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Modulation of gut microbiota and lipid metabolism in rats fed high-fat diets by *Ganoderma lucidum* triterpenoids

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ABSTRACT

Ganoderma lucidum triterpenoids (GP) have been reported to help prevent and improve hyperlipidemia. Modulation of the gut microbiota was proposed as underlying factor as well as a novel measure to prevent and treat hyperlipidemia. The effects of GP on high-fat diet (HFD)-induced hyperlipidemia and gut microbiota modulation were determined in rats. Ultra-performance liquid chromatography tandem quadrupole time-of-flight mass spectrometry (UPLC-QTOF MS-MS) indicated that GP were enriched with ganoderic acids G, B, H, A, and F. After feeding with GP supplementation, serum lipid levels including total triglyceride, total cholesterol, and low-density-lipoprotein cholesterol were significantly decreased in hyperlipidemic rats. Furthermore, administration of GP also has reversed the HFD-induced gut microbiota dysbiosis, including a significant increase in *Alloprevotella* and reduced proportion of *Blautia.* The result above suggests that GP would be developed as a functional food to ameliorate lipid metabolic disorders and hyperlipidemia.

1. Introduction

With the improvement of living standards, the prevalence of associated diseases caused by lipid metabolism disorders (LMD) has rapidly increased (Jia et al., 2021; Li et al., 2019). LMD are closely associated with obesity, hyperlipidemia, hyperglycemia, hypertension and fatty liver (Dłubek et al., 2021). Although significant progress has been achieved in the knowledge and development of hypolipidemic medications, side effects have also become apparent (Olzmann and Carvalho, 2019). Currently used hypolipidemic drugs administered to regulate LMD are suboptimal as they may cause liver or kidney damage, adverse gastrointestinal reactions, and organismic and other side effects of drug resistance (Elagina et al., 2020). As natural cholesterol-lowering active food ingredients are urgently needed, functional compounds isolated from natural foods are increasingly acknowledged to alleviate LMD (Li et al., 2019; Yang et al., 2020; Zhang et al., 2022).

The gut microbiome comprises a plethora of bacteria, viruses, archaea, fungi, and other eukaryotes inhabiting the human gut (Anto and Blesso, 2022). The total amount of gut microbiota genes exceeds that of human genes by 100-fold and is also termed the “second genome” of humans (Zhu et al., 2010). In the recent past, the gut microbiota appears to play a function in the development of LMD, according to growing research (Li et al., 2021; Tao et al., 2021; Yu et al., 2021). In the human gut, the Bacteroidetes and Firmicutes predominate, with a lower proportion of Bacteroidetes in obese persons (Ley et al., 2006). Long-term consumption of high-sugar, high-fat, and low-fiber diets can lead to increased accumulation of intestinal lipid peroxidation products (Aron-Wisnewsky et al., 2021; Deiana et al., 2017). Changes in the composition of the gut microbiota and its metabolites are the significant indicators of obesity and metabolic illnesses (Le et al., 2013; Seganfredo

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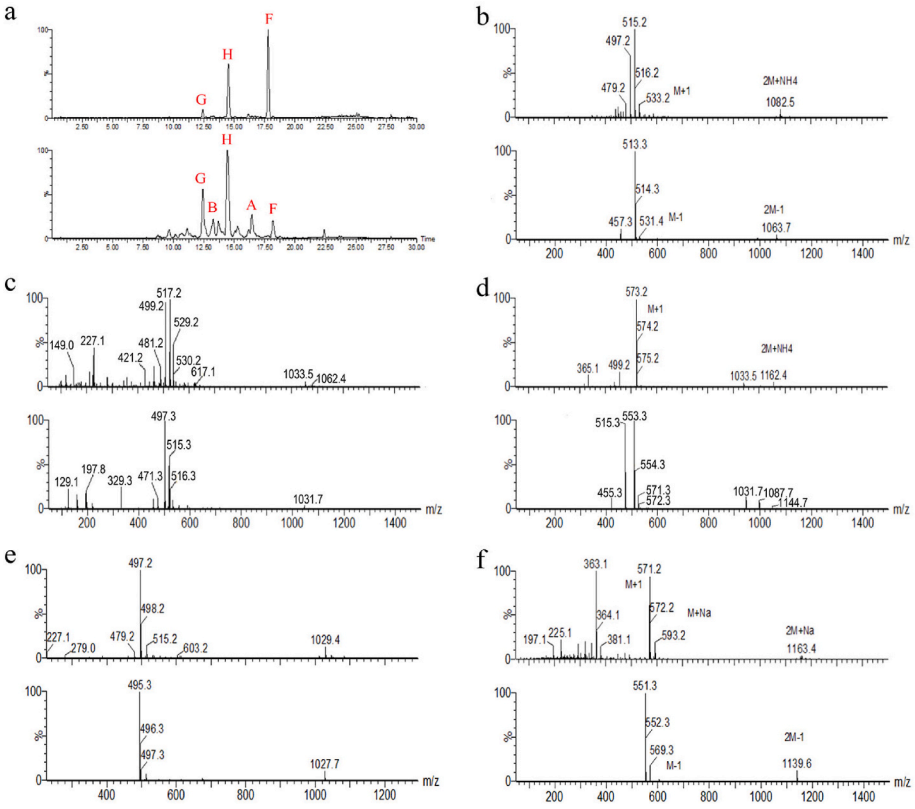

Fig. 1. UPLC-QTOF MS-MS analysis of GP identified nine major components. (a) UPLC chromatograms of 80% ethanol extract content a: $(M + H)^+$; b: $(M\text{-}H)^-$. (b–f) Mass spectrum identification results of GP (b: ganoderic acid G; c: ganoderic acid B; d: ganoderic acid H; e: ganoderic acid A; f: ganoderic acid F).

et al., 2017; Stanislawski et al., 2019). The prevalence of specific bacterial genera is positively connected with high-density lipoprotein levels but adversely correlated with body mass index (BMI) and triglyceride levels (Bock et al., 2021). Recent studies showed that the abundance of *Akkermansia* was closely related to LMD, glucose metabolism and immune response, which could improve LMD and prevent the occurrence of obesity and diabetes (Rao et al., 2021). *Bifidobacterium* can improve glucose tolerance in people with hyperglycemia or other metabolic disorders (Ming et al., 2021). Many studies have reported that the metabolic diseases were improve by regulating intestinal flora with natural active substances (Li et al., 2018; Oluwajuyitan et al., 2022; Wan et al., 2018; Yang et al., 2022). Therefore, research on health effects of gut microbiota is of considerable importance.

Ganoderma lucidum (*G. lucidum*) is one of the main edible and medicinal fungi produced at large scales in China. It is rich in active compounds including polysaccharides, triterpenes, lipids, polypeptides, proteins, alkaloids, lactones, coumarins, and trace elements (Berovic et al., 2003; Boh et al., 2007). *G. lucidum* can improve pancreatic blood circulation, reduce blood glucose, and improve the symptoms of diabetic patients, as well as reduce blood lipids and improve various symptoms of hyperlipidemia (Wang et al., 2020). *G. lucidum* triterpenoids (GP) compounds are a class of important bioactive ingredients isolated from *G. lucidum*, which exert some biological activities and show great potential in the pharmaceutical and healthcare industries (Chen et al., 2022; Guo et al., 2022; Zhao et al., 2019). Recent pharmacological studies have shown that GP exert various pharmacological activities, such as antitumor, antibacterial, antiviral, antioxidative, immune-regulative, liver-protective effects, in addition to regulating blood sugar and blood lipids (Geng et al., 2019; Li et al., 2021; Liu et al., 2020; Su et al., 2020; Zeng et al., 2021). GP have a very complex structure, and their molecular weight is generally 400–600 Da. GP mainly include *Ganoderma* acids A, C2, D, and F, among others (Liang et al., 2019). Triterpenoids can affect the absorption of cholesterol and prevent its synthesis (Min et al., 1998). At present, research on the biological activity of GP has made some achievements in different countries. The active components *Ganoderma* acid R and S can significantly reduce blood lipid levels in rats fed a HFD (Li et al., 2006). GP can also act as potential prebiotics in the gut, where they improve the abundance of *Bifidobacterium* and *Lactobacillus* in the intestines and regulate the metabolites of intestinal microorganisms such as amino acids to counteract LMDs (Chen et al., 2020). In this study, an HFD-fed rat model was used to explore hypolipidemic effects of GP. Serum parameters and fecal and intestinal content's structure were investigated with respect to LMD-associated parameters. Possible reasons for enhanced bioactivity of GP under the biotransformation of gut microbiota are discussed.

2. Materials and methods

2.1. UPLC-TOF-MS analysis of GP

G. lucidum was extracted with 80% (v/v) ethanol that refer to the previous extraction process (Guo et al., 2018; Hu et al., 2018). LC-MS analysis were conducted on an acquity UPLC system (Waters, Milford, MA, USA) coupled with MALDI SYNAPT Q-TOF mass spectrometer (Waters). The mass spectrometer and UPLC system were operated using MassLynx 4.1 software (Waters). Analyzer with C18 column (1.7 μm; 2.1 × 100 mm) (Macherey-Nagel, CA, USA) was employed for dissolution. At a flow rate of 0.3 mL/min (A: 0.1% formic acid in water, B: acetonitrile). The column temperature, and the amount of sample input were 45 °C and 1.0 μL, respectively. The scan range was 50–1000 m/z with 100 °C ion source temperature of, 300 °C desolvation temperature, 45 V cone voltage, capillary voltage 3.5 kV at ESI+, and 500 L/h nebulization gas flow.

Table 1
The results of UPLC-TOF-MS analysis of GP.

Peak no.	Rt (min)	Molecular formula	Mass data (m/z)	Identification	Reference
1	9.66	–	–	Unknown	Guo et al. (2012); Hu et al. (2018); Guo et al. (2018)
2	11.11	–	–	Unknown	
3	12.41	$C_{30}H_{44}O_8$	533.2 $[M+H]^+$,1082.5 $[2M + NH_4]^+$,531.4$[M-H]^-$, 513.3$[M-H-H_2O_2]^-$,1063.7 $[2M-H]^-$	Ganoderic acid G	
4	13.26	$C_{30}H_{44}O_7$	517.2 $[M+H]^+$,1033.5 $[2M + H]^+$,515.3 $[M-H]^-$, 497.3$[M-H-H_2O_2]^-$,1031.7 $[2M-H]^-$	Ganoderic acid B	
5	13.68	–	–	Unknown	
6	14.44	$C_{32}H_{44}O_9$	573.2 $[M+H]^+$,1162.4 $[2M + NH4]^+$,571.3$[M-H]^-$, 555.3$[M-H-H_2O_2]^-$,1144.7 $[2M-H]^-$	Ganoderic acid H	
7	15.27	–	–	Unknown	
8	16.44	$C_{30}H_{44}O_7$	515.2 $[M+H]^+$,1029.4 $[2M + H]^+$,495.3 $[M-H-H_2O_2]^-$,1027.7 $[2M-H]^-$	Ganoderic acid A	
9	18.17	$C_{32}H_{42}O_9$	571.2 $[M+H]^+$,593.2 $[M+Na]^+$,1163.4 $[2M + Na]^+$, 569.3$[M-H]^-$,551.3$[M-H-H_2O_2]^-$,1139.6 $[2M-H]^-$	Ganoderic acid F	

2.2. Experimental animals

There were 6 weeks old, 170 ± 20 g healthy Wistar rats from the Shandong Laboratory Animal Center (Jinan, China). The rats were randomly assigned to 6 treatment groups included NFD group (normal-fat diet, n = 8), HFD group (n = 8), GP group (HFD-fed rats treated with GP = 50, 100, 150 mg/kg/day, n = 8, in each), and Sym group (HFD-fed rats treated with silymarin = 30 mg/kg/day, n = 8).

2.3. Serum biochemical assays

At the 0th and 4th weeks of the study period, whole blood samples were taken via orbital blood draw using a capillary without anesthesia following 12 h fast. At the 8th week of the experiment, the rats were fasted and anesthetized, and the blood was collected from the heart of the rats. The obtained blood was centrifuged at 3500 rpm and 4 °C for 10 min to separate the serum. The obtained serum samples were stored at −80 °C until analysis. The levels of triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), alanine aminotransferase (ALT), aspartate aminotransferase (AST), free fatty acid (FFA) in rats' serum using the assay kits (Jiancheng Institute of Biotechnology, Nanjing, China).

2.4. Gut microbiota analysis

Fecal samples (0, 4 and 8 weeks) were collected. At least three fecal pellets were collected directly from the rectum of each rat, were placed in a sterile conical tube, and were immediately frozen at −80 °C until further analysis. Intestinal contents from eight rats in HFD group and NFD group were detected. Five rats were randomly selected from each experimental group for analysis of intestinal contents. Total genome DNA from samples was extracted using cetyltrimethylammonium bromide (CTAB) method. A fragment of the 16s rRNA gene (V3–V4 hypervariable regions) of the fecal and intestinal contents were amplified using specific primers (forward 5′-CCTACGGRRBGCASCAGKVRVGAAT-3′; reverse 5′-GGACTACNVGGGTWTCTAATCC-3′). Paired-end sequencing (2 × 300 bp) was performed using a MiSeq platform (Illumina, San Diego, CA, USA), and amplification bias caused by a non-official barcode was avoided. Image analysis and base calling were conducted using MiSeq control software on the MiSeq instrument. Initial taxonomy analysis was performed using Illumina's BaseSpace cloud computing platform.

2.5. Bioinformatic analyses

Sequences were filtered using Usearch (ver. 7.1, http://drive5.com/uparse/) with 3% disagreement. In order to study the species composition diversity information of the samples, for the valid sequences of all samples, the sequences were clustered into OTUs with 97% similarity. Alpha and beta diversity were used to assess the intestinal microbial composition with R software (ver. 4.1.3). The relative abundance of microorganisms in different phylum and genus were analyzed by OmicStudio (https://www.omicstudio.cn/tool). Predict the intestinal microbial functional features by Picrust (ver. 2.0). Correlation analysis through R software and visualized by network through Cytoscape (ver. 3.6.0). Relationships between gut microbiota composition and biochemical indicators in serum were determined using the Spearman's rank correlation method.

2.6. Statistical analyses

The data were expressed as means ± SD. Statistical significance was measured using one-way analysis of variance (ANOVA) and Tukey's correction. Statistics were calculated with the GraphPad Prism 7 software package. Differences were considered statistically significant at $p < 0.05$, $p < 0.01$.

3. Results

3.1. Compound analysis of GP

The UPLC-QTOF MS-MS analysis identified nine major components as shown in Fig. 1 and Table 1. The specific structure of each peak was determined by comparing those MS spectral data reported in the previous studies. Five of the detected compounds were ganoderic acids, including ganoderic acids G (peak 3), B (peak 4), H (peak 6), A (peak 8), and F (peak 9).

3.2. Effect of GP on serum lipids parameters

GP administration reduced the levels of TG, TC, and LDL-C compared with the respective results of HFD rats (Fig. 2a and b, & **2d**) ($p < 0.01$). GP150 rats with decreased serum TG, TC, and LDL-C levels (by 57.23%, 49.41%, and 19.94%, respectively) compared to the HFD group after 8 weeks of the experiment. Serum HDL-C levels of GP rats were higher than those of HFD rats (Fig. 2c). GP treatment reduced the activities of serum AST (11.25%, 18.96%, and 30.15% at dosage of 50, 100, and 150 mg/kg, respectively) and ALT (Fig. 2e and **f**). Moreover, GP treatment also reduced FFA levels by 14.26%, 19.56% and 20.29% at dosage of 50, 100, and 150 mg/kg, respectively (Fig. 2g). The results showed that GP administration markedly improved serum lipid profiles.

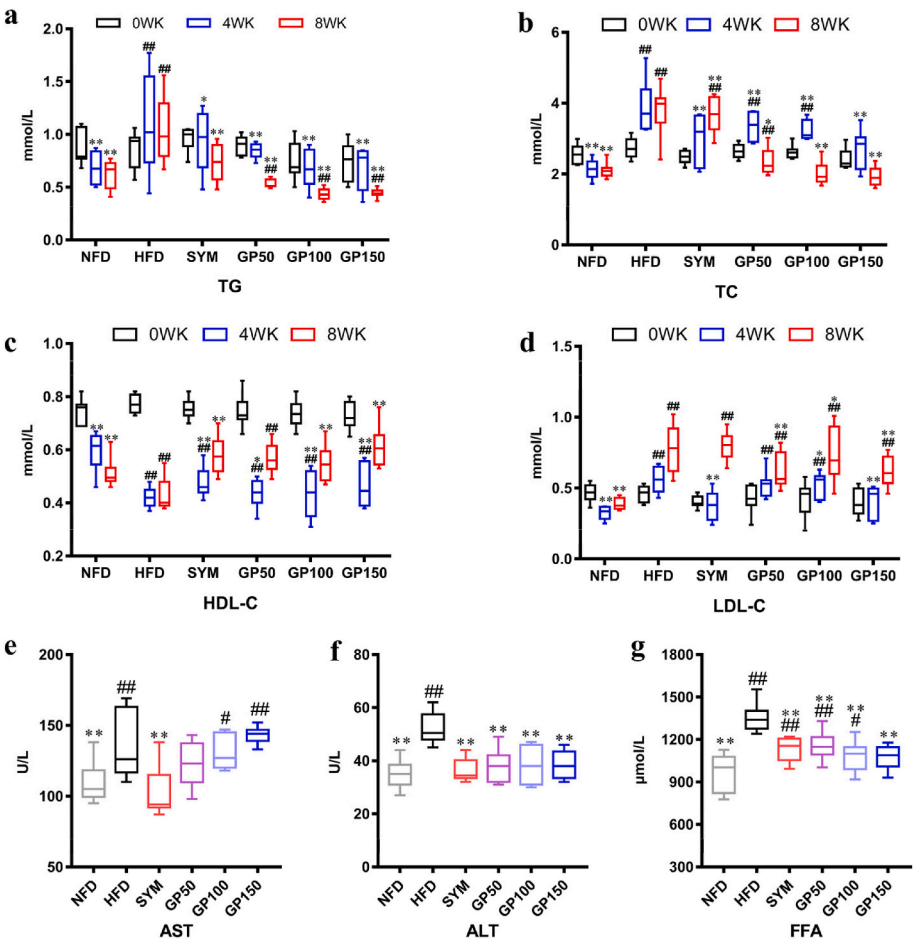

Fig. 2. Serum biological indicators of rats and histopathological analysis of liver tissue. (a) TG; (b) TC; (c) HDL-C; (d) LDL-C; (e) AST; (f) ALT; (g) FFA. The differences were assessed by ANOVA and denoted as follows: $^{*}p < 0.05$ versus the NFD group, and $^{\#}p < 0.05$ versus the HFD group; $^{**}p < 0.01$ versus the NFD group; and $^{\#\#}p < 0.01$ versus the HFD group.

3.3. GP modulates general structural changes in the intestinal contents in HFD rats

To compare bacterial diversity and species richness among treatment groups, alpha diversity indices were calculated (Fig. 3a). ACE and Chao1 indices were significantly increased in the HFD group compared with the NFD group ($p < 0.01$). HFD diet intake significantly decreased the diversity of gut microbiota with respect to Shannon indices, compared with the NFD group ($p < 0.01$). The microbial richness and diversity in the GP administration group remarkably increased diversity of gut microbiota in terms of Shannon index compared with high-fat diet intake ($p < 0.01$). Compared with the NFD group, different doses of GP helped maintain the diversity of intestinal microbial to a certain extent. The overall structure of the gut microbiota in the GP-treated groups significantly differed from that of HFD group, as analyzed by both Principal coordinate analysis (PCA), principal coordinate analysis (PCoA) and Non-metric multidimensional scaling (NMDS). Taxon-based analysis revealed marked changes in the gut microbial composition in response to GP treatment. PCA shown in Fig. 3b, the NFD group clustered separately from the HFD group, with clear differences in microbial communities; the first two axes explained 49% of the variation among groups. Compared with NFD rats, the microbial community of rats receiving GP at 50 and 150 mg/kg shifted to the NFD group along the first axis, which changed the structure and distribution of the intestinal flora. The overall structure of the gut microbiota in all the GP-treated rats significantly differed from that of HFD group, as analyzed by PCoA (Fig. 3c). The GP150 group was distant from the HFD group along PCoA1 and PCoA2, and it was closer to the NFD group, which indicated a significant change in the intestinal flora. NMDS analysis was performed to further examine differences in microbiota diversity between treatment groups; the results show are shown in Fig. 3d. A marked separation occurred between NFD and HFD rats, and differences in microbial communities were pronounced. Compared with NFD group, the microbial community of rats receiving GP at 50, 100 and 150 mg/kg shifted to the NFD group along MDS1 and MDS2, which changed the structure and distribution of the intestinal flora. These results showed significant differences in the gut microbiota between HFD and NFD rats. GP treatment helped regulate the species structure of gut microbiota and reduced differences in the gut microbiota between HFD and NFD rats.

3.4. GP modulates the intestinal contents at different taxonomic levels

Changes in the microbial community were assessed at the phylum, class, order, family, and genus levels (Fig. 4a) by comparing the relative abundances of bacterial taxa between groups after eight weeks of treatment. In all groups, Firmicutes, Bacteroidetes, Proteobacteria, and Actinobacteria were at the phylum level dominant (Fig. 4b). In addition, in the GP group, the abundances of Bacteroidetes were significant increased and Firmicutes was decreased compared to the HFD group. At the genus level, *Blautia*, *Desulfovibrio*, *Roseburia*, *Prevotella*, *Oscillibacter*, and *Alloprevotella* were the most abundant genera in all groups (Fig. 4c). Rats in the HFD group presented significantly higher abundances of *Blautia* and *Roseburia*, and significantly lower abundances of *Parasutterella*, *Oscillibacter*, and *Alloprevotella*. However, in the GP groups, the

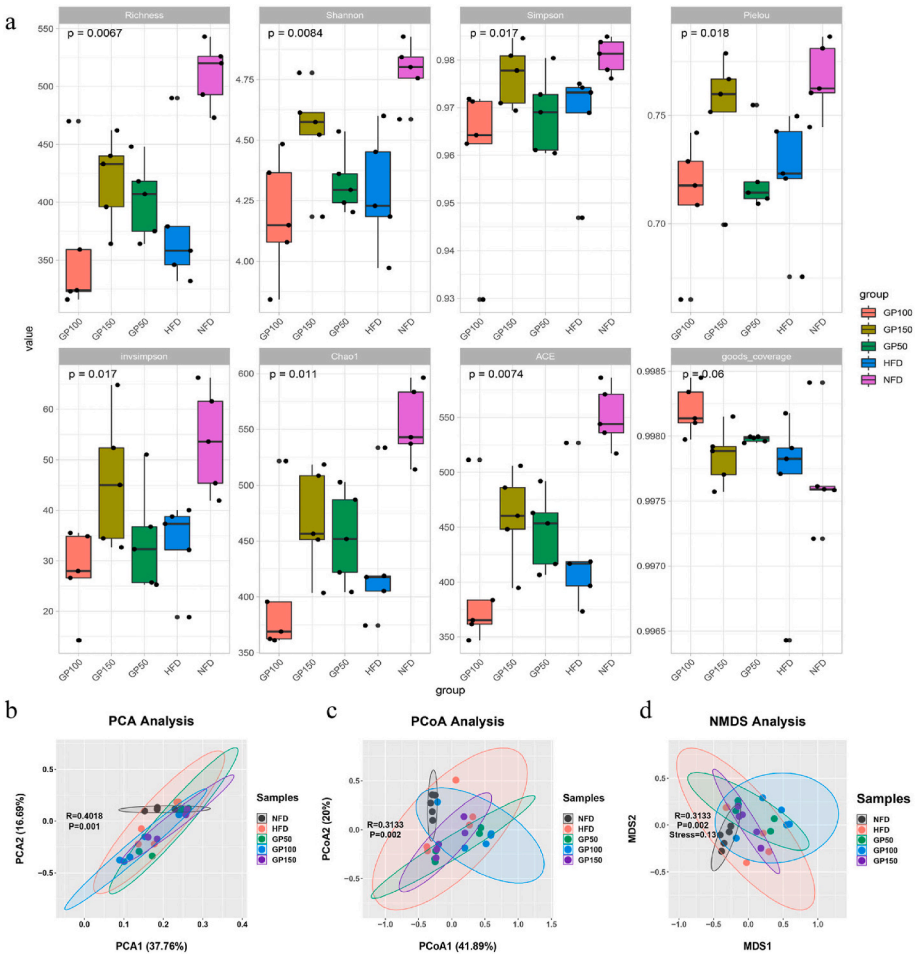

Fig. 3. Effect of GP administration on the structure of the gut microbiota. Five rats were randomly selected from each experimental group for analysis of intestinal contents. (a) Alpha diversity; (b, c) beta diversity as evaluated by PCA and PCoA score plots; (d) NMDS: Non-metric multidimensional scaling, based on Bray-Curtis distances. Each point represents one individual sample, and coloration indicates the treatment group.

abundances of *Parasutterella*, *Oscillibacter* and *Alloprevotella* were like those in the NFD group. GP prevented HFD-induced changes regarding *Blautia* and *Roseburia* abundance to a large extent.

3.5. Effects of GP on fecal microbiota composition

The rat feces at 0, 4, and 8 weeks were collected, and fecal microbial composition was analyzed at different time points to assess the effects of GP. At the genus level revealed that the HFD group changed the composition of fecal microbiota, and GP treatment prevented these effects to some extent. The diversity of individual fecal microbes was generally lower in the HFD group than in the NFD group, and HFD changed the structure of the intestinal microbiomes in rats with increasing HFD treatment time. At the phylum level, Firmicutes and Bacteroidetes were the dominant bacteria, accounting for >90% of the total gut microbiota. The abundance of Firmicutes was significantly lower in the NFD group than in the HFD group. Through 8 weeks of GP treatment, the abundance of Firmicutes decreased and the abundance of Bacteroidetes gradually increased in the GP group compared with the HFD group (Fig. 5a). At the genus level, the abundance of *Oscillibacter*, *Alloprevotella*, and *Parasutterella* gradually increased, and the abundance of *Roseburia* and *Blautia* decreased in the GP group compared with the HFD group (Fig. 5b). Differences between fecal microbiota of the NFD and HFD groups after eight weeks of treatment are shown in Fig. 5b. These results were similar to those of intestinal contents. These results suggested that HFD feeding could dysregulate gut microbiota distribution, while GP treatment could partially restore the microbiota distribution to the level of the NFD group.

3.6. Prediction of microbial metabolic functions from intestinal content

PICRUSt analysis of predictive functions of the intestinal contents was carried out, and the top 12 metabolism category in each group are shown in Fig. S1. Based on the KEGG database, the differential metabolic categories between the NFD, HFD, and GP150 groups were selected. The functional modules differed between GP150 and NFD rats, including flagellar assembly (ko 02040), fatty acid biosynthesis (ko 00061), lipopolysaccharide biosynthesis (ko 00540), peptidoglycan biosynthesis (ko 00550). Additionally, compared with the NFD group, HFD intervention significantly altered the relative abundances of the secondary bile acid biosynthesis (ko 00121), methane metabolism (ko 00540), flagellar assembly (ko 02040), pyruvate metabolism (ko 00620), among others.

3.7. Correlations of serum parameters and intestinal contents

Spearman's correlation analysis indicated that *Alistipes*, *Corynebacterium*, *Peptococcus*, *Clostridium*_sensu_stricto_1, and *Bilophila* were negatively linked with serum TG, TC, LDL-C, ALT, AST, and FFA, but positively linked with HDL-C. *Holdemania*, *Phascolarctobacterium*, *Anaerofustis*, *Streptococcus*, and *Megamonas* have the opposite effect with serum HDL-C, but the similar effect with TG, TC, LDL-C, ALT, AST, and FFA. *Barnesiella* was negatively linked with serum TC, and *Blautia* was positively linked with serum TC (Fig. 6).

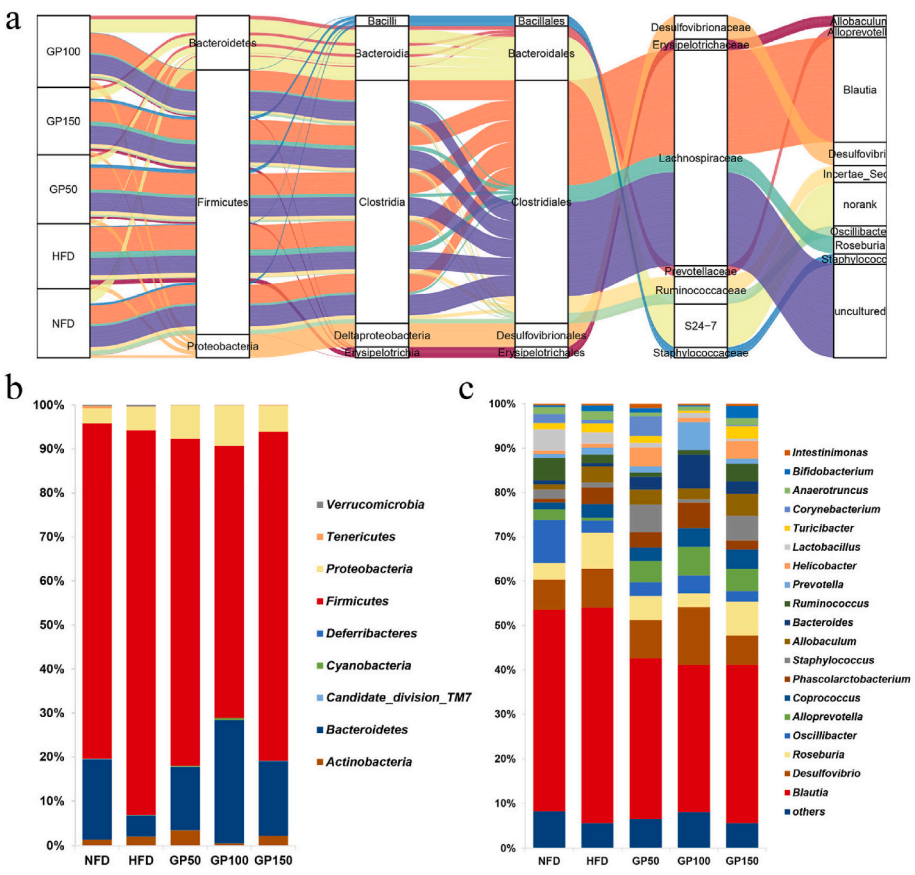

Fig. 4. Effects of GP treatment on the intestinal contents by Sankey diagram and Heatmap. Five mice were randomly selected from each group for analysis of intestinal contents. (a) Bacterial composition at the phylum, class, order, family, and genus level. (b) Bacterial composition at phylum level; (c) Bacterial composition at genus level.

4. Discussion

Hyperlipidemia is becoming one of the most health-threatening diseases in the world. Therefore, there is still an urgent need to develop effective and safe therapeutic agents. In experimental models in rats, GP are competitors due to their effective lipid-lowering effects and safety. In the study, GP administration can prevent hyperlipidemia. The results provide that gavage of different doses of GP had a certain effect on maintaining the structure and function of gut microecology in HFD rats. The main symptoms of hyperlipidemia are elevated serum levels of TC, TG and LDL-C and decreased HDL-C. Therefore, these biochemical indicators of TC, TG, LDL-C and HDL-C can be used to determine dyslipidemia (Ajeigbe et al., 2022; Jia et al., 2020). ALT and AST levels are mainly used to detect the degree of liver cell injury. After liver cell injury, ALT first enters the blood, and AST will do so with the aggravation of liver cell injury. Therefore, ALT and AST levels of patients with fatty liver are significantly increased (Do et al., 2021). ALT and AST elevations less than 5 times the upper limit of normal are considered mild elevations, values higher than 5 times the normal value are considered abnormal values (Green et al., 2002). GP treatment reduced the activities of serum AST by 11.25%, 18.96%, and 30.15% at dosage of 50, 100, and 150 mg/kg, respectively, which were much lower than the 500% ascent limit can be within the normal range for the AST. In addition, the pathological examination on the liver tissue of each group is carried out (Fig. S2) and found that the structure of the liver cord of the rats in the GP group with different administration concentrations was basically normal and radial, with a few vacuoles in the liver cells, but the lipid droplets in the cytoplasm. The number and size have been reduced to varying degrees. Especially in the GP150 group, the hepatic lobule structure was basically normal, the hepatic blood sinus was more obvious, and the hepatic cord structure basically returned to normal radial shape. Although there is no dose-response relationship on the improvement of ALT by using GP treatment, but gavage of GP to rats attenuates the level of ALT in serum, indicating that GP has a protective effect on hyperlipidemia. FFA concentrations in serum are closely related to lipid metabolism, glucose metabolism, and other functions, and the concentration of FFA increases markedly due to diabetes, LMD, and other diseases (Kim et al., 2022). The results showed that GP can improve serum FFA levels, it indicated that different doses of GP could all prevent the occurrence of diseases such as disorders of lipid metabolism in rats on HFD.

GP and the intestinal flora can produce bidirectional effects. GP can affect the gut microbiota diversity and functionality, while microorganisms can metabolize GP into metabolites to increase their bioavailability. The gut microbiota boosts its function by biotransforming and altering the structure of GP (Kai et al., 2010). The intestinal Bacterium GA A07 can use glycosylate triterpenoids and reduce the cytotoxicity of natural triterpenoids for better utilization by the organism (Chang et al., 2018). Also, GP may mitigate the loss of intestinal microflora diversity caused by HFD to some extent and played a role in preserving intestinal microecology stability.

At the phylum level, GP supplementation prevented increased Firmicutes and decreased Bacteroidetes abundance in the HFD group. The rat gut microbiome is mainly composed of Firmicutes and Bacteroidetes, and the ratio of these two phyla plays important roles for the health of the body (Nagel et al., 2016). In the GP group, the abundances of Bacteroidetes were significant increased and Firmicutes was decreased compared to the HFD group. This indicates that GP has a certain maintenance effect on the changes in the relative homeostasis of gut microbes induced by HFD, at the same time GP also relieved gut

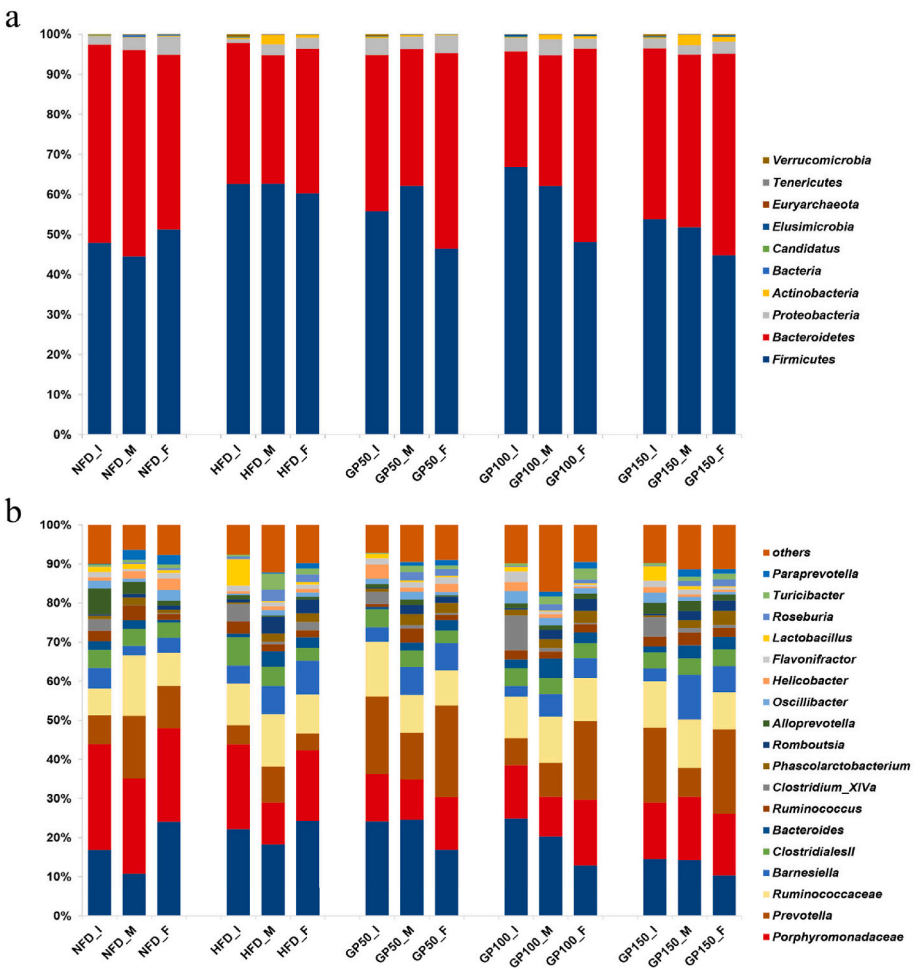

Fig. 5. Changes in the bacterial composition of rat fecal microbiota over time. (a) Bacterial composition at phylum level. (b) Composition of gut microbiota at the genus level. I, M, and F indicate zero, four, and eight weeks, respectively.

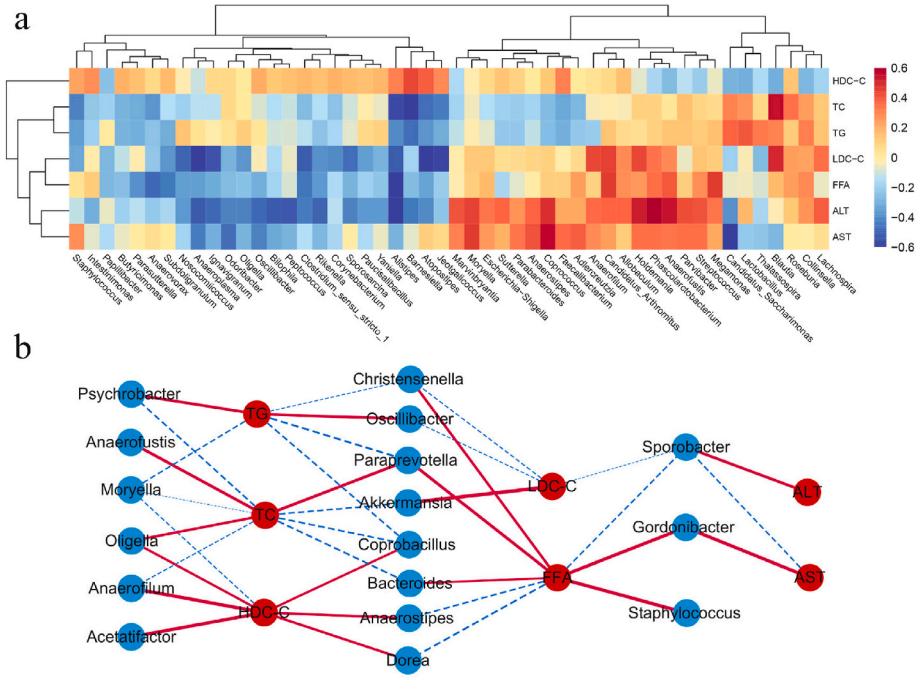

Fig. 6. Correlations between gut microbiota and serum lipid metabolic parameters. (a)The correlation network is visualized based on the partial correlation between intestinal content and LMD-related indicators; (b) Each node reflects gut microbiota genera and LMD-related characteristics. Positive and negative correlations are indicated by the solid red and dotted blue lines, respectively. The correlation's strength is indicated by the thickness of the lines. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

microbiota dysbiosis at the genus level. In this study, the HFD group presented a significantly higher level of *Blautia*, while the abundance of *Blautia* decreased in the GP group compared with the HFD group. Abundance of *Blautia* is associated with patients with nonalcoholic steatohepatitis and hyperlipidemia (Shen et al., 2017; Wang et al., 2017). The *Parasutterella* was associated with health and was a core component of the human and mouse gut microbiota, the abundance of *Parasutterella* was reduced in HFD group (Ju et al., 2019; Kreutzer et al., 2017). The abundance of the *Parasutterella* was significantly decreased, and GP helped increase its abundance *Oscillibacter* abundance was associated with obesity (Thingholm et al., 2019). Previous studies demonstrated that *G. lucidum* increased the relative abundance of *Oscillibacter* and *Alloprevotella* (Hu et al., 2018; Tong et al., 2020). In the current study, GP administration increased the abundance of beneficial bacteria, including *Oscillibacter* and *Alloprevotella*. Thus, the therapeutic effect of GP on hyperlipidemia in rats observed in the current study may be through an increase in beneficial gut bacteria.

Spearman's correlation analysis revealed significant correlations of bacteria with serum TC, serum LDL-C levels, and with other factors. Studies have shown that *Alistipes* dysbiosis can be beneficial or harmful (Parker et al., 2020). *Alistipes* has been implicated in cardiovascular disease and other potential diseases (Zuo et al., 2019), and it was negatively correlated with most serum metabolic parameters and cholesterol metabolic parameters examined in the present study. Moreover, high abundances of *Alistipes* and low abundances of *Blautia* decreased the serum TC, TG and LDL-C levels and reduced serum lipid levels, ultimately improving hyperlipidemia symptoms. *Oscillibacter* is negatively correlated with serum indicators (Zhou et al., 2019), and like these results, *Oscillibacter* previously showed a significant negative correlation with ALT, AST, and FFA. Diet can regulate the relative abundance of beneficial and harmful bacteria (Bäckhed et al., 2004). The above results demonstrate that the GP can alter the intestinal microflora communities of Hyperlipidemia rats.

5. Conclusion

Overweight and hyperlipidemia are increasingly serious problems around the world. In summary, a dietary choice of GP supplementation has a positive effect on preventing an increase in serum lipids in HFD-fed rats, which may be related to changes in the gut microbiota. In the present study, HFD rats showed decreased gut microbiota diversity and dysbiosis, and GP exhibited the potential to restore them. However, due to the Illumina MiSeq platform's low read length, the bacterial 16s rRNA gene sequence in the current study could not be sufficiently identified to assign correct taxonomic information above the genus level. On the recently created nanopore-sequencing platform, additional research into the bacterial community should be done by giving full-length 16s rRNA gene sequence data. This study suggests that GP intervention may improve hyperlipidemia. Taken together, further research is needed for better understanding the health-promoting effects of GP used as a food supplement.

CRediT authorship contribution statement

Aijun Tong: Investigation, Data curation, Writing – original draft. **Weihao Wu:** Investigation, Conceptualization, Writing – original draft. **Zhengxin Chen:** Formal analysis, Visualization. **Jiahui Wen:** Resources, Software. **Ruibo Jia:** Data curation. **Bin Liu:** Investigation, Formal analysis. **Hui Cao:** Writing – review & editing, Supervision. **Chao Zhao:** Funding acquisition, Writing – review & editing, Supervision.

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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.crfs.2022.100427.

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