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Healthy regulation of Tibetan Brassica rapa L. polysaccharides on alleviating hyperlipidemia: A rodent study

Hanyi Hua [a,b], Lin Liu [a,b], Tao Zhu [a,b], Fengyue Cheng [a,b], He Qian [a,b,*], Fanglin Shen [c,d,*], Yu Liu [e,*]

[a] School of Food Science and Technology, Jiangnan University, Wuxi 214122, China
[b] Collaborative Innovation Center of Food Safety and Quality Control in Jiangsu Province, Jiangnan University, Wuxi 214122, China
[c] Fudan University, China
[d] School of Environmental Engineering, Wuxi University, Wuxi 214105, China
[e] Departments of Orthopaedics, Wuxi 9th People's Hospital Affiliated to Soochow University, Wuxi, Jiangsu 214062, China

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ABSTRACT

Hyperlipidemia is a common metabolic disorder, which can lead to obesity, hypertension, diabetes, atherosclerosis and other diseases. Studies have shown that polysaccharides absorbed by the intestinal tract can regulate blood lipids and facilitate the growth of intestinal flora. This article aims to investigate whether Tibetan turnip polysaccharide (TTP) plays a protective role in blood lipid and intestinal health via hepatic and intestinal axes. Here we show that TTP helps to reduce the size of adipocytes and the accumulation of liver fat, playing a dose-dependent effect on ADPN levels, suggesting an effect on lipid metabolism regulation. Meantime, TTP intervention results in the downregulation of intercellular cell adhesion molecule-1 (ICAM-1), vascular cell adhesion molecule-1 (VCAM-1) and serum inflammatory factors (interleukin-6 (IL-6), interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α)), implying that TTP suppresses the progression of inflammation in the body. The expression of key enzymes associated with cholesterol and triglyceride synthesis, such as 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR), cholesterol 7α-hydroxylase (CYP7A1), peroxisome proliferator-activated receptors γ (PPARγ), acetyl-CoA carboxylase (ACC), fatty acid synthetase (FAS) and sterol-regulatory element binding proteins-1c (SREBP-1c), can be modulated by TTP. Furthermore, TTP also alleviates the damage to intestinal tissues caused by high-fat diet, restores the integrity of the intestinal barrier, improves the composition and abundance of the intestinal flora and increases the levels of SCFAs. This study provides a theoretical basis for the regulation of body rhythm by functional foods and potential intervention in patients with hyperlipidemia.

1. Introduction

Over the past two decades, the booming economy in China has greatly improved people's living standards, especially their food consumption. A survey revealed that the top ten most frequently purchased dishes were mainly fried foods and animal dishes, which resulted in excessive consumption of oil and salt and irrational dietary structure. This phenomenon has led to a rapid increase in the prevalence of overweight and obesity among Chinese residents, which has become a serious public health challenge. Diabetes, hypertension, cancer, and cardiovascular disease are closely associated with overweight and obesity. Report on Nutrition and Chronic Diseases of Chinese Residents (2020) showed that over half of the adult residents are overweight or obese (50.7%), with the prevalence of hypertension, diabetes, and hypercholesterolemia at 27.5%, 11.9%, and 8.2%, respectively. China Cardiovascular Health and Disease Report (2019) revealed that the rates of dyslipidemia, diabetes, hypertension, cardiovascular and cerebrovascular diseases and other chronic diseases of adult residents are on the rise. There is a strong connection between these chronic conditions and long-term dietary imbalances such as high intake of salt and oil. Therefore, it is imperative to identify effective methods for staying healthy in a high-fat dietary environment.

The current treatment for hyperlipidemia involves statins and fibrates. Statin therapy inhibits 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMGCR) and reduces total cholesterol (TC) and low-density lipoprotein cholesterol (LDL-C) levels by approximately 20% to 65%

* Corresponding author at: School of Food Science and Technology, Jiangnan University, Wuxi 214122, China.
E-mail addresses: 17110740031@fudan.edu.cn (F. Shen), liuyu2956@suda.edu.cn (Y. Liu).

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(Karr, 2017). However, statins may induce adverse reactions, such as respiratory tract infection and muscle pain (Taylor et al., 2017). Fibrates reduce triglyceride (TG) level by 50% and amplify high-density lipoprotein cholesterol (HDL-C) level by 15% by enhancing the peroxisome proliferator-activated receptor-α (PPARα) expression (Insua et al., 2002). However, the combination of two hypolipidemic drugs increases their side effects (Enger et al., 2010). Therefore, the use of safe, healthy and effective lipid-lowering active ingredients based on natural foods and traditional Chinese medicines is a new trend. Currently, active food ingredients such as plant polysaccharides, soluble dietary fibers, soybean protein, flavonoids and phytosterols have been confirmed to regulate blood lipids (AbuMweis, Jew, & Ames, 2010). A variety of plant polysaccharides have been shown to restore serum TG, TC and LDL-C levels in hyperlipidemic mice to normal values, which might be achieved by enhancing the activities of superoxide dismutase (SOD) and catalase (CAT) and improving insulin sensitivity (Dong et al., 2018).

Turnip is a medicinal and edible plant growing on the Tibetan plateau. Studies have shown that turnip contains a variety of active substances: glucosides, isothiocyanates (Afsharypuor & Tahmasian, 2010), flavonoids (Ferreres et al., 2006), indoles (Wu et al., 2012), sulfur-containing compounds, phenols (Cartea et al., 2010), carbohydrates (Wu et al., 2013) and volatile substances (terpenes, esters, aldehydes and ketones) (Francisco et al., 2009). Turnip extract prevents obesity and inhibits the accumulation of adipose cells; it induces the expression of β3-adrenergic receptor (β3-AR) (An et al., 2010). Studies investigating fructose-induced metabolic syndrome (MS) showed that rats in the turnip group showed significant reductions in blood lipid levels, and increased levels of reduced glutathione and liver glycogen in the blood, demonstrating that turnip has a positive effect on metabolic syndrome (Abo-Youssef & Mohammed, 2013). The anti-obesity effects of turnip ethanol extract (ETR) on 3T3-L1 adipocytes and the imprint-controlled region (ICR) mice fed a high-fat diet (HFD) were investigated. The molecular mechanisms may involve the induction of lipolysis-related gene expression in white adipocytes and the activation of cyclic adenylate-dependent protein kinase, hormone sensitive lipase (HSL) and extracellular signal-regulated kinase in 3T3-L1 cells (An et al., 2010).

Polysaccharides are carbohydrates essential to maintain life activities. In recent years, plant polysaccharides have attracted increasing attention due to their anti-cancer, hepatoprotective, anti-hypoxia, and anti-obesity effects. Because of their safety and non-toxicity, they are widely used in the fields of biochemistry and medicine. As a cost-effective medicinal food ingredient, Tibetan turnip polysaccharide (TTP) has a broad range of research and clinical applications. Thus, this study was designed to examine whether TTP can be used to relieve hyperlipidemia in rats fed with a high-fat diet.

2. Materials and methods

2.1. Materials and chemicals

Turnip were deproteinized by the sevag method [chloroform:n-butanol = 4:1 (V/V)] using ultrasonic extraction with a material to liquid ratio of 43 mL/g at 60 °C at 360 w for 55 min, precipitated by gradient ethanol precipitation, reconstituted and dialyzed in distilled water for 48 h against a dialysis bag with a molecular weight cut-off of 7000 Da. The solution inside the dialysis bag was lyophilized and the average molecular weight was measured to be 70.4 kDa, that is, TTP used experimentally. According to the previous study (Zhao et al., 2021), we found that TTP had a molecular weight of 70.4 kDa, an yield of 1.45 ± 0.11%, a neutral sugar content of 61.79 ± 3.55%, a uronic acid content of 18.30 ± 0.19%, and a protein content of 14.55 ± 3.16%. 5 mg TTP were hydrolyzed by 2 mL of TFA (4 M) in a 120 °C oil bath for 3 h in sealed test tubes, hydrolysate were injected into ion chromatography apparatus (Dionex ICS-5000, Thermo Fisher Scientific, US), the result revealed that TTP mainly consist of glucose (31.28%), galactose (25.71%), rhamnose (21.39%), mannose (19.82%), galacturonic acid (1.56%) and glucuronic acid (0.23%) and belong to neutral polysaccharides. TTP possess polysaccharide characteristic absorption peaks and a pyranose ring may be present in the structure.

The positive drug simvastatin was purchased from Merck & Dong Co., Ltd. TC, TG, HDL-C and LDL-C assay kits were purchased from Nanjing Jiancheng bio Co., Ltd. (China). Lipopolysaccharides (LPS), adiponectin (ADPN), interleukin-6 (IL-6), vascular cell adhesion molecule-1 (VCAM-1), interleukin-1β (IL-1β), intercellular cell adhesion molecule-1 (ICAM-1), tumor necrosis factor-α (TNF-α) and tight junction protein (occludin, Zona Occludens 1 (ZO-1)) assay kits were purchased from Nanjing SenBeiJia Biological Technology Co., Ltd. (China). Liver RNA and cDNA extraction kits were purchased from Nanjing Vazyme Biotech Co., Ltd. (China). SYBR Green qPCR master mix was purchased from Wuhan Seivicebio Technology Co., Ltd. (China). β-actin rabbit monoclonal, cholesterol 7α-hydroxylase (CYP7A1) rabbit polyclonal, 3-Hydroxy-3-methylglutaryl CoA reductase (HMGCR) rabbit polyclonal, acetyl-CoA carboxylase (ACC) rabbit monoclonal, fatty acid synthetase (FAS), sterol-regulatory element binding proteins-1c (SREBP-1c) and peroxisome proliferator-activated receptors γ (PPARγ) Rabbit polyclonal antibody purchased from Beyotime Biotech. Inc. (Shanghai, China).

2.2. Hyperlipidemia model

This animal experiment was approved by the ethics committee of the laboratory animal center of Jiangnan University (JN. No20210930s0900925 [357]), and the experiments were performed in strict accordance with the guide for the care and use of laboratory animals of Jiangnan University. The animals used in the experiments were SD rats, male, SPF grade, 5 weeks old, purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. (China), and housed under the condition of 12 h day night alternation, 45%–70% relative humidity, temperature 20 °C–25 °C. Normal diet and high fat diet (Supplementary Table 1) were purchased from Trophic Animal Feed High-Tech Co., Ltd. (Nantong, China) and was replaced every 48 h. Sixty rats after one week of adaptive feeding under barrier conditions were randomly divided into 2 groups and sequentially marked for each cage rat, with 10 rats randomly assigned to be set as the control group and the remaining rats as the model group, which were fed a high-fat diet. After 2 weeks, blood was collected from the tail of the rats, left undisturbed for 2 h at room temperature, and the supernatant was collected by low-temperature centrifugation at 4000 R/min for 20 min for subsequent index detection. The serum TC, TG and LDL-C levels of the model rats were significantly higher than those of the control rats ($p < 0.05$), indicating that the rats had been induced to become hyperlipidemic by a high-fat diet. Fifty rats of the model group were randomly divided and the gavage dose was shown in Table 1. Rats were continuously gavaged for five weeks and observed for growth, weighed and recorded weekly. During the gavage period, each group was continued to be fed with an equal amount of high-fat diet, except for the control group. At the end of the last gavage, rats were fasted for 12 h with free access to water. Then, rats were euthanized and organs and blood were isolated.

Table 1
Group of the experimental animals.

Group	Feed	Gavage medications	Dose (mg/kg/d)
Con	normal diet	saline	–
Mod	high fat diet	saline	–
Pos	high fat diet	simvastatin	1.042
TTP-L	high fat diet	TTP-low dose	100
TTP-M	high fat diet	TTP-medium dose	200
TTP-H	high fat diet	TTP-high dose	400

2.3. Biochemical indexes assay

TC, TG, LDL-C and HDL-C in serum were detected following the kit instructions. LPS, ADPN, VCAM-1, ICAM-1, IL-6, TNF-α, IL-1β in serum were determined by ELISA kit. A small amount of liver was weighed into prechilled saline and homogenized by a tissue grinder at 60 Hz for 45 s, repeated 3 times to make a certain proportion of tissue homogenate, centrifuged at 4000 R/min in a high-speed centrifuge at low temperature for 15 min, and the supernatant obtained after centrifugation was tested for subsequent relevant biochemical indexes. Colon tissues were assayed following the same method for the content of tight junction proteins occludin and ZO-1 et al.

2.4. Histopathological detection

Tissues were fixed with 4% paraformaldehyde solution at a volume ratio overnight, tissues from the same site were selected as much as possible, embedded, sectioned, and stained for observation and photographed.

2.5. Extraction and reverse transcription of RNA

RNA of liver tissue was extracted following the kit, its concentration and purity were determined with nanodrop, and RNA with OD260/280 at 1.8–2.2 was selected for subsequent operation. According to the reverse transcription kit instructions, the amount of RNA used in the system was adjusted so that the total amount of RNA from each sample was consistent, and the reverse transcription system was shown in Supplementary Table 2. After mixing, the following reaction program was performed: 50 °C for 15 min, 85 °C for 5 s. After completion of reverse transcription, products were stored at −20 °C, used as soon as possible and avoid repeated freeze thawing.

2.6. qRT-PCR analysis

The cDNA solution obtained from 2.5 was diluted to appropriate multiples, qPCR was performed according to the kit, the reaction system was shown in Supplementary Table 3. Primers for each gene were designed using the NCBI database and their specificity were verified. Primer sequences were shown in Supplementary Table 4. β-Actin was used as an internal reference gene for each sample, 2-ΔΔCt was used to represent the calculated results of each target gene.

2.7. Western blot analysis

Liver tissues were weighed, and proteins were extensively denatured by adding RIPA lysate at a ratio of 1:15 (w/V). Electrophoresis was stopped after the protein has been run to the lowest layer. Subsequently, PVDF was transmembrane at 250 mA and low temperature. After the transmembrane was completed, the membranes were immersed in 5% nonfat milk for 1 h and subsequently co-incubated with ACC, PPARγ, HMGCR, CYP7A1, and β-Actin monoclonal antibodies overnight at 4 °C. The membranes were incubated with secondary antibodies for 1 h and the results were analyzed using Image J software.

2.8. Determination of short chain fatty acids (SCFAs) in colonic contents

The in vitro fermentation products (100–200 mg) were weighed, 1 mL dimethyl carbonate, 0.1 mL saturated solution of potassium bisulfate and 0.1 mL internal standard solution of 2-hydroxybutyric acid (0.1 mg/mL) were added, mixed evenly by vigorous shaking for 10 min, and centrifuged at 4000 R/min for 10 min to obtain the dimethyl carbonate phase, which was known as SCFAs. Standard solutions of SCFAs were prepared by the same method with a concentration gradient of 0.05 mg/mL–5.0 mg/mL. Machine detection by GC–MS after sample preparation. Standard solutions of SCFAs were prepared by the same method with a concentration gradient of 0.05 mg/ml–5.0 mg/ml. Machine detection by GC–MS after sample preparation. Ionization mode was EI, emission current of 1 mA, electron energy of 70 eV. Column: DB wax UI, 30 m × 0.25 mm × 0.25 mm. The initial temperature of 60 °C was held for 1 min, and it was held for 10 min after increasing to 240 °C. The structure and composition of flora were analyzed by R Studio software.

2.9. 16S rRNA gene sequencing analysis

DNA from rat feces was extracted using a Genomic DNA Kit (Omega Bio-tek, GA, USA.). The primer pair (341 F, 5′-CCTAYGGGRBGCASCAG-3′; 806R, 5′-GGACTACHVGGGTWTCTAAT-3′) was used to amplify the 16S rRNA sequencing genes (V3–V4 regions) from the whole genome of bacteria. Quantification, pooling, and sequencing of amplicons were carried out on an Illumina MiSeq machine. FLASH (v1.2.8) program was used to stitch the double-ended sequences, and Vsearch (v2.3.4) filter was applied to eliminate unqualified sequences. Sequences with 97 % similarity are classified as OTUs. Based on systemic affinities of ITS2 gene sequence of RDP and Unite database, the 16S rRNA gene sequences were distributed into distinct taxonomic categories. PICRUSt software and KEGG database (https://huttenhower.sph.harvard.edu/galaxy/) were used to predict and analyze gene functions.

2.10. Statistical analysis of data

Statistics results were expressed in terms of "mean ± standard deviation" (mean ± SD). Graphpad prism 9 software was used to plot the data, and one-way ANOVA followed by *t*-test was used to compare the data between groups, with $p < 0.05$ indicating a significant difference, and the experiments were repeated three times.

3. Results

3.1. TTP alleviates symptoms of hyperlipidemia in rats

Rats were fed with high-fat diet for 2 weeks, followed by blood collection from the tail vein. Four indices of blood lipids were evaluated. The concentrations of serum TC, TG and LDL-C in the rats (Fig. 1A) belonging to the Mod group were increased, implying successful development of the model. Five groups of rats were randomly selected and treated intragastrically (i.g.) by gavage for 5 weeks.

As shown in Fig. 1B, the body weight of each group continued to increase after gavage. A slight difference existed between the TTP-L/M and the Mod groups. The body weights in the Pos and the TTP-H groups were slightly higher than in the Con group, indicating that the weight gain in TTP-H group was effectively controlled after the high-fat diet intake. As the main site of the body's metabolism, liver plays a key role in glucose and lipid metabolism. The liver index is changed when it is overworked. As shown in Fig. 1C, except for the Pos group, the liver coefficients of the other groups were enhanced compared with the Con group. Intragastric administration of TTP led to a significant variation in the liver coefficients between the Mod and the TTP-H groups, implying that TTP alleviated liver damage. The four blood lipid parameters were determined after 35 days of gavage, and the results are shown in Fig. 1D–1G. Exposure to high-fat diet led to a significant increase in the levels of TC, TG and LDL-C in the Mod group compared with the Con group. However, this trend was reversed by TTP intervention, especially in the TTP-H group. Additionally, the HDL-C levels of the five high-fat diet groups did not differ significantly.

3.2. TTP improved the organ shape of hyperlipidemic rats

To analyze the effect of TTP on liver fat accumulation, the liver morphology of hyperlipidemic rats was assessed (Fig. 1H). The livers of the rats in the Con group showed normal morphology, uniform arrangement and distribution, clear texture, normal reddish-brown

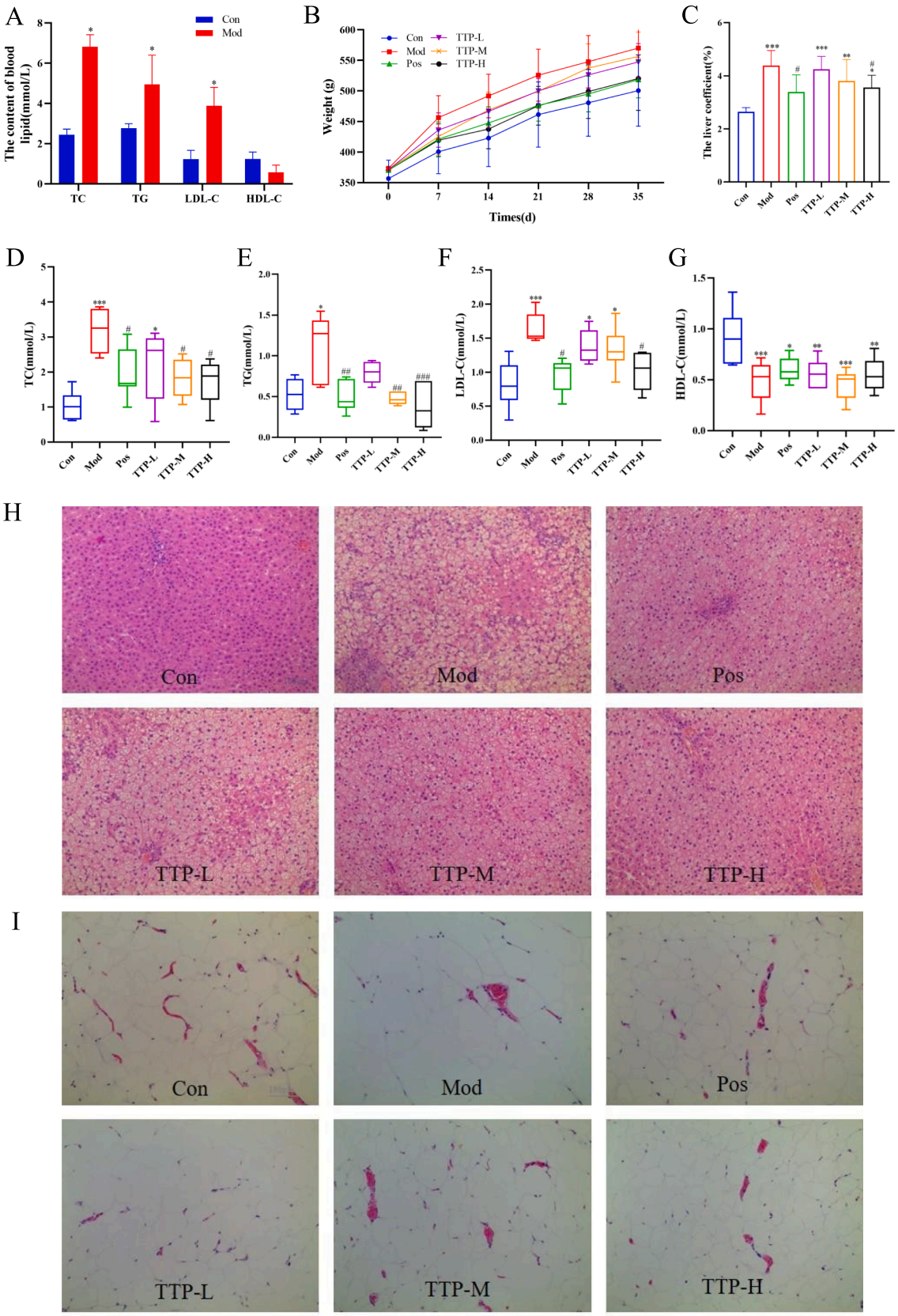

Fig. 1. Effects of TTP on body weight, liver index, serum lipids, liver and adipocyte histopathologyin rat. (A) Hyperlipidemia model indicators. (B) Body weights. (C) Liver coefficient. (D) TC levels. (E) TG levels. (F) LDL-C levels. (G) HDL-C levels. (H) HE staining of liver tissues (×200). (I) HE staining of adipocytes (×200). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ *vs.* the Con group; #$p < 0.05$, ##$p < 0.01$, ###$p < 0.001$ *vs.* the Mod group, n = 10 per group.

color, and absence of fat vacuoles in the cells. After feeding with high-fat diet, both the liver size and weight in the Mod group were enlarged. In addition to brownish-yellow color, the morphology of the liver was disrupted. The cells were swollen, and inflammatory cells infiltrated around the central vein of the liver, along with fat vacuoles in the cytoplasm. Notably, the liver color, size and weight were palpably improved in the TTP and the Pos groups. Further, the inflammatory cell infiltration and fat vacuoles in the TTP-M and the TTP-H groups were reduced, which was consistent with the results in the Pos group. It indicated that TTP reduced liver fat accumulation and alleviated liver damage caused by high-fat diet.

To further analyze the effect of TTP on fat accumulation induced by high-fat diet, hematoxylin-eosin (HE) staining of the white fat was performed. As shown in Fig. 1I, the size of adipocytes in the Mod group increased markedly, which was consistent with the increased body weight in rats. Conversely, the TTP group showed a decrease in the size of adipocytes and weight loss, indicating that TTP regulated the tissue morphology and blood lipids in rats induced by high-fat diet.

3.3. Effects of TTP on serum biochemical indices and inflammation in hyperlipidemic rats

The serum ADPN content was determined by ELISA (Fig. 2A). TTP showed a dose-dependent effect on the ADPN levels, which implied that the TTP regulated the lipid metabolism in the body, suggesting a potential anti-obesity effect. The ICAM-1 results of the Mod group showed (Fig. 2B) that a high-fat diet enhanced the adhesion of macrophages to endothelial cells, which resulted in inflammation.

Instead, the TTP-H group showed downregulation of ICAM-1 level, suggesting that TTP indirectly inhibited the inflammation in the body. As shown in Fig. 2C, the serum VCAM-1 level was amplified in the Mod group, while TTP-H showed an opposite trend. Next, the expression of LPS and serum inflammatory factors (TNF-α, IL-6 and IL-1β) was elevated (Fig. 2D–2G). Exposure to high-fat diet increased the LPS level remarkably in the Mod group; however, the TTP-H and the Pos interventions decreased the LPS levels to comparable levels, with significant difference compared with the Mod group. A similar phenomenon was observed in the levels of inflammatory factors. The intake of high-fat diet induced the three inflammatory factors in the Mod group, while the TTP group dose-dependently attenuated their expression and thereby alleviated the inflammatory response.

3.4. Regulation of liver inflammation and lipid metabolism genes by TTP

To further investigate the effect of TTP on inflammation induced by high-fat diet, a qPCR analysis of liver mRNA expression of three inflammatory factors was conducted. As shown in Fig. 3A–3C, the mRNA levels of TNF-α, IL-6 and IL-1β in the Mod groups were considerably increased after administration of high-fat diet, while TTP intervention led to a dose-dependent inhibition, especially in the TTP-H group. The outcome suggested that TTP had a specific anti-inflammatory effect, based on the serum levels.

In order to study the molecular mechanism of TTP in regulating the blood lipids, we evaluated the difference between target genes associated with lipid metabolism. The genes levels of HMGCR, SREBP-1c, FAS, ACC, PPARγ, CYP7A1 in the liver were shown in Fig. 3D–3I. High-fat diet intervention significantly elevated the levels of the six genes associated with lipid metabolism in the Mod group. TTP-H significantly downregulated the expression of HMGCR (Fig. 3D) and SREBP-1c (Fig. 3E) and PPARγ (Fig. 3H). The FAS level was reduced in all groups of TTP (Fig. 3F). Nonetheless, no significant differences were found between the groups. The levels of ACC (Fig. 3G) in the TTP-M and TTP-H groups were greatly diminished. The expression of CYP7A1 in the TTP-H group was upregulated to 1.63 ± 0.20 (Fig. 3I), which was consistent with the regulation of CYP7A1 in the liver of hyperlipidemic rats by Laminaria japonica (Zhang et al., 2020).

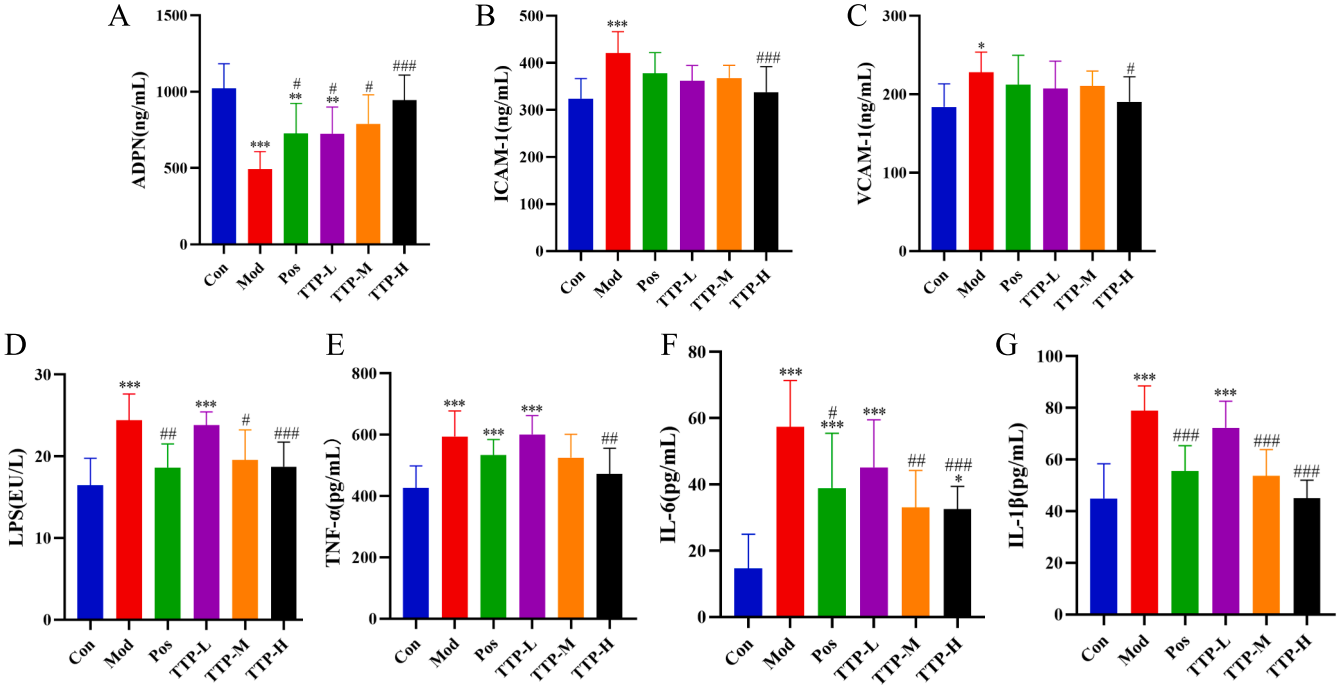

Fig. 2. Effects of TTP on ADPN, ICAM-1, VCAM-1 and inflammatory factors in rat exposed to high-fat diets. (A) ADPN levels. (B) ICAM-1 levels. (C) VCAM-1 levels. (D) LPS levels. (E) TNF-α levels. (F) IL-6 levels. (G) IL-1β levels. Each group comprised 10 animals (n = 10). $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ *vs.* the Con group; $\#p < 0.05$, $\#\#p < 0.01$, $\#\#\#p < 0.001$ *vs.* the Mod group.

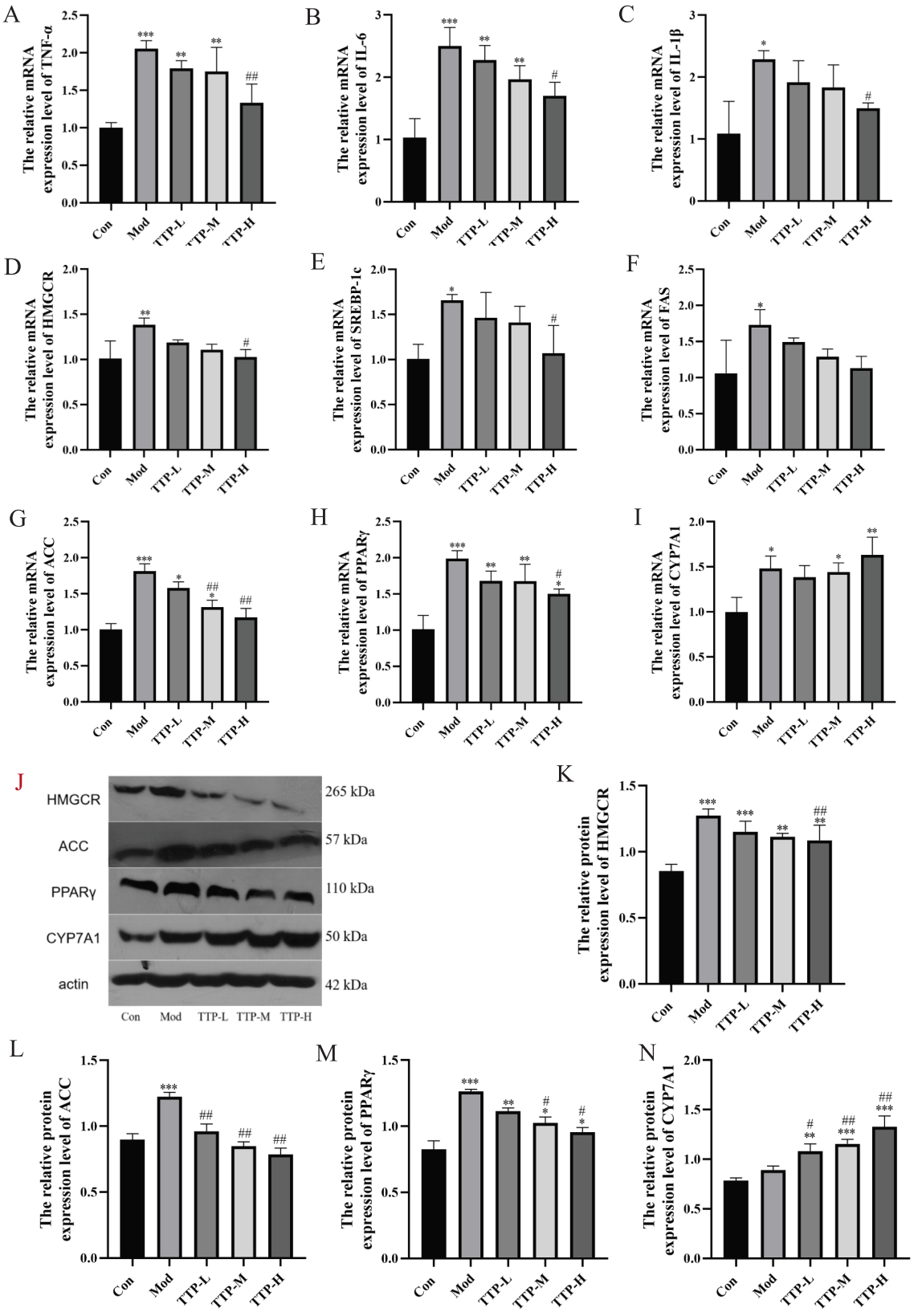

Fig. 3. Effects of TTP on inflammation, cholesterol and fatty acid metabolism-related genes in rat fed with high-fat diets. (A) TNF-α levels. (B) IL-6 levels. (C) IL-1β levels. (D) HMGCR levels. (E) CYP7A1 levels. (F) PPARγ levels. (G) ACC levels. (H) FAS levels. (I) SREBP-1c levels. (J) Representative western blots of key proteins. β-Actin was used as an internal control. (K)–(N) Relative band intensities of ACC (K), PPARγ (L), HMGCR (M) and CYP7A1 (N). Each group included 10 animals (n = 10). *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ *vs.* the Con group; #$p < 0.05$, ##$p < 0.01$ *vs.* the Mod group.

3.5. Effect of TTP on the translation of key genes in lipid metabolism of hyperlipidemic rats

To study the molecular mechanism of TTP in regulating hyperlipidemia, key protein levels were analyzed (Fig. 3J). Compared with the Mod group, the TTP group showed inhibition of HMGCR (Fig. 3K), ACC (Fig. 3L) and PPARγ (Fig. 3M) proteins in a dose-dependent manner, which was consistent with the mRNA levels. In addition, the TTP group showed significant increase in the CYP7A1 level (Fig. 3N), suggesting that TTP may accelerate the conversion of cholesterol into bile acids and interfere with the accumulation of cholesterol in the liver.

3.6. Protective intestinal effect of TTP in hyperlipidemic mice

To determine the protective effect of TTP on the intestinal barrier, HE-stained paraffin sections of colon were pathologically analyzed (Fig. 4A). Compared with the Con mice, the Mod mice showed disrupted colon goblet cells and mucosal layer. However, this phenomenon was reversed by TTP intervention, especially in the TTP-H group. Changes in the ZO-1 (Fig. 4B) and occludin (Fig. 4C) levels reflect the effect of high-fat diet on intestinal barrier. Compared with Con group, the Mod group showed a drastic reduction in ZO-1 levels. However, TTP-M and TTP-L treatment vastly enhanced ZO-1 content. A similar trend was observed in the levels of occludin.

The SCFA levels (mmol/L) in the colon of hyperlipidemic rats were determined. As shown in Table 2, compared with the Con group, the Mod group carried a significantly lower level of SCFAs. However, TTP improved the levels of SCFAs in hyperlipidemic rats, and the concentrations of acetic acid, propionic acid and isovaleric acid were substantially higher than in the Mod group. A 16S rRNA gene sequence analysis of colon was performed to measure the effect of TTP on gut microbiota. Alpha diversity results are shown in Fig. 5A–5D, high-fat diet intervention significantly decreased the levels of Chao1, faith-pd, observed-OTUs and Shannon indices compared with the Con group, suggesting that the intestinal microbial diversity of rats in the Mod group was diminished. However, all four indices were elevated after TTP administration, indicating that TTP improves the alpha diversity of intestinal flora in high-fat-induced rats.

16S rRNA gene sequencing analysis was used to evaluate the changes in intestinal flora, and the composition of the intestinal flora was analyzed at the gate level using histograms. As shown in Fig. 6A and 6B, the abundance of *Firmicutes*, *Verrucomicrobia* and *Proteobacteria* in the Mod group increased compared with the Con group, while the abundance of *Bacteroidetes* and *Actinobacteria* decreased. However, TTP intervention decreased the abundance of *Firmicutes* and enhanced the abundance of *Verrucomicrobia*, *Proteobacteria*, *Bacteroidetes* and *Actinobacteria*. The *Firmicutes*-to-*Bacteroidetes* (F/B) ratio is commonly used to measure the health status. As shown in Fig. 6C, the F/B ratio in the Mod group was significantly higher than in the Con group, and was effectively reduced by TTP intervention, suggesting that TTP alleviated the imbalance in intestinal flora of hyperlipidemic rats, thereby reducing the risk of obesity. Further, a genus-level analysis of intestinal flora was conducted (Fig. 6D) including mainly *Blautia, Akkermansia, Dorea, Bacteroides, Lactobacillus, Ruminococcus, Turicibacter*, *Ruminococcaceae* and *Coprococcus*. The genera with substantial variation were selected for further analysis, and the results are shown in Fig. 6E. Compared with the Con group, the Mod group carried a higher relative abundance of *Blautia*, *Akkermansia*, *Dorea*, *Lactobacillus*, *Ruminococcus*, and *Turicibacter*. By contrast, the levels of *Bacteroides*, *Ruminococcaceae* and *Coprococcus* were reduced. Notably, the relative abundance of *Blautia*,

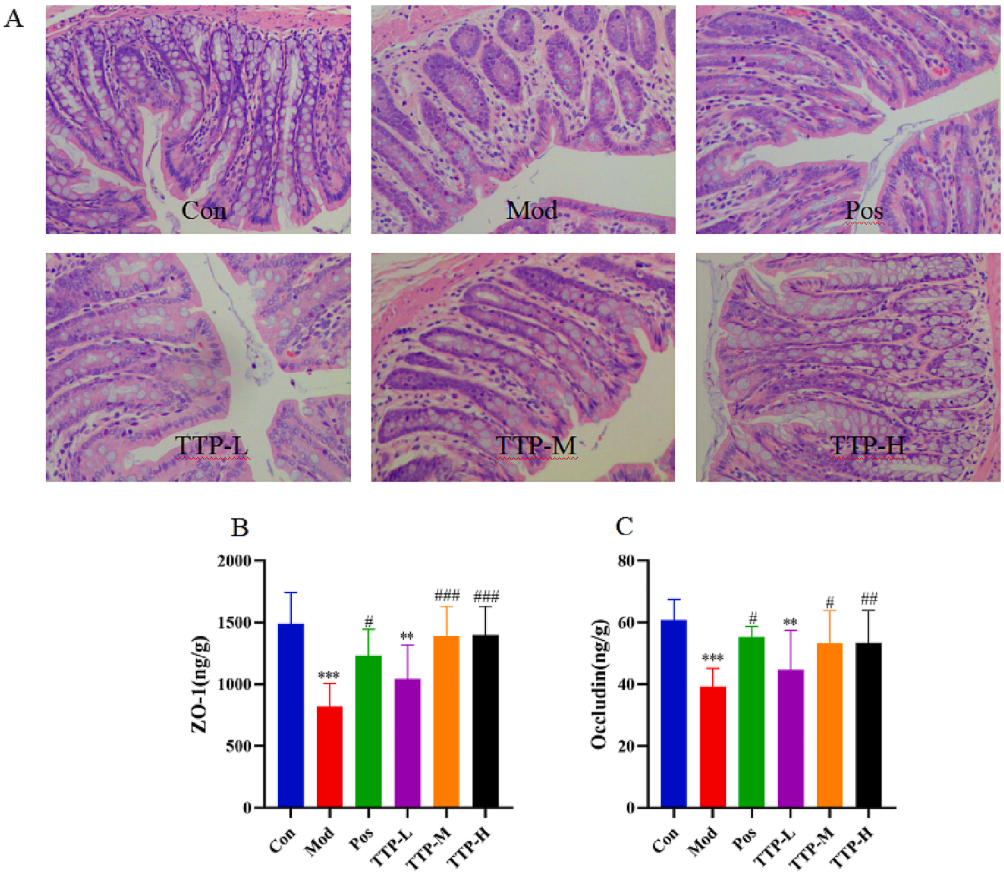

Fig. 4. Effects of TTP on colon and colon barrier integrity in rats treated with high-fat diets. (A) HE staining of the colon. (B) ZO-1 levels. (C) Occludin levels. $*p < 0.05$, $**p < 0.01$, $***p < 0.001$ *vs.* the Con group; $\#p < 0.05$, $\#\#p < 0.01$, $\#\#\#p < 0.001$ *vs.* the Mod group.

Table 2
Effects of TTP on SCFAs in rats treated with high-fat diets.

Group	Acetic acid (mmol/L)	Propionic acid (mmol/L)	n-butyric acid (mmol/L)	Isobutyric acid (mmol/L)	n-valeric acid (mmol/L)	Isovaleric acid (mmol/L)
Con	28.02 ± 5.36	7.60 ± 1.62	10.46 ± 2.86	1.48 ± 0.34	1.11 ± 0.39	0.76 ± 0.27
Mod	18.43 ± 6.71*	4.19 ± 1.96*	2.58 ± 0.81**	0.81 ± 0.12**	0.35 ± 0.11**	0.57 ± 0.15
TTP	29.33 ± 4.02#	7.61 ± 2.61#	3.88 ± 1.42**	1.03 ± 0.24*	0.50 ± 0.10*	0.90 ± 0.18#

*$p < 0.05$, **$p < 0.01$ *vs.* the Con group; #$p < 0.05$ *vs.* the Mod group.

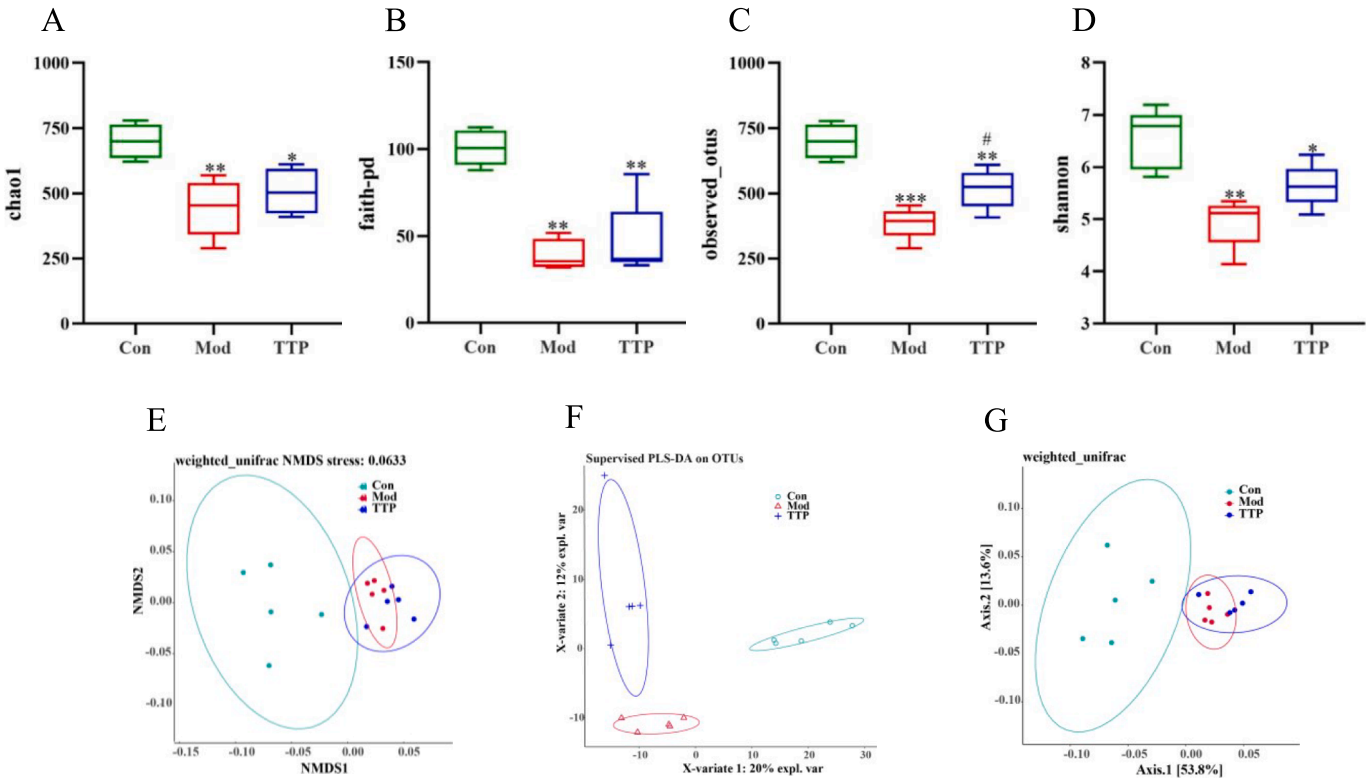

Fig. 5. TTP affects intestinal flora diversity in high-fat diet-fed rats. (A) Chao 1 index. (B) Faith-pd index. (C) Observed-OTU index. (D) Shannon index. (E) NMDS analysis. (F) PLS-DA analysis. (G) PCoA analysis. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ *vs.* the Con group; #$p < 0.05$ *vs.* the Mod group.

Lactobacillus, and *Turicibacter* in the TTP group was reduced, while that of *Akkermansia*, *Dorea*, *Bacteroides*, *Ruminococcus*, *Ruminococcaceae* and *Coprococcus* was elevated compared with the Mod group.

4. Discussion

Hyperlipidemia, a chronic disease caused by abnormal blood lipid metabolism, is widespread in modern society. The causes of hyperlipidemia are complex, usually associated with environmental (eating disorders, lack of exercise, and geographical location) and genetic factors (familial hypercholesterolemia and diabetes) (Aguilar-Salinas et al., 2002). LDL-C, TC, TG and HDL-C levels are commonly used to screen hyperlipidemia. The upregulated ICAM-1 levels are a sign of inflammation, which is closely related to hypertension, coronary heart disease and other chronic diseases (Singh et al., 2021). Anti-inflammatory therapies also target VCAM-1, which is highly expressed in inflammatory states by the stimulation of TNF-α, IL-6, IL-1β, and Toll-like receptors (TLR). As a product of gut microbes, lipopolysaccharide (LPS) is enhanced by high-fat diet intake, thereby activating TLRs, leading to local inflammation mediated via cytokine signaling pathways, resulting in systemic inflammatory response (Leigh & Morris, 2020).

ADPN is a plasma protein secreted by adipocytes. As an intracellular energy sensor, it can phosphorylate AMPK, oxidize fatty acids in the liver, and increase glucose uptake, exhibits anti-inflammatory activity (Sabio & Davis, 2014), thereby regulating lipid metabolism and improving atherosclerosis in the body (Gamberi et al., 2018). Phosphorylated AMPK regulates lipid metabolism in the body through a variety of pathways, including: (1) Inhibiting the expression of HMGCR (Jiang et al., 2018), which further inhibits TC synthesis (Notarnicola et al., 2010); (2) Reducing the level of SREBP-1c (Lin et al., 2005), which in turn indirectly reduces the level of downstream FAS and inhibits the synthesis of TG; (3) Inhibiting ACC activity, promoting fatty acid oxidation and reducing TG synthesis. Serum ADPN levels increased in a dose-dependent manner in hyperlipidemia rats after TTP intervention, and ADPN content in the TTP-H group could be restored to almost the same level as Con group. Further studies showed that TTP down-regulated the protein levels of HMGCR, SREBP-1c, FAS and ACC in the liver, and reduced the expression of serum TG and TC, indicating that TTP could improve hyperlipidemia by activating three pathways of AMPK signaling pathway.

PPARγ is a transcription factor associated with adipocyte differentiation and participates in lipogenesis. Excessive fat accumulation activates the expression of PPARγ and NF-κB, leading to increased release of inflammatory cytokines (Zhang et al., 2016). CYP7A1 belongs to the cytochrome P450 family, it is the rate-limiting enzyme in the bile acid synthesis pathway, regulated by FXR, LXR, and PPARs. Upregulation of CYP7A1 expression in the liver improves chronic diseases such as hypercholesterolemia in animal models (Huang et al., 2018). Our results

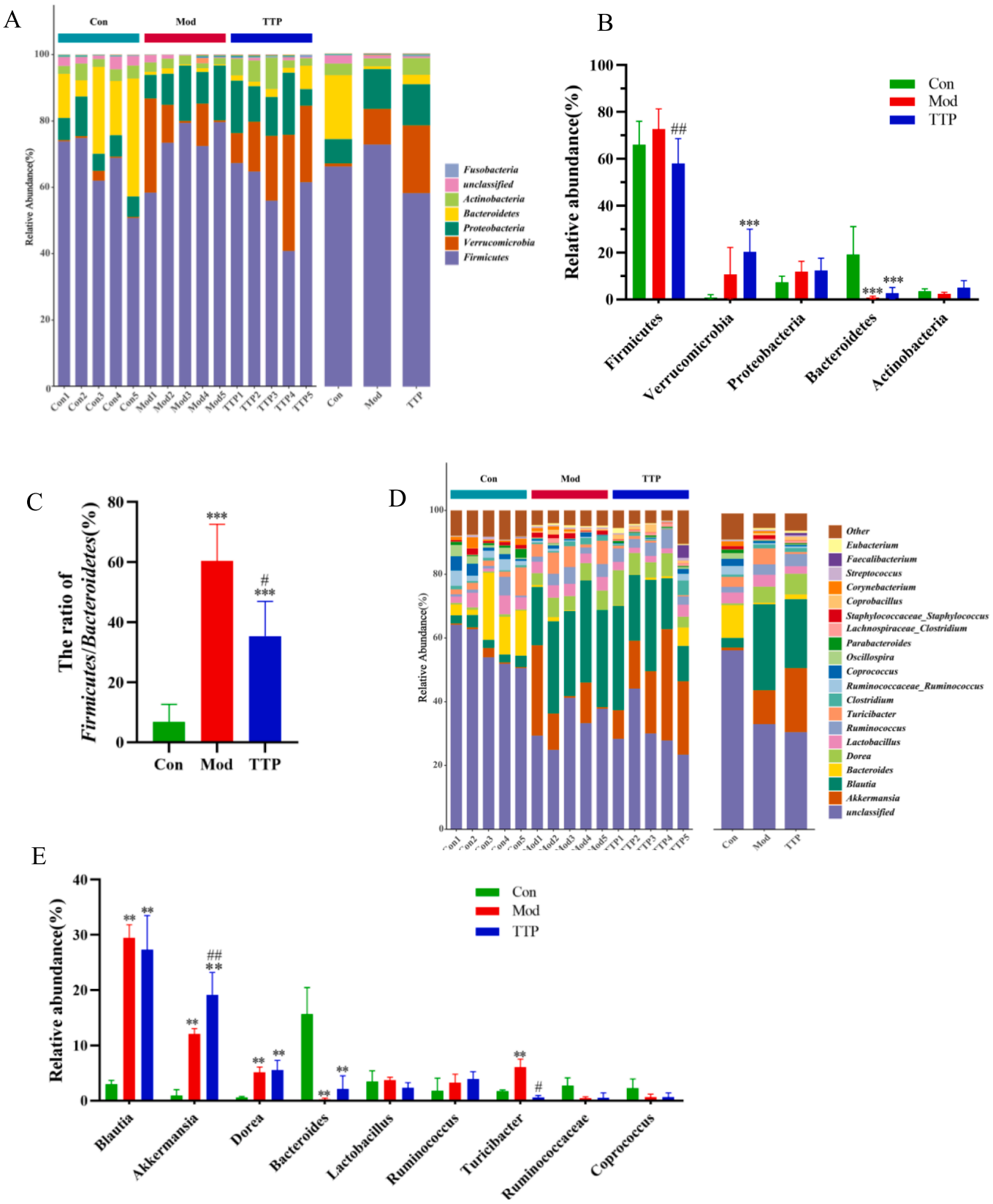

Fig. 6. TTP affects intestinal flora at the phylum and genus levels in rats fed with a high-fat diet. (A) and (B) Relative abundance of the key microorganisms at phylum level. (C) The ratio of Firmicutes to Bacteroidetes. (D) and (E) Relative abundance of the key microorganisms at genus level. $^{**}p < 0.01$, $^{***}p < 0.001$ *vs.* the Con group; $\#p < 0.05$, $\#\#p < 0.01$ *vs.* the Mod group.

showed that TTP restored PPARγ to normal level in the liver of HFD-induced hyperlipidemia rats and enhanced the expression of rate-limiting enzyme CYP7A1, indicating that TTP promoted cholesterol reverse transport and played an anti-hyperlipidemia role by activating PPARγ/CYP7A1 pathway.

The function of polysaccharides depends on their molecular weight and structure. According to our previous study, the Mw of TTP did not change significantly after gastrointestinal digestion, indicating that TTP was not easily degraded (Liu et al., 2022). In the intestine, anaerobes use glucose (31.28%) and mannose (19.82%) in TTP to produce acetic acid. Additionally, acetic acid decreases the intestinal pH, inhibits the colonization of intestinal pathogens, and maintains the integrity of the intestinal barrier (Schulthess et al., 2019). Except for a small amount of propionic acid remaining in the intestinal tract, most of the propionic acid enters the peripheral circulation and participates in liver metabolism, thereby reducing the concentration of fatty acids in the plasma and liver, and improving the insulin sensitivity of the tissue (Al-Lahham et al., 2010). Butyric acid and propionic acid exhibit contrasting metabolic features. The main metabolic site of butyric acid is located in the intestine, which enhances the mechanical support of the intestinal barrier and exerts an anti-inflammatory effect by producing anti-inflammatory cytokines and inhibiting the activity of histone deacetylase (HDAC) (Cresci et al., 2017).

High-fat diet affects the composition and metabolic activities of the intestinal flora, and the metabolites damage the intestinal barrier. In addition, the accumulation of lipids in the intestine triggers oxidative stress response and damages the intestinal barrier. Intestinal mucosa blocks the entry of pathogens within the body. The tight junction proteins play a key protective role, including intracellular proteins such as members of the zonula occludens family (ZO) and transmembrane proteins such as occludins (Ronaghan et al., 2016). Our study showed that TTP improved the intestinal damage induced by high-fat diet and enhanced the integrity of intestinal barrier.

SCFAs are predominantly synthesized by gut microorganisms via fermentation of various indigestible polysaccharides. Hyperlipidemia occurs due to the lack of SCFA-producing microorganisms (Ding, Pu, Kan, 2017). As the dominant genus of intestinal flora, *Blautia* can rapidly digest glucose and generate an abundance of acetic acid, thus regulating the metabolic pathway of the body and reducing the occurrence of chronic diseases such as obesity (Liu et al., 2021). *Akkermansia* protects the integrity of the intestinal mucosal barrier and epithelial cells by degrading mucins (Naito, Uchiyama, & Takagi, 2018). *Dorea* is frequently encountered in patients with colitis, and is closely related to pro-inflammatory cytokines, which aggravate obesity (Han et al., 2019). *Lactobacillus* species occur widely in yogurt and other foods, which protect the host from pathogens but also reduce blood lipids and hyperlipidemia (Guan et al., 2017). *Ruminococcus* species have been reported to produce SCFAs by fermenting polysaccharides, which are negatively correlated with metabolic disorders in liver tissue (Shang et al., 2017). *Turicibacter* species belong to the phylum Sclerochaeta, which suppress intestinal health and serum metabolic parameters (Hu et al. 2018). Further, they are negatively correlated with insulin resistance (Velazquez et al., 2019). *Ruminococcaceae* species are strongly related to triglycerides in VLDL particles, small HDL particles and medium HDL particles; they are involved in the conversion of primary bile acids into secondary bile acids and/or SCFAs, which are strongly correlated with the levels of circulating acetic acid (Vojinovic et al., 2019).

Our study demonstrated that TTP increased the levels of SCFAs in hyperlipidemic rats, and further enhanced the levels of ZO-1 and occludin in intestines. TTP intervention decreased the abundance of *Firmicutes*, *Blautia*, *Lactobacillus*, and *Turicibacter*, while that of *Verrucomicrobia*, *Proteobacteria*, *Bacteroidetes* and *Actinobacteria* was elevated compared with the Mod group. The *Firmicutes*-to-*Bacteroidetes* (F/B) ratio was effectively reduced by TTP intervention, suggesting that TTP alleviated the imbalance in intestinal flora of hyperlipidemic rats, thereby reducing the risk of obesity. Notably, TTP intervention effectively increased the relative abundance of *Akkermansia*, *Dorea*, *Ruminococcus* and *Ruminococcaceae*, which was consistent with its effect on occludin and ZO-1 levels. These results suggested that TTP might be prebiotic in improving metabolic disorders and protecting the intestinal barrier. *Coprococcus* and *Bacteroides* species maintain intestinal homeostasis by producing SCFAs. High-fat diet disrupts the balance in intestinal flora, by decreasing the relative abundance of Coprococcus and Bacteroides significantly. However, TTP intervention reversed this phenomenon, thereby reducing the weight and improving hyperlipidemia and liver steatosis. In brief, the regulatory effects of TTP on blood lipids may be mediated via altered composition and content of intestinal flora. Thus, TTP may be developed as a functional food to improve lipid metabolism disorder and hyperlipidemia.

5. Conclusion

In this analysis, we showed that TTP intervention effectively alleviated hyperlipidemia in rats. The findings relating to abnormal metabolism of blood lipids, inflammatory response and metabolic disorders could been explained by its mechanism. Simultaneously, TTP reduced intestinal damage associated with a high-fat diet and improved the integrity of intestinal barrier. Also, it provided an potent effect on regulating the intestinal flora and SCFAs in hyperlipidemic rats, which suggested that liver diseases may be mediated in part by the microbiota-gut-liver axis.

CRediT authorship contribution statement

Hanyi Hua: Conceptualization, Methodology, Investigation, Formal analysis, Data curation, Writing – original draft, Visualization. **Lin Liu:** Validation, Formal analysis. **Tao Zhu:** Validation, Formal analysis. **Fengyue Cheng:** Validation, Formal analysis. **He Qian:** Conceptualization. **Fanglin Shen:** Writing – review & editing. **Yu Liu:** 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.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.fochms.2023.100171.

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