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Original Article

Protective effects of Shenlingbaizhu powder against non-alcoholic fatty liver disease in rats through activation of SIRT3/AMPK/ACC signaling pathways

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Abstract: The aim of this study was to investigate the effects of Shenlingbaizhu Powder (SL) on silent mating type information regulation 2 homolog 3 (SIRT3)/adenosine 5'-monophosphate-activated protein kinase (AMPK)/acetyl-coenzyme A carboxylase (ACC) signaling pathways in non-alcoholic fatty liver disease (NAFLD) rats. Thirty-two Sprague-Dawley (SD) male rats were randomly divided into four groups, including the normal control (NC) group, high-fat diet (HFD) group, low-dose Shenlingbaizhu powder (LSL) group (ig, 10.0 g/kg/d), and high-dose Shenlingbaizhu powder (HSL) group (ig, 30.0 g/kg/d). There were 8 rats in each group. The rat model of NAFLD was induced by feeding with a high-fat diet for 16 weeks. Rats in the LSL and HSL groups were simultaneously fed SL at low and high doses, respectively. Sixteen weeks later, the rats were sacrificed. Blood and liver samples were then collected. Liver pathology changes were inspected using hematoxylin and eosin (H&E), Oil red O staining, and transmission electron microscopy. Next, serum biochemical indices and parameters of oxidative stress were measured. Protein expression of SIRT3/AMPK/ACC signaling pathways was detected via Western blotting. Results showed that SL supplementation significantly decreased hepatic steatosis, lipid peroxidation, and lipid profiles in rats fed with HFD, especially in the HSL group. Furthermore, SL supplementation induced an increase in SIRT3, p-AMPK, and p-ACC expression, compared with the HFD group. In conclusion, these experiments indicate that SL significantly improved lipid metabolisms and oxidative stress levels of livers in NAFLD rats, possibly related to activation of hepatic SIRT3/AMPK/ACC pathways.

Keywords: Shenlingbaizhu powder, non-alcoholic fatty liver disease, SIRT3/AMPK/ACC signaling pathway, lipid metabolism

Introduction

Non-alcoholic fatty liver disease (NAFLD) is defined by no significant alcohol consumption, but evidence of hepatic steatosis, either by imaging or by histology [1]. NAFLD is the most common liver disease in the world, with a prevalence of 25.24%. The highest prevalence is in the Middle East and South America, while the lowest is in Africa. Furthermore, incidence of NAFLD has grown at alarming rates in children and adolescents [2, 3]. NAFLD, ranging from simple steatosis to inflammation with associated fibrosis to cirrhosis, and non-alcoholic steatohepatitis (NASH) have been predicted to become the primary causes of liver-related morbidity and mortality in Western countries in the coming decade [4, 5]. More importantly, an increasing amount of clinical and epidemiological studies have suggested that NAFLD is related to an increased danger of developing both cardiovascular disease and type 2 diabetes [6].

Although knowledge concerning the pathogenesis of NAFLD is far from complete, the "two-hit" theory is generally accepted. The "two-hit" theory suggests that the "first hit" is hepatic lipid accumulation caused by insulin resistance, a high-fat diet (HFD) and obesity. The "second hit" is oxidative stress and fibrosis caused by the "first hit" [7]. Currently, lipid metabolism research is an emphasis in NAFLD. Excessive uptake of fatty acids, raised hepatic *de novo* lipogenesis, suppression of fatty acid oxidation, and secretion of very low-density lipoproteins (VLDL) causes hepatic lipid metabolism disor-

Table 1. Composition of SL

Chinese Name	Family Name	Latin Name	Ratio
Renshen	Panax L	Ginseng Radix et Rhizoma	5
Fuling	Poria cocos	Poria	5
Baizhu	Compositae	Atractylodis Macrocephalae Rhizoma	5
Shanyao	Dioscoreaceae	Dioscorea Rhizoma	5
Baibiandou	Leguminosae	Lablab Semen Album	4
Lianzi	Nelumbo nucifera Gaertn	Nelumbinis Semen	3
Zhigancao	Leguminosae	Glycyrrhizae Radix et Rhizoma	3
Yiyiren	Gramineae	Coicis Semen	3
Jiegeng	Campanulaceae	Platycodonis Radix	2
Sharen	Zingibrceae	Amomi Fructus	2

ders, turning fatty acid synthesis into triglycerides (TGs) in hepatocytes, thereby leading to the development of steatosis [8]. Therefore, exploration of the mechanisms of hepatic lipid metabolism disorders in NAFLD is significant in the prevention and treatment of NAFLD.

Silent mating type information regulation 2 homolog 3 (SIRT3) is a member of the sirtuin family of protein deacetylases. Most of these proteins exist in the mitochondria and participate in cell energy metabolism balance regulation [9]. Research results of Kendrick [10] and Hirschey [11] showed that SIRT3 regulates hepatic lipid metabolism. SIRT3 regulates adenosine 5'-monophosphate-activated protein kinase (AMPK), another metabolism of regulating the synthesis of adenosine triphosphate (ATP). AMPK is a cell energy receptor widely found in various types of eukaryotic cells. Activation of AMPK improves hepatic lipid metabolism. Mechanisms may be related to inhibiting liver fat synthesis and enhancing the effects of fatty acid oxidation [12]. Acetyl-coenzyme A carboxylase (ACC) is a rate-limiting enzyme for the synthesis of fatty acids. It promotes acetyl-coenzyme A transformation into malonyl-coenzyme A [13]. Carnitine palmitoyl transferase 1 (CPT-1) is the rate-limiting enzyme responsible for mitochondrial oxidation. It takes charge of the transportation of long-chain acyl-coenzyme A in the oxidation of the mitochondria. Its activity is subjected to negative control of malonyl-coenzyme A [14]. In summary, SIRT3 activates the phosphorylation of AMPK and expression of downstream proteins, affecting regulation of lipid metabolism disorders [15].

At present, there are no approved pharmacological therapies for NAFLD [16]. Lifestyle modifications, through diet and physical activity, to treat NAFLD, are generally recommended [17]. However, NAFLD sufferers often find these hard to maintain. For thousands of years, Traditional Chinese Medicines have been used to prevent human diseases. Currently, many long-term studies about herbal extracts or organic products have found that herbal products have potent effects against NAFLD [18]. Furthermore, lots of Traditional Chinese herbal formulas have been reported to have obvious effects against NAFLD [19], such as Gegenqinlian decoction [20] and Baihujiarenshen decoction [21]. The present research team previously reported that SL had beneficial effects on attenuating hepatic steatosis and lipid metabolism [22]. However, the exact mechanisms of SL against NAFLD are not clear. Hence, the current study investigated whether SL regulates lipid metabolism disorders through SIRT3/AMPK/ACC pathways, aiming to provide new evidence for its effects against NAFLD.

Materials and methods

Preparation of SL

SL consists of the ten dried crude herbs listed in **Table 1**. All herbs were provided by the Guangdong Province Engineering Technology Research Institute of Traditional Chinese Medicine, China. Formula granules were dissolved in distilled water and stored at 4°C in a refrigerator.

Quantification of SL

For quality control (**Figure 1**), representative active components, including Ginsenosides, Glycyrrhizinate, Atractylodes lactone III, Atra-

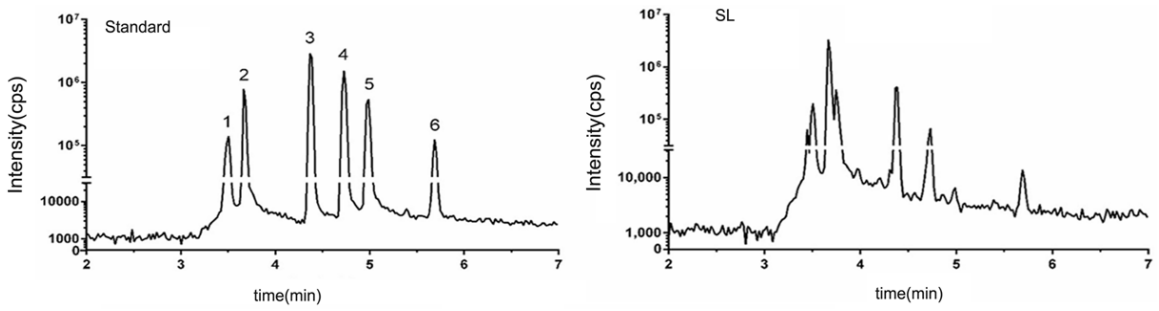

Figure 1. HPLC-MS chromatograms of SL. Peaks 1: Ginsenosides Rc; 2: Glycyrrhizinate; 3: Atractylodes lactone III; 4: Atractylodes lactone II; 5: Atractylodes lactone I; 6: Poria acid.

ctylodes lactone II, Atractylodes lactone I, and Poria acid, were submitted for compositional analysis using high-performance liquid chromatography-mass spectrometry (HPLC-MS) methods, as recommended by Chinese Pharmacopoeia (Chinese Pharmacopoeia Commission, 2015). The standard products (Ginsenosides Rc; Glycyrrhizinate; Atractylodes lactone I, II, and III; Poria acid) were dissolved in anhydrous methanol to prepare a 50 μg/L standard solution. Researchers then weighed 10 g of SL granules dissolved in 1 L of anhydrous formaldehyde, after filtration with a 0.25 μm microporous membrane. This was then diluted 200 times with anhydrous formaldehyde, creating the 200 μL sample solution. The sample solution and standard solution were separated using the Poroshell 120 EC-C_{18} column (Agilent Technologies, USA) (mobile phase: phase A 0.5% formic acid-water; phase B acetonitrile; gradient elution conditions: 90% A (0 min), 0% A (1.5 min), 0% A (10 min), 90% A (10.1 min), 90% A (15 min); flow rate 500 μL/min; column temperature: 20°C; injection volume: 5 μL). A liquid chromatography mass spectrometer (Agilent Technologies, USA) was used as well. Samples were analyzed using multistage reaction monitoring scanning and positive and negative ion detection modes.

Animals

Thirty-two male Sprague-Dawley (SD) rats (6 or 7 weeks old, 200 ± 20 g) were obtained from the Laboratory Animal Research Center of Guangzhou University of Traditional Chinese Medicine (Animal License no. SCXK (Yue) 2013-0034, China). The rats were separately housed in a temperature-controlled room (24 ± 2°C on a 12 h: 12-hour light-dark cycle) in the Animal Administration Laboratory, Jinan University, China. All experiments were approved by and performed according to guidelines of the Jinan University Animal Experiment Ethics Committee. The experimental process was strictly in accordance with experimental animal ethical requirements.

Grouping and modeling

After acclimatization for one week, 32 male SD rats were randomly divided into four groups, with eight rats in each group. Groups included the normal control (NC) group, HFD group, high-dose Shenlingbaizhu powder (HSL) group, and low-dose Shenlingbaizhu powder (LSL) group. The NC group received a standard diet, while HFD and HSL groups received a high-fat diet (HFD diet, which is 88% regular chow plus 1.5% cholesterol, 10% lard, and 0.5% bile salt). LSL and HSL groups were dosed with SL, orally, at a dose of 10 g/kg/d or 30 g/kg/d. NC and the HFD groups were dosed with an equal volume of distilled water. Body weights were assessed once per week. Sixteen weeks later, the rats were sacrificed after fasting overnight. Abdominal aorta blood and liver samples were then collected for assay.

Histopathological examination

Liver samples were taken from the right lobe edge of rat liver tissues and placed in neutral-buffered formalin. After embedding in paraffin, the samples were sliced into 4 μm sections. The sections were stained with hematoxylin for 5 minutes and eosin solution for 2 minutes. After being cleared by xylene, the sections were mounted by neutral resin. The frozen liver tissue (10 mm thick) was fixed with OCT fixative for 15 minutes and sliced into 10 μm slices. After staining with Oil red O (Jiancheng, Bioen-

Table 2. Body weight, liver weight, and liver index of rats (mean ± SD, $n = 8$)

Groups	Body weight (g)		Liver weight (g)	Liver Index
	Initial	Final		
NC	208.4 ± 15.6	485.8 ± 69.2*	13.4 ± 2.7**	0.027 ± 0.003**
HFD	212.4 ± 15.2	583.5 ± 26.3	23.8 ± 2.6	0.041 ± 0.006
LSL	217.0 ± 20.2	504.9 ± 24.4**	20.1 ± 2.7	0.040 ± 0.006
HSL	211.5 ± 19.3	500.1 ± 25.4**	18.2 ± 3.7**	0.037 ± 0.008

*$P < 0.05$, **$P < 0.01$ versus the HFD group.

gineering Institute, Nanjing, China) for 10 minutes and hematoxylin for 2 minutes, the sections were mounted with water-soluble sealant. All sections were observed under a light microscope (Leica Microsystems, Germany).

Transmission electron microscopy in liver tissues

Liver sections, with a size less than 1 mm^3, were fixed in 2.5% glutaraldehyde in phosphate buffer at 4°C. The tissues were then fixed in 1% osmium tetroxide for 1 hour, dehydrated, and infiltrated with ethanol and acetone. Next, the tissues were sliced into 50-60 nm sections, after embedding with pure acetone and embedding medium. Sections were then observed under a transmission electron microscope (TECNAI-10, Philips, Netherlands).

Serum biochemical parameter analysis

After abdominal aorta blood collection, serum was separated by centrifugation at 2355 × g for 10 minutes at 4°C. Total cholesterol (TC), triglycerides (TG), alanine aminotransferase (ALT), aspartate aminotransferase (AST), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) were determined by an automatic biochemical analyzer (Hitachi, Japan).

Detection of oxidative stress

Activities of superoxide dismutase (SOD) and malondialdehyde (MDA), as well as the content of glutathione peroxidase (GSH-Px), were determined, in accordance with manufacturer instructions (Jiancheng, Bioengineering Institute, Nanjing, China).

Western blotting analysis

SIRT3, AMPK, p-AMPK, ACC, p-ACC, and CPT-1A proteins in the liver tissues were measured via Western blotting. HRP-conjugated GAPDH (1:10,000; cat. no. KC-5G5, KangChen Biotech Co., Ltd., Shanghai, China) was used as an internal control. Liver tissues, treated as described above, were collected and lysed in radio immunoprecipitation assay lysis buffer (cat. no. P0013, Beyotime Biotech Co., Ltd., Shanghai, China) on ice for 30 minutes. Protein was extracted by lysis buffer and transferred into an EP tube, then centrifuged at 13,000 × g for 10 minutes at 4°C. The supernatant was immediately transferred into an EP tube and marked as the total protein sample. Protein concentrations were determined using a BCA protein content test kit (cat. no. KGPBCA; KeyGEN BioTECH Co., Ltd., Nanjing, China). Liver denatured protein liquid was separated by 10% SDS gel electrophoresis (SDS-PAGE). The Immobilon-P Transfer (PVDF) membrane (0.45 μm; cat. no. IPVH-00010; Millipore Corporation, USA) was blocked with 5% skim milk (Whiga Technology Co., Ltd., Guangzhou, China) in Tris-Buffered Saline Tween-20 (TBST) at room temperature for 1 hour. Specific primary antibodies were then added, followed by incubation at 4°C overnight. SIRT3 antibody (1:1,000; cat. no. 2627), AMPK antibody (1:1,000; cat. no. 2532), p-AMPK antibody (1:1,000; cat. no. 2535), ACC antibody (1:1,000; cat. no. 3676), and p-ACC antibody (1:1,000; cat. no. 3661) were purchased from Cell Signaling Technology (USA), while the CPT-1A antibody (1:1,000; cat. no. 176320) was purchased from Abcam (UK). After PVDF membranes were washed for ten minutes, for a total of three times in TBST, HRP-conjugated Goat Anti-Rabbit IgG antibody (1:20,000; cat. no. 4050-05; Southern Biotech, USA) was added. It was incubated at 37°C for 1 hour. PVDF membranes were washed again, placed into the developer, and exposed to X-ray film (12.7 × 17.8 cm; cat. no. XBT-1; Kodak, USA). The gel image processing system was used for analysis of films.

Statistical analysis

SPSS 20.0 Software (International Business Machines, Corp., Armonk, NY, USA) was used to analyze all data. Measurement data are expressed as mean ± standard deviation. GraphPad Prism 6.0 (GraphPad Software, Inc., CA, USA) was used for drawing. One-way analysis of vari-

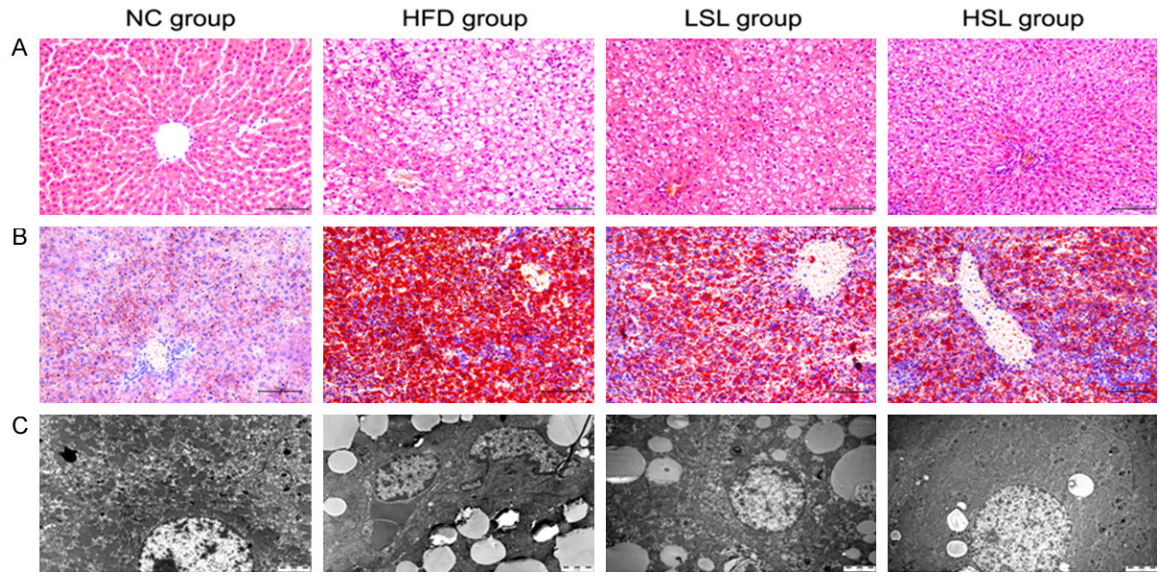

Figure 2. Hepatic steatosis in the NC, HFD, LSL, and HSL groups: A. H&E staining. Magnification, × 200. B. Oil red O staining. Magnification, × 200. C. Transmission electron microscopy. Magnification, × 6200.

ance (ANOVA) was used to determine the statistical significance of differences. When $P < 0.05$, results are considered significantly different.

Results

Body weight, liver weight, and liver indexes of rats

As shown in **Table 2**, after 16 weeks, body weight, liver weight, and liver indexes of rats in the HFD group were higher than in the NC group ($P < 0.05$ or $P < 0.01$). HSL and LSL groups had a lower body weight than the HFD group ($P < 0.01$), while the HSL group had a lower liver weight than the HFD group ($P < 0.01$). However, liver indexes in LSL and HSL groups showed no obvious differences from the HFD group ($P > 0.05$). Present results show that HFD increased body weights and liver weights in rats and SL decreased body weights and liver weights in NAFLD rats.

Observation of the morphology of liver tissues in NAFLD rats

As shown in **Figure 2A**, for the NC group, the hepatocyte had a clear structure. The cell nucleus was found in the center of the cell. The liver rope to the central vein axis was arranged radically. The hepatic lobule's structure was clear and there was a small number of lipid droplets. For the HFD group, different sizes of lipid droplets accumulated in the hepatocyte. Some of them fused into a big vacuole. The nuclei were close to the edge. Liver cells were swelled and some of them were ballooned. The hepatic lobule and the liver rope's structure were not clear. Also, part of the portal tract had an inflammatory infiltrate. Compared with the HFD group, the liver rope and hepatocyte form improved, while the number of lipid droplets decreased significantly in the two SL therapy groups. The HSL group showed a significant impact on liver histopathology. Oil red O staining (**Figure 2B**) showed that few obvious orange lipid droplets were seen in the NC group. For the HFD group, many orange lipid droplets existed in the hepatic cells. Part of the lipid droplets fused into one piece, while most of the cell nuclei were pushed toward the edge of the cell. The hepatocyte had a disorganized structure. Lipid droplets decreased in HSL and LSL groups, especially in the HSL group. Results indicate that SL provided protection against HFD-induced morphological damage of liver tissues in NAFLD rats.

Ultrastructure of liver tissues in NAFLD rats

As shown in **Figure 2C**, for the NC group, a normal hepatic parenchyma was observed. The

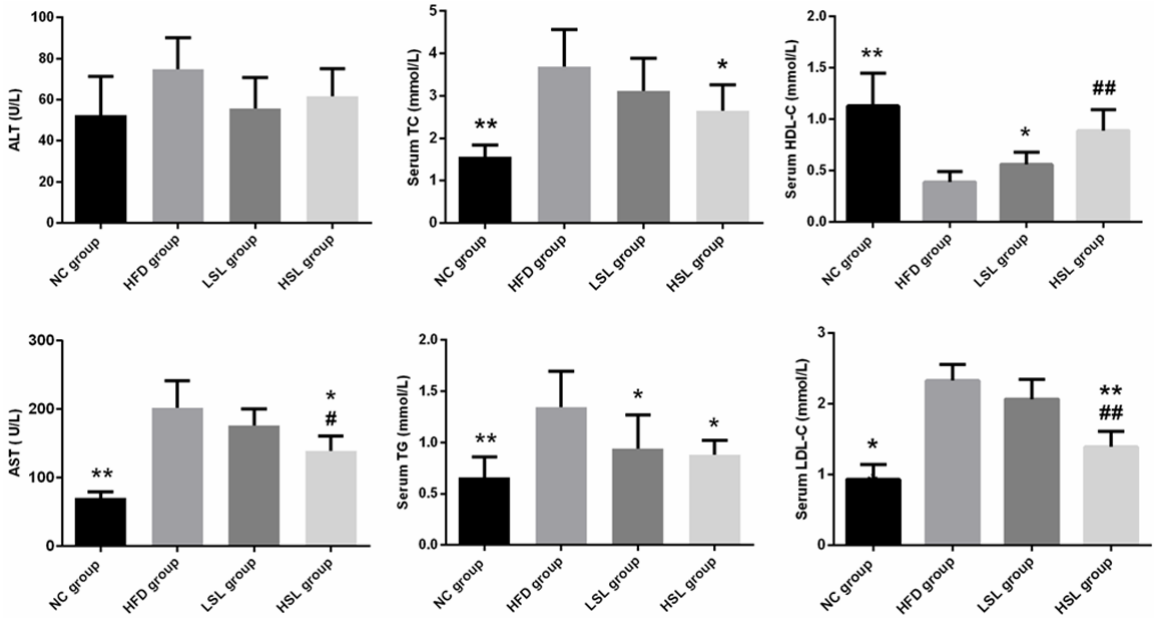

Figure 3. Levels of the abdominal aorta serum ALT, AST, TC, TG, HDL-C, and LDL-C in the NC, HFD, LSL, and HSL groups. $^{*}P < 0.05$, $^{**}P < 0.01$ versus the HFD group. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, LSL group vs HSL group.

round nucleus of the hepatocyte was surrounded by a large number of mitochondria, with cristae that were well organized. For the HFD group, the ultrastructure of the hepatocyte was damaged, as the nucleus showed an irregular shape. The mitochondria were rare and swollen, showing disarranged cristae. In addition, many lipid droplets appeared in the HFD group, implying that lipid metabolism declined. HSL and LSL groups had round nuclei and a larger number of mitochondria. Lipid droplet accumulation was mitigated, compared with the HFD group, especially in the HSL group. Results suggest that SL improved the ultrastructural changes of liver tissues in NAFLD rats.

Levels of AST and ALT in the serum and the effects of SL

As shown in **Figure 3**, serum AST levels significantly increased in the HFD group, compared to the NC group ($P < 0.01$), while serum ALT levels showed no significant changes. Serum AST and ALT levels in HSL and LSL groups were reduced, to different degrees, compared with the HFD group. Serum AST levels in the HSL group decreased significantly ($P < 0.05$). Serum AST levels in the HSL group decreased significantly, compared to the LSL group ($P < 0.05$). Results suggest that SL reduced serum AST levels in NAFLD rats, while the roles of SL present a certain dose-effect relationship.

Levels of TC, TG, HDL-C, and LDL-C in liver tissues and the effects of SL

As shown in **Figure 3**, compared with the NC group, TC, TG, and LDL-C values in the abdominal aorta serum of the HFD group increased, while HDL-C values significantly decreased ($P < 0.01$). TC, TG, and LDL-C values in HSL and LSL groups were reduced, to different degrees, compared with the HFD group. HDL-C values increased, to different degrees, especially for TC, TG, HDL-C and LDL-C values in the HSL group, with significant differences ($P < 0.05$, $P < 0.01$). TG values of the LSL group were lower than those in the HFD group ($P < 0.05$), while TC, HDL-C, and LDL-C values showed no obvious changes. Compared with the HSL group, LDL-C values in the LSL group increased and HDL-C in the LSL group decreased, with significant differences shown ($P < 0.01$). TG and TC values did not show significant differences. Results suggest that SL improved lipid metabolism in NAFLD rats, while the roles of SL present a certain dose-effect relationship.

Parameters of oxidative stress in NAFLD rats

As shown at **Table 3**, activities of SOD and GSH-Px in the HFD group were lower than the NC group ($P < 0.01$). In contrast, MDA levels in the HFD group were increased significantly, com-

Table 3. SOD, MDA, and GSH-Px of rats (mean ± SD, *n* = 8)

Groups	n	SOD (U/mg)	MDA (nmol/mg)	GSH-Px (U/mg)
NC	8	118.12 ± 18.27**	10.60 ± 1.70**	26.72 ± 2.84**
HFD	8	79.39 ± 7.77	40.11 ± 10.39	15.87 ± 3.03
LSL	8	92.01 ± 5.41*	28.79 ± 3.41	17.85 ± 3.95
HSL	8	105.92 ± 9.90**	18.21 ± 3.13**	21.44 ± 2.71**

*$P < 0.05$, **$P < 0.01$ versus the HFD group.

pared with the NC group. Activities of SOD in the LSL and HSL group increased, to different degrees, with significant differences ($P < 0.05$, $P < 0.01$). MDA levels in the HSL group were lower than in the HFD group ($P < 0.01$). The activity of GSH-Px in the HSL group was higher than in the HFD group ($P < 0.01$). Results suggest that SL improved oxidative stress in NAFLD rats.

Hepatic SIRT3/AMPK/ACC protein pathway expression in NAFLD rats

As shown in **Figure 4A** and **4B**, values of SIRT3, p-AMPK, and CPT-1A protein in the HFD group were lower than in the NC group ($P < 0.01$). Compared with the HFD group, not only did SIRT3, p-AMPK, and p-ACC protein levels in the LSL group increase ($P < 0.05$ or $P < 0.01$), but SIRT3 and p-AMPK protein levels also increased in the HSL group ($P < 0.05$ or $P < 0.01$). There were no significant differences in levels of AMPK and ACC protein in each group ($P > 0.05$). Results suggest that SL activated SIRT3/AMPK/ACC signaling pathways in NAFLD rats.

Discussion

The current study used HFD for 16 weeks to replicate an NAFLD model in rats, aiming to evaluate the protective effects of SL on NAFLD. For rats in the HFD groups, serum TG, TC, LDL-C, and AST levels were higher than the NC group, while HDL-C levels in the HFD group were lower, compared to the NC group. Currently, liver biopsy is the gold standard for diagnosis and prognosis of NAFLD [23]. The current study observed that model rat liver tissues diffused into steatosis from H&E staining. Many lipid droplets were fused into one piece, based on the Oil red O staining. In addition, the hepatic ultrastructure was damaged, with marked steatosis and reduced mitochondria, causing hepatocyte nuclear modifications. Results indicate that the NAFLD model was replicated successfully by HFD.

A previous study found that SL significantly improved serum and liver lipids in NAFLD rats [24]. Using these NAFLD model rats, the current study demonstrated that SL decreased levels of TC, TG, LDL-Cm and AST, suggesting that SL had certain effects on improving liver lipid metabolism disorders and reducing liver damage. Levels of ALT in HSL and LSL groups also had the same trend as AST but did not show significant differences. This might be because liver damage was not serious, though the liver had steatosis. Histopathological analysis showed that SL decreased the number of lipid droplets. The HSL group has less lipid droplets, compared with LSL and HFD groups. In addition, according to transmission electron microscopy results, SL made a significant improvement on the hepatic ultrastructure. Results indicate that SL improved liver steatosis and accumulation of fat. Specifically, the HSL group showed better effects, indicating a dose-dependent relationship between SL and anti-NAFLD.

SOD and GSH-Px are anti-oxidants that control the formation of ROS and repair oxidative damage to hepatic cells. MDA is an important detection index of cellular oxidative damage and lipid peroxidation [25]. In this study, activities of SOD and GSH-Px increased and levels of MDA decreased in both HSL and LSL groups. Results indicate that SL improved lipid peroxidation, thereby reducing hepatic cell damage and even cell death.

Many studies have indicated that SIRT3 participates in liver lipid metabolism and influences the development and progression of NAFLD [26, 27]. There was an absence of SIRT3 in liver results in the reduction in fatty acid oxidation observed in mice. This situation is alleviated by exogenous SIRT3 overexpression [11]. Moreover, the inhibition of fatty acid oxidation increases hepatic lipid accumulation, which may cause development of NAFLD [28]. In the present study, TG and TC values decreased, while expression of SIRT3 increased significantly in HSL and LSL groups, compared with the HFD group. Therefore, it was hypothesized that upregulation of SIRT3 expression decreased hepatic lipid accumulation by enhancing fatty acid oxidation, thus preventing development of NAFLD.

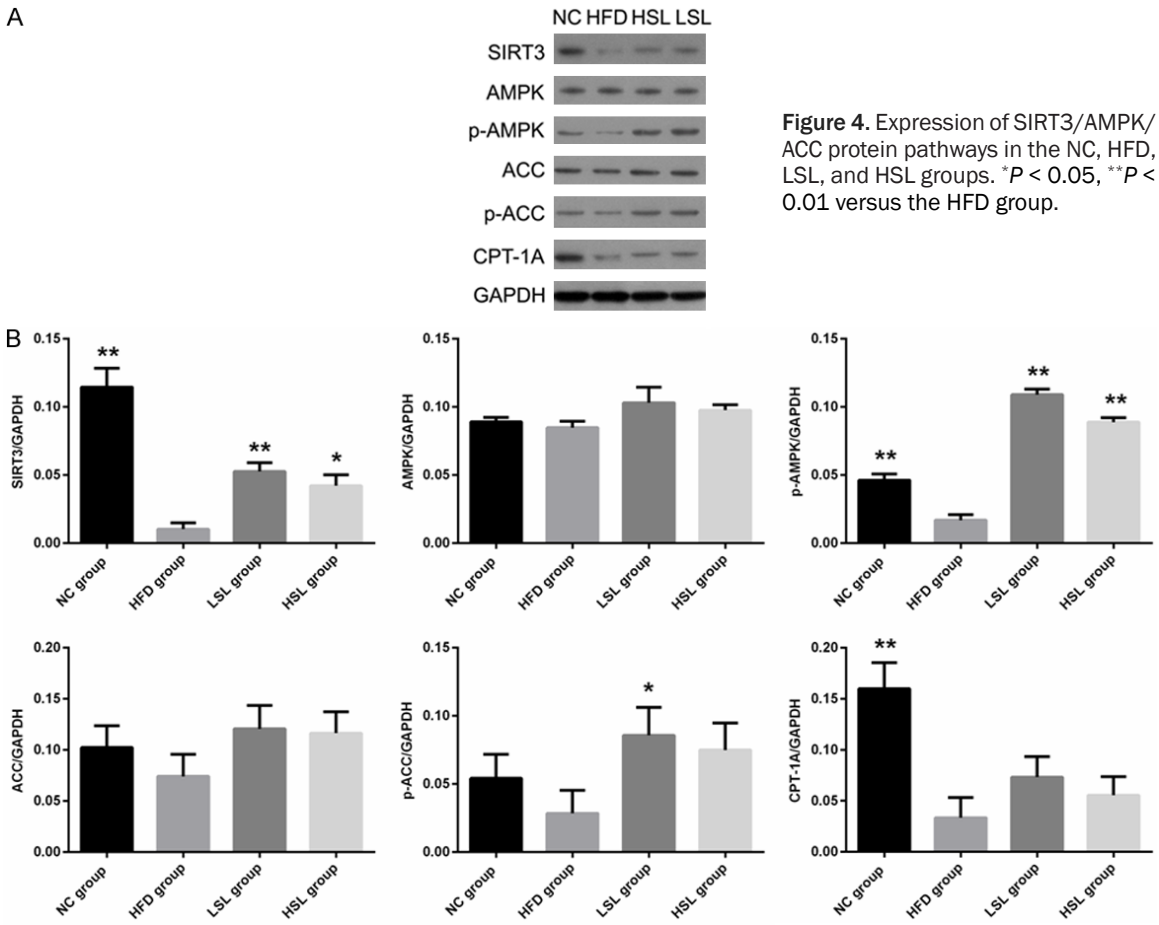

Figure 4. Expression of SIRT3/AMPK/ACC protein pathways in the NC, HFD, LSL, and HSL groups. $^{*}P < 0.05$, $^{**}P < 0.01$ versus the HFD group.

Several studies have proven that SIRT3 might be involved in lipid metabolism by activating AMPK/ACC pathways [11, 26]. Tong's study [29] demonstrated that SIRT3 overexpression inhibited lipid synthesis through AMPK-dependent phosphorylation and inhibition of ACC. Upregulating AMPK pathways reduces hepatic lipogenesis and improves fatty-acid oxidation, thereby reducing hepatic lipid accumulation [30, 31]. ACC is one of the first enzymes shown to be an AMPK target. Activation of AMPK inhibits its synthesis of ACC and lowers the production of malonyl-coenzyme A. Malonyl-coenzyme A, a regulator of fatty acid synthesis, is an allosteric inhibitor of CPT-1. Furthermore, it promotes the synthesis of fatty acids and inhibits β-oxidation of fatty acids [32]. In addition, activation of CPT-1 protects NAFLD development. In clinic, serum markers of liver damage (AST, ALT, bilirubin, and mtDNA) in treated NAFLD patients are decreased by CPT-1 activation [33]. Therefore, activation of AMPK diminishes the synthesis of ACC by phosphorylation, then activates CPT-1 by reducing malonyl-coenzyme A. This alleviates hepatic lipid accumulation by inhibiting the synthesis of lipids and enhancing the oxidation of fatty acids. Hence, the current study assessed expression of AMPK and related downstream proteins in the livers of rats, aiming to explore whether improvements in NAFLD, caused by SL, were associated with activation of AMPK. The present study found that expression of p-AMPK, p-ACC, and CPT-1A in livers of the HFD group decreased obviously, compared with the NC group, and expression of p-AMPK, p-ACC, and CPT-1A in the livers increased distinctly. Furthermore, hepatic lipid accumulation was reduced by intervention with high-doses and low-doses of SL. As a result, it was hypothesized that another mechanism of the improvement of hepatic lipid accumulation, caused by SL, might be because ACC phosphorylation is caused by activation of AMPK phosphorylation, thereby upregulating expression of CPT-1 to improve liver lipid metabolism disorders. Thus, results suggest that SL may improve liver lipid

metabolism disorder in NAFLD rats by upregulating expression of p-AMPK, p-ACC and CPT-1A by SIRT3 upregulation.

To summarize, the current study showed that activation of SIRT3/AMPK/ACC signaling pathways might be one of the mechanisms for improvement of hepatic lipid accumulation and anti-NAFLD by SL.

Acknowledgements

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Disclosure of conflict of interest

None.

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