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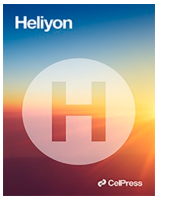

Protective effects of methyl protodioscin against lipid disorders and liver injury in hyperlipidemic gerbils

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ABSTRACT

Methyl protodioscin (MPD) is the main component of total diosgenin, which was reported to reduce cholesterol and triglyceride levels potentially. This study aimed to investigate the beneficial effects of MPD against lipid disorder in hyperlipidemic gerbils induced by a high-fat diet (HFD). Hyperlipidemia was induced in gerbils by feeding them with HFD for six weeks, and a daily oral dose of MPD solution (25 and 50 mg/kg/day) was administered. This study investigated blood lipid levels and hepatic lipid accumulation in hyperlipidemic gerbils. The potential mechanism of MPD was explored by detecting the expression level of genes, including SREBPs, ACC, FASN, HMGCR, PCSK9, and LDL-R. The results showed that MPD treatment decreased the body weight, the relative weight of the liver, blood lipid, and hepatic lipid levels of gerbils fed with HFD. The administration of MPD alleviates liver steatosis and injury in gerbils fed with an HFD. MPD treatment reduced the expression of HMGCR, increased the expression of LDL-R, and decreased the expression of PCSK9 for cholesterol reduction. Additionally, MPD treatment reduced the expression of hepatic ACC and FASN for triglycerides reduction. The underlying mechanisms for these effects are attributed to MPD-induced inhibition of protein expression of LXR, SREBP1, and SREBP2. This study demonstrates that MPD protects gerbils against lipid disorders and liver injury by suppressing hepatic SREBPs expression.

1. Introduction

The incidence rates of arteriosclerotic cardiovascular disease (ASCVD) and non-alcoholic fatty liver disease (NAFLD) have been increasing annually with the improvement of living standards, changes in dietary habits, and an increasingly aging population [1]. NAFLD is rapidly emerging as a global health issue and is currently the world's most common chronic liver disease. ASCVD is the primary cause of death among patients with NAFLD [2]. The common pathogenesis basis of ASCVD and NAFLD is lipid metabolism disorder, such as increased low-density lipoprotein cholesterol (LDL-C), total cholesterol (TC), and triglyceride (TG), as well as the abnormal high-density lipoprotein cholesterol (HDL-C) function. The primary treatment of these two diseases is to facilitate the outflow of arterial plaque lipids and regulate the metabolism of blood and liver lipids. Common lipid-lowering drugs used in clinical

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practice include statins, bezafibrate, and niacin (etc.), which can regulate blood lipids by inhibiting the synthesis of cholesterol and triglycerides [3,4]. Despite their potent cholesterol-lowering and lipid-lowering effects, these medicines are limited by their side effect (hepatotoxicity and aggravating liver fat accumulation) [5]. There is an urgent need for drugs or treatments to decrease blood lipid levels and enhance hepatic lipid metabolism to prevent liver damage.

Sterol regulatory element binding proteins (SREBPs) are nuclear transcription factors that play critical roles in maintaining intracellular cholesterol balance by regulating the activities of cholesterol and triglyceride-synthesis-related enzymes. There are three subtypes of SREBPs, namely SREBP1a, SREBP1c, and SREBP2 [6]. SREBP1 mainly regulates the genes related to fatty acid *de novo* synthesis: acetyl-CoA carboxylase (ACC) and fatty acid synthase (FASN), which control the rate of lipid synthesis. The inhibition of ACC and FASN expression can reduce the hepatic synthesis of fatty acids, thus reducing the accumulation of hepatic lipids and plasma TG levels [7]. SREBP2 mainly affects cholesterol synthesis by regulating the activity of the key enzyme of cholesterol synthesis: 3-hydroxy-3-methyl-glutaryl-coenzyme A reductase (HMGCR) [8]. Thus, reducing the expression of SREBPs can inhibit the synthesis of triglycerides and cholesterol.

Approximately 70 % of circulating cholesterol and LDL-C is removed by LDL-receptor (R)-mediated endocytic circulation [9]. Transcriptional and posttranscriptional factors regulate LDL-R expression. Additionally, proprotein convertase subtilisin kexin type 9 (PCSK9) can hinder LDL-R circulation in cells, thereby triggering the abnormal accumulation of LDL-C in plasma [10]. Inhibitors of PCSK9 have been developed to treat familial hypercholesterolemia caused by functional acquired variation in the PCSK9 gene [11]. Furthermore, both PCSK9 and LDL-R genes contain sterol regulatory elements, which SREBP2 can regulate at the transcriptional level [12].

Methyl protodioscin (MPD) is the main component of total diosgenin in the water extract of dioscorea nipponica [13]. Diosgenin exhibits diverse pharmacological effects, including improving the symptoms of atherosclerosis and protecting vascular endothelial function, reducing the ischemia/reperfusion injury of the heart, brain, and kidney, reducing blood sugar, inhibiting liver fibrosis, improving menopausal osteoporosis, anti-inflammation and anti-virus activity [14,15]. Previous studies have demonstrated that MPD could decrease fatty acid and cholesterol synthesis by reducing the expression of HMGCR, ACC, and FASN, promote cholesterol efflux by improving the expression of ATP binding cassette protein 1 (ABCA1), and inhibit the expression of SREBPs by reducing LDL-C and TG levels *in vitro* [16]. These results implied that MPD has the potential to regulate cellular lipid metabolism at the cellular level, but the lipid-lowering effect of MPD has yet to be verified *in vivo*. Mongolian gerbils easily acquired hyperlipidemia after being fed a high-fat/high-cholesterol diet over four weeks, which resembled mixed hyperlipidemia and NAFLD in humans [17]. This study investigated the effect of MPD on blood lipid and hepatic lipid accumulation in hyperlipidemic gerbils. The potential mechanism of MPD was explored by detecting the expression level changes of genes SREBPs, ACC, FASN, HMGCR, PCSK9, and LDL-R. This research will establish the experimental foundation for using MPD to treat hyperlipidemia.

2. Materials and methods

2.1. Methyl protodioscin and simvastatin preparation

Methyl protodioscin (catalog number: 14-0528-20; 98 % pure by HPLC) was obtained from Beijing Xinrong Technology Co., Ltd (Beijing, China). The MPD was prepared at concentrations of 0.8 % suspension with 0.5 % sodium hydroxymethyl cellulose solution; simvastatin was prepared at concentrations of 0.4 % suspension.

2.2. Animals and treatments

This study protocol was approved by the Animal Care and Use Committee of Guangdong Medical University (ID number: GDY2002241; Zhanjiang, China).

Male sand gerbils (Meriones Unguiculatus, six weeks, 40–60 g, specific pathogen-free) were acquired from the Laboratory of Animal Center of Zunyi Medical University [production license: SYXK (Qian) 2011-0003]. The gerbils were housed in a controlled environment, free of any possible pathogens, with the temperature maintained at $24 \pm 2\ ^{\circ}C$ and humidity at 50 ± 5 %, with a standard 12 h light/dark cycle. A total of 50 gerbils were randomly assigned into five treatment groups, including the control group (gerbils were given a standard chow diet), the HFD treatment group (gerbils were given a diet containing 1.25 % cholesterol, 20 % fat, and 0.5 % cholic acid for six weeks), HFD with a low-MPD group (25 mg/kg/day), HFD with a high-MPD group (50 mg/kg/day), and simvastatin group (8 mg/kg/day). Each of these groups consisted of 10 gerbils. The MPD and simvastatin solution were administered orally daily for six weeks at a calculated dose based on drug concentration and individual animal weight (at 9:00 a.m.). Gerbils' feed and water were provided ad libitum throughout the experiment.

2.3. Record of body weight and relative weight of the liver

The body weight of gerbils was recorded every week to calculate body weight changes. The final liver weight was recorded, and the results were reported relative to body weight (g/kg).

2.4. Blood lipid and liver function assay

Approximately 1 mL of blood samples were extracted from the angular vein of mice under anesthesia by pentobarbital. Serum

samples were collected by centrifugation at 1790×*g* for 10 min at room temperature and subsequently preserved in microtubes at −80 °C. The levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), TG, TC, HDL-C, and LDL-C were determined using commercially available kits purchased from Nanjing Jiancheng Bioengineering Institute (C009-2-1, C010-2-1, A110-1-1, A111-1-1, A112-1-1, A113-1-1, Nanjing, Jiangsu, China) following the manufacturer's instructions.

2.5. Hepatic lipid determination and histological analysis

After being rinsed with ice-cold sterile saline solution to remove blood contamination, the liver of each gerbil was extracted and stored in a cryogenic vial. A homogenate was prepared using 20 mg of liver tissue to determine hepatic lipid contents. The minced tissue was homogenized in 1 mL of ice-cold 80 % methanol solution using a glass homogenate tube and was centrifuged at 1,2000×*g* for 10 min at 4 °C. Approximately 300 μL of supernatant was collected and mixed with 1 mL of methyl *tert*-butyl ether (MTBE) by vertexing for 20 min, then 300 μL of distilled water was added and vortexed for 30 s. The oil phase was collected by centrifuging at 1,2000×*g* for 10 min at 4 °C and kept at −80 °C until further analysis [18]. TG and TC levels in each sample were determined using a commercially available kit purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China) under the manufacturer's instructions. The protein concentrations in tissue homogenates were measured using the bicinchoninic acid assay.

For histological analysis, liver tissues were preserved in 4 % paraformaldehyde in PBS for 10 min at room temperature. The fixed liver samples were placed into 30 % sucrose solution for dehydration and then embedded into optimal cutting temperature (OCT) compound. Subsequently, 5 slices were prepared from each sample, each slide containing 3 sections (10 μm thickness). The sections were stained with hematoxylin and eosin (H&E) and sealed with neutral resin.

The liver slides were stained for 10 min with freshly made oil red O solution for oil red O staining. Subsequently, the slides were washed three times with distilled water and imaged using an inverted light microscope (Axio Vert. A1, Carl Zeiss, Germany). ImageJ 1.45 software (NIH, United States) calculated the oil-red O ratio based on the average proportion of lipid droplets accumulated in hepatocytes in each field at 200 magnifications in 20 random regions.

For Masson's trichrome staining, the liver slides were stained with Masson lichen red acidic compound solution for 10 min, rinsed with running water, and air-dried through shaking. The samples were then differentiated for 5 min with 1 % phosphomolybdate aqueous solution. After removing the phosphomolybdate, the slices were directly stained with aniline blue for 5 min and rinsed with water. Then, the slices were washed and dried with 95 % alcohol, anhydrous alcohol, and transparent xylene in sequence. Each slice was sealed with neutral gum. All images were acquired using a light microscope (magnification, × 500; Nikon Corporation) and analyzed using Image J 1.45 (US National Institutes of Health, Bethesda, MD, USA).

2.6. Western blotting

Liver samples were frozen in liquid nitrogen, ground into powder in a mortar, and lysed with the addition of RIPA lysis buffer (Beyotime Biotechnology, Jiangsu, China). Following the complete lysis of samples, the supernatant was centrifuged at 12,000×*g* for 15 min at 4 °C. Bicinchoninic Acid Protein Kit (Meilun Biotechnology, Dalian, China) was used to quantify total protein supernatants; 50 mg of whole protein samples were resolved with 8, 10, or 15 % polyacrylamide gel (depending on the molecular size of the proteins to be examined). Immunoblotting was performed by transferring resolved proteins onto polyvinylidene difluoride (PVDF) membranes in a *trans*-buffer at 100 V for 1 or 2 h, depending on the molecular size of the protein. PVDF membranes were blocked with 5 % skimmed milk or 5 % bovine serum albumin (for phosphorylated protein) in TBST buffer [Tris–HCl (20 mM), pH 7.5; sodium chloride (150 mM); 0.05 % Tween 20] for 2 h. Subsequently, the membranes were washed thrice with TBST buffer for 5 min each time and then incubated overnight with primary antibodies targeting HMGCR (catalog number: SAB4200529, Sigma), PCSK9 (PAB17045, Abnova), liver X receptor (LXR; ab176323, Abcam), SREBP1 (ab3259, Abcam), SREBP2 (ab30682, Abcam), LDL-R (PAB8804, Abnova), ACC (ab45174, Abcam), and FASN (SAB4300700, Sigma) at 4 °C. Subsequently, PVDF membranes were washed three times with TBST buffer for 5 min each time and incubated with anti-rabbit (or anti-mouse) secondary antibodies conjugated to horseradish peroxidase (1:2000 dilution) for 2 h at room temperature. Blots were developed using an electrochemiluminescence detection kit under dark conditions. The developed blots underwent densitometric analysis using ImageJ 1.45 (US National Institutes of Health, Bethesda, MD, USA). For the normalization of expression, glyceraldehyde 3-phosphate dehydrogenase (GAPDH; MA1-16757, Thermo Fisher Scientific) was employed as the internal control.

2.7. Immunohistochemistry

The expressions of HMGCR, LDL-R, PCSK9, ACC, and FASN in the liver were evaluated by immunohistochemistry. Briefly, liver slides were prepared by paraffin-embedded, sliced, and deparaffinized as follows: xylene I (10 min)-xylene II (10 min)-xylene III (10 min) 100 % alcohol I (5 min)-95 % alcohol I (5 min)-85 % alcohol I (5 min)-75 % alcohol II (5 min). The slides were antigen-repaired in 0.1 mol/L sodium citrate solution heated in a microwave oven for 20 min, followed by 1 % hydrogen peroxide (to eliminate endogenous peroxidase activity), and blocked with 2 % goat serum for 1 h. Then, slides were incubated with primary antibodies HMGCR, LDL-R, PCSK9, ACC, and FASN at 1:200 dilution overnight at 4 °C, followed by incubation with fluorescein isothiocyanate conjugated goat anti-rabbit immunoglobin G (1:500 dilution; Beyotime Biotechnology) for 2 h at room temperature. Nuclei were stained by counterstaining slides with 4′,6-diamidino-2-phenylindole for 5 min. Coverslips were mounted on slides, then visualized under a light microscope (magnification, × 500; Nikon Corporation) and analyzed using ImageJ 1.45 (US National Institutes of Health, Bethesda, MD, USA).

2.8. Statistical analysis

Quantitative data are presented as the mean ± SD of three independent experiments. The results were assessed using one-way ANOVA followed by Tukey's multiple-comparison test, and the statistical analysis was conducted using Prism 6 (GraphPad, San Diego, CA, USA). $P < 0.05$ was considered significant.

3. Results

3.1. MPD treatment decreased the body weight and blood lipid level of HFD-fed gerbils

The gerbils were fed with HFD for six weeks to induce hyperlipidemia, followed by a six-week treatment of MPD (Fig. 1A). Fig. 1B showed that the gerbils in the HFD group had a higher body weight than the control group ($P < 0.05$). However, the body weight of HFD-gerbils decreased significantly upon high-MPD treatment. The concentration of TG in the serum of HFD-gerbils showed a significant rise as compared to the control group ($P < 0.05$) (Fig. 1C). However, serum TG level was significantly reduced by a low and high concentration of MPD ($P < 0.05$) (Fig. 1C). HFD-fed gerbils had a higher serum level of TC and LDL-C than the control group. However, the serum levels of TC and LDL-C were significantly reduced with low and high concentrations of MPD supplementation (Fig. 1D and E). The HFD significantly reduced the serum HDL-C level, while a low concentration of MPD resulted in a significant

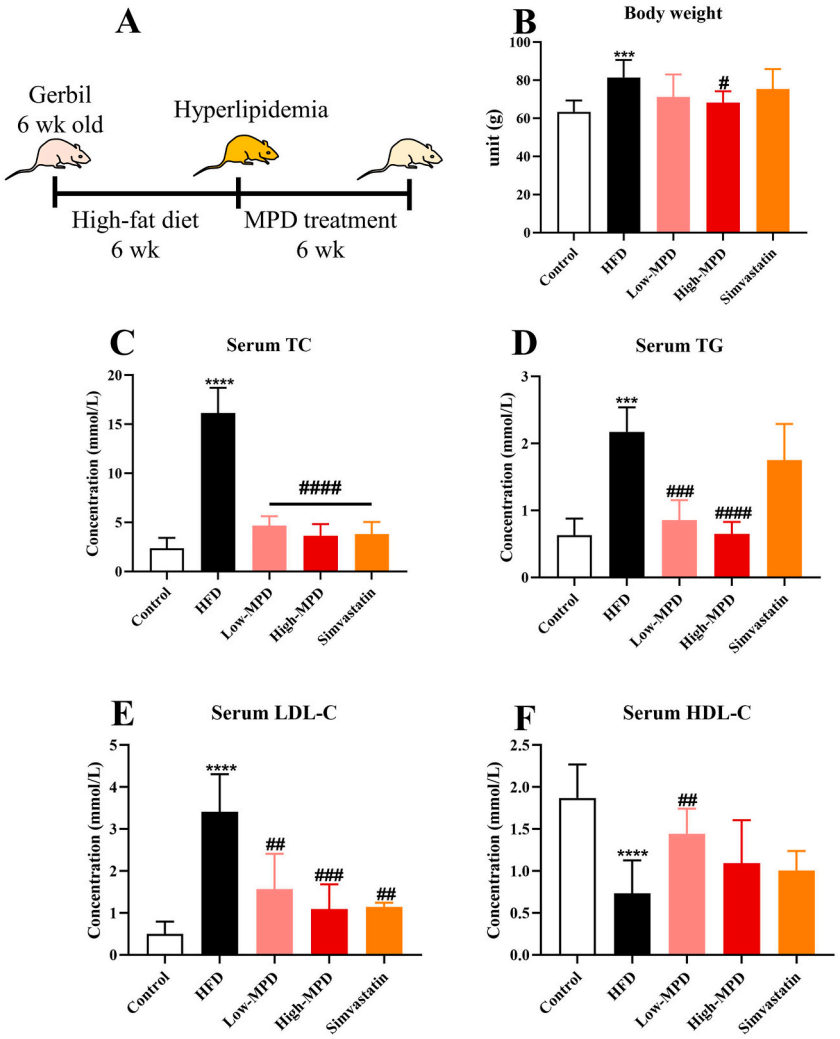


Fig. 1. Methyl protodioscin decreases blood lipid levels in HFD-fed mice. (A) Body weight changes; (B) Relative weight of liver; (C) Serum total cholesterol (TC) level; (D) Serum triglycerides (TG) level; (E) Serum low-density lipoprotein cholesterol (LDL-C) level; (F) Serum high-density lipoprotein cholesterol (HDL-C) level. Data are expressed as mean ± SD (n = 6). *P < 0.05, **P < 0.01, ***P < 0.001 (* represents HFD groups compared with the Control group). #P < 0.05, ##P < 0.01, ###P < 0.001 (# represents MPD and Simvastatin treatments compared with the HFD group). HFD: high-fat diet; MDP: methyl protodioscin.

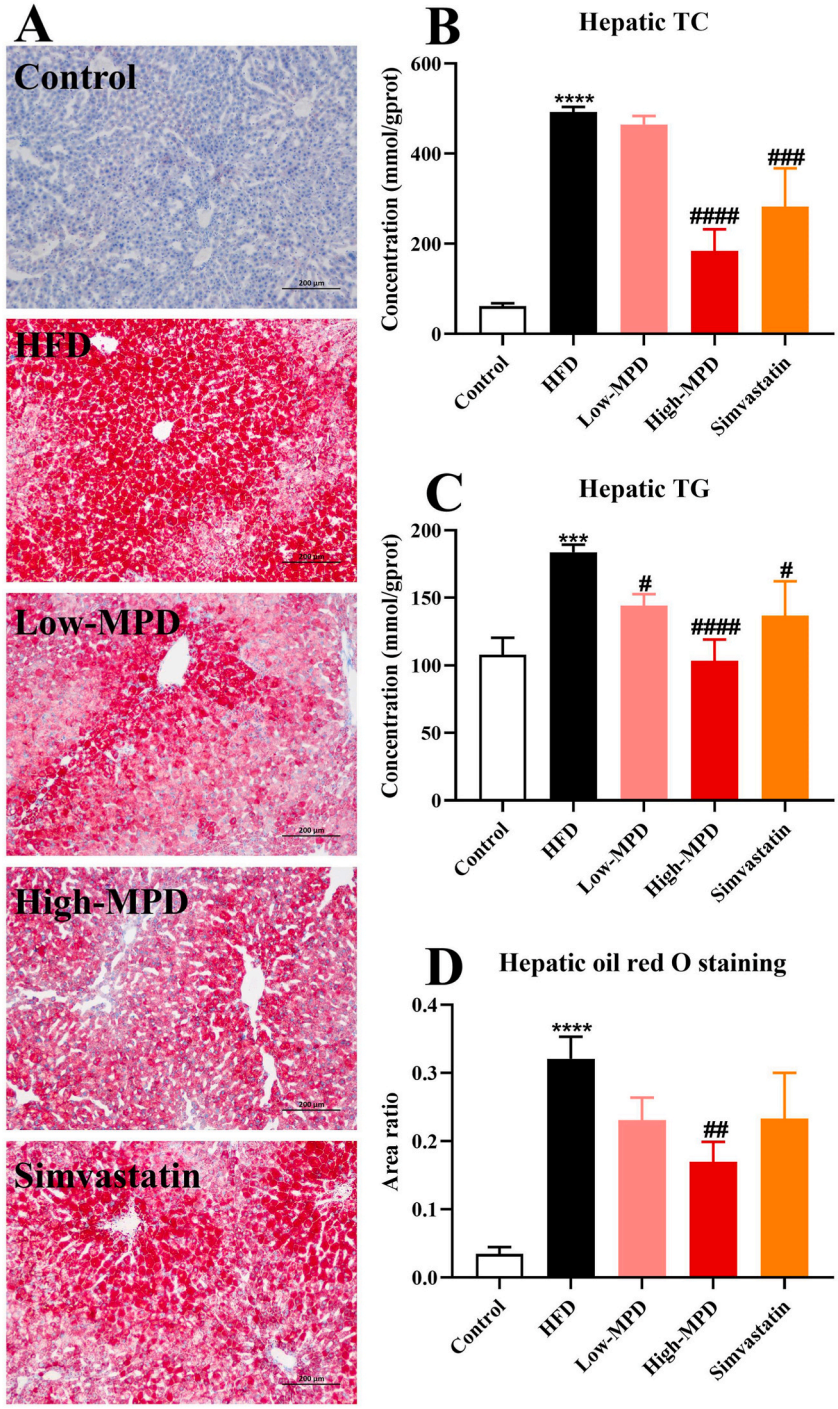
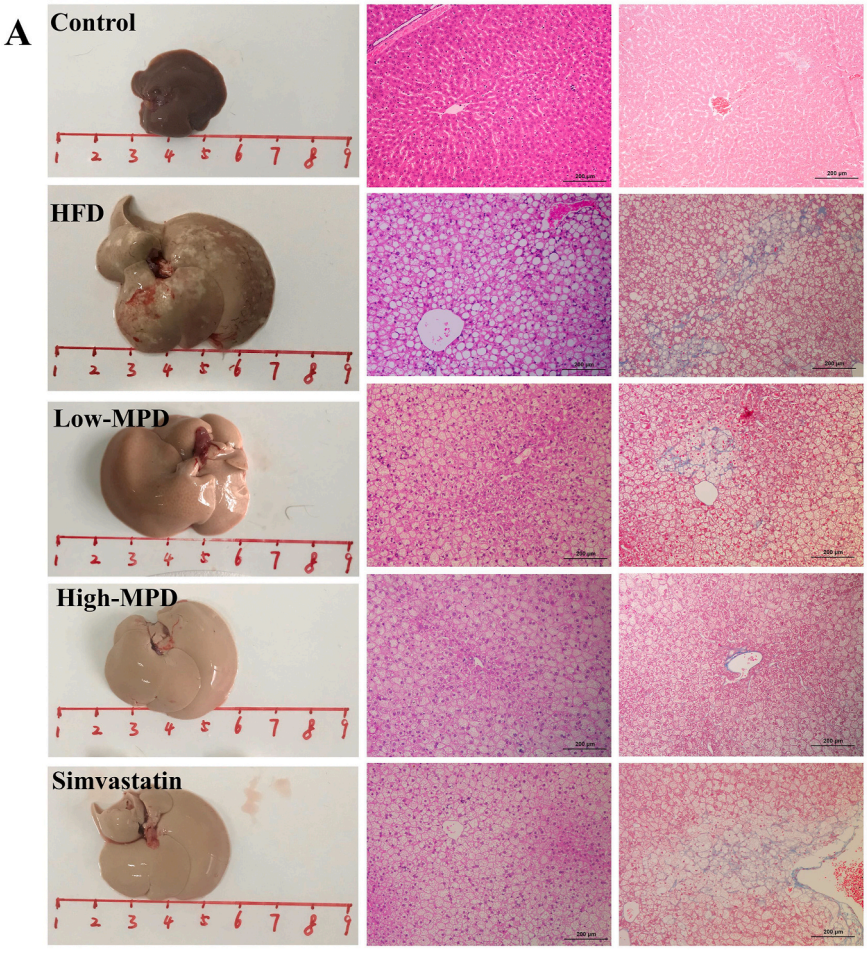


Fig. 2. Methyl protodioscin decreases hepatic steatosis in HFD-fed mice. (A) Oil red O staining of the liver; (B) Hepatic total cholesterol (TC) level; (C) Hepatic triglycerides (TG) level; (D) Area ratio of Oil red staining in the liver. Data are expressed as mean ± SD (n = 6). *P < 0.05, **P < 0.01, ***P < 0.001 (* represents HFD groups compared with the Control group). #P < 0.05, ##P < 0.01, ###P < 0.001 (# represents MPD and Simvastatin treatments compared with the HFD group). HFD: high-fat diet; MDP: methyl protodioscin. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)



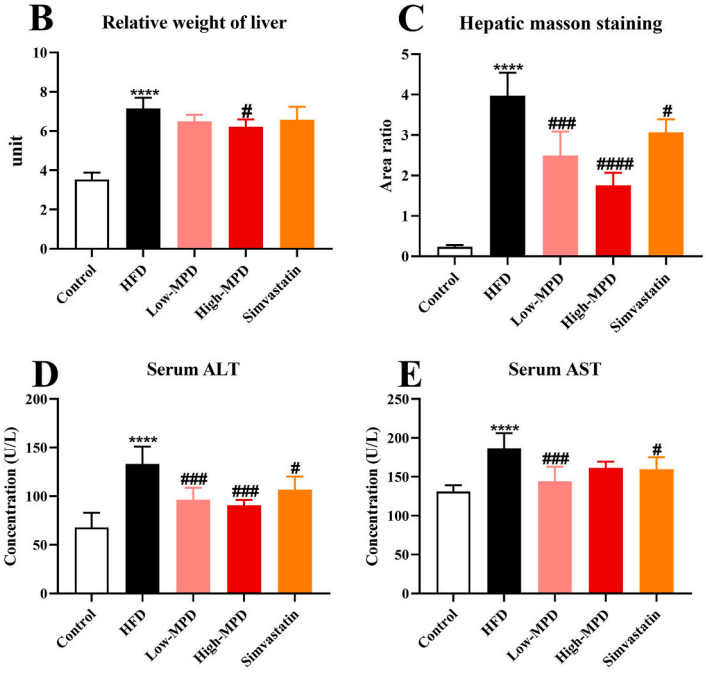


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Fig. 3. Methyl protodioscin alleviates liver injury in HFD-fed mice. (A) The fresh liver sample, H&E staining, and Masson staining of the liver; (B) Relative weight of liver; (C) Area ratio of Masson staining in the liver; (D) Hepatic alanine aminotransferase (ALT) level; (E) Hepatic aspartate aminotransferase (AST) level. Data are expressed as mean ± SD (n = 6). *P < 0.05, **P < 0.01, ***P < 0.001 (* represents HFD groups compared with the Control group). #P < 0.05, ##P < 0.01, ###P < 0.001 (# represents MPD and Simvastatin treatments compared with the HFD group). HFD: high-fat diet; MDP: methyl protodioscin.

increase in serum HDL-C level (Fig. 1F). Moreover, simvastatin therapy compromised the higher serum TC and LDL-C induced by the HFD (Fig. 1D and E).

3.2. MPD treatment decreased hepatic lipid level

The intracellular lipid was examined using oil red O staining-lipid droplets (LDs; Fig. 2A), and the results revealed that LDs in rat liver (the ratio of oil red O is 32.1 %; Fig. 2B) was significantly higher than that the control group of gerbils (the ratio of oil red O is 3.5 %; Fig. 2B). The LDs in HFD-fed gerbils were reduced in the presence of MPD (Fig. 2B), and the area ratio of oil red O is 23.13 % for low-MPD and 17 % for high-MPD. A high concentration of MPD significantly reduced the hepatic TC level in HFD-fed gerbils ($P < 0.05$) (Fig. 2C). In addition, both low and high concentrations of MPD were found to decrease hepatic TG ($P < 0.05$) (Fig. 2D).

3.3. MPD treatment alleviates liver steatosis and injury in HFD-fed gerbils

In the control group, the liver volume of gerbils was of normal size, reddish brown, and the capsule exhibited no signs of tension. However, the liver of gerbils in the HFD group was diffusely enlarged, grayish white, with a tight capsule and a greasy feeling (Fig. 3A; left column). MPD and simvastatin treatment decreased the volume size of the liver, but the color was still gray-white and darker than that of the HFD group (Fig. 3A; left column); high concentration of MPD decreased the relative weight of the liver than the HFD group (Fig. 3B). H&E staining demonstrated that the liver of gerbils in the HFD group had vacuoles of different sizes, hepatocyte swelling, cell nucleus deviation, and unclear cell boundaries, indicating clear hepatic (Fig. 3A; middle column). MPD and simvastatin treatment decreased the hepatic steatosis rate per unit under the light microscope (Fig. 3A; middle column). Masson's trichrome staining analyzed liver fibrosis, and the results revealed that hepatic fibrosis of HFD-fed gerbils was observed in perisinusoidal or periportal space (Fig. 3A; right column), and approximately area ratio of 3.97 fibrosis was measured (Fig. 3C). MPD treatment reduced hepatic fibrosis in low (area ratio of 2.49) and high (area ratio of 1.75) concentrations ($P < 0.05$) (Fig. 3C). The HFD-fed gerbils exhibited a substantial increase in serum ALT and AST. In contrast, the ALT and AST levels in the MPD group were substantially lower than in the HFD group (Fig. 1D and E).

3.4. Effect of methyl protodioscin on HMGCR, LDL-R, and PCSK9 in the liver of HFD-fed gerbils

As shown in Fig. 4A, low and high concentrations of MPD treatment decreased the level of HMGCR protein compared with the HFD-fed group ($P < 0.05$). Immunohistochemistry (IHC) staining revealed the localization of HMGCR on the liver (Fig. 3D; up channel), but no significant effect of MPD was observed ($P > 0.05$) (Fig. 3E). HFD reduced the protein level of LDL-R and increased the protein expression of PCSK9 in the liver. MPD treatment resulted in a significant increase in the protein expression of LDL-R at both low and high concentrations ($P < 0.05$) (Fig. 4B). The protein expression of PCSK9 was increased by a high concentration of MPD (Fig. 4C). IHC analyzed the LDL-R and PCSK9 staining; LDL-R was located on the plasma membrane (Fig. 4D; Middle channel) and PCSK9 in the cytoplasm (Fig. 4D; Down channel). MPD increased the positive area ratio of LDL-R in low and high concentrations ($P < 0.05$) (Fig. 4F), whereas a high concentration of MPD decreased the positive area ratio of PCSK9 ($P < 0.05$) (Fig. 4F). In addition, Simvastatin therapy significantly reduced the protein expression of HMGCR (Fig. 4A) and increased the protein level of LDL-R (Fig. 4B and F).

3.5. Effect of methyl protodioscin on ACC and FASN in the liver of HFD-fed gerbils

High-fat diet increased the hepatic protein level of ACC and FASN more than the control group ($P < 0.05$) (Fig. 5A and B). MPD treatment decreased the protein expression of ACC and FASN, both in low and high concentrations (Fig. 5A and B). IHC analysis revealed that ACC and FASN are in the cytoplasmic compartment (Fig. 5C). High-fat diet increased the positive area ratio of ACC and FASN. As compared with the HFD group, a high concentration of MPD decreased the positive area ratio of ACC ($P < 0.05$) (Fig. 5D); the positive area ratio of FASN was decreased by the low and high concentration of MPD (Fig. 5E). In addition, Simvastatin therapy significantly reduced the protein expression of ACC (Fig. 5A) and FASN (Fig. 5B).

3.6. Effect of methyl protodioscin on SREBP1, SREBP2, and LXR in the liver of HFD-fed gerbils

High-fat diet increased hepatic protein levels of SREBP1, SREBP2, and LXR. In contrast, in low and high concentrations, MPD decreased the protein expression of SREBP1 (Fig. 6A) and SREBP2 (Fig. 6B). The hepatic protein level of LXR decreased significantly at a high concentration of MPD (Fig. 6C). In addition, Simvastatin therapy significantly reduced the protein expression of SREBP1 (Fig. 6A), SREBP2 (Fig. 6B), and LXR (Fig. 6C).

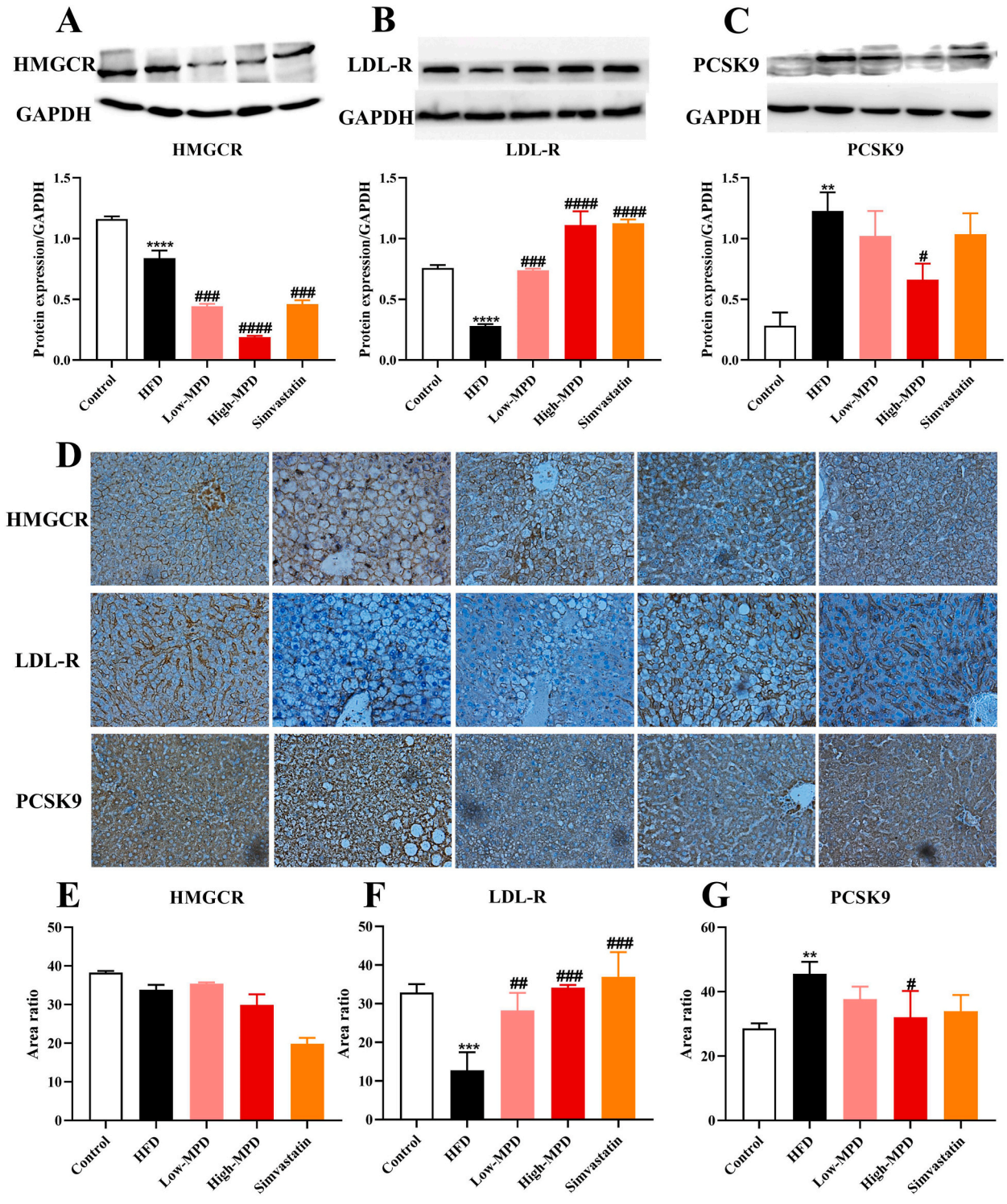

Fig. 4. Effect of methyl protodioscin on HMGCR, LDLR, and PCSK9 in the liver of HFD-fed mice. (A), (B), and (C) Relative protein expressions of HMGCR, LDL-R, and PCSK9 determined by Western blotting; (D) Representative immunohistochemical image of HMGCR, LDL-R, and PCSK9 in livers; (E), (F), and (G) Positive area ratio of HMGCR, LDL-R, and PCSK9. Data are expressed as mean ± SD (n = 6). *P < 0.05, **P < 0.01, ***P < 0.001 (* represents HFD groups compared with the Control group). #P < 0.05, ##P < 0.01, ###P < 0.001 (# represents MPD and Simvastatin treatments compared with the HFD group). HFD: high-fat diet; MDP: methyl protodioscin.

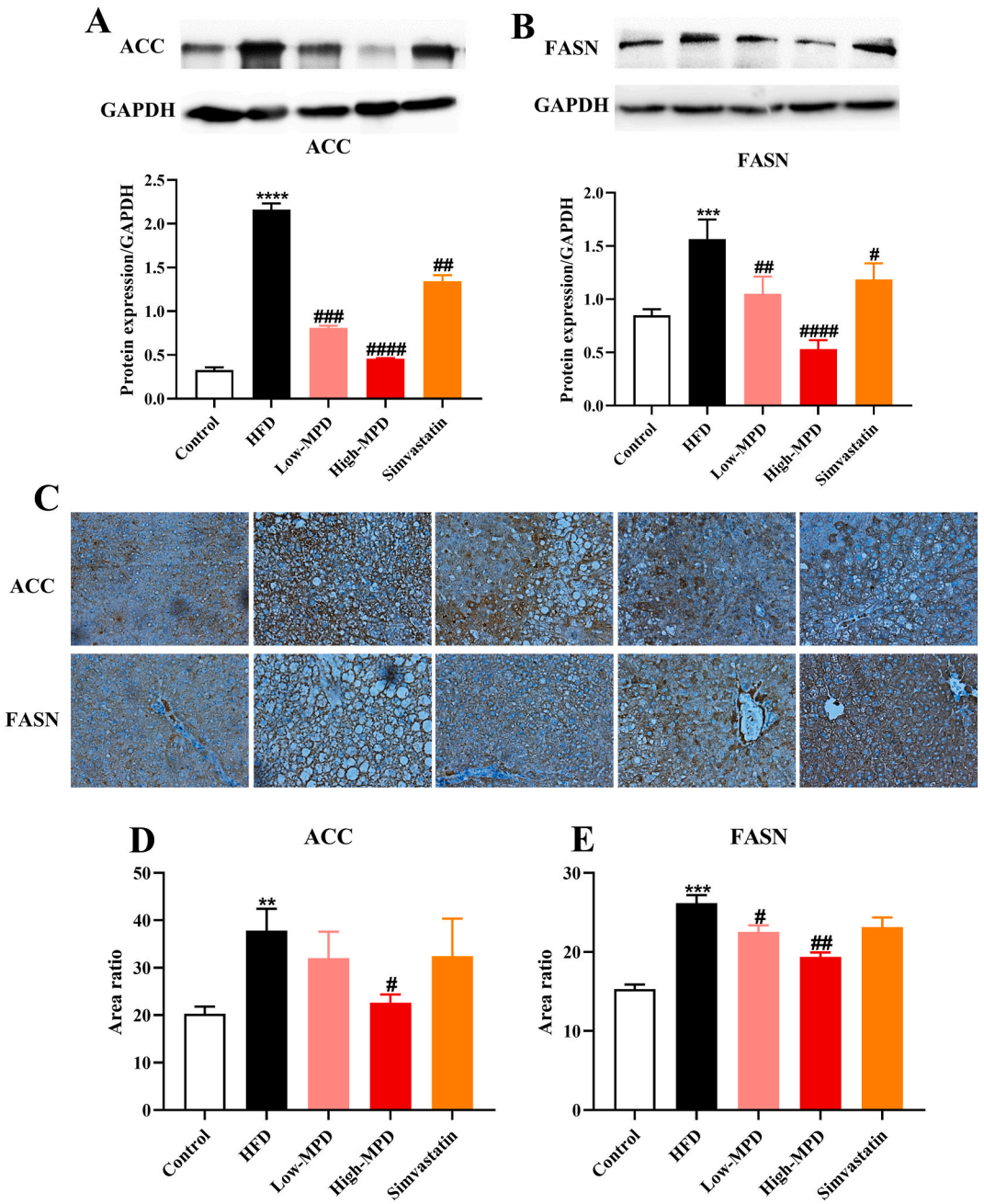

Fig. 5. Effect of methyl protodioscin on ACC and FASN in the liver of HFD-fed mice. (A) and (B) Relative protein expressions of ACC and FASN determined by Western blotting; (C) Representative immunohistochemical image of ACC and FASN in livers; (D) and (E) Positive area ratio of ACC and FASN. Data are expressed as mean ± SD (n = 6). *P < 0.05, **P < 0.01, ***P < 0.001 (* represents HFD groups compared with the Control group). #P < 0.05, ##P < 0.01, ###P < 0.001 (# represents MPD and Simvastatin treatments compared with the HFD group). HFD: high-fat diet; MDP: methyl protodioscin.

4. Discussion

Currently, chemosynthetic drugs used in clinical settings have a relatively direct impact on regulating lipid disorders. Statins can strongly inhibit cholesterol synthesis by inhibiting HMGCR, reducing TC and LDL-C in plasma. However, the potential adverse effects, such as liver injury, myalgia, and myositis, have limited their clinical application [19]. Fibrates usually decrease plasma TG levels but do not significantly reduce total cholesterol and LDL cholesterol levels [20]. In addition, caution should be exercised when administering a combination of statins and fibrates to patients with mixed hyperlipidemia, as it can lead to liver and muscle toxicity. With its

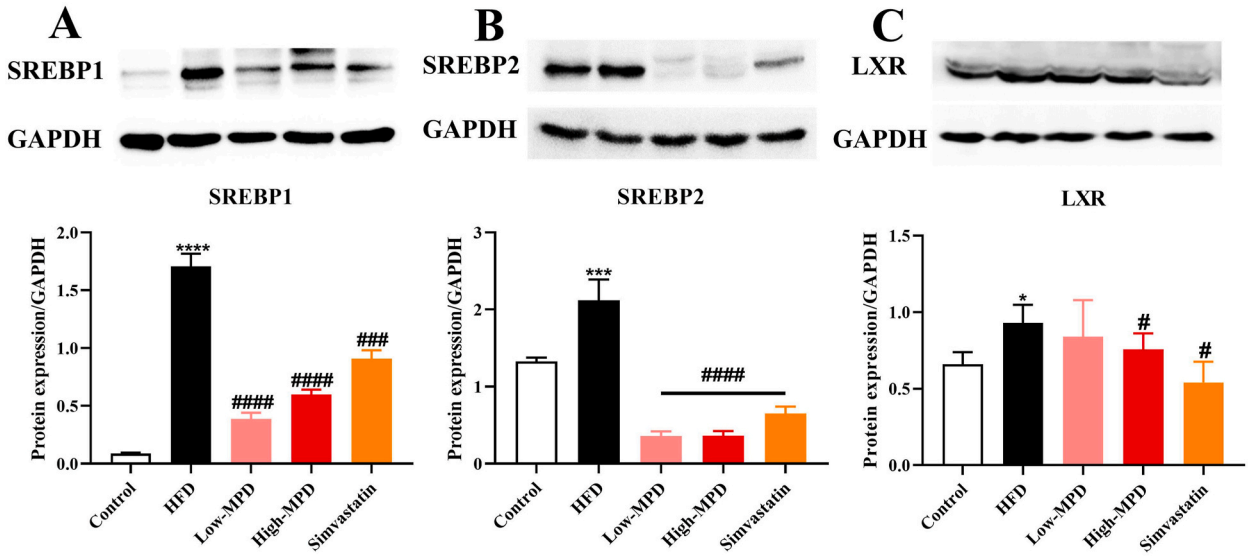

Fig. 6. Effect of methyl protodioscin on SREBP1, SREBP2, and LXR in the liver of HFD-fed mice. (A), (B), and (C) Relative protein expressions of SREBP1, SREBP2, and LXR were determined by Western blotting. Data are expressed as mean ± SD (n = 6). *P < 0.05, **P < 0.01, ***P < 0.001 (* represents HFD groups compared with the Control group). #P < 0.05, ##P < 0.01, ###P < 0.001 (# represents MPD and Simvastatin treatments compared with the HFD group). HFD: high-fat diet; MDP: methyl protodioscin.

long history, Traditional Chinese Medicine plays a unique role in the treatment of ASCVD. It exhibits outstanding regulatory effects on hyperlipidemia, with minimal toxicity, side effects, and a hepatoprotective effect [21]. MPD is an important steroidal saponin extracted from the dioscorea nipponica, which is one of the main components of traditional Chinese patent medicines [22]. It can be used to synthesize steroid hormones and contraceptives and has anti-inflammatory and hypolipidemic functions [14,15]. LDL-C, TC, and TG are the main risk factors of ASCVD and are also the leading indicators for the clinical evaluation of the efficacy of lipid-lowering drugs. A previous study showed MPD could potentially increase HDL cholesterol while reducing LDL cholesterol and triglycerides *in vitro*, which indicated that MPD may simultaneously reduce three major lipid risk factors [16]. However, the lipid-lowering effect (*in vivo*) and the underlying effect remain unclear.

The findings of this study reveal that MPD is as effective as simvastatin in significantly reducing serum levels of TC and LDL-C. Furthermore, serum TG level also decreased by administration of MPD, and the TG level was lowered to close to the level of gerbils in the control group. These results implied that MPD could reduce TC, TG, and LDL-C simultaneously, and its efficacy is better than chemically synthesized lipid-lowering drugs in clinical practice. After six weeks of the high-fat diet, the serum HDL-C level was observed to be lower compared to the control group., However, a low concentration of MPD significantly increased the serum HDL-C level. It is suggested that MPD can potentially promote cholesterol efflux, which can be validated through enhanced expression of ABCA1 and cholesterol efflux in ox-LDL-treated THP-1 and HepG2 cells [16].

The liver performs a crucial role in both lipid and drug metabolism. Most lipid-lowering drugs can potentially harm liver function, raising serum AST and ALT [23]. In this study, treatment with MPD for six weeks significantly reduced the relative weight of livers and the liver enlargement in gerbils fed with HFD. H&E staining and Masson three-color staining showed that various fat vacuoles and fibrosis were observed in the liver of HFD-fed gerbils. However, MPD treatment reduced the hepatic TG and TC levels, density of fat vacuoles, and the degree of fibrosis in the liver, suggesting that MPD could alleviate hepatic steatosis. Furthermore, MPD treatment significantly decreased the serum AST and ALT levels, which suggested the decreased level of hepatocellular injury induced by virus, steatosis, alcohol abuse, and drugs [24]. All these results implied that MPD could reduce lipid accumulation and improve the liver's non-alcoholic steatosis while protecting the liver against the injury induced by HFD.

SREBP2 plays a significant role in cholesterol metabolism as it binds to sterol regulatory elements on HMGCR to facilitate crucial cholesterol synthesis [25]. In this study, MPD treatment significantly decreased hepatic SREBP2 and HMGCR expression. Considering the lower TC levels in both serum and liver, it may be speculated that MPD inhibits cholesterol *de novo* synthesis by reducing the expression of HMGCR and SREBP2. The clearance of LDL-C in serum is mainly through binding to LDLR on the surface of hepatocytes. MPD treatment significantly increased hepatic LDLR expression, which leads to decreased serum LDL-C levels. For the mechanism, MPD decreases PCSK9 expression in the liver, increasing hepatic LDLR levels and promoting LDL-C metabolism.

SREBP1 mainly regulates fatty acid synthesis in hepatocytes, and its primary target genes are ACC and FASN. This study demonstrated that MPD significantly decreased hepatic expression of SREBP1, ACC, and FASN, which suggested that MPD reduces TG levels in the liver and serum by inhibiting SREBP1 expression, reducing ACC and FASN expression.

As a nuclear receptor, the liver X receptor (LXR) plays a crucial role in liver lipid metabolism by regulating the expression of SREBPs [26]. This study found that MPD treatment decreased the expression of LXR. Therefore, the lipid-lowering effect of MPD is primarily achieved by inhibiting the expression of LXR and reducing the function of SREBP1 and SREBP2.

In summary, this study demonstrates that MPD has a protective effect on gerbils against lipid disorders and liver injury by reducing hepatic expression of LXR, SREBP1, and SREBP2, then decreasing their downstream gene targets that control *de novo* synthesis of TG and TC, clearance of LDL-C, and elevation of HDL-C. It is suggested that LXR may be the potential target of MPD. However, our experiment was conducted only on HFD-fed gerbils, and further experiments are needed to clarify the mechanism of action of MPD in more animal models, such as LXR knockout or overexpression mice. The present study proved the lipid-lowering effect of MPD for managing and mitigating dyslipidemia and NAFLD, providing a fundamental experimental and theoretical basis for the clinical use of MPD.

Data availability statement

Data will be made available on request.

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CRediT authorship contribution statement

Xiaojia Chen: Project administration. **Pengfei Zhang:** Writing – original draft. **Weilie Ma:** Methodology. **Haiqiang Pan:** Methodology. **Weitao Hong:** Investigation. **Gengji Chen:** Methodology. **Hang Ding:** Validation. **Wanze Tang:** Validation. **Guorong Lin:** Writing – review & editing. **Zhizhen Zhang:** Writing – review & editing.

Declaration of competing interest

The authors declare that the research was conducted without commercial or financial relationships construed as a potential conflict of interest.

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