Hindawi
Journal of Food Biochemistry
Volume 2023, Article ID 7473668, 12 pages
https://doi.org/10.1155/2023/7473668

Research Article

Sparassis latifolia Polysaccharide Attenuates Cholesterol in Rats Maintained on a High-Fat, High-Cholesterol Diet

Feier Cheng,[1] Yaru Yang,[1] Shaojun Yun,[1,2] Jinling Cao,[1,2] Mingchang Chang,[1,3] Yanfen Cheng,[1,2] and Cuiping Feng[1]

[1]*College of Food Science and Engineering, Shanxi Agricultural University, Minxian Street No. 1, Taigu 030801, Shanxi, China*
[2]*Shanxi Key Laboratory of Edible Fungi for Loess Plateau, Taigu 030801, Shanxi, China*
[3]*Shanxi Research Station for Engineering Technology of Edible Fungi, Minxian Street No. 1, Taigu 030801, Shanxi, China*

Correspondence should be addressed to Cuiping Feng; ndfcp@163.com

Received 10 February 2023; Revised 18 June 2023; Accepted 7 July 2023; Published 7 August 2023

Academic Editor: Akhilesh K. Verma

Sparassis latifolia polysaccharide (SLP) has broad application prospects as a raw material of functional food. This study aimed to investigate the effects of SLP on liver cholesterol metabolism and its mechanism. Sprague–Dawley rats were fed a high-fat, high-cholesterol diet, and various assessments were conducted including hematoxylin and eosin staining, measurement of liver and metabolic indices, and antioxidant analysis. In addition, fluorescence quantitative reverse transcription-polymerase chain reaction was performed to analyze mRNA expression, and gas chromatography-mass spectrometry was employed for screening differential metabolites. The results demonstrated that SLP reduced the abnormal increase in the liver index caused by high fat and cholesterol and improved liver tissue morphology, metabolism level, and antioxidant-related enzyme activities. The polysaccharide regulated the liver sterol metabolism pathway, decreased the mRNA expression levels of 3-hydroxy-3-methylglutaryl-CoA reductase (HMG-CoAR) and sterol O-acyltransferase 1 (SOAT1), upregulated the mRNA expression levels of cholesterol 7α hydroxylase (CYP7α-1) and ABCA1, and increased the contents of propionic acid and butyric acid in the liver. These findings indicated a novel pathway through which SLP prevented hepatic lipid deposition and ameliorated sterol metabolism via regulating the HMG-CoAR-CYP7α-1 axis.

1. Introduction

Cholesterol is an essential structural component of the cell membrane and a precursor for synthesizing bioactive molecules such as bile acids, fat-soluble vitamins, and sterols. When the cholesterol intake exceeds its biotransformation and excretion capacity, it can lead to abnormal accumulation of cholesterol in the body; high plasma cholesterol level is an essential cause of atherosclerosis. In addition, the disorder of cholesterol metabolism is closely related to the occurrence and development of fatty liver, diabetes, gallstones, and other diseases [1].

The liver is the core organ involved in human metabolism and maintains a steady state of sugar and lipid metabolism [1]. Dietary cholesterol is ingested by the liver as chylomicron residues via very-low-density lipoprotein (VLDL) receptors or low-density lipoprotein (LDL) receptors (LDLRs), while endogenous cholesterol is secreted by the liver as part of VLDL and returned to the liver as intermediate-density lipoprotein or LDL. Excessive synthesis, uptake, or outflow of cholesterol in liver cells not only results in abnormal accumulation of cholesterol in the liver but also leads to hypercholesterolemia and then induces atherosclerosis and other cardiovascular diseases, posing a serious threat to human health. Liver cholesterol metabolism is essential for the balance of cholesterol metabolism.

The uptake of liver cholesterol mainly depends on the endocytosis pathway mediated by the LDLR family and the selective uptake pathway mediated by scavenger receptor class BI (SR-BI) [2, 3]. Downregulated LDLR expression can cause lipid accumulation in the blood, leading to hypercholesterolemia [4, 5]. The decrease in the SR-BI expression level in the liver leads to impaired absorption of high-density lipoprotein (HDL) by the liver, resulting in the abnormal

accumulation of cholesterol esters in plasma [6]. In the liver, acetyl-coenzyme A (CoA) is used as a raw material and 3-hydroxy-3-methylglutaryl-CoA reductase (HMGCR) is used as the rate-limiting enzyme to catalyze the biotransformation of mehydroxyvalonate. This enzymatic reaction represents the rate-limiting step of cholesterol biosynthesis [7, 8]. The rate-limiting enzyme cholesterol 7α hydroxylase (CYP7α-1) plays a key role in cholesterol biotransformation in the liver. It is a cytochrome P450 enzyme located and expressed only in the endoplasmic reticulum of liver cells, catalyzing the biotransformation of free cholesterol into bile acids. Its expression and activity are regulated by the feedback of bile acids in the final product [9–11]. The export of cholesterol from the liver is necessary to maintain hepatic cholesterol homeostasis. ATP-binding cassette transporterA1 (ABCA1) and ATP-binding cassette G1 (ABCG1) are key proteins mediating liver cholesterol output [12–14].

The long-term intake of a high-fat, high-cholesterol diet can lead to disorders of human lipid metabolism, causing chronic metabolic diseases such as obesity and hyperlipidemia and hence damaging human health. Polysaccharides are considered good at preventing and controlling such diseases. Studies have found that natural polysaccharides can reduce triglyceride and cholesterol levels by regulating a series of signaling pathways [15]. Gu et al. [16] found that polygonatum polysaccharide relieved inflammation and prevented glucose and lipid metabolism disorders. Mingx et al. [17] showed that *Lycium barbarum* polysaccharides significantly inhibited lipid peroxidation, thus preventing the occurrence of cardiovascular and cerebrovascular diseases. Li et al. [18] found that *Agaricus blazei* acidic polysaccharide exerted a lipid-lowering effect via activating related cholesterol metabolism pathways. Several studies have demonstrated the extensive biological activities of the polysaccharides from edible fungi [15–18]. *Sparassis latifolia* is an edible and medicinal fungus. Its sub-body contains a variety of active substances, with polysaccharide content up to 39.3%–43.6% [19–21]. Studies showed the anti-inflammatory, antioxidant, antitumor, antiaging, immunity-improving, antibacterial, and other activities of *Sparassis latifolia* polysaccharide (SLP). This study aimed to investigate the cholesterol-lowering mechanism of *Sparassis latifolia* polysaccharide by giving certain doses to hypercholesterolemic rats.

2. Materials and Methods

2.1. Ethical Approval. All animal treatments were performed following the provisions and general recommendations of the Chinese Experimental Animal Administration Legislation. The protocol was approved by the College of Food Science and Engineering, Shanxi Agricultural University (SWAU-EAW-2018R0304001).

2.2. Animals. Six-week-old female and clean-grade Sprague–Dawley (SD) rats were purchased from Beijing Weitong Lihua Experimental Animal Technology Co., Ltd. (license number: SXAU-EAW-2018R0304001). After adaptive feeding for 1 week, 50 female SD rats were randomly allocated into 5 groups ($n = 10$/group): normal control (NC), hyperlipidemia model (HFCM), HFC + LD, HFC + MD, and HFC + HD groups. The rats in the latter three groups were administered 100, 200, and 400 mg/kg of SLP, respectively, by daily gavage. In contrast, all control-group rats (NC and HFCM) were administered with the same amount of 0.9% saline. The rats were allowed free access to feed and water. Picric acid was applied to different parts to distinguish rats. The rats were housed in groups of three in a cage under 12 h light/dark cycles at 22 ± 2°C. The rats in the NC group were fed an essential diet. In contrast, the rats in the other four groups were fed a high-fat, high-cholesterol supplemented formula (essential feed 88.8%, lard 10%, cholesterol 1% (Shanghai Aladdin Biotechnology Co., Ltd.), and bile salt 0.2% (Beijing Solarbio Technology Co., Ltd.)).

2.3. Materials. *Sparassis latifolia* was purchased from Shanxi Taihe Mushroom Cultivation Base, and SLP was prepared as previously described [22]. In brief, the fruiting body powder was added to distilled water in the ratio of 1 : 40 (w/v), mixed, soaked, and extracted at 75°C for 3 h. The aqueous extract was isolated by filtration and concentrated before precipitation with three volumes of absolute ethanol at 4°C for 12 h. The resulting precipitate was collected by centrifugation and washed sequentially with acetone and ether before using the savage complex enzyme method to remove proteins. After that, the mixture was further purified using macroporous resin (HZ-830) absorption chromatography, and the polysaccharide fraction was subjected to lyophilization, yielding dry SLP with a purity of 85.7% [22]. The polysaccharide structure was investigated according to the methods described by Zhengqi. The molecular weight of SLP was estimated to be 1.04×10^4 Da based on high-performance gel permeation chromatography analysis. SLP comprised galactose, glucose, and xylose in a molar ratio of 1 : 4 : 1; the main chain was β-(1⟶3)-D-glucan, and its branched chain was β-(1⟶6)-D-glucan with a branching frequency of approximately three (Figure 1(a)).

2.4. Rat Liver Index and Histology. The rats were sacrificed after 8 weeks of the prescribed diet and treatment. They were anesthetized using isoflurane (0.41 mL/min at 4 L/min fresh gas flow), ensuring every possible measure was taken to minimize their suffering. The livers were dissected and weighed to determine the liver index (liver weight (g)/body weight (g)). Part of the liver tissue was fixed with 10% formaldehyde, and the morphological changes in the liver tissue were examined using hematoxylin and eosin staining.

2.5. Enzyme-Linked Immunosorbent Assay. Total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), and total bile acid (TBA) levels were measured following the manufacturer's protocols (Nanjing Jiancheng Institute of Bioengineering). Superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), and catalase (CAT) activities and the

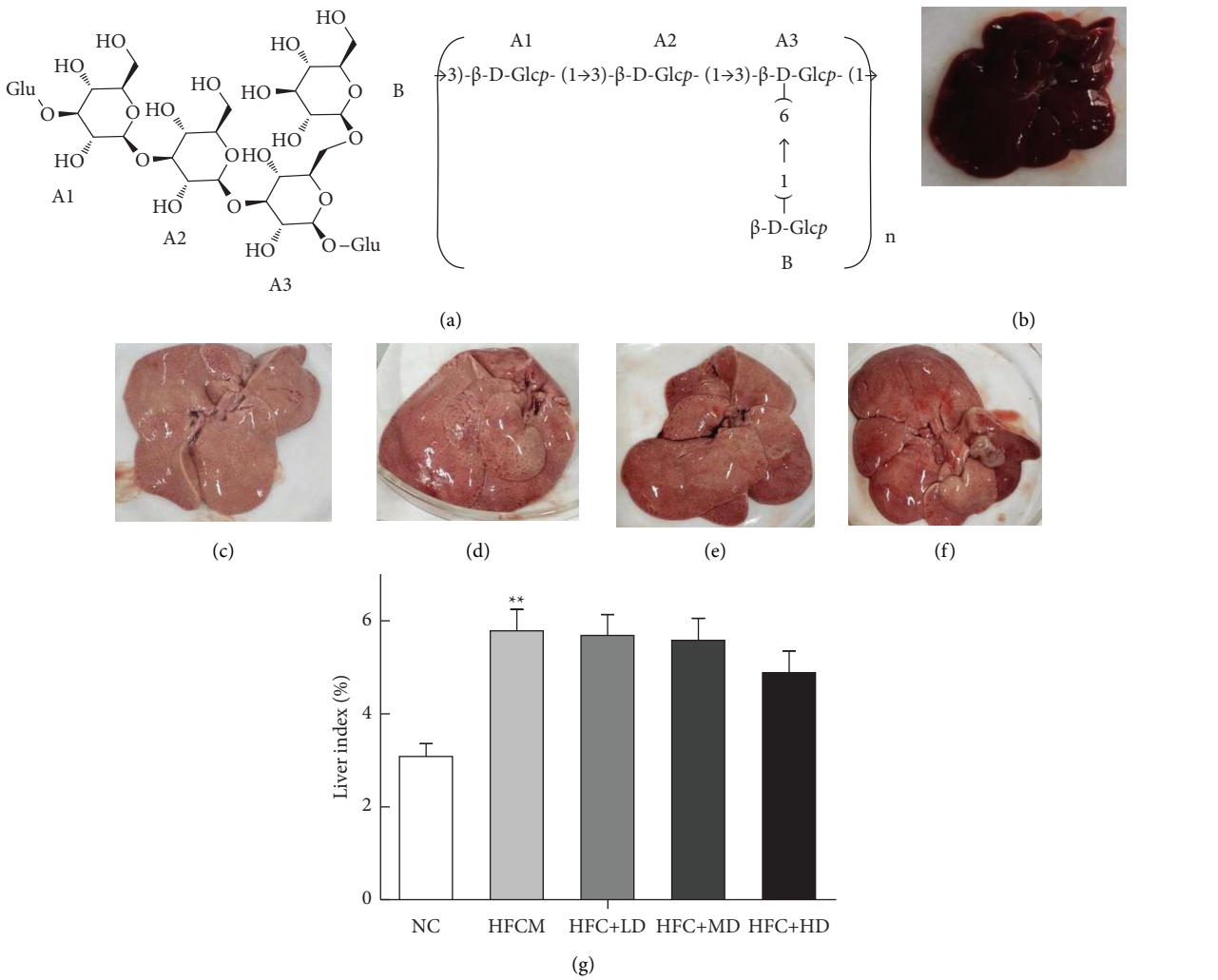


Figure 1: Effects of *Sparassis latifolia* polysaccharide on the liver index of rats fed a high-fat, high-cholesterol diet. Note: (a) the structure of SLP and molecular formula of SLP, (b) normal control group, (c) high-fat and high-cholesterol model group, (d) high-fat and high-cholesterol + low-dose group, (e) high-fat and high-cholesterol + middle-dose group, (f) high-fat and high-cholesterol + high-dose group, and (g) liver index. $^{**}P < 0.01$ compared with the NC group.

contents of T-AOC and malondialdehyde (MDA) were also determined following the recommended protocols (Nanjing Jiancheng Institute of Bioengineering).

2.6. Real-Time Polymerase Chain Reaction. Trizol (TaKaRa, Japan) was used to isolate total RNA from the liver samples. After cDNA synthesis, quantitative reverse transcription-polymerase chain reaction is carried out with the primers listed in Table 1.

2.7. Western Blot Analysis. Anti-lecithin-cholesterol acyltransferase (anti-LCAT), anti-PPARα (Abcam, MA, USA), anti-SOAT1, anti-HMGCR, anti-SCARB1, anti-CYP7A1 (Cell Signaling Technology, Inc., MA, USA), and other antibodies (Bioworld Technology, Co., Ltd., Nanjing, China), as well as a Molecular Imager ChemiDOC XRS System (Bio-Rad, Shanghai, China), were used for Western blot analysis. In brief, the cell and liver samples were rinsed with phosphate-buffered saline and lysed in radioimmunoprecipitation assay (RIPA) buffer (Beyotime Institute of Biotechnology) containing 1 mmol/L of phenylmethylsulfonyl fluoride (Beyotime Institute of Biotechnology). Subsequently, the cell and tissue lysates were separated on 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and then transferred onto poly(vinylidene fluoride) membranes (Millipore, MA, USA). After incubation with primary and secondary antibodies, the membranes were stained with chemiluminescent reagents (WesternBright Enhanced Chemiluminescence Kit, Solarbio, Beijing, China). Finally, the Bio-Rad ChemiDoc imaging system (Bio-Rad, Shanghai, China) was used to visualize the protein bands.

2.8. Content of Two Short-Chain Fatty Acids' Analysis. Gas chromatography-mass spectrometry (GC-MS) was used to measure tissue metabolite levels. The liver sample (0.1 g) in each group was mixed with nine times the volume of normal saline and centrifuged to obtain the supernatant. The

Table 1: Primer sequence, corresponding polymerase chain reaction product size, and position.

No.	Gene	Primers (5′-3′)	Primer location (start)	Product size (bp)	GenBank no.
1	*β-actin*	CGTTGACATCCGTAAAGAC TAGGAGCCAGGGCAGTA	858 951	110	NM_031144.3
2	*HMG-CoAR*	TGCGTGTCCCTGGTCCTA TTGGGTTACTGGGTTTGG	643 745	120	NM_013134.2
3	*CYP7α-1*	CTGGCTGAGGGATTGAA ATAGCGAGGTGCGTCTT	696 813	134	NM_012942.2
4	*PPARα*	GTGGAGTCCTGGAACTGAAGC ACAGAGCACCAATCTGTGATG	90 351	262	NM_013196.1
5	*LCAT*	ACCTCCTTCTGGCTCCTCAAT GACAACCCTGGTGTTATCAATCC	74 325	252	NM_017024.2
6	*ABCA1*	GCGAGCTGCGTTCTAACATG CAACGTTGTGGTGGCTTCAG	500 603	104	NM_178095.2
7	*SR-BI*	TCGAACAGAGCGGGATGATG TTGGCTTCTTGCAGTACCGT	1449 1725	277	NM_031541.1
8	*SOAT 1*	TCTTAGCATACACGCTGCCC AACCGTGCTCGATTTCTCCT	804 943	140	NM_031118.1

neutral sterols in rat liver samples were extracted and derived. The contents of differential metabolites in the liver were detected using GC-MS. In brief, the liver sample (0.1 g) was mixed with 50 μL of 1 mg/mL 5α-cholestane chloroform solution (Aladdin Biotechnology Company, Shanghai, China) as the internal standard and 2 mL of methanol: chloroform solution (v/v = 1: 2), shaken well and used for layering. After removing the lower layer of the liquid, the mixture was dried with nitrogen (nitrogen-blowing instrument N-EVAP-111, Organomation, MA, USA), mixed with 2 mL of 1 mol/L methanolic KOH solution, and maintained in a water bath at 50 °C for 60 min. Subsequently, the mixture was mixed with 2 mL of ultrapure water and 5 mL of n-hexane and dried with nitrogen. Furthermore, the dissolved solution in 100 μL of pyridine was filtered into a sample bottle through a 0.22 μm filter, mixed with 50 μL of silanization reagent (Aladdin Biotechnology Company), and sealed to react for 30 min at room temperature. Finally, the solution was put in an ice water bath to stop the reaction, and the cholesterol contents in the liver were detected by GC-MS (Trace IQS, Thermo, MA, USA).

2.9. Statistical Analysis. All experiments were performed at least in triplicate. The data were presented as the mean ± standard error of the mean of at least six independent experiments. The differences in the measurement results between control and treatment groups were compared with one-way analysis of variance, followed by Tukey's test (GraphPad Prism 6). P values less than 0.05 (significant) and 0.01 (extremely significant) indicated statistically significant differences.

3. Results

3.1. Liver Index Was Reduced by SLP in Rats Fed a High-Fat, High-Cholesterol Diet. As expected, the livers in the NC group appeared bright red compared with those in the HFCM group, which seemed dull with a coarse surface and hard texture (Figures 1(a)–1(e)). The livers in the groups treated with SLP showed a change in color from whitish in the HFCM group to bright red, with smooth and shiny surfaces and a soft texture (Figures 1(c)–1(e)). These observations suggested that SLP maintained healthy liver status in rats fed a high-fat, high-cholesterol diet. The comparison of the liver index values indicated that damage significantly increased in the HFCM group compared with the NC group ($P < 0.01$, Figure 1(f)). The liver indexes of polysaccharide-treated rats decreased compared with those in the HFCM group, but the differences were not significant (Figure 1(f)).

The liver histology revealed that the hepatocytes in the NC group were arranged orderly with round-shaped nuclei and well-distinguishable nucleoli (Figure 2). The single layer of hepatocytes was aligned, forming hepatic chords located around the central vein and radially extended to the far end (Figure 2(a)). However, a high degree of liver abnormality was noted in the HFCM group, with hepatocytes swollen and disordered, containing large numbers of cytoplasmic drip-shaped fat vacuoles (Figure 2(b)). In comparison, the hepatocytes in the polysaccharide-treated groups showed lower degrees of swelling, fewer fatty vacuoles, and more neatly arranged tissue structure, indicating much fewer liver abnormalities and damage (Figures 2(c)–2(e)). The results demonstrated that SLP could reverse liver abnormalities and liver damage induced by a high-fat, high-cholesterol diet.

3.2. Effect of SLP on Lipid Parameters in Rat Liver. Compared with the NC, the HFCM-treated rats showed significantly higher levels of liver TC, TG, and LDL-C (all $P < 0.01$, Figure 3). In contrast, the rats in all polysaccharide-treated groups showed decreased TC, TG, and LDL-C levels compared with HFCM-treated rats in a noticeable dose-dependent manner. The lower levels of TC, TG, and LDL-C also exhibited a higher statistical significance in the high-dose-treated group than in the other

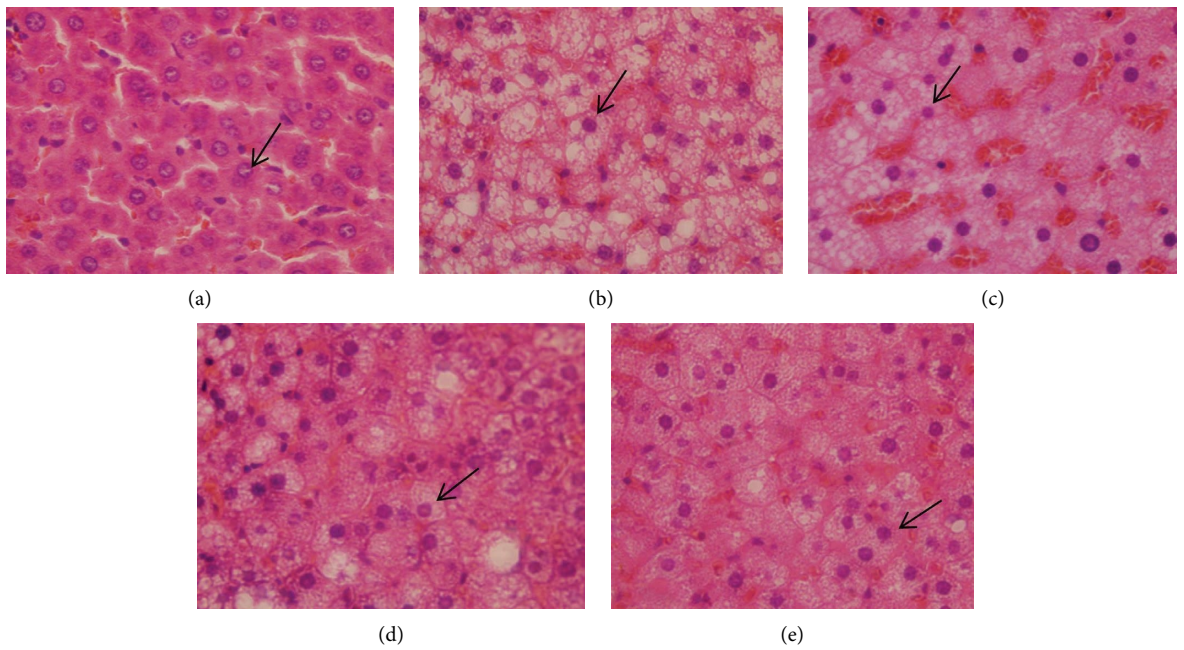


Figure 2: Effects of *Sparassis latifolia* polysaccharide on the liver histology of rats fed a high-fat, high-cholesterol diet. (100×). Note: (a) normal control group, (b) high-fat and high-cholesterol model groups, (c) high-fat and high-cholesterol + low-dose groups, (d) high-fat and high-cholesterol + middle-dose group, and (e) high-fat and high-cholesterol + high-dose group. The arrow shows liver cells.

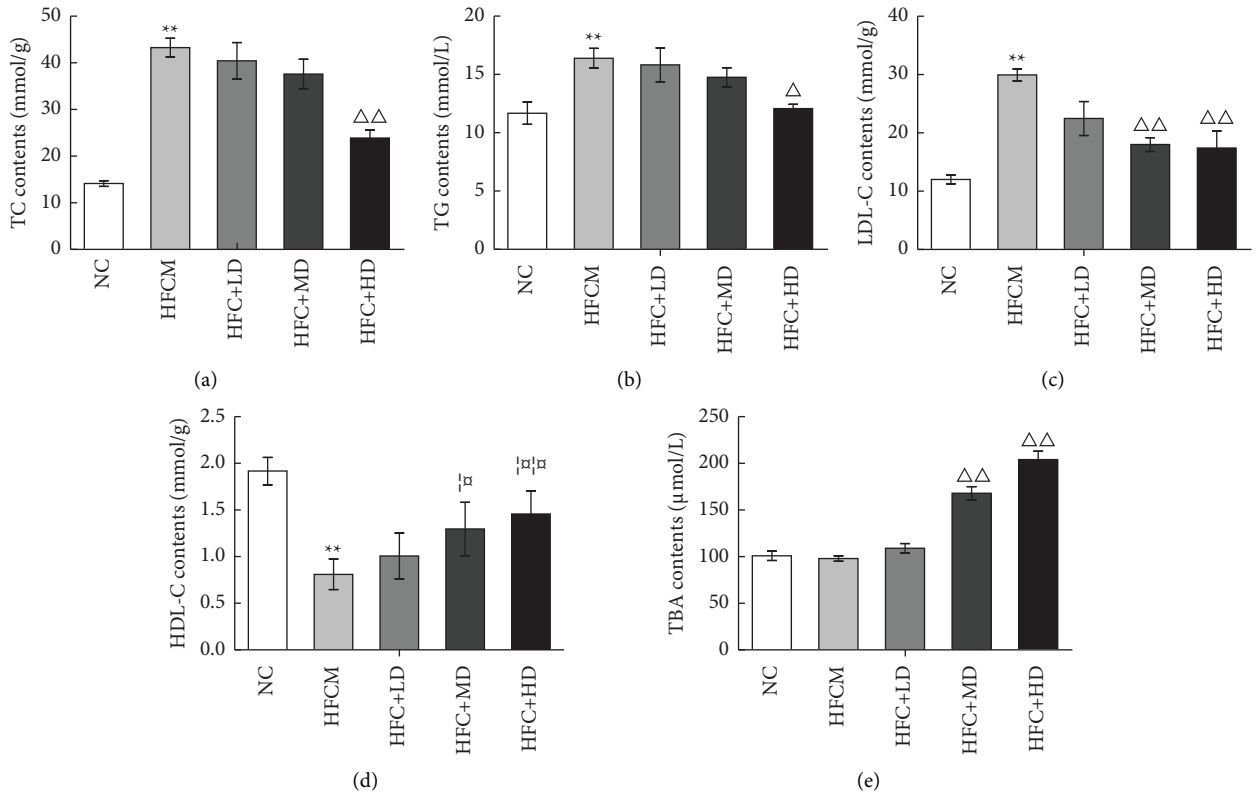


Figure 3: Effects of *Sparassis latifolia* polysaccharide on the liver of rats fed a high-fat, high-cholesterol diet: (a) TC, (b) TG, (c) LDL-C, (d) HDL-C, and (e) TBA. Note: $^{**}P < 0.01$, compared with the NC group;$^{\triangle\triangle}P < 0.01$ compared with the HFCM group.

groups ($P < 0.01$). These results indicated that SLP reduced liver TC, TG, and LDL-C levels in rats fed a high-fat, high-cholesterol diet. Moreover, compared with the NC, the HFCM-treated rats showed significant decreases in the levels of HDL-C and TBA (all $P < 0.01$). In contrast, compared with the HFCM group, all polysaccharide-treated groups

showed increased HDL-C and TBA levels (all $P < 0.01$). These indicated that SLP counteracted the changes in liver HDL-C and TBA levels caused by the high-fat, high-cholesterol diet.

3.3. Effect of SLP on Antioxidant Capacity. The activities of liver T-AOC, SOD, GSH-Px, and CAT significantly decreased in the HFCM group compared with the NC group (all $P < 0.01$). The activities of T-AOC, SOD, GSH-Px, and CAT significantly increased in all polysaccharide-treated groups, especially in the HFC + HD group, compared with the HFCM group (all $P < 0.01$, Figure 4). SLP could improve the activities of T-AOC, SOD, GSH-Px, and CAT in rats fed a high-fat, high-cholesterol diet. The liver MDA content increased in the HFCM group compared with the NC group, but with no statistically significant difference. Furthermore, the polysaccharide-treated groups showed decreased liver MDA levels compared with the HFCM group, though without any statistically significant difference.

3.4. Effect of SLP on Liver mRNA Expression in Signaling Pathways Related to Cholesterol Metabolism. The liver mRNA levels of HMG-CoAR and SOAT1 increased in the HFCM-treated group compared with the NC group (all $P < 0.01$, Figure 5). The polysaccharide treatment resulted in decreased HMG-CoAR and SOAT1 mRNA expression compared with the HFCM treatment, with the high-dose group showing statistically significant differences ($P < 0.05$). Thus, SLP could inhibit the expression of HMG-CoAR and SOAT1 mRNA in a dose-dependent manner. Also, CYP7α-1, PPARα, LCAT, SR-BI, and ABCA1 mRNA expressions decreased significantly in the HFCM group compared with the NC group (all $P < 0.05$, Figure 5). In contrast, their expression increased in the polysaccharide-treated groups compared with the HFCM group in a dose-dependent manner. The high-dose group showed significant differences in enhancing CYP7α-1, PPARα, LCAT, SR-BI, and ABCA1 mRNA expressions (all $P < 0.05$).

3.5. SLP Affected Liver Cholesterol-Related Protein Expression. Western blot analyses conducted on rat liver homogenates showed significant decreases in liver CYP7A1, SCARB1, HMGCR, SOAT1, PPAR-α, and LCAT protein expressions in the HFCM group compared with the NC group (all $P < 0.01$, Figure 6). Notably, all polysaccharide-treated groups exhibited elevated levels of SCARB1 and SOAT1 protein expression in a dose-dependent manner compared with the HFCM group; especially, the levels in the HFC + HD group increased by 58.1% and 64.2%, respectively. HMGCR and LCAT protein expression levels were significantly higher in the HFC + HD group than in the HFCM group. CYP7A1 and PPARα protein expression levels were significantly higher in the HFC + HD group than in the HFCM group. Together, these data indicated that SLP could enhance the levels of CYP7A1, SCARB1, HMGCR, SOAT1, and PPARα in rats fed a high-fat, high-cholesterol diet.

3.6. Qualitative Analysis of Liver Two Short-Chain Fatty Acids Analysis. The serum samples were collected parallel with the liver tissues and subjected to GC-MS detection. The peak output time of propionic acid and butyric acid was 4.96 min and 6.32 min, respectively (Figure 7). The presentation curve was finally obtained according to different concentrations of mixed standard solution and target peak area: propionic acid, $Y = 1.336 \times 1010x - 1.605 \times 10^7$; butyric acid, $Y = 4.203 \times 1010x - 1.990 \times 10^7$, and $R^2 = 0.9993$. The qualitative analysis of various short-chain fatty acids (SCFAs) in rat liver contents was carried out using the peak time and standard curve, as shown in the figure. The contents of propionic acid and butyric acid were significantly lower in the HFCM group than those in the NC group. The SCFAs in the HFC + HD group displayed an increasing trend, and the difference was significant ($P < 0.05$).

4. Discussion

This study provided a theoretical framework for expanding the research and developing functional polysaccharide products from *S. crispa* as cholesterol-reducing genes. Metabolic abnormalities are closely related to liver damage, and the degree of liver damage can be inferred by the changes in the liver index. Moreover, malfunction in cholesterol metabolism is mainly responsible for hyperlipidemia, characterized by cholesterol accumulation, degeneration, and necrosis of liver cells. This study found that a high-fat, high-cholesterol diet increased the liver index in rats. In contrast, increasing doses of SLP reversed these increases, albeit in a nonsignificant manner. Furthermore, the pathological assessment of the corresponding liver tissues revealed a protective effect of the polysaccharides, with reductions in the size and number of lipid droplets and vacuoles within the hepatocytes and reduced swelling of hepatocyte cell bodies [23]. These phenotypic changes showed that treatment with SLP resulted in the repair or alleviation of liver tissue damage associated with the high-fat, high-cholesterol food intake. Moreover, given the linkages between liver damage and cholesterol metabolism, this result suggested that SLP could repair or alleviate liver damage through mechanisms affecting cholesterol metabolism.

This study showed that treatment with higher doses of SLP caused significant reductions in liver TC, TG, and LDL-C levels. The possible mechanisms might involve enhancing the transformation of liver cholesterol, which might then be more efficiently converted into bile acids, further interrupting the enterohepatic circulation of bile acids and leading to lower cholesterol levels in the liver [24, 25]. Furthermore, the TBA content significantly increased in the middle- and high-dose polysaccharide groups, which was in agreement with our speculation that bile acid levels would rise.

Oxidative stress and metabolic disorders can be induced by a high-fat, high-cholesterol diet [26]. This study showed that SLP also significantly enhanced the activities of liver SOD, GSH-Px, and T-AOC. Increasing the activities of SOD, GSH-Px, and T-AOC promoted the elimination of free

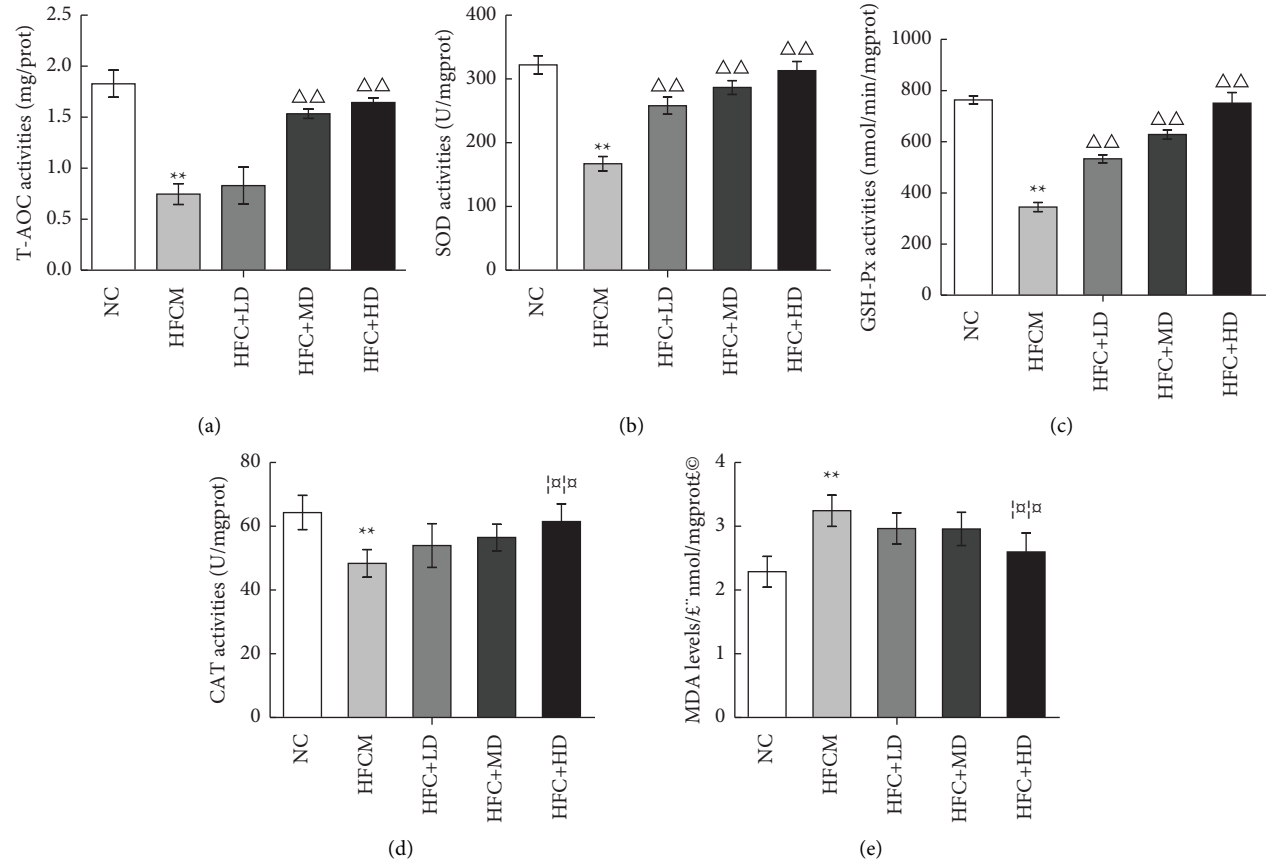


Figure 4: Effects of *Sparassis latifolia* polysaccharide on the liver of rats fed a high-fat, high-cholesterol diet: (a) T-AOC, (b) SOD, (c) GSH-Px, (d) CAT, and (e) MDA. Note: $^{**}P < 0.01$ compared with the NC group; $^{\triangle\triangle}P < 0.01$ compared with the HFCM group.

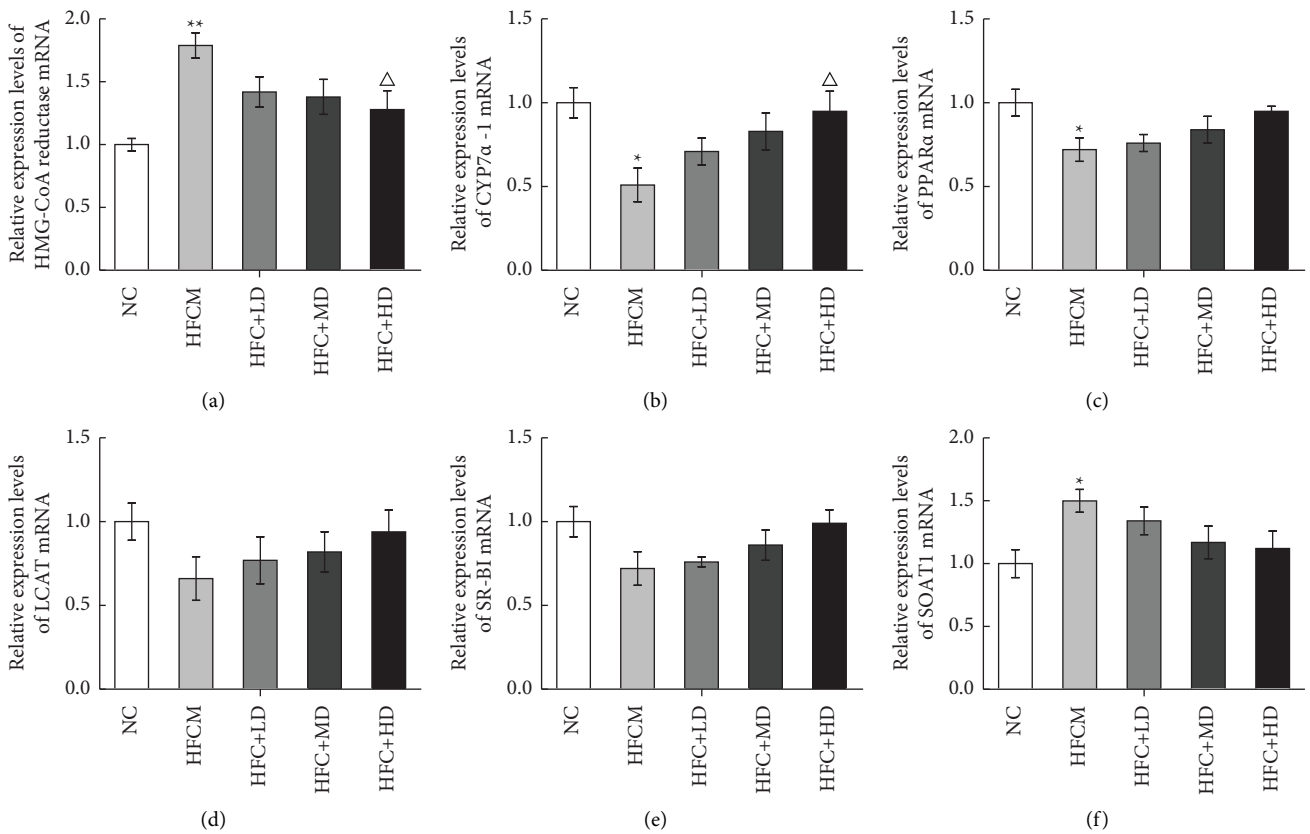
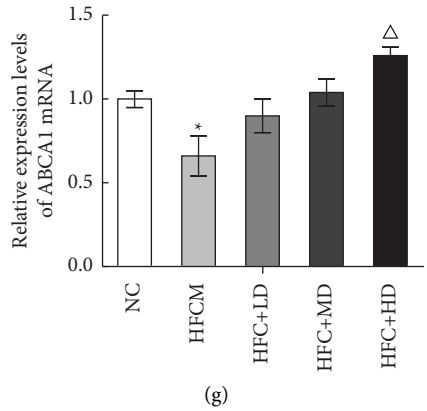
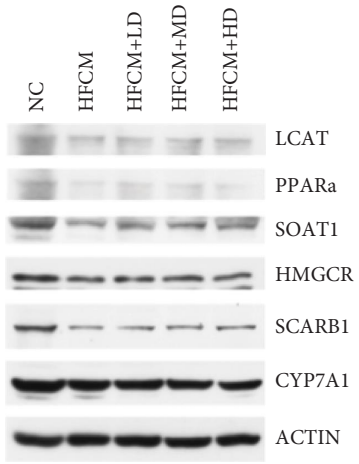


Figure 5: Continued.

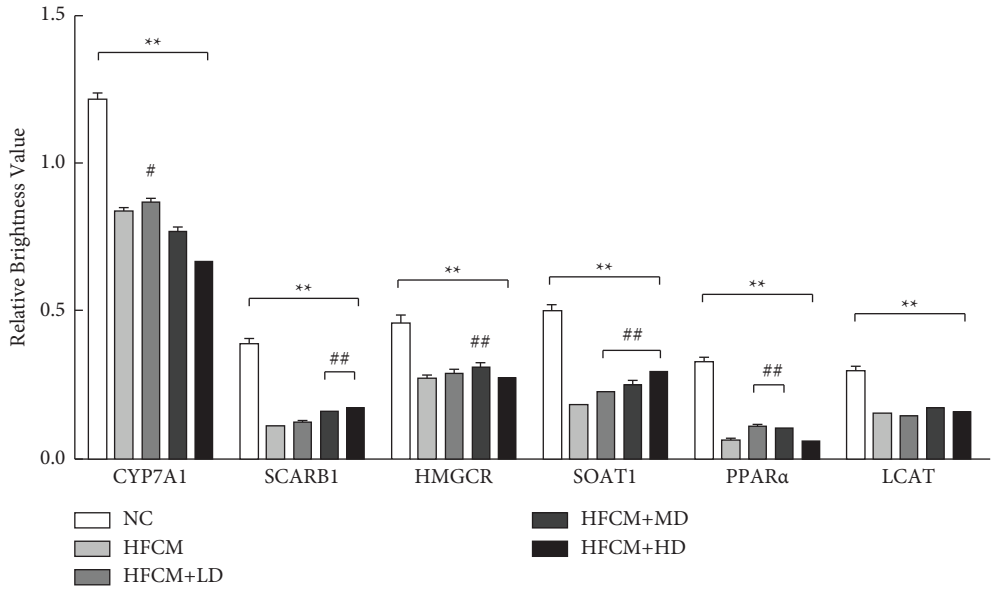


(g)

Figure 5: Relative expression levels of genes related to cholesterol metabolism pathway in the liver: (a) HMG-CoAR, (b) CYP7α-1, (c) PPARα, (d) LCAT, (e) SR-BI, (f) SOAT1, and (g) ABCA1. Note: $^{*}P < 0.05$ and $^{**}P < 0.01$ compared with the NC group; $^{\triangle}P < 0.05$ and $^{\triangle\triangle}P < 0.01$ compared with the HFCM group.



(a)



(b)

Figure 6: Effects of Sparassis latifolia polysaccharide on cholesterol metabolism in the livers of rats fed a high-fat diet.(A) LCAT,PPAR α,SOAT1,HMGCR,CYP7A1 protein expression; and (B) representative images of Western blots showing CYP7A1, SCARB1, HMGCR, SOAT1, PPAR α, and LCAT proteins.Values are means ± Standard Error of Mean, SEM. $^{*}P < 0.05$, $^{**}P < 0.01$ vs normal control diet (NC); $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ vs high-fat diet (H); $n = 9$ for each group.

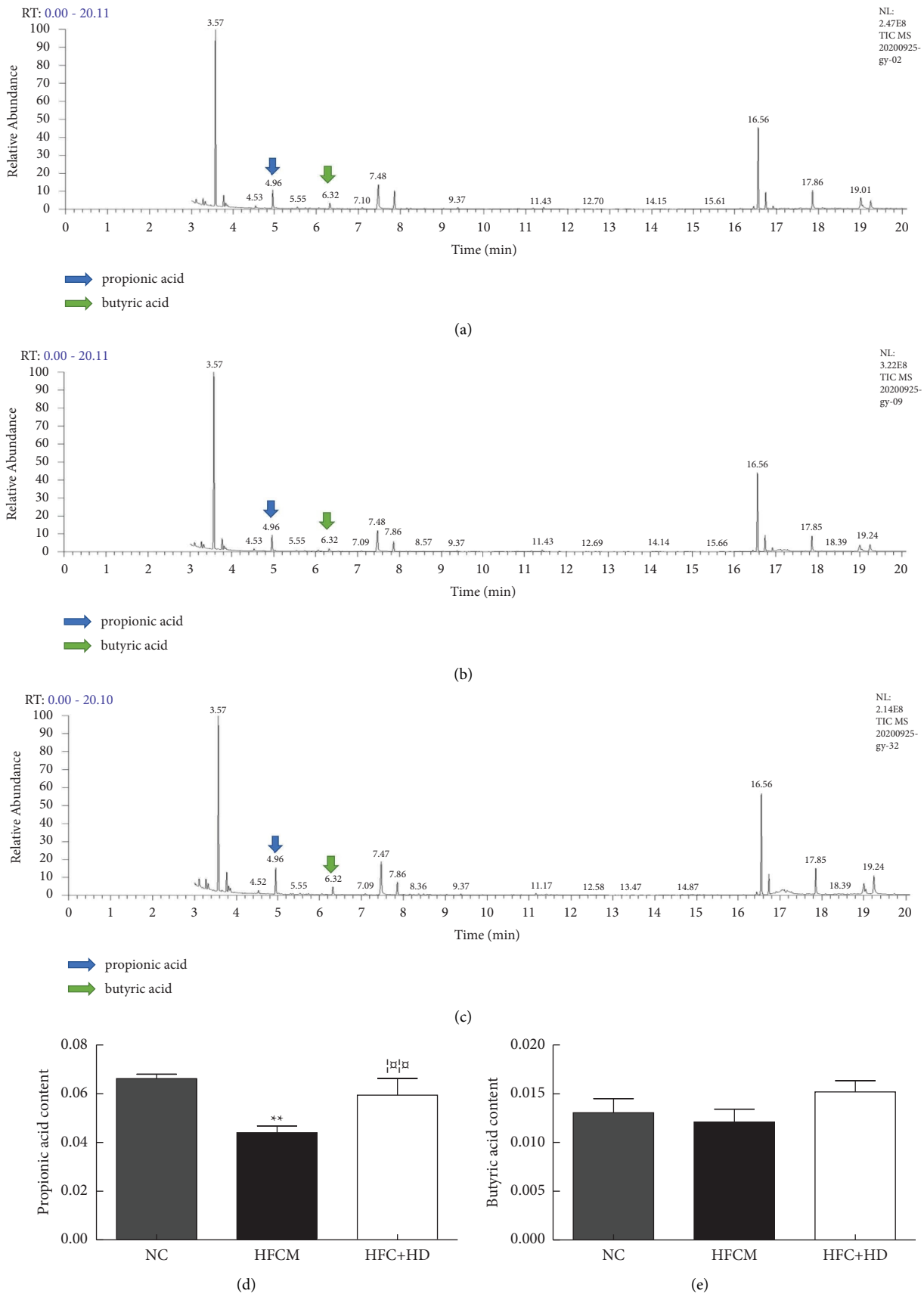


FIGURE 7: Effects of *Sparassis latifolia* polysaccharide on the content of short-chain fatty acids in the livers of rats: (a) normal control group, (b) high-fat, high-cholesterol model group; (c) high-fat and high-cholesterol + high-dose groups, (d) propanoic acid content, and (e) butyric acid content. Values are means ± SEM. $^{*}P < 0.05$ and $^{**}P < 0.01$ vs. normal control diet (NC); $^{\#}P < 0.05$ and $^{\#\#}P < 0.01$ vs. high-fat diet (H); $n = 9$ for each group.

radicals and lipid peroxides that otherwise caused cell damage, indicating that SLP also possessed a defensive antioxidant ability in the livers of rats fed the high-fat, high-cholesterol diet [27]. Moreover, SLP increased CAT activity and reduced MDA levels, perhaps due to the overall high levels of free radicals attributed to the limited antioxidant effects of the intervention [28].

Cholesterol metabolism is mainly balanced through synchronized regulation of cholesterol synthesis, absorption, reverse transport, and excretion [29]. This study demonstrated that SLP elicited changes in critical regulators of these processes at the mRNA level. Notably, the intervention significantly inhibited the expression of HMG-CoAR and SOAT1 mRNA but enhanced the expressions of CYP7α-1 and ABCA1 mRNA. These four genes (*HCG-COA R*, *CYP7α-1*, *SOAT1*, and *ABCA1*) were involved in cholesterol synthesis, promoted cholesterol reverse transport, inhibited cholesterol absorption, and accelerated cholesterol conversion into bile acids, indicating that *S. crispa* might have a mechanism for lowering cholesterol levels [25, 30]. Polysaccharides from the perennial water plant *Brasenia schreberi* were found to upregulate hepatic CYP7A1 mRNA expression to increase hepatic bile acid synthesis in hamsters [31], which was consistent with our findings. Polysaccharides from *Cyclocarya paliurus* leaves exerted therapeutic effects on rats with hyperlipidemia through the induction of PPARα and the downregulation of HMGCR [32]. Thus, to a certain extent, SLP could restore the balanced interactions between these gene products to help lower the level of cholesterol raised with high-fat food intake (30). Moreover, some reports showed that polysaccharides with cholesterol-lowering effects could also bind to bile acids [33, 34]. Therefore, the study speculated that these polysaccharides could combine with bile acids to form polymers and enhance their intestinal excretion, stimulating the conversion of cholesterol into bile acids in the liver and hence reducing TC levels. We also observed that the expressions of PPARα, LCAT, and SR-BI mRNA increased after treatment, but the changes were not statistically significant. Nonetheless, further studies are required to determine to what extent SLP alters the protein expression of the genes mentioned earlier. Moreover, our analysis was not exhaustive, as a considerable number of other genes related to cholesterol synthesis, degradation, reverse transport, and absorption were not included in the study.

GC-MS analysis was conducted to examine the SCFAs, namely, propionic acid and butyric acid, which also played regulatory roles [35]. Usually, SCFAs are generated via the activity of colonic anaerobic bacteria, fermentation of glycoproteins secreted from intestinal epithelial cells, and undigested and unabsorbed carbohydrate species such as oligosaccharides, resistant starch, nonstarch polysaccharides, and sugar alcohols [36]. This study demonstrated that SLP significantly increased SCFA levels and at least partially alleviated hepatic steatosis induced by high serum levels of TC, TG, and LDL in rats fed a high-fat diet.

5. Conclusions

The present study aimed to lessen inflammation and oxidative stress, reduce liver cell apoptosis or lipid deposition, and lower circulatory lipid levels, thus improving liver cholesterol metabolism using SLP. Moreover, our analysis was not exhaustive, considering that large numbers of other genes related to cholesterol synthesis, degradation, reverse transport, and absorption were not analyzed.

Data Availability

The data used to support the findings of this study are included within the article.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Authors' Contributions

Feng Cuiping conceptualized the study; Cheng Feier proposed the methodology; Cheng Yanfen performed formal analysis and investigated the data; Yang Yaru, Yun Shaojun, and Cao Jinling prepared the original draft; Cheng Feier and Yang Yaru reviewed and edited the manuscript; Cheng Feier and Feng Cuiping were responsible for funding acquisition; Chang Mingchang was responsible for resources; Feng Cuiping supervised the study.

Acknowledgments

The authors would like to thank Shanxi Key Laboratory of Edible Fungi in Loess Plateau. This research was funded by the Incentive Funding Research Program for Doctor Graduates Working in Shanxi Province, grant number SXYBKY2020004; Science and Technology Innovation Project of Shanxi Agricultural University, grant number 2020BQ82; key project of Shanxi Provincial Key R&D Program, grant number 201603D21106; Shanxi Nonggu Construction Scientific Research Project, grant number SXNGJSKYZX201903; Edible Mushroom Shanxi Province Science and Technology Innovation Key Team Project, grant number 201805D131009.

References

[1] J. Luo, H. Yang, and B. L. Song, "Mechanisms and regulation of cholesterol homeostasis," *Nature Reviews Molecular Cell Biology*, vol. 21, no. 4, pp. 225–245, 2020.

[2] H. Li, G. Liu, X. Wan et al., "The zinc finger and BTB domain containing protein ZBTB20 regulates plasma triglyceride metabolism by repressing lipoprotein lipase gene transcription in hepatocytes," *Hepatology*, vol. 75, no. 5, pp. 1169–1180, 2022.

[3] W. J. Shen, S. Azhar, and F. B. Kraemer, "SR-B1: a unique multifunctional receptor for cholesterol influx and efflux," *Annual Review of Physiology*, vol. 80, no. 1, pp. 95–116, 2018.

[4] A. Alabi, X. D. Xia, H. M. Gu et al., "Membrane type 1 matrix metalloproteinase promotes LDL receptor shedding and

accelerates the development of atherosclerosis," *Nature Communications*, vol. 12, p. 1889, 2021.

[5] B. Lauzier, S. Delemasure, B. Collin et al., "Effect of a chronic cholesterol-rich diet on vascular structure and oxidative stress in LDLR^{-/-} mice," *Cellular Physiology and Biochemistry*, vol. 27, no. 1, pp. 31–36, 2011.

[6] O. Briand, V. Touche, S. Colin et al., "Liver X receptor regulates triglyceride absorption through intestinal down-regulation of scavenger receptor class B, type 1," *Gastroenterology*, vol. 150, no. 3, pp. 650–658, 2016.

[7] M. Danielli, J. Marrone, A. M. Capiglioni, and R. A. Marinelli, "Data of H(2)O(2) release from AQP8-knockdown rat hepatocyte mitochondria," *Data in Brief*, vol. 23, Article ID 103722, 2019.

[8] M. M. Schumacher and R. A. DeBose-Boyd, "Post-translational regulation of HMG CoA reductase, the rate-limiting enzyme in synthesis of cholesterol," *Annual Review of Biochemistry*, vol. 90, no. 1, pp. 659–679, 2021.

[9] K. F. Chambers, P. E. Day, H. T. Aboufarrag, and P. A. Kroon, "Polyphenol effects on cholesterol metabolism via bile acid biosynthesis, CYP7A1: a review," *Nutrients*, vol. 11, p. 2588, 2019.

[10] T. Li, E. Owsley, M. Matozel, P. Hsu, C. M. Novak, and J. Y. L. Chiang, "Transgenic expression of cholesterol 7α-hydroxylase in the liver prevents high-fat diet-induced obesity and insulin resistance in mice," *Hepatology*, vol. 52, no. 2, pp. 678–690, 2010.

[11] C. R. Pullinger, C. Eng, G. Salen et al., "Human cholesterol 7α-hydroxylase (CYP7A1) deficiency has a hypercholesterolemic phenotype," *Journal of Clinical Investigation*, vol. 110, no. 1, pp. 109–117, 2002.

[12] M. Liu, S. Chung, G. S. Shelness, and J. S. Parks, "Hepatic ABCA1 deficiency is associated with delayed apolipoprotein B secretory trafficking and augmented VLDL triglyceride secretion," *Biochimica et Biophysica Acta (BBA) Molecular and Cell Biology of Lipids*, vol. 1862, no. 10, pp. 1035–1043, 2017.

[13] Y. He, G. E. Ronsein, C. Tang et al., "Diabetes impairs cellular cholesterol efflux from ABCA1 to small HDL particles," *Circulation Research*, vol. 127, no. 9, pp. 1198–1210, 2020.

[14] Y. Sun, J. Wang, T. Long et al., "Molecular basis of cholesterol efflux via ABCG subfamily transporters," *Proceedings of the National Academy of Sciences of the U S A*, vol. 118, no. 34, Article ID e2110483118, 2021.

[15] Q. Wu, Q. Wang, J. Fu, and R. Ren, "Polysaccharides derived from natural sources regulate triglyceride and cholesterol metabolism: a review of the mechanisms," *Food & Function*, vol. 10, no. 5, pp. 2330–2339, 2019.

[16] W. Gu, Y. Wang, L. Zeng et al., "Polysaccharides from Polygonatum kingianum improve glucose and lipid metabolism in rats fed a high fat diet," *Biomedicine & Pharmacotherapy*, vol. 125, Article ID 109910, 2020.

[17] M. Ming, L. Guanhua, Y. Zhanhai, C. Guang, and Z. Xuan, "Effect of the Lycium barbarum polysaccharides administration on blood lipid metabolism and oxidative stress of mice fed high-fat diet in vivo," *Food Chemistry*, vol. 113, no. 4, pp. 872–877, 2009.

[18] Y. Li, Y. Sheng, X. Lu et al., "Isolation and purification of acidic polysaccharides from Agaricus blazei Murill and evaluation of their lipid-lowering mechanism," *International Journal of Biological Macromolecules*, vol. 157, pp. 276–287, 2020.

[19] T. Kimura, "Natural products and biological activity of the pharmacologically active cauliflower mushroom Sparassis crispa," *BioMed Research International*, vol. 2013, Article ID 982317, 9 pages, 2013.

[20] H. H. Kim, S. Lee, T. S. Singh, J. K. Choi, T. Y. Shin, and S. H. Kim, "Sparassis crispa suppresses mast cell-mediated allergic inflammation: role of calcium, mitogen-activated protein kinase and nuclear factor-κB," *International Journal of Molecular Medicine*, vol. 30, no. 2, pp. 344–350, 2012.

[21] J. M. Han, E. K. Lee, S. Y. Gong, J. K. Sohng, Y. J. Kang, and H. J. Jung, "Sparassis crispa exerts anti-inflammatory activity via suppression of TLR-mediated NF-κB and MAPK signaling pathways in LPS-induced RAW264.7 macrophage cells," *Journal of Ethnopharmacology*, vol. 231, pp. 10–18, 2019.

[22] H. Zhengqi, *Structural Characterization, Gel Rheological Properties and Antioxidant and Immune Function of Polysaccharides from Spirococcus Aureus*, Shanxi Agricultural University, Jinzhong, China, 2018.

[23] D. H. Ipsen, J. Lykkesfeldt, and P. Tveden-Nyborg, "Molecular mechanisms of hepatic lipid accumulation in non-alcoholic fatty liver disease," *Cellular and Molecular Life Sciences*, vol. 75, no. 18, pp. 3313–3327, 2018.

[24] G. Simon, V. Heckmann, D. Tóth, D. Pauka, K. Petrus, and T. F. Molnár, "The effect of hepatic steatosis and fibrosis on liver weight and dimensions," *Legal Medicine*, vol. 47, Article ID 101781, 2020.

[25] K. Paunovska, A. J. Da Silva Sanchez, C. D. Sago et al., "Nanoparticles containing oxidized cholesterol deliver mRNA to the liver microenvironment at clinically relevant doses," *Advances in Materials*, vol. 31, no. 14, Article ID e1807748, 2019.

[26] J. S. Bhatti, G. K. Bhatti, and P. H. Reddy, "Mitochondrial dysfunction and oxidative stress in metabolic disorders a step towards mitochondria based therapeutic strategies," *Biochimica et Biophysica Acta Molecular Basis of Disease*, vol. 1863, no. 5, pp. 1066–1077, 2017.

[27] V. Rani, G. Deep, R. K. Singh, K. Palle, and U. C. Yadav, "Oxidative stress and metabolic disorders: pathogenesis and therapeutic strategies," *Life Sciences*, vol. 148, pp. 183–193, 2016.

[28] C. Hou, L. Chen, L. Yang, and X. Ji, "An insight into anti-inflammatory effects of natural polysaccharides," *International Journal of Biological Macromolecules*, vol. 153, pp. 248–255, 2020.

[29] J. Q. Liang, N. Teoh, L. Xu et al., "Dietary cholesterol promotes steatohepatitis related hepatocellular carcinoma through dysregulated metabolism and calcium signaling," *Nature Communications*, vol. 9, no. 1, p. 4490, 2018.

[30] T. D. Nguyen, O. Prykhodko, F. F. Hållenius, and M. Nyman, "Monobutyrin reduces liver cholesterol and improves intestinal barrier function in rats fed high-fat diets," *Nutrients*, vol. 11, no. 2, p. 308, 2019.

[31] H. Kim, Q. Wang, C. F. Shoemaker, F. Zhong, G. E. Bartley, and W. H. Yokoyama, "Polysaccharide gel coating of the leaves of Brasenia schreberi lowers plasma cholesterol in hamsters," *Journal of Traditional and Complementary Medicine*, vol. 5, no. 1, pp. 56–61, 2015.

[32] Z. W. Yang, K. H. Ouyang, J. Zhao, H. Chen, L. Xiong, and W. J. Wang, "Structural characterization and hypolipidemic effect of Cyclocarya paliurus polysaccharide in rat," *International Journal of Biological Macromolecules*, vol. 91, pp. 1073–1080, 2016.

[33] L. Gong, H. Zhang, Y. Niu et al., "A novel alkali extractable polysaccharide from Plantago asiatic L. Seeds and its radical-scavenging and bile acid-binding activities," *Journal of*

Agricultural and Food Chemistry, vol. 63, no. 2, pp. 569–577, 2015.

[34] J. Gao, L. Lin, B. Sun, and M. Zhao, "Comparison study on polysaccharide fractions from laminaria japonica: structural characterization and bile acid binding capacity," *Journal of Agricultural and Food Chemistry*, vol. 65, no. 44, pp. 9790–9798, 2017.

[35] E. Sozen, B. Yazgan, A. Sahin, U. Ince, and N. K. Ozer, "High cholesterol diet-induced changes in oxysterol and scavenger receptor levels in heart tissue," *Oxidative Medicine and Cellular Longevity*, vol. 2018, Article ID 8520746, 13 pages, 2018.

[36] D. J. Morrison and T. Preston, "Formation of short chain fatty acids by the gut microbiota and their impact on human metabolism," *Gut Microbes*, vol. 7, no. 3, pp. 189–200, 2016.