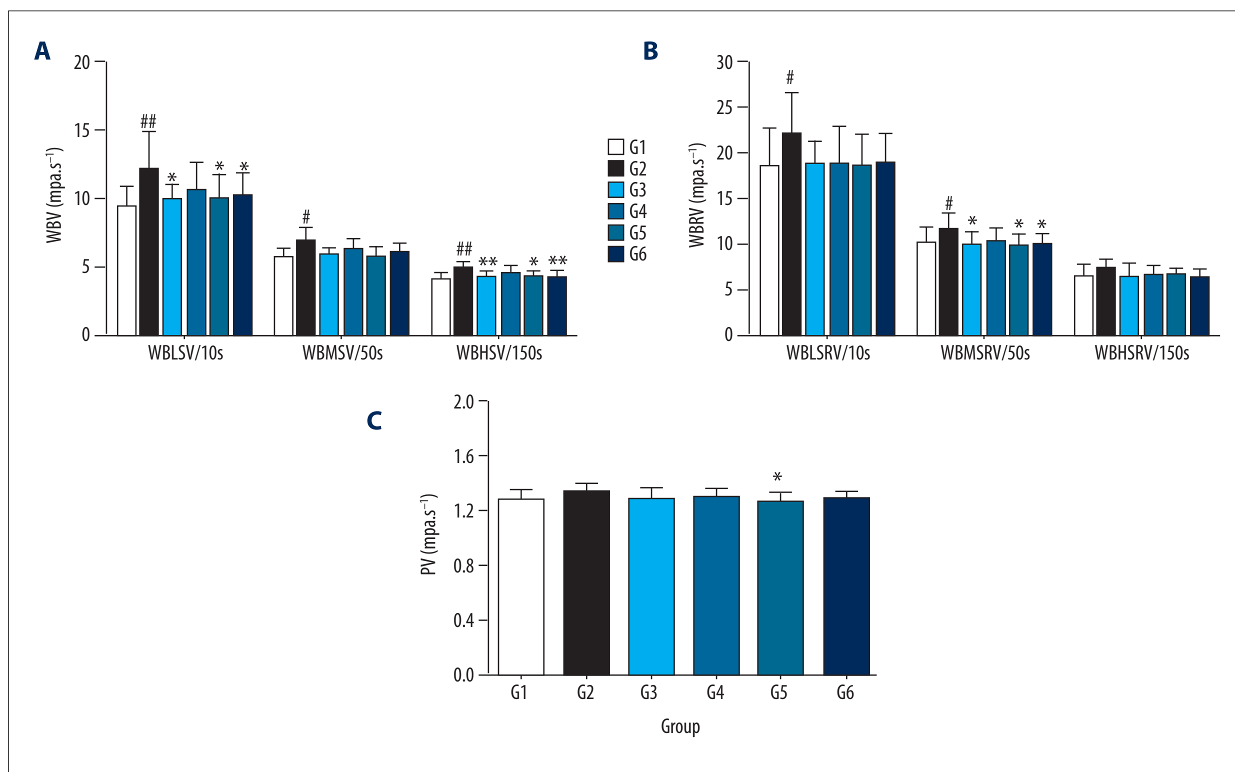

Figure 5. Effect of paeoniflorin treatment on hemorheology. (**A**) The effect on whole blood viscosity (WBV). The data are expressed as mean ±SD (n=10). (**B**) The effect on whole blood reductive viscosity (WBRV). The data are expressed as mean ±SD (n=10). (**C**) The effect on plasma viscosity (PV). The data are expressed as mean ±SD (n=10). G1=NC: normal control group; G2=HC: high cholesterol group; G3=HC-S: high cholesterol with simvastatin group; G4=HC-PNH: high cholesterol with 500 mg/kg paeoniflorin group; G5=HC-PNM: high cholesterol with 300 mg/kg paeoniflorin group; G6=HC-PNL: high cholesterol with 100 mg/kg paeoniflorin group. # $P<0.05$, ## $P<0.01$ *vs.* NC group, * $P<0.05$, ** $P<0.01$ *vs.* HC group.

As shown in Figure 8C, simvastatin resulted in no obvious effect on hepatic steatosis. In addition, as presented in Figure 8E and Figure 8F, the quantity of lipid droplets and inflammatory cells in the HC-PNM and HC-PNL group (300 mg/kg body weight and 100 mg/kg body weight per day) were reduced when compared with the HC group, suggesting that paeoniflorin could reduce the accumulation of lipid droplets and inhibit inflammation, thereby having a protective effect on the liver.

Implications of these results on targets of paeoniflorin in the reduction of hyperlipidemia

The results of this study can be summarized, based on the understanding that HMG-CoAR is the rate-limiting enzyme in the synthesis of cholesterol, while CYP7A1 is the rate-limiting enzyme in the classic pathways of bile acid enzymes. The Western blotting results for HMG-CoAR, LDLR, PPARα and CYP7A1 are presented in Figure 9A. The protein expressions of PPARα and LDLR were found to be significantly lower in the HC group compared with the NC group. HMG-CoAR was markedly upregulated. However, paeoniflorin treatment significantly inhibited the expression of HMG-CoAR (Figure 9B), as well as increasing the activation of LDLR (Figure 9C), PPARα (Figure 9D), and CYP7A1(Figure 9E). The results of immunohistochemistry analysis of the protein expressions of Nrf2, PPARα and CYP7A1 are presented in Figure 10, which were lower in the HC group compared with the NC group. However, paeoniflorin treatment upregulated the expression of Nrf2, PPARα and CYP7A1. Figure 11 shows that paeoniflorin could regulate synthesis and metabolism of cholesterol, hemodynamic and oxidative stress, as well as prevent hepatic injury.

Discussion

Paeoniflorin is a monoterpene glycoside extracted from the roots of *Paeonia lactiflora* and is used in Chinese herbal medicine to treat hyperlipidemia. In this study, in a hyperlipidemic rat model, serum and hepatic cholesterol, hepatic steatosis and the products of cholesterol metabolism were reduced by paeoniflorin treatment, which also reduced the activity of 3-hydroxy-3-methylglutharyl-coenzyme A reductase (HMG-CoAR) and upregulated the expression of low-density lipoprotein receptor (LDLR), peroxisome proliferator-activated receptors

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

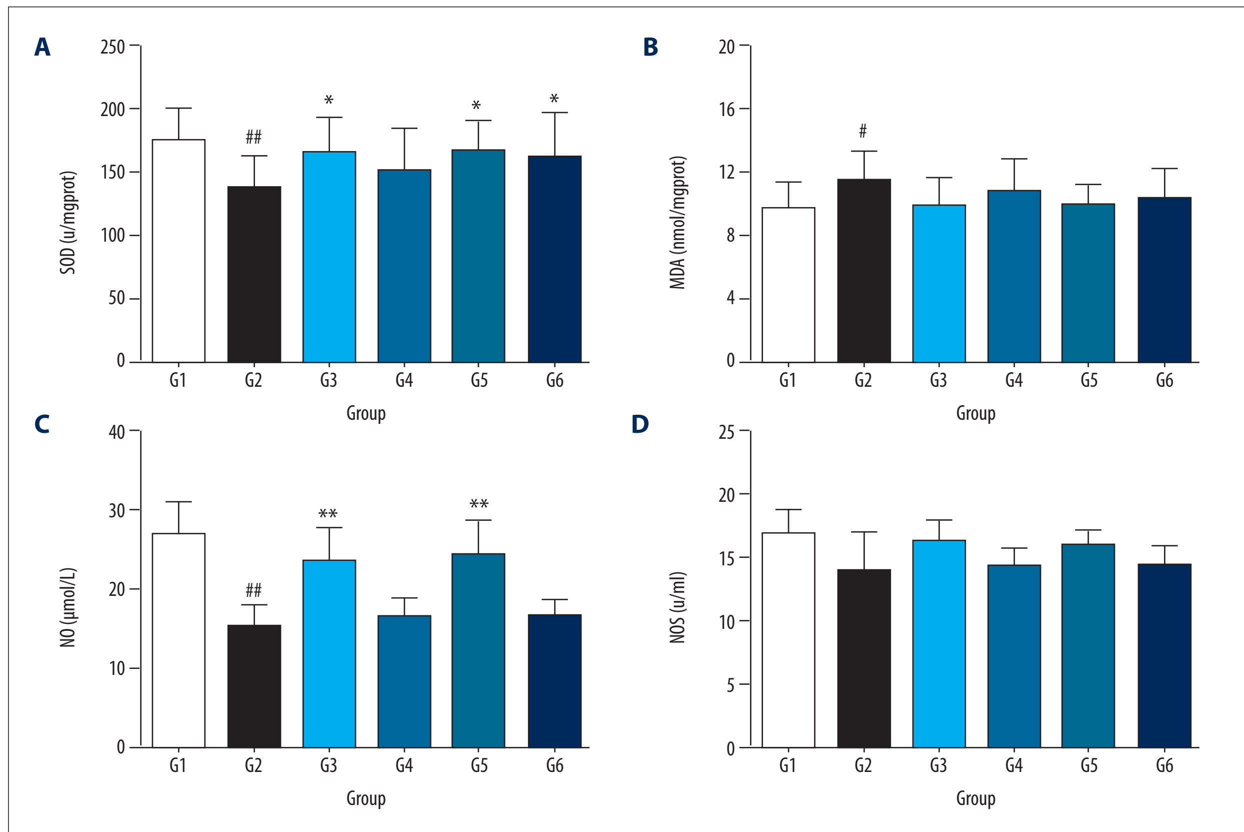

Figure 6. Effect of paeoniflorin treatment on antioxidant profiles and vasoactive substance in rats. (**A**) The effect on plasma superoxide dismutase (SOD). The data are expressed as mean ±SD (n=10). (**B**) The effect on plasma malondialdehyde (MDA). The data are expressed as mean ±SD (n=10). (**C**) The effect on serum nitric oxide (NO). The data are expressed as mean ±SD (n=10). (**D**) The effect on serum nitric oxide synthase (NOS). The data are expressed as mean ±SD (n=10). G1=NC: normal control group; G2=HC: high cholesterol group; G3=HC-S: high cholesterol with simvastatin group; G4=HC-PNH: high cholesterol with 500 mg/kg paeoniflorin group; G5=HC-PNM: high cholesterol with 300 mg/kg paeoniflorin group; G6=HC-PNL: high cholesterol with 100 mg/kg paeoniflorin group. $^{\#}$ *P*<0.05, $^{\#\#}$ *P*<0.01 *vs.* NC group, * *P*<0.05, ** *P*<0.01 *vs.* HC group.

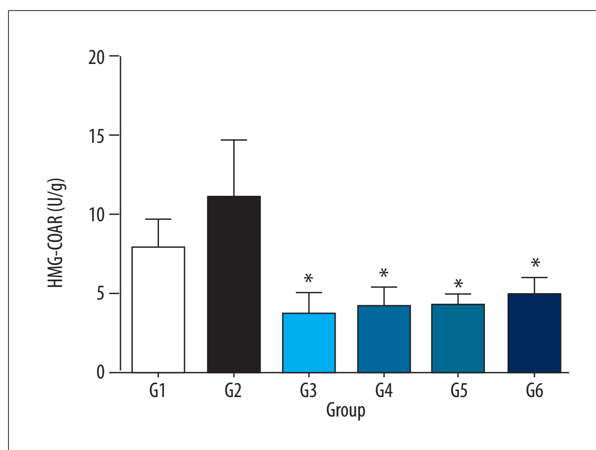

Figure 7. Effect of paeoniflorin on activity of 3-hydroxy-3-methylglutharyl-coenzyme A reductase (HMG-CoAR). The data are expressed as mean ±SD (n=7–8). G1=NC: normal control group; G2=HC: high cholesterol group; G3=HC-S: high cholesterol with simvastatin group; G4=HC-PNH: high cholesterol with 500 mg/kg paeoniflorin group; G5=HC-PNM: high cholesterol with 300 mg/kg paeoniflorin group; G6=HC-PNL: high cholesterol with 100 mg/kg paeoniflorin group. $^{\#}$ *P*<0.05, $^{\#\#}$ *P*<0.01 *vs.* NC group, * *P*<0.05, ** *P*<0.01 *vs.* HC group.

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

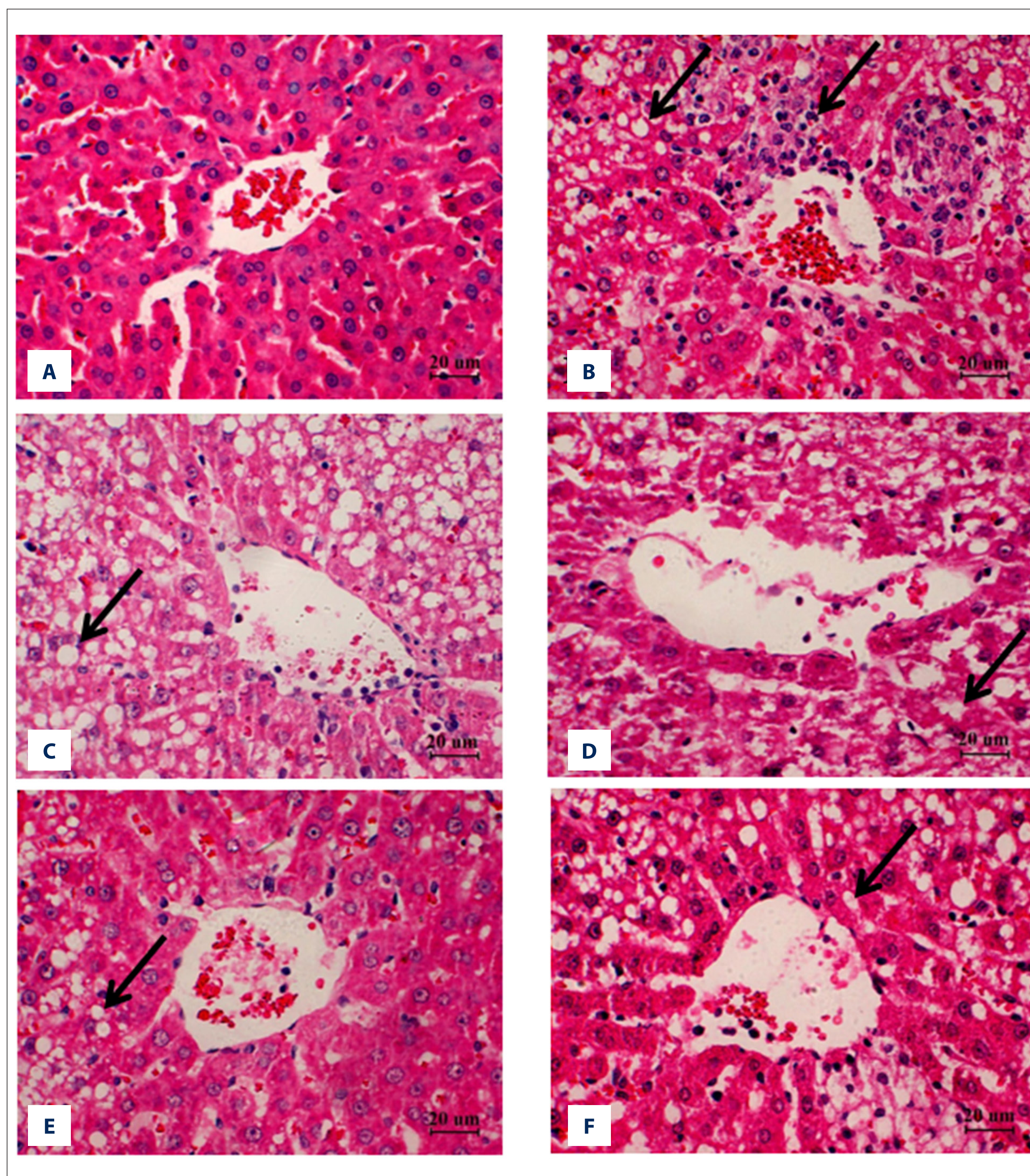

Figure 8. Effect of paeoniflorin on hepatic tissue morphology in rats fed a high-cholesterol diet. Photomicrographs of liver tissue sections stained with hematoxylin and eosin (H&E). The arrow in the pictures indicates fat droplets or inflammatory cells. Liver histology is shown from the following groups: (**A**) Normal control group (NC). (**B**) High cholesterol group (HC). (**C**) High cholesterol with simvastatin group (HC-S). (**D**) High cholesterol with 500 mg/kg paeoniflorin group (HC-PNH). (**E**) High cholesterol with 300 mg/kg paeoniflorin group (HC-PNM). (F) High cholesterol with 100 mg/kg paeoniflorin group (HC-PNL).

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

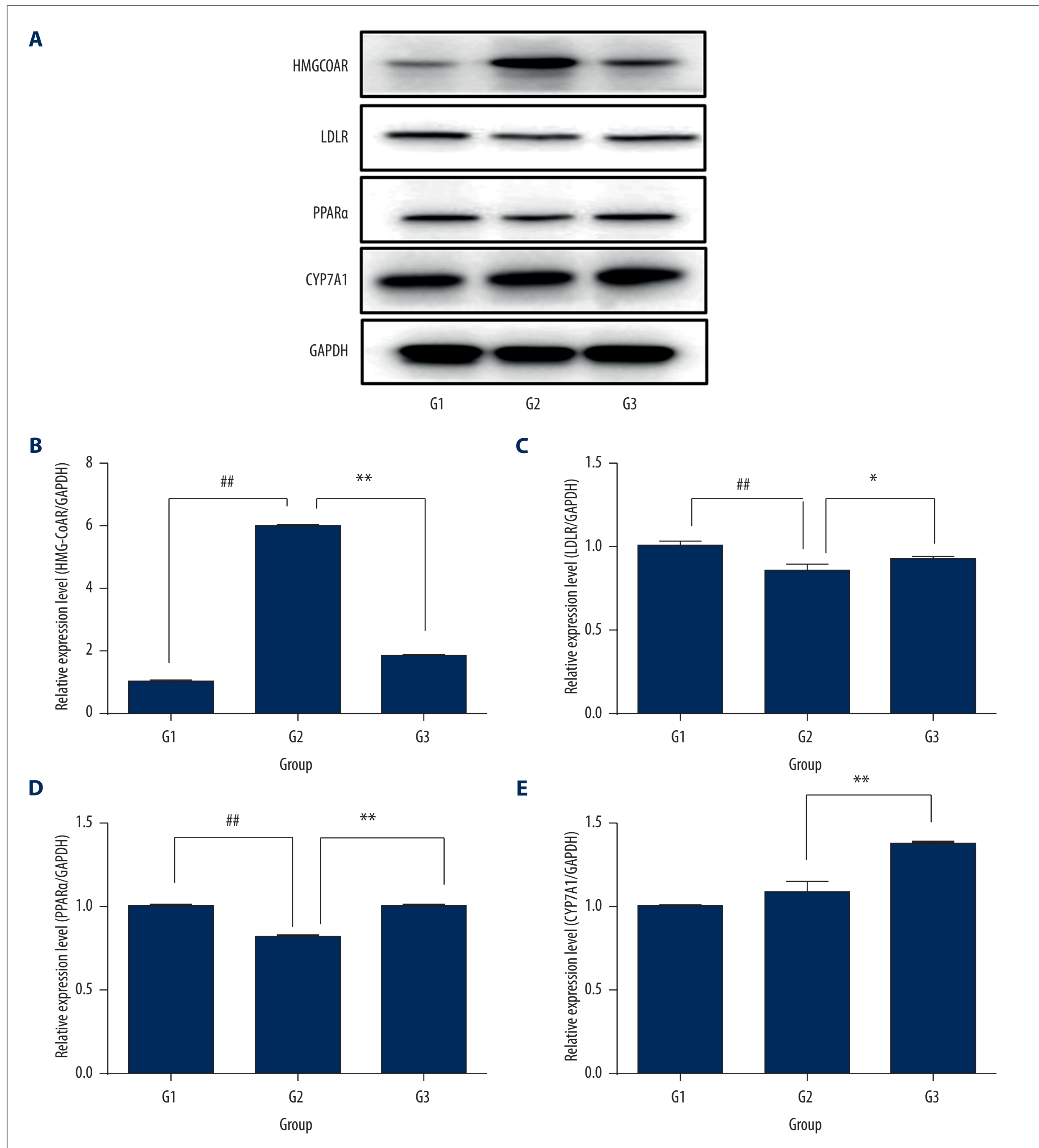

Figure 9. The potential drug targets for regulating blood lipids by paeoniflorin. (**A**) The expression of protein. (**B**) The quantification analysis of Western blot. The data are expressed as mean ±SD (n=3). G1 – normal control group; G2 – model control group; G3 – paeoniflorin group. $^{\#}$ *P*<0.05, $^{\#\#}$ *P*<0.01 *vs.* normal control group, * *P*<0.05, ** *P*<0.01 *vs.* model control group.

(PPAR)-α, and cytochrome P450 7A1 (CYP7A1) expression, increased superoxide dismutase (SOD), decreased malondialdehyde (MDA), and upregulated nuclear factor erythroid 2-related factor 2 (Nrf2) expression.

A high cholesterol diet leading to hyperlipidemia is regarded as an important factor in the development of ischemic heart disease, and so far the focus has mainly been on the systemic and coronary vascular effects of cholesterol [31,32]. Hyperlipidemia is the major cause of cardiovascular disease [33], which is the leading cause of mortality in industrialized countries and has

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

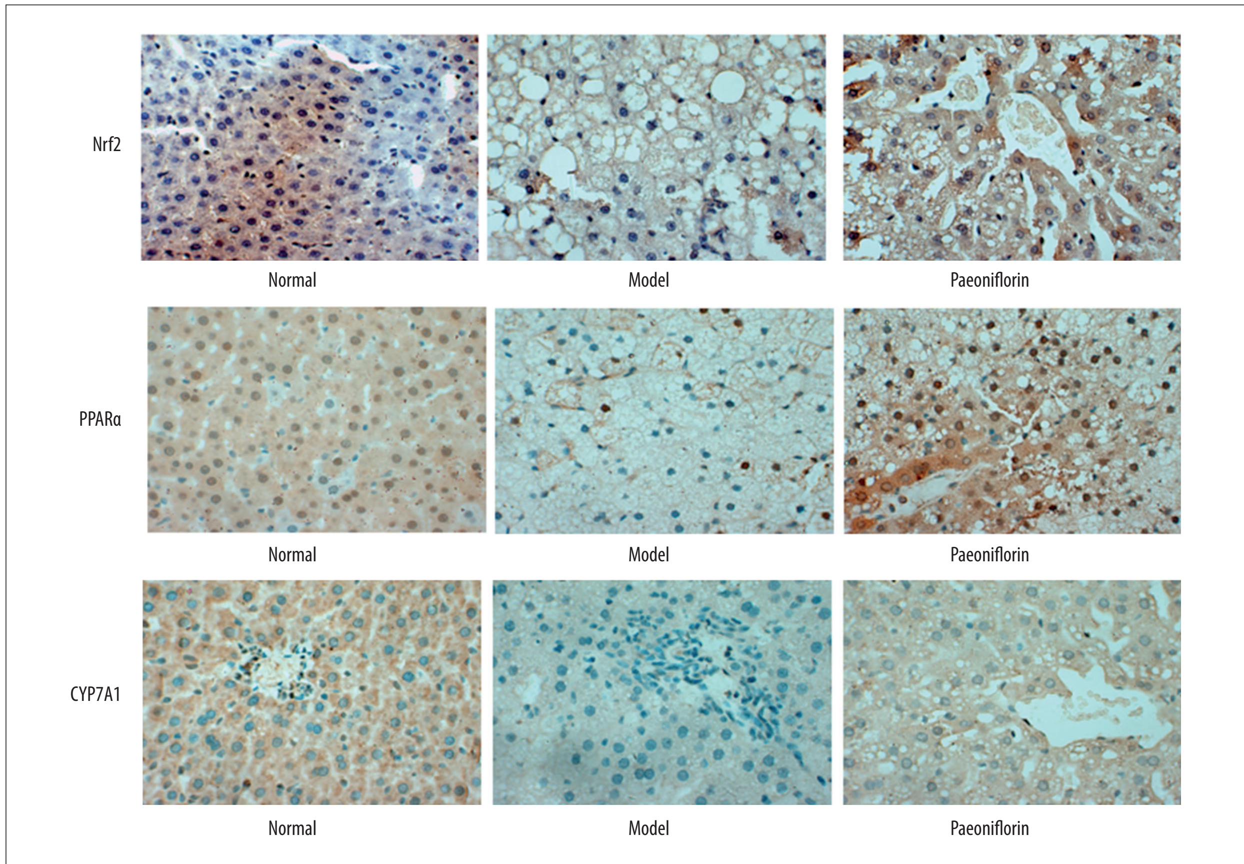

Figure 10. Effect of nuclear factor erythroid 2–related factor 2 (Nrf2), peroxisome proliferator-activated receptors (PPAR)-α, and cytochrome P450 7A1 (CYP7A1) exposure on hepatic cells in the hyperlipidemia rat model. G1 – normal control group; G2 – model control group; G3 – paeoniflorin group.

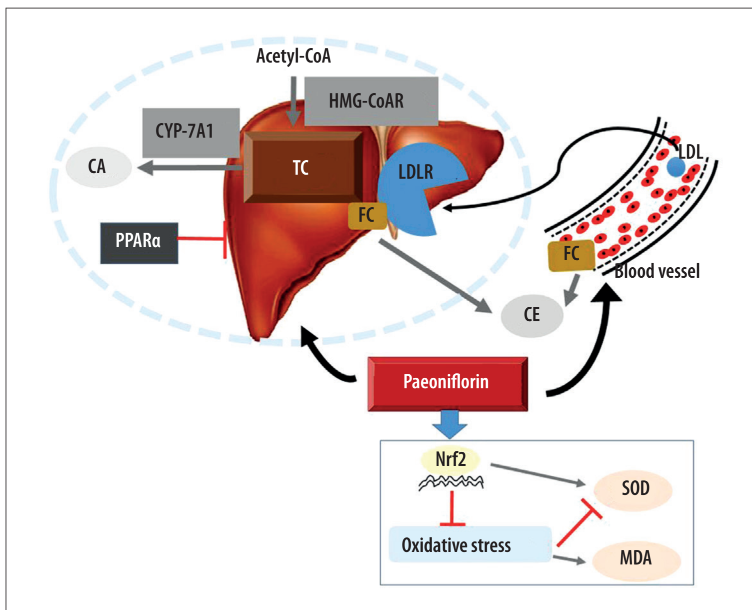

Figure 11. Regulation of the synthesis and metabolism of cholesterol, and the effects of paeoniflorin enriched extract on hemodynamic and oxidative stress.

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

become a global problem [34]. In China, medicinal plants that reduce hyperlipidemia may be useful in decreasing the risk of the development of cardiovascular disease.

In the establishment of hyperlipidemia animal model, the relatively common modeling methods include feeding a high-cholesterol diet [35], a high-lipid emulsion [36], intraperitoneal injection of egg yolk emulsion [12], and intravenous infusion of the lipoprotein lipase inhibitor, Triton WR1339 [37,38]. In this study, a hyperlipidemic rat model was chosen to investigate the effects of the traditional Chinese herbal medicine, paeoniflorin, which is used to treat hyperlipidemia. The advantages of this model over other models of hyperlipidemia, including egg yolk emulsion and Triton WR1339, is that our model more closely mimics dietary hyperlipidemia seen in humans. The most appropriate animal model of hyperlipidemia should be similar to human disease, have a high reproducibility, and should be simple to perform. Therefore, a high-cholesterol diet was used to establish an animal model of hyperlipidemia in this study.

Previous studies have shown that rats who were fed a high-cholesterol diet for 16 weeks showed a significant increase in serum levels of total cholesterol (TC) and low-density lipoprotein cholesterol (LDLC) [39], which was consistent with the findings of our study, demonstrating that the hypercholesterolemic model was successfully established. Hyperlipidemia is a serious condition that requires treatment in humans, because it is strongly associated with the development of atherosclerosis, atherothrombosis, and ischemic heart disease (IHD), including myocardial infarction (MI) [34,40]. Our study demonstrates that paeoniflorin could effectively reduce the levels of serum and hepatic TC in rats fed a high cholesterol diet. HGowever, this study did not show that paeoniflorin had a dose-dependent effect, and the reasons for this are unclear.

The enzyme 3-hydroxy-3-methylglutharyl-coenzyme A reductase (HMG-CoAR) is the key enzyme in the biosynthesis of cholesterol, and its inhibitors are very effective in reducing serum cholesterol on most animal species, including humans [41–43]. In our study, when compared with the high cholesterol group, the activity of HMG-CoAR was reduced by 62.42%, 61.99%, and 56.40%, respectively in the three groups of paeoniflorin treated rats with different treatment doses. Paeoniflorin treatment significantly inhibited the expression of HMG-CoAR, indicating that the decrease in cholesterol levels of animals administrated with paeoniflorin might be partially attributable to the attenuation of the hepatic HMG-CoAR activity. During the consumption of stored hepatic cholesterol, the increase in the number of low-density lipoprotein receptors (LDLR) and activity in liver cells. Our results demonstrated that paeoniflorin treatment significantly upregulated the expression of LDLR, reducing the levels of TC and LDL-C.

The acceleration of cholesterol metabolism could effectively reduce cholesterol levels [44]. Bile acids are synthesized from cholesterol in the liver and are eventually released into the small intestine, recovered, and then returned to the liver for reuse [45]. The hepatic conversion of cholesterol into bile acids and then the subsequent excretion of bile acids into the feces represent the major route for cholesterol excretion that is important in whole-body sterol homeostasis [46]. CYP7A1 is the rate-limiting enzyme for classic synthetic pathways of bile acid enzymes that play an important systemic role in maintaining the body cholesterol metabolism balance. In our study, we discovered that the administration of paeoniflorin could increase the concentrations of fecal TC and decrease the concentrations of plasma FC and CE, and significantly upregulated the expression of CYP7A1, indicating that paeoniflorin could inhibit the absorption of cholesterol in the gut to lower the concentrations of FC and CE.

Liver cells are involved in many stages of lipid metabolism, and it is possible that hyperlipidemia drives the development of fatty liver (steatosis) [47,48]. PPARα plays a significant role in the pathogenesis of hepatic steatosis. Our results indicated that the concentration of hepatic TC was lowered by 30.77% in paeoniflorin treatment group (300 mg/kg body weight per day), as well as significantly upregulating the expression of PPARα. We also observed that the quantity of lipid droplets and inflammatory cells in the HC-PNM group were significantly lower than those of HC group, suggesting that paeoniflorin plays an important role in preventing hepatic steatosis of rats fed a high-cholesterol diet.

Oxidative stress is the unifying mechanism for many cardiovascular diseases risk factors, especially in atherogenesis [49,50]. In the plasma, an antioxidant defense system normally exists that consists of enzymes and non-enzymatic compounds that prevent oxidative damage of lipoproteins [51]. SOD is one of the key enzymes involved in the oxidative defense system [52]. Nrf2 and the antioxidant responsive element system act as essential antioxidant pathways that play crucial roles in various diseases. Cholesterol-rich diets have varying effects on lipid peroxidation, cholesterol oxides, and antioxidant enzymes [53]. Following a high cholesterol diet, oxidative changes in the plasma and cardiovascular system have been shown to play an additive role in the formation of atheromatous plaques in rabbit aorta [54]. A previously reported study has shown that extracts from Ajuga iva increased the antioxidant enzyme activity in red blood cells of rats fed a cholesterol-rich diet [55]. MDA is derived from lipid peroxides, and changes in MDA concentration reflects changes in lipid oxidation level which may be predictive of atherosclerotic events [56]. Our results demonstrated that paeoniflorin could recover the activity of SOD and lowered the levels of MDA in the liver, as well as significantly upregulating the expression of Nrf2, demonstrating that

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

it has an inhibitory role in inhibiting oxidative stress induced by a high cholesterol diet.

Nitric oxide (NO) plays a regulating function of the cardiovascular system and maintains hemodynamic balance; vascular injury associated with atherogenesis that can be inhibited by nitric oxide [57]. A high-cholesterol diet decreases nitric oxide synthase (NOS) activity in erythrocytes from rats [58]. Plasma viscosity and whole-blood viscosity were related to prevalent cardiovascular disease, triglyceride, HDL cholesterol, and cholesterol with very low-density lipoprotein (VLDL) [59]. Our results suggest that paeoniflorin at a dose of 300mg/kg body weight per day could improve blood viscosity of rats fed a high-cholesterol diet. In our study, we found that paeoniflorin at a dose of 300mg/kg body weight per day could significantly enhance the levels of NO in serum, which suggests that paeoniflorin could also have a protective role in the vasculature.

Conclusions

The findings of this study in a rat model of hyperlipidemia have shown that paeoniflorin regulates hepatic cholesterol synthesis and metabolism and may also protect the liver from oxidative injury.

Conflict of interest

None.

References:

1. Zeman M, Vecka M, Perlik F et al: Niacin in the treatment of hyperlipidemias in light of new clinical trials: Has niacin lost its place? Med Sci Monit, 2015; 21: 2156–62
2. Zhang PR, Guo GH: [The pathogenesis and classification of hyperlipidemia.] Chinese Journal For Clinicians, 2012; 40: 18–20 [in Chinese]
3. Chen GY, Li L, Dai F et al: Prevalence of and risk factors for type 2 diabetes mellitus in hyperlipidemia in China. Med Sci Monit, 2015; 21: 2476–84
4. Grover SA, Abrahamowicz M, Joseph L et al: The benefits of treating hyperlipidemia to prevent coronary heart disease. Estimating changes in life expectancy and morbidity. JAMA, 1992; 267: 816–22
5. Nelson RH. Hyperlipidemia as a risk factor for cardiovascular disease. Prim Care, 2013; 40: 195–211
6. Qian L, Xu LH, Lin Y et al. Statins and beta-cell function. Med Sci Monit, 2010; 16(1): HY1–2
7. Moosmann B, Behl C: Selenoprotein synthesis and side-effects of statins. Lancet, 2004; 363: 892–94
8. Bjornsson E, Jacobsen EI, Kalaitzakis E: Hepatotoxicity associated with statins: Reports of idiosyncratic liver injury post-marketing. J Hepatol, 2012; 56: 374–80
9. Norata GD, Tibolla G, Catapano AL: Statins and skeletal muscles toxicity: From clinical trials to everyday practice. Pharmacol Res, 2014; 88: 107–13
10. Zhang YH, Lou H, Zhang W et al. [Content determination of paeoniflorin in radix Paeoniae Rubraextract by HPLC.] Chinese Journal of Spectroscopy Laboratory, 2013; 30: 2424–27 [in Chinese]
11. Jin SN, Wen JF, Wang TT et al: Vasodilatory effects of ethanol extract of Radix Paeoniae Rubra and its mechanism of action in the rat aorta. J Ethnopharmacol, 2012; 142: 188–93
12. Hu HM, Chen SH, Lv GY et al. [Effect of Shenshao Hypolipidemic Tablet on blood lipid levels and liver function of hyperlipidemia mice.] Pharmacology and Clinics of Chinese Materia Medica, 2011; 27: 113–14 [in Chinese]
13. Yang HO, Ko WK, Kim JY et al: Paeoniflorin: An antihyperlipidemic agent from *Paeonia lactiflora*. Fitoterapia, 2004; 75: 45–49
14. Kikuchi T, Orihara K, Oikawa F et al. Intestinal CREBH overexpression prevents high-cholesterol diet-induced hypercholesterolemia by reducing Npc1l1 expression. Mol Metab, 2016; 5: 1092–102
15. Osaki Y, Nakagawa Y, Miyahara S et al: Skeletal muscle-specific HMG-CoA reductase knockout mice exhibit rhabdomyolysis: A model for statin-induced myopathy. Biochem Biophys Res Commun, 2015; 466: 536–40
16. Goldstein J, Brown M: The LDL receptor. Arterioscler Thromb Vasc Biol, 2009; 29: 431–38
17. Pyper SR, Viswakarma N, Yu S et al: PPARalpha: Energy combustion, hypolipidemia, inflammation and cancer. Nucl Recept Signal, 2010; 8: e002
18. Choi HS, Do KM, Park YB et al: Effect of naringin supplementation on cholesterol metabolism and antioxidant status in rats fed high cholesterol with different levels of vitamin E. Ann Nutr Metab, 2001; 45: 193–201
19. Chiang JY: Regulation of bile acid synthesis. Hepatology, 1998; 13: 590–600
20. Wang Y, Han Y, Chai F et al: The antihypercholesterolemic effect of columbamine from Rhizoma Coptidis in HFHC-diet induced hamsters through HNF-4α/FTF-mediated CYP7A1 activation. Fitoterapia, 2016; 115: 111–21
21. Zhu RG, Sun YD, Hou YT et al: Pectin penta-oligogalacturonide reduces cholesterol accumulation by promoting bile acid biosynthesis and excretion in high-cholesterol-fed mice. Chem Biol Interact, 2017; 272: 153–59
22. Lei YC, Li YP: [The index analysis of hemorheology in patients with hyperlipidemia.] People's Military Surgeon, 2007; 50: 276–77 [in Chinese]
23. Li H, Horke S, Forstermann U: Vascular oxidative stress, nitric oxide and atherosclerosis. Atherosclerosis, 2014; 237: 208–19
24. Omari-Siaw E, Wang Q, Sun C et al: Tissue distribution and enhanced *in vivo* anti-hyperlipidemic-antioxidant effects of perillaldehyde-loaded liposomal nanoformulation against Poloxamer 407-induced hyperlipidemia. Int J Pharm, 2016; 513: 68–77
25. Wang MM, Mei ZD, Zhang M et al: [Effects of pueraria extract on blood lipid and antioxidant activity in hyperlipidemia rats.] Science and Technology of Food Industry, 2015; 36: 369–72 [in Chinese]
26. Lambros ML, Plafker SM: Oxidative stress and the Nrf2 anti-oxidant transcription factor in age-related macular degeneration. Springer International Publishing, 2016; 67–72
27. Wang L, Yao Y, He R et al: Methane ameliorates spinal cord ischemia-reperfusion injury in rats: Antioxidant, anti-inflammatory and anti-apoptotic activity mediated by Nrf2 activation. Free Radic Biol Med, 2016; 103: 69–86
28. Yu J, Nashunbayaer, Yang HY et al. [Effects of alfalfa extract on experimental hyperlipemia in rats.] Laboratory Animal and Comparative Medicine, 2008; 28: 171–73 [in Chinese]
29. Wei YJ, Li KA, Tong SY: A linear regression method for the study of the Coomassie brilliant blue protein assay. Talanta, 1997; 44: 923–30
30. Katiyar D, Singh B, Lall AM et al: Efficacy of chitooligosaccharides for the management of diabetes in alloxan induced mice: A correlative study with antihyperlipidemic and antioxidative activity. Eur J Pharm Sci, 2011; 44: 534–43
31. Csont T, Balogh G, Csonka C et al: Hyperlipidemia induced by high cholesterol diet inhibits heat shock response in rat hearts. Biochem Biophys Res Commun, 2002; 290: 1535–38
32. Hiroyuki Y, Yukio M: High cholesterol diet is a risk factor for ischemic heart failure independent to coronary stenotis severity. Journal of Cardiac Failure, 2004; 10: S155
33. Tiwari V, Khokhar M: Mechanism of action of anti-hypercholesterolemia drugs and their resistance. Eur J Pharmacol, 2014; 741: 156–70
34. Berenson GS, Srinivasan S: Cholesterol as a risk factor for early atherosclerosis: The Bogalusa Heart Study. Progress in Pediatric Cardiology, 2003; 17: 113–22

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]

35. Jo SP, Kim JK, Lim YH: Antihyperlipidemic effects of stilbenoids isolated from Morus alba in rats fed a high-cholesterol diet. Food Chem Toxicol, 2014; 65: 213–18
36. Umar A, Iskandar G, Aikemu A et al: Effects of *Cydonia oblonga Miller* leaf and fruit flavonoids on blood lipids and anti-oxydant potential in hyperlipidemia rats. J Ethnopharmacol, 2015; 169: 239–43
37. Harnafi H, Serghini Caid H, el Houda Bouanani N et al: Hypolipemic activity of polyphenol-rich extracts from *Ocimum basilicum* in Triton WR-1339-induced hyperlipidemic mice. Food Chemistry, 2008; 108: 205–12
38. Hu HM, Zhu YC, Zhu QQ et al: Analysis on animal models of experimental hyperlipidemia. China J Chin Mater Med, 2016; 41: 3709–14
39. Fki I, Bouaziz M, Sahnoun Z et al: Hypocholesterolemic effects of phenolic-rich extracts of *Chemlali olive* cultivar in rats fed a cholesterol-rich diet. Bioorg Med Chem, 2005; 13: 5362–70
40. Sloop GD: A critical analysis of the role of cholesterol in atherogenesis. Atherosclerosis, 1999; 142: 265–68
41. Burg JS, Espenshade PJ: Regulation of HMG-CoA reductase in mammals and yeast. Prog Lipid Res, 2011; 50: 403–10
42. Larsson O: HMG-CoA reductase inhibitors role in normal and malignant cells. Hematology, 1996; 22: 197–212
43. Pazzucconi F, Dorigotti F, Gianfranceschi G et al: Therapy with HMG CoA reductase inhibitors characteristics of the long-term permanence of hypocholesterolemic activity. Atherosclerosis, 1995; 117: 189–98
44. Ma H, Patti ME: Bile acids, obesity, and the metabolic syndrome. Best Pract Res Clin Gastroenterol, 2014; 28: 573–83
45. Zwicker BL, Agellon LB: Transport and biological activities of bile acids. Int J Biochem Cell Biol, 2013; 45: 1389–98
46. de Aguiar Vallim TQ, Tarling EJ, Edwards PA: Pleiotropic roles of bile acids in metabolism. Cell Metab, 2013; 17: 657–69
47. Tessari P, Coracina A, Cosma A et al: Hepatic lipid metabolism and non-alcoholic fatty liver disease. Nutr Metab Cardiovasc Dis, 2009; 19: 291–302
48. Lee HS, Nam Y, Chung YH et al: Beneficial effects of phosphatidylcholine on high-fat diet-induced obesity, hyperlipidemia and fatty liver in mice. Life Sci, 2014; 118: 7–14
49. Singh U, Jialal I: Oxidative stress and atherosclerosis. Pathophysiology, 2006; 13: 129–42
50. Harrison D, Griendling KK, Landmesser U et al: Role of oxidative stress in atherosclerosis. Am J Cardiol, 2003; 91: 7–11
51. Araujo FB, Barbosa DS, Hsin CY et al: Evaluation of oxidative stress in patients with hyperlipidemia. Atherosclerosis, 1995; 117: 61–71
52. Kanth BK, Jnawali HN, Niraula NP et al: Superoxide dismutase (SOD) genes in *Streptomyces peucetius*: Effects of SODs on secondary metabolites production. Microbiol Res, 2011; 166: 391–402
53. Mahfouz MM, Kummerow FA: cholesterol-rich diets have different effects on lipid peroxidation, cholesterol oxides, and antioxidant enzymes in rats and rabbits. J Nutr Biochem, 2000; 11: 293–302
54. Balkan J, Doğru-Abbasoğlu S, Aykaç-Toker G et al: The effect of a high cholesterol diet on lipids and oxidative stress in plasma, liver and aorta of rabbits and rats. Nutr Res, 2004; 24: 229–34
55. Bouderbala S, Prost J, Lacaille-Dubois MA et al: Iridoid extracts from Ajuga iva increase the antioxidant enzyme activities in red blood cells of rats fed a cholesterol-rich diet. Nutr Res, 2010; 30: 358–65
56. Lykkesfeldt J: Malondialdehyde as biomarker of oxidative damage to lipids caused by smoking. Clin Chim Acta, 2007; 380: 50–58
57. Raman KG, Gandley RE, Rohland J et al: Early hypercholesterolemia contributes to vasomotor dysfunction and injury associated atherogenesis that can be inhibited by nitric oxide. J Vasc Surg, 2011; 53: 754–63
58. Devrim E, erguder IB, Ozbek H et al: High-cholesterol diet increases xanthine oxidase and decreases nitric oxide synthase activities in erythrocytes from rats. Nutr Res, 2008; 28: 212–15
59. Slyper A, Le A, Jurva J et al: The influence of lipoproteins on whole-blood viscosity at multiple shear rates. Metabolism, 2005; 54: 764–68

This work is licensed under Creative Common Attribution-NonCommercial-NoDerivatives 4.0 International (CC BY-NC-ND 4.0)

Indexed in: [Current Contents/Clinical Medicine] [SCI Expanded] [ISI Alerting System] [ISI Journals Master List] [Index Medicus/MEDLINE] [EMBASE/Excerpta Medica] [Chemical Abstracts/CAS] [Index Copernicus]