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Physicochemical characteristics and anti-hyperlipidemic effect of polysaccharide from BaChu mushroom (*Helvella leucopus*)

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ABSTRACT

To investigate the effects of *Helvella leucopus* (*H. leucopus*) on hyperlipidemic mice, polysaccharide (HLP) was prepared from *H. leucopus* by hot water extraction and alcohol precipitation. HLP was primarily contained glucose, rhamnose, galactose and mannose, with an average molecular weight of 7.34×10^4 Da. It was a complex and irregular network with a few pores by scanning electron microscopy. Meanwhile, oral administration of HLP at 60 mg/kg decreased levels of total cholesterol, triglycerides, and low-density lipoprotein cholesterol in serum while increased that of high-density lipoprotein cholesterol in high fat diet (HFD) mice. Furthermore, HLP significantly reversed the serum aspartate transaminase, alanine transaminase, and free fatty acid levels in HFD mice. Moreover, HLP treatment markedly regulated the mRNA levels of PPAR-α, ACS, and CPT-1α compared to the model group. Thus, these findings support that the supplement of *Helvella leucopus* polysaccharide is a novel complementary alternative strategy for management of hyperlipidemia.

1. Introduction

The disorder of lipid and carbohydrate metabolism in human is caused by many variables, such as faulty lipid/glucose synthesis, breakdown, absorption, and other metabolic processes in the body, resulting in excessive cholesterol, insulin resistance, and liver damage (Li et al., 2020). More and more individuals are suffering from lipid and glucose disorder and its complications as their lifestyle and environment have changed. Therein, hyperlipidemia is one of the primary risk factors for vascular disease, which is a widespread metabolic syndrome across the world. Mostly, hyperlipidemia is clinically characterized by high total cholesterol (TC), triglyceride (TG), and low-density lipoprotein cholesterol (LDL-C) levels in the blood (De Costa & Park, 2017). Although considerable progress has been made in the development of medications to treat hyperlipidemia, the side effects of conventional treatments are becoming more apparent as time goes on (Harvey, Edrada-Ebel, & Quinn, 2015). Therefore, considerable efforts have been devoted to developing new sources from natural foods for alleviating the disorder of lipid and carbohydrate metabolism.

Helvella leucopus, also named Bachu mushroom, is a common edible fungus in Xinjiang province, China (Hyde et al., 2016). It has attracted a lot of interest in recent years because some species offer high economic, nutritional, and health values (Hyde et al., 2016). *H. leucopus*'s

therapeutic benefits included antitumor, antioxidant, hypolipidemic, and immunomodulating capabilities (Hou & Chen, 2008). Polysaccharide from *H. leucopus* play an important role in their biological activity. Some studies have shown their health-promoting functions. For example, crude polysaccharide from the fruiting bodies of *H. leucopus* had immunoenhancement effect on cyclophosphamide-induced immunosuppressive mice (Zhang et al., 2020). Two fractions from *H. leucopus* polysaccharide separated by DEAE-cellulose column exhibited strong antioxidant activity and inhibited HepG2 cell proliferation (Zeng & Zhu, 2018). Hyperlipidemic causes the smooth muscle cells in arteries to produce superoxide anion radicals. Recently, It was found that enzymatic-extractable mycelia zinc polysaccharides from *Pleurotus eryngii* var. *tuoliensis* had antioxidant and hepatoprotective properties in hyperlipidemic mice (Xu et al., 2017). Administration of mushroom to hypercholesterolemic rats up-regulated CoA synthetase (ACS), carnityl palmityl transferase-1 (CPT-1) and peroxisome proliferator activator receptor-α (PPAR-α) (Ismail, Soliman, Nassan, & Mohamed, 2015). All those genes involved in hepatic lipid uptake and oxidation. However, there are few studies on the anti-hyperlipidemia effects of *H. leucopus* polysaccharide. It is therefore quite necessary and significative to explore polysaccharide from *H. leucopus* ability to modulate cholesterol-related gene expression in relieving hyperlipidemia induced by the high-fat diet.

Therefore, the emphases in this study were on the physicochemical characterization of *H. leucopus* polysaccharide (HLP), and its anti-hyperlipidemia effect and modulation of cholesterol-related genes (PPAR-α, ACS and CPT-1α) in high fat diet mice. Results will help to explore the *H. leucopus* polysaccharide as a potential functional food to alleviate hyperlipidemia.

2. Material and methods

2.1. Material and reagents

The mature fruiting body samples were purchased in Bachu county, Xinjiang province, China and were identified by Guangdong Institute of Microbiology to be *Helvella leucopus.* Petroleum ether, anhydrous ethanol, and trichloromethane were procured from Sinopharm Chemical Reagent (Chemical Reagent Co., ltd., Beijing, China). The standards fucose, rhamnose, arabinose, galactose, glucose, xylose, mannose, fructose, and ribose were acquired from Sigma-Aldrich (St. Louis, MO, USA). In addition, RNA-easy Isolation Reagent, HiScript III-RT Super-Mix, and ChamQ Universal SYBR qPCR Master Mix (Vazyme Biotech Co. ltd. Nanjing, Jiangsu, China) were used in this study.

2.2. HLP preparation

The polysaccharide was prepared from *H. leucopus* by hot water extraction. Briefly, the dried *H. leucopus* powder was extracted with deionized distilled water at 95 ℃ in a 1:3 (w/v) ratio for 4 h. The extracts were centrifuged at 5000 × g for 10 min (Centrifuge 5424R, Eppendorf, Germany), then precipitated with triple its volume of anhydrous ethanol at 4 ℃ for 12 h. The precipitate was collected after centrifugation at 10000 × g for 10 min and re-dissolved in distilled water. The protein was removed by Sevage method (chloroform: butanol, 4:1, v/v) (Huang, Chen, Yang, & Huang, 2021). The aqueous phases were collected and freeze-dried (Christ Alpha 1–2 LD plus, Germany) to obtain HLP.

2.3. Structural characteristics of HLP

2.3.1. Determination of total sugar, moisture, protein and ash content

The phenol–sulfuric acid method was employed to determine the total sugar content of HLP (Chen et al., 2020). Briefly, phenol–sulfuric acid reagent was added to HLP samples and standard glucose (10, 20, 30, 40, 60, 80 and 100 μg/mL). The mixture was heated at 90 ℃ water bath for 10 min and cooled to room temperature. The absorbances of the standard and sample solution were determined at the wavelength of 490 nm (Multiskan GO, Thermo Fisher Scientific, Waltham, USA). The amount of total sugar in HLP was calculated using a standard curve calibrated with glucose. The protein content was determined using Bradford method (Bradford, 1976) and bovine serum albumin was used as reference standard for test. Then, the oven drying method was used to determine the moisture content. The loss weight obtained after drying was used to calculate the moisture content (Ballesteros, Cerqueira, Teixeira, & Mussatto, 2018). Moreover, the ash content was determined using Manzi method (Manzi, Aguzzi, & Pizzoferrato, 2001). One gram of sample was placed in a known weight crucible and heated to 550 ℃ for 6 h in a Gallenkamp furnace. The crucibles were weighed after cooling in a desiccator.

2.3.2. Molecular weight determination

The average molecular weight was determined by a size-exclusion chromatography coupled with multiple detectors, *viz.*, a RID and a MALLS (Wyatt Technology, CA, USA), which consisted of a DAWN HELEOS-II laser photometer (Wyatt Technology, Santa Barbara, CA, USA). Three tandem size-exclusion columns (300 mm × 8 mm, Shodex OH-pak SB-805, 804 and 803; Showa Denko K.K., Tokyo, Japan) were used. The lyophilized HLP samples were dissolved (1 mg/mL) in 0.1 M NaNO₃. A volume of 100 μL was injected into the separation system. The elution was performed with 0.1 M NaNO₃ aqueous solution at a flow rate of 0.4 mL/min. Data were acquired and processed using ASTRA software (Version 6.1, Wyatt Technology Corporation, USA).

2.3.3. Monosaccharide composition

High-performance liquid chromatography (HPLC, ICS5000, Thermo Fisher Scientific, USA) was used for the determination of mono-saccharide composition of HLP (Chen et al., 2020). Briefly, HLP was hydrolyzed by 2 M trifluoroacetic (TFA) at 121 ℃ for 2 h in a sealed tube. TFA was removed by extraction with 1 mL of methanol three times. The methanol was evaporated with a stream of nitrogen at room temperature. The hydrolyzed sample was dissolved in deionized water and filtered before injections. Nine standards (fucose, rhamnose, arabinose, galactose, glucose, xylose, mannose, fructose, ribose) were similarly dissolved in deionized water. The injection volume of hydrolysate was 5 μL. The temperature was set at 30 ℃. The retention time of each monosaccharide standard in the mixtures was confirmed by the analysis of each monosaccharide.

2.3.4. Scanning electron microscopy

After coating lyophilized HLP with a thin layer of gold, the morphological features of HLP were observed by using a scanning electron microscopy instrument (Zeiss Merlin Compact, Germany) in accelerating voltage of 3.0 kV at a 2000-fold magnification of the image (Chen, You, Ma, Zhao, & Kulikouskaya, 2021).

2.4. Fourier transform infrared spectroscopy

The functional groups of HLP were determined by the Fourier transforms infrared spectrometer (Nicolet iZ-10, Thermo fisher Scientific Inc., USA) in the frequency range of 4000–400 cm^{-1} by pressing HLP (2 mg) and KBr (200 mg) into a pellet (Ye, Ji, You, Zhou, Zhao, & Brennan, 2018).

2.5. Animal study

Six weeks old male Kunming mice were used after obtaining approval from Guangdong Medical Laboratory Animal Center (Foshan, Guangdong, China). All animals were maintained in an SPF environment (controlled temperature: 25 ± 1 ℃, 12 h/12 h light–dark cycle; humidity 60 ± 10 %) with free access to water and food. All experimental procedures were authorized by the Animal Management Committee and the Animal Ethics Committee of the Experimental Animal Center, South China Agricultural University. After a week acclimation period, the mice were randomly divided into six groups and fed for six weeks as follow: (1) normal (normal diet); (2) model (high fat diet); (3) AT (feeding HFD and oral gavage atorvastatin; 10 mg/kg) (4) HLP-L (feeding HFD and oral gavage HLP; 15 mg/kg); (5) HLP-M (feeding HFD and oral gavage HLP; 30 mg/kg); and (6) HLP-H (feeding HFD and oral gavage HLP; 60 mg/kg). The mice were treated with atorvastatin or HLP in the same volume of corresponding solution (0.1 mL/10 g body weight) once per day in the morning. The control and model groups were treated with the same volume of sterile saline solution. After 6 weeks of experimental administration, all mice were fasted overnight with free access to water. The mice were sacrificed by cervical dislocation after carbon dioxide absorption anesthesia. The blood samples were collected, then centrifuged (2000 × g, 10 min, 25 ℃) to separate serum and stored at −20 ℃. Liver and epididymal fat tissues were collected and weighted before being stored at −80 ℃ for further analysis. Liver index or epididymal fat index were calculated as the percentage of liver weight or epididymal fat weight to mice body weight.

2.6. Biochemical analysis

The levels of total cholesterol (TC), triglyceride (TG), high-density

lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), aspartate aminotransferase (AST), alanine aminotransferase (ALT), free fatty acids (FFA), and the blood urea nitrogen (BUN) in serum were determined according to the instruction provided by the enzyme-linked immunosorbent assay (ELISA) kit (Nanjing Jiancheng Bioengineering Inc., Nanjing, Jiangsu, China).

2.7. Real-time quantitative PCR (RT-qPCR) analysis

Total RNA was isolated by homogenizing the liver in RNA-easy Isolation Reagent following the manufacturer's instructions and was quantified by Nanodrop (Thermo Fisher Scientific, Inc., Wilmington, DE, USA). Reverse transcription was performed using the HiScript® III RT SuperMix. And qRT-PCR was performed using ChamQ Universal SYBR® qPCR Master Mix on the StepOne Plus (Thermo Fisher Scientific, USA) following the manufacturer's protocol. The list of primers (Sangon Biotech, Shanghai, China) used for real-time PCR analysis is described in Table 1. Levels of PPARA, ACS, and CPT1a mRNA were subsequently normalized to GAPDH mRNA levels, the calculation method was $2^{-\triangle\triangle CT}$.

2.8. Statistical analysis

All the values obtained were (Table 2) shown as the mean ± standard deviations (S.D.) and were calculated through SPSS version 19 (IBM software, NY, USA). Differences between experimental groups were carried out by one-way ANOVA statistical method and *$p < 0.05$ was deemed as a significant difference after repeating each experiment three times.

3. Results and discussion

3.1. Physical and chemical characterization of HLP

Since the polysaccharide extracted from *Helvella leucopus* has not been characterized previously, the results in this study can be considered as a trailblazing step to explore the potential of HLP. The hot water extraction used in this study resulted in a higher yield of 30 % which was higher than that of the same genus mushroom as previously reported (Hou & Chen, 2008; Hou, Zhang, Xiong, Li, & Yang, 2008). HLP was found to contain 19.01 % total sugar. Other composition in HLP were water (21.67 %), protein (8.75 %) and ash (46.23 %). Similar quantities of total sugar content have been documented in other edible mushroom extraction, like *Auricularia auricula-judae* (Sawangwan, Wansanit, Pattani, & Noysang, 2018). Over the past decades, edible mushrooms have confirmed utmost significance in the pharmaceutical and food industries (Yang, Belwal, Devkota, Li, & Luo, 2019). The molecular weights of biopolymers affects their bioactivity characteristics (Yang, Jiang, Zhao, Shi, & Wang, 2008). The average molecular weight of HLP was determined by using HPSEC-MALLS-RID.

Due to differences in molecular weight, branching and monosaccharides composition, mushroom polysaccharides behave in different biological activities while they interact with different receptors (Yang

Table 1
Primers of gene for qRT-PCR.

Gene	Primer sequence		Product size (bp)
GADPH	Forward	AGGAGCGAGACCCCACTAACA	247
	Reverse	AGGGGGGCTAAGCAGTTGGT	
PPARα	Forward	GAGCTGCAAGATTCAGAAGAAG	171
	Reverse	GAATCTTTCAGGTCGTGTTCAC	
ACS	Forward	ATGTACGATGGCTTCCAGAGGG	254
	Reverse	GGGACGACCACCATTGAGTAAGA	
CPT1a	Forward	TCAAGAATGGCATCATCACTGG	177
	Reverse	CGATGTTCTTCGTCTGGCTTG	

Table 2
Chemical composition of *Helvella leucopus* polysaccharides.

Index	Yield	Total sugar	Water	Protein	Ash	Average molecular weight (Da)
Values	30.07 %	19.01 %	21.67 %	8.75 %	46.23 %	7.34×10^4

et al., 2019). The polysaccharide extracted from *H. leucopus* was further analyzed its monosaccharide composition by using HPLC. The presence of monosaccharide peaks in HLP was confirmed by comparing with standard chromatograms. The result showed that monosaccharide from *H. leucopus* was composed of glucose, rhamnose, galactose, and mannose in a molar ratio of 4.58: 0.46: 8.51: 10.25 (Fig. 1A). Some polysaccharides were composed of the same monosaccharides but in a different ratio and showed strong capacity in improving blood lipid metabolism, liver lipid levels and antioxidant status (Yang et al., 2018).

3.2. Microstructure of HLP

The surface microscopic structure of HLP was determined by Scanning Electronic Microscopy (SEM). As shown in Fig. 1B, the HLP displayed a complex and irregular network with a few pores. The results were in agreement with the previous reports of Fu and others, which showed that polysaccharides extracted from mushroom *Inonotus obliquus* exhibited the surface of the pores covering with smooth small particles (Fu, Chen, Dong, Zhang, & Zhang, 2010). By comparison with others, different microstructure of mushroom polysaccharides can be found. The surface of *Lactarius volemus Fr.* showed ovoid-shaped particles that distributed throughout its randomly (Huang, Yu, Huang, Liu, & Liang, 2020). Moreover, the microstructure of the most mushroom and mycelial particles species turned compact and particulate aggregates to more porous and flattened fragments after ultrasound-assisted extraction (Cheung, Siu, & Wu, 2013). Therefore, the surface microscopic structure of polysaccharide was closely associated with its extraction method.

3.3. FT-IR spectra analysis of HLP

The Fourier Transform Infrared (FT-IR) spectra of *H. leucopus* polysaccharide was presented in Fig. 1C. The intense absorption band between 3600 cm^{-1} to 3200 cm^{-1} was associated with the stretching vibration of O—H. Stretch vibrations of O—H and saturated C—H are seen in the infrared spectra at 3419.2 cm^{-1} and 2931.6 cm^{-1}, respectively (Tian, Zhao, Zeng, Zhang, & Zheng, 2016). The stretching vibrations of C=O and C—O in ester and carboxyl groups were ascribed to the absorbance peaks at 1730.1 cm^{-1} and 1249.9 cm^{-1}, indicating that HLP contained uronic acid (Pu et al., 2016). The absorption band at 1026 cm-1 in the FT-IR belongs to the tensile vibration of the pyranose ring, which indicated the presence of glucan in HLP (Zhao, Gao, Wang, Liu, Jin, & Xing, 2016). However, HLP did not show any absorbance peak at this region, indicating that it did not contain uronic acid, which was consistent with the results in monosaccharides composition. The absorbance peaks at 815 cm^{-1} corresponding to the presence of D-mannose in the polysaccharide (Hu, Kong, Yang, & Pan, 2011).

3.4. HLP alleviate body weight, liver index, and epididymal fat index in in HFD-fed mice

The body weight change of mice was described in Table 3. During the 42 days study, HFD mice presented a significant increase in body weight as compared to the normal group (p < 0.01). Compared with model group, the body weight of mice in HLP-H (p < 0.05) and AT (p < 0.01) treatment were slowly increased. These results indicated that high dose of HLP treatment had capacity to inhibit the weight gain induced by HFD. However, there was no significant difference in liver index among
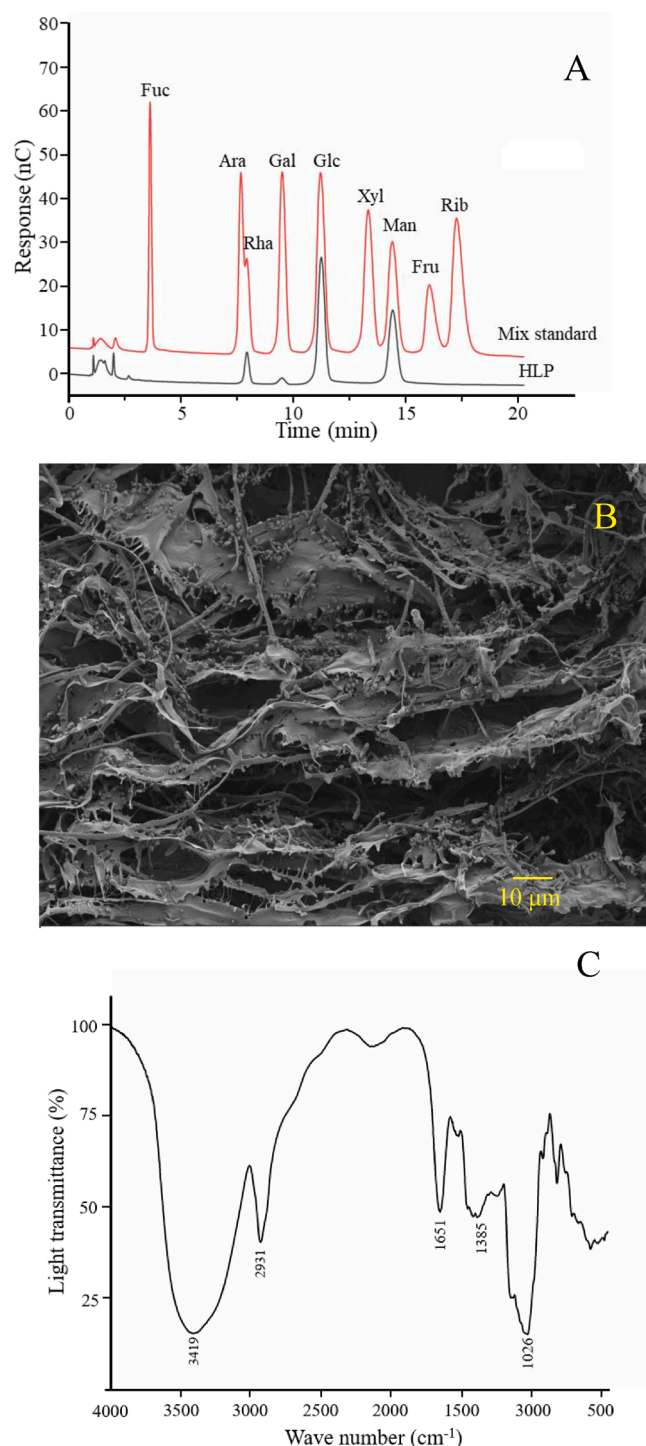
Fig. 1. The physicochemical characteristics of polysaccharide from *H. leucopus*. Typical chromatograms of mixed standards (including Fuc, Ara, Rha, Gal, Xyl, Man, Fru, and Rib) and monosaccharides composition of polysaccharide from *H. leucopus* analyzed by HPLC (A), microstructure of HLP under scanning electron microscopy: zoomed 2000 times (B), Fourier Transform Infrared spectra of HLP (C).

the six experimental groups ($p > 0.05$). HFD mice displayed a significant increase in epididymal fat index compared with the normal group ($p < 0.05$), which could be alleviated by HLP and AT treatment.

Table 3
Effect of model group with HLP on body weight, liver index, and adipose tissue.

Group	Body weight (g) in day 7	Body weight (g) in day 42	Liver index (%)	Epididymal fat weight index (%)
Normal	34.78 ± 0.83	41.93 ± 1.98^b	3.69 ± 0.17	2.28 ± 0.42^b
Model	33.62 ± 1.07	48.93 ± 2.45^a	4.01 ± 0.23	3.39 ± 0.50^a
AT	33.08 ± 0.92	43.96 ± 2.31^b	3.87 ± 0.14	2.17 ± 0.51^b
HLP-L	34.27 ± 0.48	45.26 ± 1.01^{ab}	3.96 ± 0.10	2.27 ± 0.65^b
HLP-M	33.81 ± 1.10	45.40 ± 1.40^{ab}	3.86 ± 0.17	2.12 ± 0.13^b
HLP-H	33.40 ± 1.04	43.86 ± 1.30^b	3.98 ± 0.07	1.91 ± 0.03^b

Values are expressed as means $\pm$ SD (n = 10). [b] $p < 0.05$ compare to the model group.

3.5. HLP ameliorated hyperlipidemia in HFD-fed mice

Hyperlipidemia is a dyslipidemia condition that affects lipid metabolism (Nelson, 2013). It has been found to induce oxidative stress and could cause cellular damage (Amal & Sanaa, 2012). Meanwhile, elevated levels of blood lipids are risk factors for cardiovascular disease (Nelson, 2013). Some studies showed that patients with high levels of TC, LDL-C, and TG were at increased risk of coronary heart disease (Ghosian Moghaddam, Roghani, & Maleki, 2016). In other words, the levels of serum TC and TG are important in evaluating hyperlipidemia effects. After 6 weeks in vivo animal study, the levels of TC and TG in HFD mice (Fig. 2A - B) reached up to 3.4 and 1.8 mmol/L, respectively. Supplementation with HLP at high dosages, the levels of TC and TG were decreased, close to those of control and atorvastatin treated groups. The TC can be transported to peripheral tissues leading to unnormal gather of atherosclerotic plaque lesion in the blood vessel walls by LDL-C (Wang et al., 2015). Conversely, TC also can be transferred from peripheral tissues to the liver by the HDL-C and affords potential protection against many cardiac problems (Sathivel, Raghavendran, Srinivasan, & Devaki, 2008). In this study, a marked increase in serum LDL-C level, along with a decrease in serum HDL-C level were observed in HFD induced hyperlipidemic mice. The treatment of high dose of HLP tended to delay increased level of LDL-C in serum, and the decrease level of that in serum HDL-C (Fig. 2C-D). The effect of high HLP dosage is comparable to Atorvastatin treatment which induced increases in the concentration of HDL-C correlated significantly with the reduction in serum TG. Furthermore, free fatty acids (FFA) are closely linked to blood lipid metabolism and many diseases including metabolic syndrome (Niu et al., 2012). Blood urea nitrogen (BUN) is an important index in the kidney, which can be considered as an indicator of kidney function. Model group had higher level of FFA ($p < 0.05$) and BUN ($p > 0.05$) compared with control group (Fig. 2E - F). Low dose of HLP supplementation significantly decreased serum FFA level ($p < 0.05$). All doses of HLP maintained at similar levels with control group in serum BUN. Moreover, ALT and AST enzyme activities in serum were commonly used for the investigation of early liver damage. Elevated levels of ALT and AST enzymes indicate cellular leakage and cell membrane functional integrity loss in the liver. In the present study, the levels of AST ($p < 0.01$) and ALT ($p < 0.05$) in the serum of the model group significantly increased compared to those in normal group. The administration of HLP improved the liver function by decreasing the serum AST and ALT in the model group. This indicates that HLP protected liver cell by decreasing lipid levels efficiently. Those interesting results indicated that HLP could lower the serum lipids and restore lipid metabolic disturbance induced by high fat diet through attenuating fatty acids synthesis.
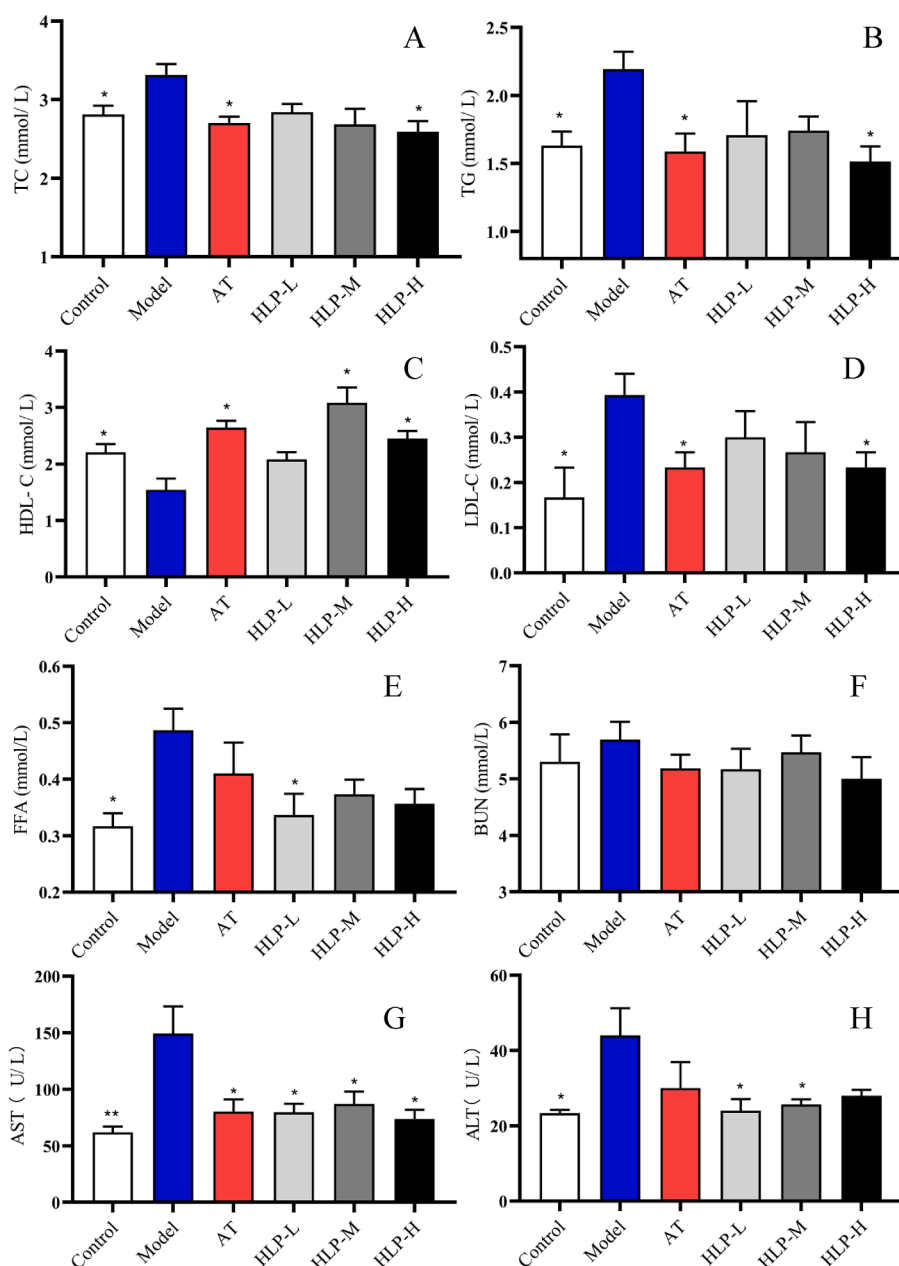
Fig. 2. Effects of HLP on serum index and hepatic function in HFD-induced hyperlipidemia mice. (A) total cholesterol (TC), (B) triglycerides TG, (C) high-density lipoprotein cholesterol HDL-C, (D) low-density lipoprotein cholesterol LDL-C, (E) free fatty acid (FFA), (F) blood urea nitrogen (BUN), (G) aspartate aminotransferase (AST), (H) alanine aminotransferase (ALT). The values were reported as the Mean $\pm$ S.D, n = 10, *$p < 0.05$, **$p < 0.01$ compared to model group.

3.6. HLP regulated mRNA expressions levels of genes involved in lipid metabolism

In order to elucidate the mechanism of hyperlipidemia effect of HLP, the hepatic mRNA expressions of some key genes involved in lipid metabolism were evaluated. PPAR was a transcription factor that could modulate tissue lipolysis and blood lipoprotein metabolism by regulating its gene expression (Bougarne et al., 2018). PPAR-α is a type of PPAR and its activation leads to the improvement of liver steatosis, inflammation, and fibrosis in mice (Staels et al., 2013). Moreover, PPAR-α is a known transcriptional regulator of CPT-1 that catalyzes the transfer of long-chain fatty acids from acyl-CoA to carnitine, allowing them to pass mitochondrial membranes. Previous study has shown that PPAR played a key role in fatty acids β-oxidation (Burri, Thoresen, & Berge, 2010). As a result, it is possible that PPAR activation could result in increasing ACS expression, allowing for more conjugated acyl-CoAs to

be used as fuels via the fatty acids β-oxidative pathway.

As depicted in Fig. 3, there was a significant decrease in PPAR-α ($p <$ 0.05), ACS ($p < 0.05$) and CPT-1α ($p < 0.01$) mRNA expression levels in the liver tissue of model group compare with normal group. In different doses of HLP treatment, the mRNA expression levels of PPAR-α were significantly up-regulated ($p < 0.05$) and closed to that of normal group. PPARα gene could regulate fatty acid oxidation and lipolysis (Kim, Jeong, Kang, Kim, Song, & Seo, 2017). Thus, the level of hepatic PPAR-α in HLP treatment mice were remarkably higher than that in the model group, implicating that HLP might reduce lipid deposition in the liver. Furthermore, HLP significantly up-regulated the CPT-1α mRNA expression ($p < 0.05$) comparing with model group while its level still lower than normal group. It could result in promoting lipolysis in HFD mice through promoting hepatic fatty acid catabolism (Guo, Zhu, Li, Feng, Wu, & Zeng, 2021). Besides, the mRNA expression levels of ACS in liver in HLP-M and HLP-H groups were higher than that in the model
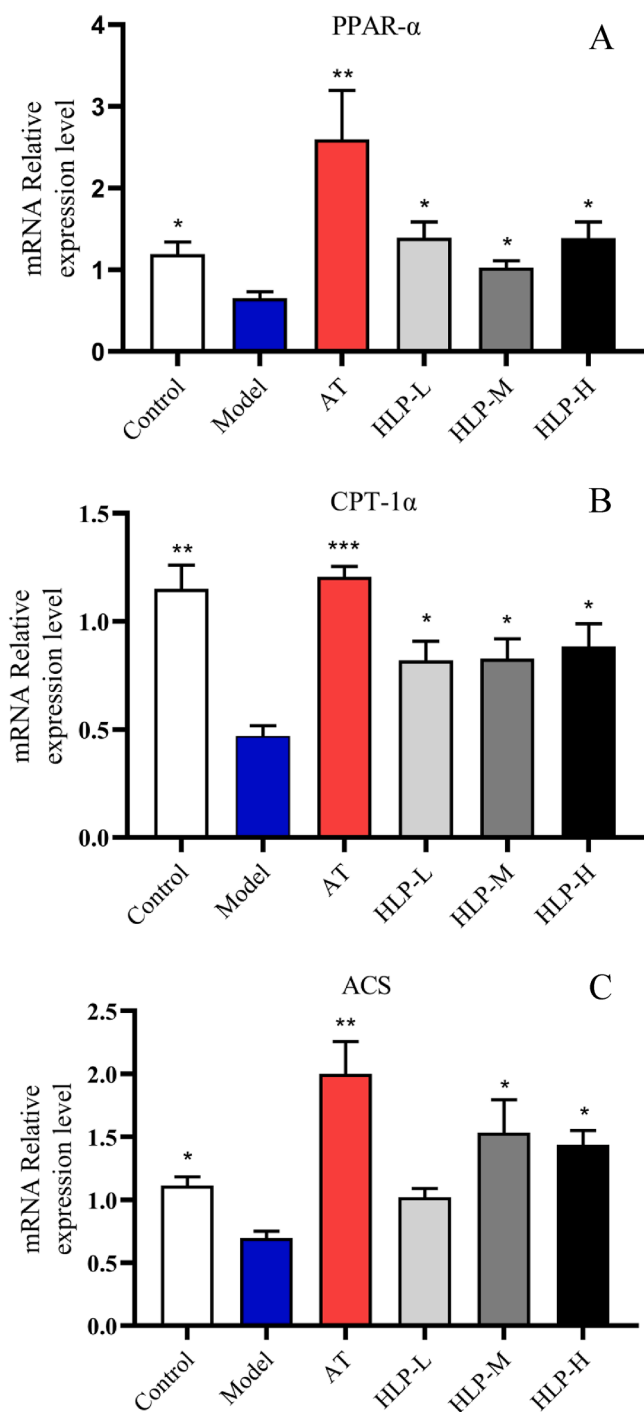
Fig. 3. Effects of HLP on hepatic expression of genes involved in fatty acid metabolism at the end of the intervention. HLP increased the gene expression of PPAR-α (A), CPT-1α (B), and ACS (C). The values were reported as the Mean ± S.D, n = 10, *p < 0.05, **p < 0.01, ***p < 0.001 compared to model group.

group. Several in vitro studies have proven the efficacy of ACSL1 in increasing TG and phospholipid production. The mRNA expression of ACSL1 in the liver is upregulated by PPAR-α ligands (Martin, Schoonjans, Lefebvre, Staels, & Auwerx, 1997).

High dosage of HLP supplementation might contribute to regulating lipid metabolism involved in genes in the liver, and finally, prevent abnormal levels of serum indexes in HFD mice. Therefore, the anti-hyperlipemia effect of HLP might be because it could decrease lipid lipogenesis and enhance fat catabolism and oxidation.

4. Conclusion

This is the first study to report the structural characteristics and anti-hyperlipidemic effect of *H. leucopus* polysaccharide (HLP). HLP was found to be a heteropolysaccharide with an average molecular weight of 7.34×10^4 Da. HLP could alleviate hyperlipidemic symptoms through relieving the body weight gain and regulating the levels of TC, TG, LDL-C, and HDL-C in serum. Furthermore, HLP could catalyze the transfer of long-chain fatty acids from acyl-CoA to carnitine by upgrading the activity and gene expression of PPAR-α, CPT-1A, and ACS in the liver. Thus, these findings could pave the foundation for the high utilization of *H. leucopus*.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

Amal, A. F., & Sanaa, T. (2012). Effect of Allium sativum extract on serum lipid and antioxidant status in hypercholesterolemic rabbits. *Life Science Journal, 9*(3), 187–196.

Ballesteros, L. F., Cerqueira, M. A., Teixeira, J. A., & Mussatto, S. I. (2018). Production and physicochemical properties of carboxymethyl cellulose films enriched with spent coffee grounds polysaccharides. *International Journal of Biological Macromolecules, 106*, 647–655. https://doi.org/10.1016/j.ijbiomac.2017.08.060

Bougarne, N., Weyers, B., Desmet, S. J., Deckers, J., Ray, D. W., Staels, B., & De Bosscher, K. J. E. r. (2018). Molecular Actions of PPAU± in Lipid Metabolism and Inflammation. *39 5*, 760-802.

Bradford, M. M. (1976). A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Analytical Biochemistry, 72*(1), 248–254. https://doi.org/10.1016/0003-2697(76)90527-3

Burri, L., Thoresen, G. H., & Berge, R. K. (2010). The Role of PPAR Activation in Liver and Muscle. *PPAR Research, 2010*, Article 542359. https://doi.org/10.1155/2010/542359

Chen, X., You, L., Ma, Y., Zhao, Z., & Kulikouskaya, V. (2021). Influence of UV/H2O2 treatment on polysaccharides from Sargassum fusiforme: Physicochemical properties and RAW 264.7 cells responses. *Food and Chemical Toxicology, 153*, Article 112246. https://doi.org/10.1016/j.fct.2021.112246

Chen, X., Zhang, R., Li, Y., Li, X., You, L., Kulikouskaya, V., & Hileuskaya, K. (2020). Degradation of polysaccharides from Sargassum fusiforme using UV/H2O2 and its effects on structural characteristics. *Carbohydrate Polymers, 230*, Article 115647. https://doi.org/10.1016/j.carbpol.2019.115647

Cheung, Y.-C., Siu, K.-C., & Wu, J.-Y. (2013). Kinetic Models for Ultrasound-Assisted Extraction of Water-Soluble Components and Polysaccharides from Medicinal Fungi. *Food and Bioprocess Technology, 6*(10), 2659–2665. https://doi.org/10.1007/s11947-012-0929-z

De Costa, G., & Park, A. (2017). Hyperlipidaemia. *Medicine, 45*(9), 579–582. https://doi.org/10.1016/j.mpmed.2017.06.002

Fu, L., Chen, H., Dong, P., Zhang, X., & Zhang, M. (2010). Effects of ultrasonic treatment on the physicochemical properties and DPPH radical scavenging activity of polysaccharides from mushroom Inonotus obliquus. Journal of food science, 75(4), C322-327. Doi: 10.1111/j.1750-3841.2010.01590.x.

Ghosian Moghaddam, M. H., Roghani, M., & Maleki, M. (2016). Effect of Hypericum perforatum Aqueous Extracts on Serum Lipids, Aminotransferases, and Lipid Peroxidation in Hyperlipidemic Rats. *Research in Cardiovascular Medicine, 5*(2), e31326–e. https://doi.org/10.5812/cardiovascmed.31326

Guo, W., Zhu, S., Li, S., Feng, Y., Wu, H., & Zeng, M. (2021). Microalgae polysaccharides ameliorates obesity in association with modulation of lipid metabolism and gut microbiota in high-fat-diet fed C57BL/6 mice. *International Journal of Biological Macromolecules, 182*, 1371–1383. https://doi.org/10.1016/j.ijbiomac.2021.05.067

Harvey, A. L., Edrada-Ebel, R., & Quinn, R. J. (2015). The re-emergence of natural products for drug discovery in the genomics era. *Nature Reviews Drug Discovery, 14*(2), 111–129. https://doi.org/10.1038/nrd4510

Hou, X., & Chen, W. (2008). Optimization of extraction process of crude polysaccharides from wild edible BaChu mushroom by response surface methodology. *Carbohydrate Polymers, 72*(1), 67–74. https://doi.org/10.1016/j.carbpol.2007.07.034

Hou, X., Zhang, N., Xiong, S., Li, S., & Yang, B. (2008). Extraction of BaChu mushroom polysaccharides and preparation of a compound beverage. *Carbohydrate Polymers, 73*(2), 289–294. https://doi.org/10.1016/j.carbpol.2007.11.033

Hu, C., Kong, Q., Yang, D., & Pan, Y. (2011). Isolation and structural characterization of a novel galactomannan from Eremurus anisopterus (Ker. et Kir) Regel roots. *Carbohydrate Polymers, 84*(1), 402–406. https://doi.org/10.1016/j.carbpol.2010.11.054

Huang, G., Chen, F., Yang, W., & Huang, H. (2021). Preparation, deproteinization and comparison of bioactive polysaccharides. *Trends in Food Science & Technology, 109*, 564–568. https://doi.org/10.1016/j.tifs.2021.01.038

Huang, Y.-Y., Yu, J.-J., Huang, J., Liu, D.-M., & Liang, M.-H. (2020). Structural characterization of a novel Lactarius volemus Fr. polysaccharide and its immunity activity in BALB/c mice. *RSC Advances, 10*(51), 30254–30264. https://doi.org/10.1039/D0RA05176D

Hyde, Kevin, ., D., Zhao, Qi, Zhu, . . . Sulayman. (2016). Species clarification of the culinary Bachu mushroom in western China. *Mycologia*.

Ismail, T. A., Soliman, M. M., Nassan, M. A., & Mohamed, D. I. (2015). Antihypercholesterolemic Effects of Mushroom, Chrysin, Curcumin and Omega-3 in Experimental Hypercholesterolemic Rats. *Journal of Food Nutrition Research, 3*(2), 77–87. http://pubs.sciepub.com/jfnr/3/2/1.

Kim, D.-H., Jeong, D., Kang, I.-B., Kim, H., Song, K.-Y., & Seo, K.-H. (2017). Dual function of Lactobacillus kefiri DH5 in preventing high-fat-diet-induced obesity: Direct reduction of cholesterol and upregulation of PPAR-α in adipose tissue. *Molecular Nutrition & Food Research, 61*(11), 1700252. https://doi.org/10.1002/mnfr.201700252

Li, Y., Chen, D., Xu, C., Zhao, Q., Ma, Y., Zhao, S., & Chen, C. (2020). Glycolipid metabolism and liver transcriptomic analysis of the therapeutic effects of pressed degreased walnut meal extracts on type 2 diabetes mellitus rats. *Food & Function, 11*(6), 5538–5552. https://doi.org/10.1039/D0FO00670J

Manzi, P., Aguzzi, A., & Pizzoferrato, L. (2001). Nutritional value of mushrooms widely consumed in Italy. *Food Chemistry, 73*(3), 321–325. https://doi.org/10.1016/S0308-8146(00)00304-6

Martin, G., Schoonjans, K., Lefebvre, A. M., Staels, B., & Auwerx, J. (1997). Coordinate regulation of the expression of the fatty acid transport protein and acyl-CoA synthetase genes by PPARalpha and PPARgamma activators. *The Journal of Biological Chemistry, 272*(45), 28210–28217. https://doi.org/10.1074/jbc.272.45.28210

Nelson, R. H. (2013). Hyperlipidemia as a risk factor for cardiovascular disease. *Primary Care, 40*(1), 195–211. https://doi.org/10.1016/j.pop.2012.11.003

Niu, Y., Li, S., Na, L., Feng, R., Liu, L., Li, Y., & Sun, C. (2012). Mangiferin Decreases Plasma Free Fatty Acids through Promoting Its Catabolism in Liver by Activation of AMPK. *PLOS ONE, 7*(1), e30782.

Pu, X., Ma, X., Liu, L., Ren, J., Li, H., Li, X., … Fan, W. (2016). Structural characterization and antioxidant activity in vitro of polysaccharides from angelica and astragalus. *Carbohydrate Polymers, 137*, 154–164. https://doi.org/10.1016/j.carbpol.2015.10.053

Sathivel, A., Raghavendran, H. R. B., Srinivasan, P., & Devaki, T. (2008). Anti-peroxidative and anti-hyperlipidemic nature of Ulva lactuca crude polysaccharide on d-galactosamine induced hepatitis in rats. *Food Chemical Toxicology, 46*(10), 3262–3267.

Sawangwan, T., Wansanit, W., Pattani, L., & Noysang, C. (2018). Study of prebiotic properties from edible mushroom extraction. *Agriculture and Natural Resources, 52*(6), 519–524. https://doi.org/10.1016/j.anres.2018.11.020

Staels, B., Rubenstrunk, A., Noel, B., Rigou, G., Delataille, P., Millatt, L. J., … Hanf, R. (2013). Hepatoprotective effects of the dual peroxisome proliferator-activated receptor alpha/delta agonist, GFT505, in rodent models of nonalcoholic fatty liver disease/nonalcoholic steatohepatitis. *Hepatology, 58*(6), 1941–1952. https://doi.org/10.1002/hep.26461

Tian, Y., Zhao, Y., Zeng, H., Zhang, Y., & Zheng, B. (2016). Structural characterization of a novel neutral polysaccharide from Lentinus giganteus and its antitumor activity through inducing apoptosis. *Carbohydrate Polymers, 154*, 231–240. https://doi.org/10.1016/j.carbpol.2016.08.059

Wang, L., Xu, N., Zhang, J., Zhao, H., Lin, L., Jia, S., & Jia, L. (2015). Antihyperlipidemic and hepatoprotective activities of residue polysaccharide from Cordyceps militaris SU-12. *Carbohydrate Polymers, 131*, 355–362.

Xu, N., Ren, Z., Zhang, J., Song, X., Gao, Z., Jing, H., … Jia, L. (2017). Antioxidant and anti-hyperlipidemic effects of mycelia zinc polysaccharides by Pleurotus eryngii var. tuoliensis. *International Journal of Biological Macromolecules, 95*, 204–214.

Yang, B., Jiang, Y., Zhao, M., Shi, J., & Wang, L. (2008). Effects of ultrasonic extraction on the physical and chemical properties of polysaccharides from longan fruit pericarp. *Polymer Degradation and Stability, 93*(1), 268–272. https://doi.org/10.1016/j.polymdegradstab.2007.09.007

Yang, M., Belwal, T., Devkota, H. P., Li, L., & Luo, Z. (2019). Trends of utilizing mushroom polysaccharides (MPs) as potent nutraceutical components in food and medicine: A comprehensive review. *Trends in Food Science & Technology, 92*, 94–110. https://doi.org/10.1016/j.tifs.2019.08.009

Yang, Z., Wang, J., Li, J., Xiong, L., Chen, H., Liu, X., … Wang, W. (2018). Antihyperlipidemic and hepatoprotective activities of polysaccharide fraction from Cyclocarya paliurus in high-fat emulsion-induced hyperlipidaemic mice. *Carbohydrate Polymers, 183*, 11–20. https://doi.org/10.1016/j.carbpol.2017.11.033

Ye, Y., Ji, D., You, L., Zhou, L., Zhao, Z., & Brennan, C. (2018). Structural properties and protective effect of Sargassum fusiforme polysaccharides against ultraviolet B radiation in hairless Kun Ming mice. *Journal of Functional Foods, 43*, 8–16. https://doi.org/10.1016/j.jff.2018.01.025

Zeng, D., & Zhu, S. (2018). Purification, characterization, antioxidant and anticancer activities of novel polysaccharides extracted from Bachu mushroom. *International Journal of Biological Macromolecules, 107*, 1086–1092. https://doi.org/10.1016/j.ijbiomac.2017.09.088

Zhang, W., Gong, L., Liu, Y., Zhou, Z., Wan, C., Xu, J., … Chen, Y. (2020). Immunoenhancement effect of crude polysaccharides of Helvella leucopus on cyclophosphamide-induced immunosuppressive mice. *Journal of Functional Foods, 69*, Article 103942. https://doi.org/10.1016/j.jff.2020.103942

Zhao, C., Gao, L., Wang, C., Liu, B., Jin, Y., & Xing, Z. (2016). Structural characterization and antiviral activity of a novel heteropolysaccharide isolated from Grifola frondosa against enterovirus 71. *Carbohydrate Polymers, 144*, 382–389. https://doi.org/10.1016/j.carbpol.2015.12.005