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Research Article

Protective Effects of Honey-Processed *Astragalus* on Liver Injury and Gut Microbiota in Mice Induced by Chronic Alcohol Intake

Jingxuan Zhou [ID],[1] **Nanhai Zhang** [ID],[1] **Liang Zhao** [ID],[2] **Mohamed Mohamed Soliman** [ID],[3] **Wei Wu,**[4] **Jingming Li,**[5] **Feng Zhou** [ID],[1] and **Liebing Zhang** [ID][1]

[1]*Beijing Key Laboratory of Functional Food from Plant Resources, College of Food Science and Nutritional Engineering, China Agricultural University, 17 Tsinghua East Road, Beijing 10083, China*
[2]*Beijing Engineering and Technology Research Center of Food Additives, Beijing Technology and Business University (BTBU), 11 Fucheng Road, Beijing 100048, China*
[3]*Clinical Laboratory Sciences Department, Turabah University College, Taif University, Taif, Saudi Arabia*
[4]*College of Engineering, China Agricultural University, 17 Tsinghua East Road, Beijing 10083, China*
[5]*Center for Viticulture and Enology, College of Food Science and Nutritional Engineering, China Agricultural University, 17 Tsinghua East Road, Beijing 10083, China*

Correspondence should be addressed to Feng Zhou; zf@cau.edu.cn and Liebing Zhang; lbzhang@cau.edu.cn

Honey-processed *Astragalus* (HPA) is a mixture of *Astragalus* and honey, which is a processed product of Chinese medicine. It has the active ingredients of *Astragalus* and the unique effects of honey. However, the mechanism of HPA for improving alcoholic liver disease (ALD) is not clear. The purpose of this study is to explore the ameliorating effect and mechanism of HPA (4 and 8 g/kg bw) on alcoholic liver injury. Two doses of HPA were orally administered to alcohol-treated mice for four weeks. The results showed that HPA could effectively reduce triglycerides (TG) by 59% and free fat acid (FFA) and total cholesterol (TC) in serum and hepatic were reduced by least 25.9%. HPA could cause a decrease in serum low-density lipoprotein cholesterol (LDL-C) from 0.145 mM to 0.117 mM, and the serum high-density lipoprotein cholesterol (HDL-C) was increased. After alcohol-treated mice were supplemented with HPA, antioxidant markers (superoxide dismutase (SOD), catalase (CAT), glutathione (GSH), and Glutathione peroxidase (GSH-Px)), liver function index (alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP)), proinflammatory cytokines (tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β)), and liver tissue were all significantly improved. This is related to the fact that HPA can promote the expression of oxidative stress-related genes and inhibit the expression of inflammation-related genes. In addition, HPA could also regulate the disturbance of the intestinal microflora. In general, HPA could significantly improve the accumulation of serum and liver lipids caused by alcohol and the imbalance of intestinal flora in mice. It could also improve liver function, oxidative stress, and inflammation.

1. Introduction

Alcohol-related diseases are one of the most common preventable diseases in the world [1]. More than 3 million people around the world die from alcoholism every year [2]. Alcohol injury can cause damage to many end-organs and systems in the body, and alcoholic liver disease (ALD) is an important manifestation of liver injury [3]. ALD can develop from asymptomatic to alcoholic fatty liver, liver cirrhosis, and even alcoholic hepatitis and hepatocellular carcinoma [2, 4]. There is evidence that the development mechanism of ALD is closely related to oxidative stress, inflammatory development, and intestinal microflora disorders [5]. Although drugs such as disulfiram, naltrexone, and corticosteroids are used in abstinence treatment, some drugs are not approved for ALD treatment. Corticosteroids are effective only in the short term in 50% patients with ALD [6, 7]. Due to the personal differences of patients and adverse reactions

caused by drugs still exist, so it is common to choose natural products with low side effects and good tolerance to achieve the improvement of ALD.

Astragalus (Huangqi, Astragalus membranaceus Beg. var. mongholicus (Beg.)) appears in people's field of vision as a common healthcare traditional Chinese medicine because of its antioxidant, anti-inflammatory, and other biological activities [8–10]. Astragalus extracts can improve lipo-polysaccharide-induced liver injury by inhibiting the formation of lipid peroxides and proinflammatory factors [11]. Our previous studies showed that Astragalus polysaccharides and Astragalus saponins could improve liver injury in ALD mice [12]. As a safe alternative sweetener, honey could reduce triglycerides (TG), total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C) [13]. Data showed that honey could improve liver injury by regulating oxidative stress markers: catalase (CAT), glutathione (GSH), malondialdehyde (MDA), superoxide dismutase (SOD), and liver function [14, 15]. Honey-processed Astragalus (HPA) is a mixture of astragalus and honey [16]. Some studies have shown that after the combination of honey and Astragalus, the structure of the active components of Astragalus has changed, and the efficacy of HPA was stronger than that of Astragalus [16, 17]. In addition, HPA had a certain anti-inflammatory effect [16, 17]. HPA could not only improve the inflammatory response but also regulate the gut microbial flora diversity of colitis mice [18].

However, the mechanism and research of HPA alleviating ALD are still poorly understood. In view of the above biological activities of HPA, we speculate that HPA may have a beneficial effect on improving ALD. Therefore, this study aims to evaluate the improvement effect of HPA (Hunyuan, Shanxi Province) on alcohol-induced liver injury in mice and the effect of reducing serum and hepatic lipids. In addition, explore the mechanism of inhibiting oxidative stress, inflammation, and the influence of intestinal flora in alcohol-treated mice.

2. Materials and Methods

2.1. Materials and Chemicals.
Honey-processed Astragalus was provided by Ze Qingqi Industry Development Co., Ltd. (Shanxi, China). Edible alcohol was purchased from Henan Xinheyang Alcohol Co., Ltd. (Henan, China). The assay kits of CAT, SOD, GSH, MDA, γ-glutamyl transpeptidase (γ-GT), glutathione peroxidase (GSH-Px), and free fat acid (FFA) and the commercial enzyme-linked immunosorbent assay (ELISA) kits of tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), and interleukin-1β (IL-1β) were bought from Beijing Sinouk Institute of Biological Technology (Beijing, China). The commercial assay kits of TG, TC, high-density lipoprotein cholesterol (HDL-C), LDL-C, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) were purchased from Biosino Bio-Technology and Science Inc. (Beijing, China). FastQuant RT Kit and SuperReal PreMix Plus with SYBR Green were purchased from Tiangen Biotech Co. Ltd. (Beijing, China). E.Z.N.A.® soil DNA Kit was bought from Omega Bio-tek (Norcross, GA, USA). AxyPrep DNA Gel Extraction Kit was provided by Axygen Biosciences (Union City, CA, USA). Other reagents, such as ethanol, hydrochloric acid, chloroform, acetone, and NaOH, were all of analytical grade.

2.2. Animals and Experimental Design.
Forty-eight 5-week-old male ICR mice (20 ± 1 g of weight) were all bought from Beijing Vital River Laboratory Animal Technology Co., Ltd. (Beijing, China; Certificate no. SCXK (Beijing) 2016-0006). The mice were kept in separate cages with fed food and water ad libitum. The mice were housed in a controlled environment with 40–55% humidity and a 12 h light/dark cycle at 22–24°C. All animal procedures act up to the Animal Ethics Committee of the Beijing Key Laboratory of Functional Food from Plant Resources and the guidelines for the care and use of laboratory animals of the National Institutes of Health.

All mice were randomly divided into normal group (NG), model group (MG), HPA low-dose group (HPAL), and HPA high-dose group (HPAH) after adaption for 1 week (n = 12 each group). The mice in the NG and MG were orally distilled water for the following 4 weeks. After 1 hour of treatment with distilled water, the MG were given orally 10 mL/kg body weight (bw) of 50% alcohol. In the HPAL and HPAH, HPA was formulated into a solution with a mass fraction of 40% and 80% (HPA was dissolved in distilled water). First, all mice were given orally the same dose alcohol as MG. Then, the HPAL and HPAH were administrated with 40% and 80% of HPA solution by oral route respectively (10 mL/kg bw) after treatment with 50% alcohol 1 hour later. The mice were weighted every three days, and the perfusion volume was adjusted based on the body weight. After the fourth week of feeding, the mice were weighed and recorded for the last time. The mice were fasted for 12 hours with water freely before the blood samples were taken. The blood samples were taken from the orbital venous plexus of the mice and stored at 4°C for 12 hours. The liver tissue was weighed immediately after the mice were killed and the liver index was calculated [liver index (%) = liver weight (g)/final body weight (g) × 100%]. The liver sample was divided into two parts: one part was immersed in 10% formalin solution for histopathology; the other part was used for biochemical determination and stored at −80°C. Colon contents were collected and stored at −80°C for intestinal microbiota analysis.

2.3. Analysis of Biochemical Indicators in Serum and Hepatic.
The serum was obtained after the blood samples centrifuged at 4°C with 4000 g for 15 minutes. According to the test kits, the levels of serum TC, TG, FFA, HDL-C, LDL-C, AST, ALT, APL, and γ-GT were tested by the Mindray BS-420 automatic biochemical analyzer (Shenzhen Mindray Biomedical Electronics Co., Ltd.). According to the method of Zhao et al. [19], the liver sample was homogenized, and then liver lipids were extracted from liver homogenate. The hepatic TC, TG, and FFA were determined by the same corresponding detection kits for detection with serum TC, TG, and FFA. The activities of SOD, CAT, GSH, GSH-Px, and the level of MDA

in liver were detected using the corresponding assay kits. The contents of TNF-α, IL-6, and IL-1β were measured following the instructions of the corresponding ELISA kits. The BCA kit was used to measure the concentration of total protein in liver homogenate.

2.4. Analysis of Real-Time PCR.

The total liver RNA was extracted by using TRIpure reagent according to the instruction and reverse-transcribed into cDNA by using FastQuant RT Kit. The levels of mRNA expression were measured according to the kit manufacturer's instruction by SuperReal PreMix Plus with SYBR Green [20]. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) as the internal reference was measured to normalize mRNA expression. The appropriate primers are shown in Table 1. The relative mRNA expression was calculated by the $2^{-\Delta\Delta Ct}$ method (relative expression = $2^{-\Delta\Delta Ct}$ (ΔCt (test) = Ct (target, test) − Ct (ref, test), ΔCt (calibrator) = Ct (target, calibrator) − Ct (ref, calibrator), $\Delta\Delta Ct$ = ΔCt (test) − ΔCt (calibrator))) [21].

2.5. Histological Analysis.

The extracted livers were fixed in 10% (v/v) formalin solution for 24 h, before the samples were cut into slices. Then, the slices were embedded in paraffin. The slices were stained with hematoxylin and eosin (H&E) and Masson. Finally, the sections were placed under a light microscope (BA-9000, Osaka, Japan) for observation.

2.6. Analysis of Gut Microbiota.

The genome of fecal flora was extracted by using the DNA extraction kit, and the DNA samples were determined by NanoDrop2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). The V3-V4 region of the bacterial 16S rDNA genes was amplified using the barcoded universal primers (338F: 5′-ACTCCTACGGGAGGCAGCAG-3′; 806R: 5′-GGAC-TACHVGGGTWTCTAAT-3′). The amplified products were confirmed by agarose gel electrophoresis for completeness and purified by AxyPrep DNA Gel Extraction Kit. The amplified products library was paired-end-sequenced on Illumina Miseq platform (Illumina, San Diego, CA, USA). Gut microbiota analysis was completed by Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China).

2.7. Statistical Analysis.

The results of the animal experiment were presented as mean ± standard deviation (SD). SPSS 25.0 was used to evaluate the statistical analysis between model and other groups by independent-samples T test using SPSS 25.0 (SPSS Inc., Chicago, USA). The figures were drawn by GraphPad Prism version 8.0 (La Jolla, CA, USA). $p < 0.05$ was recognized as statistically significant and $p < 0.01$ were regarded as highly statistically significant.

3. Result

3.1. Effect of HPA on Food Intake, Body Weight, and Hepatic Index in ALD Mice.

As shown in Table 2, the final body weight and food intake of alcohol-treated mice were significantly lower than the NG ($p < 0.01$). The hepatic index in the MG was lower than that in the NG ($p < 0.05$). Only the high-dose HPA could clearly decrease the hepatic index compared to the MG ($p < 0.05$).

3.2. Effect of HPA on Lipids of Serum and Hepatic in ALD Mice.

Figures 1(a)–1(e) present the influence of HPA on the serum lipids (TC, TG, FFA, HDL-C, and LDL-C). Compared with the NG, the levels of serum of TC, TG, FFA, and LDL-C in alcohol-treated mice were apparently increased ($p < 0.01$). Serum HDL-C in the MG was significantly reduced by 25.3% (versus NG, $p < 0.01$). As displayed in Figures 1(f)–1(h), the contents of hepatic lipids (TC, TG, and FFA) in the MG were obviously higher than that in the NG ($p < 0.01$). The levels of serum and hepatic lipids in all treatment groups were improvement apparently (versus MG, $p < 0.01$). The contents of serum TG and hepatic TG in the HPAH could reduce by more than 59% (versus MG, $p < 0.01$). As Figures 1(a)–1(h), the high-dose HPA had the best improvement effect on the lipids of serum and hepatic in ALD mice.

3.3. Effect of HPA on Hepatic Function in ALD Mice.

Figure 2 shows that the activities of ALT, AST, ALP, and γ-GT in the MG were apparently higher than that in the NG ($p < 0.01$). The HPA could reduce the levels of these indexes (versus MG, $p < 0.01$). In particular, the improvement effect of high dose HPA was the best, reducing AST (18.82%), ALT (23.67%), ALP (31.76%), and γ-GT (26.66%), respectively.

3.4. Effect of HPA on the Hepatic Oxidative Stress in ALD Mice.

As shown in Figures 3(a)–3(e), there was a significant plummet in the CAT, SOD, GSH, and GSH-Px contents that occurred in alcohol-treated mice, and the MDA level had an apparently elevation (versus NG, $p < 0.01$). After treatment with HPA, the content of oxidation markers in the liver of ALD mice was significantly improved (versus MG, $p < 0.01$). In terms of SOD, GSH-Px, and MDA, the improvement effect of the HPAH was better than that of the HPAL. Especially, high dose of HPA could reduce the MDA level in ALD mice to 60% of the original (Figure 3(e), $p < 0.01$). In addition, HPAL had the best improvement effect on CAT and GSH, and low dose of HPA could increase the level of CAT in ALD mice by 0.27 times (versus MG, $p < 0.01$).

3.5. Effect of HPA on Inflammation Response in ALD Mice.

As shown in Figures 4(a)–4(c), the effect of HPA on the level of inflammatory factors in ALD mice was evaluated. The level of inflammatory factors (IL-1β, IL-6, and TNF-α) increased significantly in alcohol-treated mice (versus NG, $p < 0.01$). The three indexes in the treatment groups were obviously decreased (versus MG, $p < 0.01$). In the levels of IL-1β and TNF-α, the HPAH had the best improvement effect. The HPAH not only reduced the values of IL-1β from 7.77 to 3.86 pg/mg pro but also reduced the TNF-α index by 4.12 pg/mg pro (versus MG, $p < 0.01$). Compared with the MG, the content of IL-6 in the HPAL was only 0.67 times that in the MG (Figure 4(b)).

TABLE 1: Primer sequences of genes used for qRT-PCR.

Gene	Forward primer (5′-3′)	Reverse primer (5′-3′)
Gapdh	TCTCCTGCGACTTCAACA	TGTAGCCGTATTCATTGTCA
Keap1	CAGATTGACAGCGTGGTT	GCAGTGTGACAGGTTGAA
Nfe2l2	GTGCTCCTATGCGTGAAT	TCTTACCTCTCCTGCGTATA
Hmox1	AGGTCCTGAAGAAGATTGC	TCTCCAGAGTGTTCATTCG
Nqo1	ATGAAGGAGGCTGCTGTA	AGATGACTCGGAAGGATACT
Tlr4	TGACATTCCTTCTTCAACCA	CACAGCCACCAGATTCTC
Myd88	CCGTGAGGATATACTGAAGG	TTAAGCCGATAGTCTGTCTG
Nfkb1	AGACAAGCAGCAGGACAT	CCAGCAACATCTTCACATC

TABLE 2: Effects of AP and AS on body weight, food intake, and liver index of mice.

Groups	Initial weight (g)	Final weight (g)	Food intake (g/d)	Hepatic index (%)
NG	28.38 ± 1.17	34.09 ± 2.97	4.92 ± 0.46	3.63 ± 0.22
MG	28.55 ± 1.49	28.98 ± 2.00**	3.63 ± 0.66**	3.99 ± 0.11*
HPAL	28.90 ± 1.69	28.98 ± 1.17	3.28 ± 0.73	3.82 ± 0.16
HPAH	28.74 ± 1.32	28.63 ± 1.98	3.20 ± 0.68	3.73 ± 0.22#

Data were expressed as mean ± SD ($n = 12$). ** $p < 0.01$ and * $p < 0.05$, versus NG; # $p < 0.05$, versus MG.
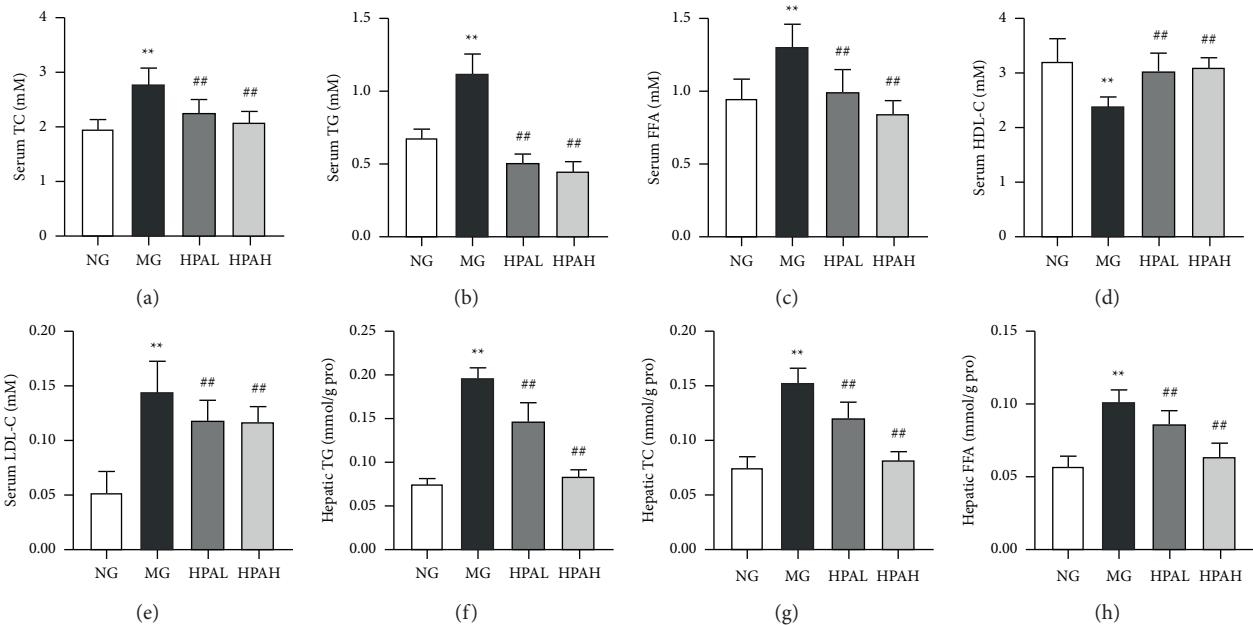

FIGURE 1: Effect of HPA on the serum and liver lipids in ALD mice. (a) Serum TG; (b) serum TC; (c) serum HDL-C; (d) serum LDL-C; (e) serum FFA; (f) hepatic TG; (g) hepatic TC; (h) hepatic FFA. Data were expressed as mean ± SD ($n = 12$). ** $p < 0.01$, versus NG; # $p < 0.05$ and ## $p < 0.01$, versus MG.

3.6. Effect of HPA on Oxidative Stress and Inflammation-Related Gene Expression in ALD Mice.

Figure 5 shows the effect of HPA on gene expression in inflammatory signal pathway (TLR4 (Tlr4), Myd88 (Myd88), and NF-κB (Nfkb1)) and oxidative stress signal pathway (Kelch-like ECH-associated protein 1 (Keap1 (Keap1)), NF-E2-related factor 2 (NRF2 (Nfe2l2)), heme oxygenase 1 (HO-1 (Hmox1)), and NADPH quinone dehydrogenase 1 (NQO1 (Nqo1)) in alcohol-treated mice. After the mice were treated by alcohol, the mRNA expression of Keap1, Nfe2l2, Hmox1, and Nqo1 was significantly downregulated, while the mRNA expression of Tlr4, Myd88, and Nfkb1 was significantly upregulated (versus NG, $p < 0.01$). It has been observed that the HPA could obviously reduce the expression of Keap1, Nfe2l2, Hmox1, and Nqo1 and clearly increased the expression of Tlr4, Myd88, and Nfkb1 (vs. MG, $p < 0.01$). Compared with the MG, the genes related to oxidative stress in the HPAH increased more than 2 times, and the genes related to inflammation in the HPAH decreased by more than 36.6%. Therefore, it can be seen that HPAH reduced the expression of inflammation-related genes and at the same time promotes the expression of antioxidant-related genes.

3.7. Effect of AP and AS on Histopathological Variations of Livers in ALD Mice.

In order to confirm that the HPA has improvement effect on ALD, hepatic sections were stained
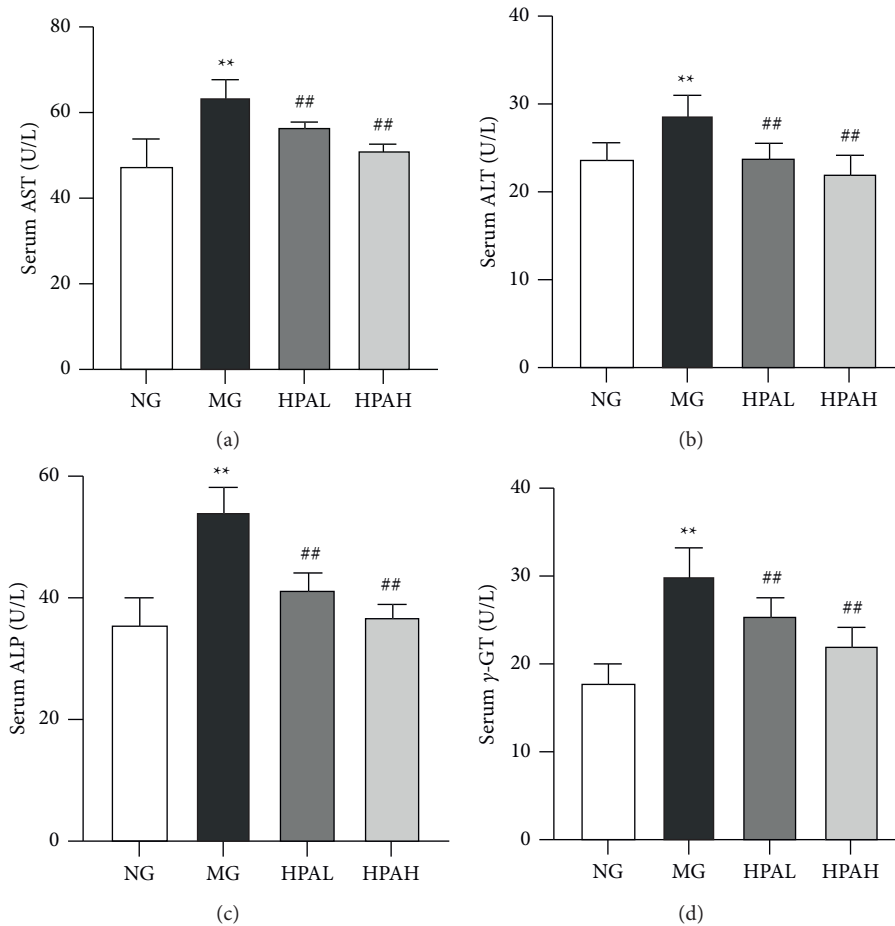
FIGURE 2: Effect of HPA on the hepatic function in ALD mice. (a) Serum AST; (b) serum ALT; (c) serum ALP; (d) serum γ-GT. Data were expressed as mean ± SD ($n = 12$). $^{**}p < 0.01$, versus NG; $^{#}p < 0.05$ and $^{##}p < 0.01$, versus MG.

by H&E and Masson and pathological observations were performed. As presented in Figure 6, the structure of hepatocytes and the arrangement of hepatic cords in the NG were normal, and there was no edema and inflammatory infiltration. At the same time, there was no accumulation of collagen fibers in liver tissue. However, the structure of hepatocytes and hepatic cords of alcohol-fed mice were disordered, and there was obvious inflammatory cell infiltration. At the same time, hepatocytes were accompanied by severe accumulation of collagen fibers. After the intervention of HPA, the structure of hepatocytes and hepatic cords was not arranged neatly; there were a small amount of inflammatory cell infiltration and collagen fibers accumulated. But compared with the MG, it had obvious improvement effect.

3.8. Effects of HPA on the Changes of the Gut Microbiota Composition in ALD Mice. In Figure 7(a), the Venn chart shows that the NG, MG, HPAL, and HPAH had a total of 104 OTUs at the genus level. Figures 7(b) and 7(c) show the composition of intestinal flora in each group of mice at the genus and phylum levels. At the genus level (Figure 7(c)), the dominant bacteria in the NG mice were

norank_f_Muribaculaceae and *Lactobacillus*, accounting for 29.36% and 21.25% respectively. While, in the MG, the dominant bacteria were *Lactobacillus* (35.24%), *norank_f_Muribaculaceae* (25.77%), and *Faecalibaculum* (5.80%). After being treated with HPA, the abundance of *Feacalibaculum* and *norank_f_Muribaculaceae* were decreased to some extent, and the abundance of *Prevotellaceae* was increased (versus MG). At the phylum level (Figure 7(b)), the abundance of Firmicutes in MG was significantly increased, and the content of *Bacteroidota* was decreased (versus NG). Compared with the MG, the abundance of Firmicutes was decreased, and the *Verrucomicrobiota* was increased.

The correlation between gut microbial flora and biochemical indicators is shown in Figure 7(d). There were both positive and negative correlations between serum lipids indexes and each colony. The *Romboutsia* was positively correlated with AST and hepatic TC ($p \leq 0.001$). The relative abundance of *Turicibacter* was significantly positively correlated with IL-6, and it was negatively correlated with SOD ($p \leq 0.001$). Both *unclassified_k__norank_d__Bacteria* and *Bifidobacterium* showed positive correlation with serum LDL-C, while *norank_f__Erysipelotrichpaceae*, *norank_-f__Lachnospiraceae,* and *Colidextribacter* were negatively
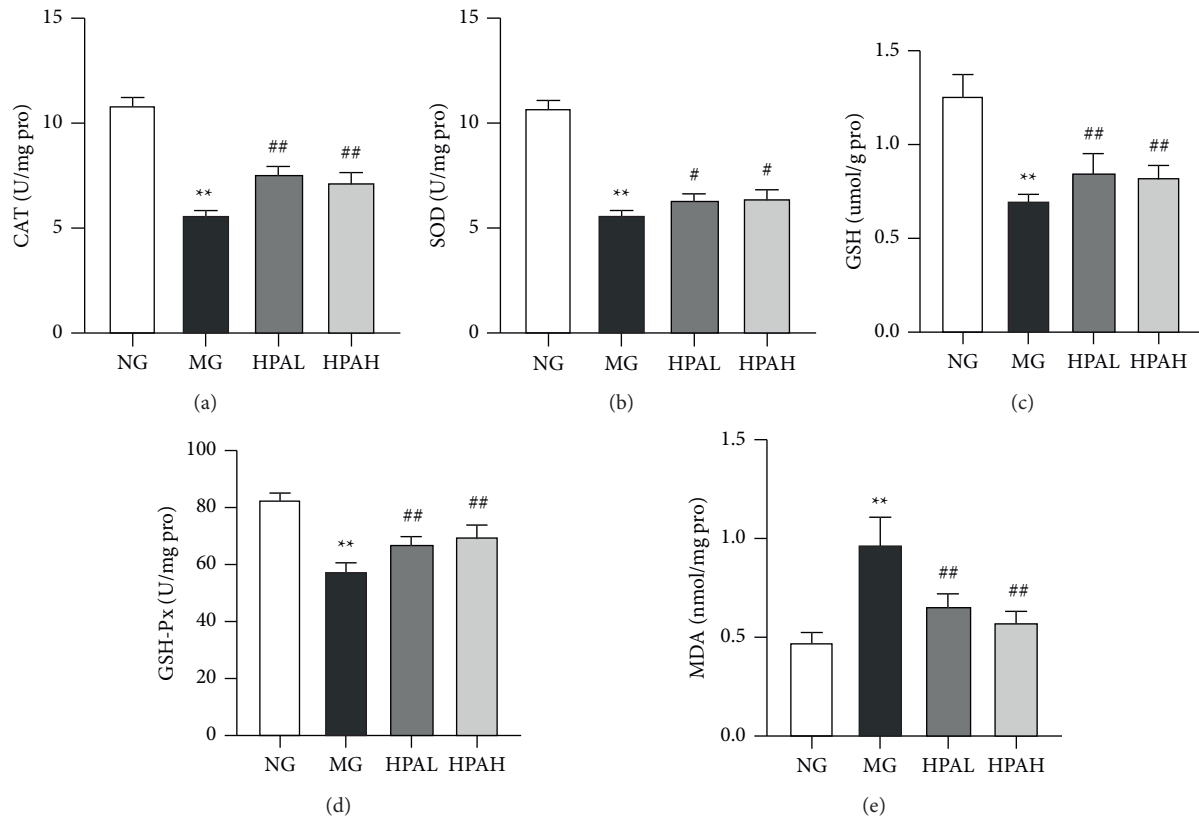
FIGURE 3: Effect of HPA on the antioxidant capacities in ALD mice. (a) CAT activity; (b) SOD activity; (c) GSH activity; (d) GSH-Px activity; (e) MDA level. Data were expressed as mean ± SD ($n = 12$). $^{**}p < 0.01$, versus NG; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$, versus MG.
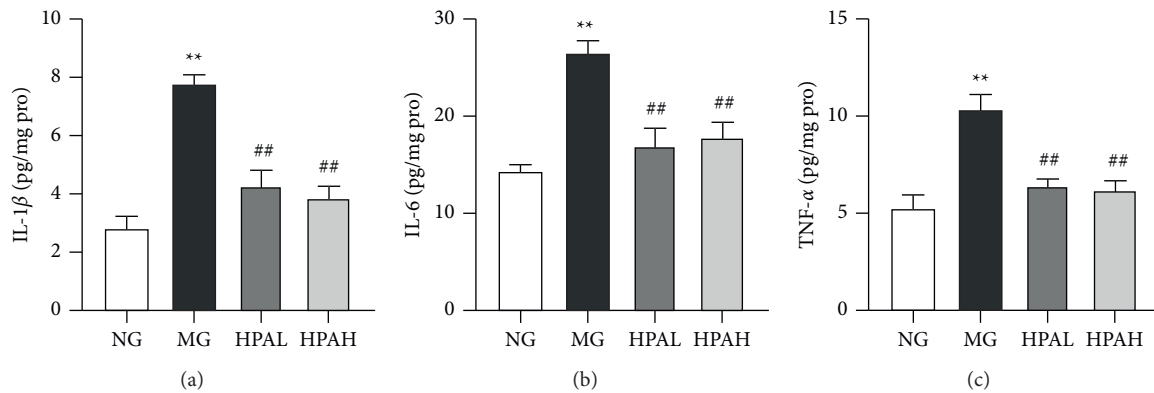

FIGURE 4: Effect of HPA on inflammation response in ALD mice. (a) Hepatic IL-1β; (b) hepatic IL-6; (c) hepatic TNG-α. Data were expressed as mean ± SD ($n = 12$). $^{**}p < 0.01$, versus NG; $^{\#}p < 0.05$ and $^{\#\#}p < 0.01$, versus MG.

correlated with serum LDL-C ($p \leq 0.001$). *Colidextribacter* was negatively correlated with TNF-α and hepatic FFA ($p \leq 0.001$). The relative abundance of *Roseburia* was obviously negatively correlated with ALP ($p \leq 0.001$).

4. Discussion

ALD is the leading cause of global liver disease, and its risk increases with increased dose and duration of alcohol consumption [22, 23]. Some scholars have pointed out that oxidative stress and inflammatory cytokines could directly

or indirectly make the development of ALD more serious [24, 25]. In this study, the alcohol-induced ALD mice model was established. The purpose of experiment was to explain the mechanism of HPA to improve ALD in mice by studying detected liver index, blood lipids, oxidation markers, and inflammatory factors. In addition, the regulatory effect of HPA on intestinal microflora in ALD mice was also investigated.

The final weight, food intake, and hepatic index in the MG were significantly different from those in the NG (Table 1). After treatment with high-dose HPA, the liver
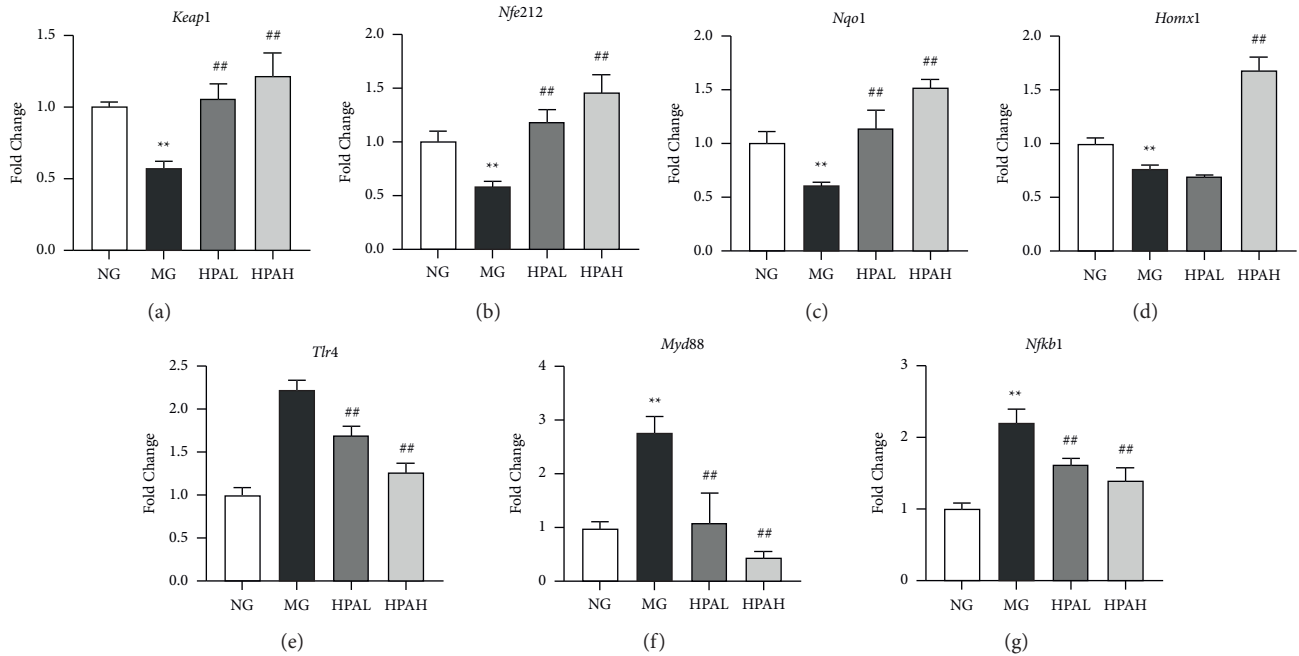
FIGURE 5: Effect of HPA on oxidative stress and inflammation-related gene expression in ALD mice. (a) *Keap*1; (b) *Nfe*2l2; (c) *Nqo*1; (d) *Homx*1; (e) *Tlr*4; (f) *Myd*88; (g) *Nfkb*1. Data were expressed as mean ± SD ($n = 12$). $^{**}p < 0.01$, versus NG; $^{##}p < 0.01$, versus MG.
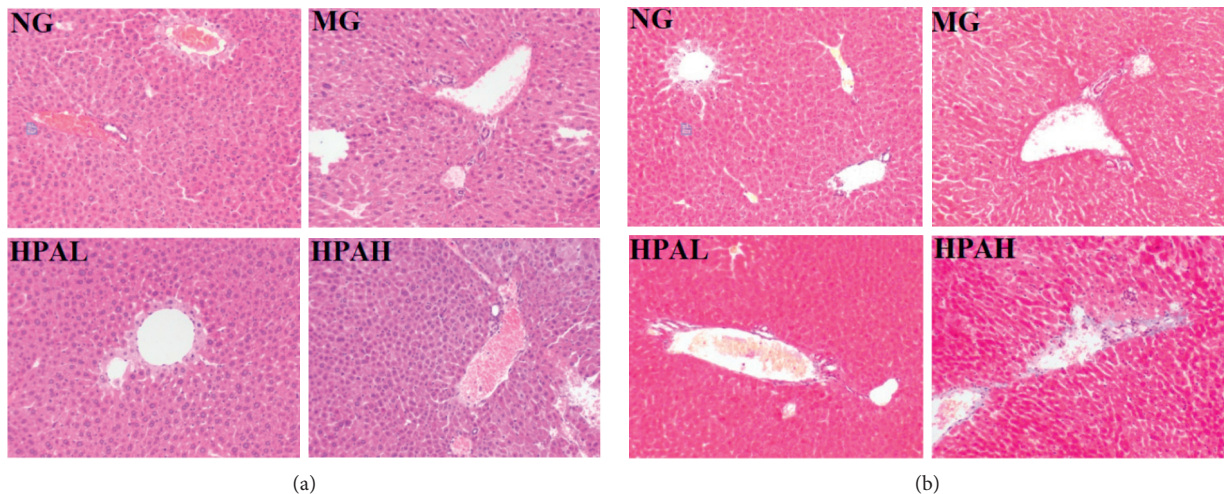

FIGURE 6: Histopathological detection of livers in ALD mice. (a) H&E staining in liver (×200 magnification); (b) Masson staining in liver (×200 magnification).
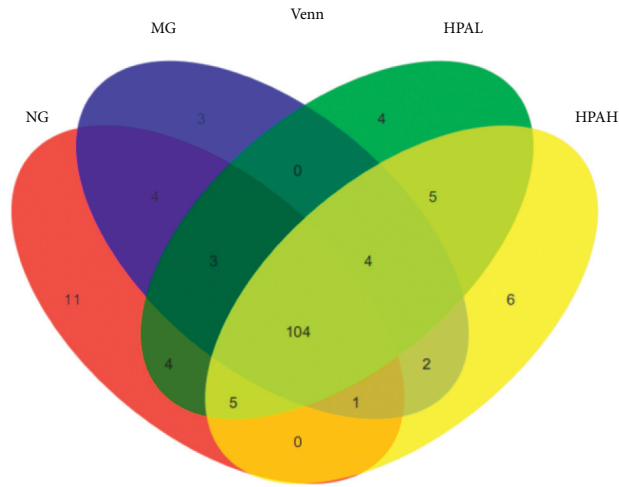
index was obviously reduced (versus MG). However, there was no significant change in these indexes between the HPAL and the MG. This shows that the liver index decreases with the increase of the HPA.

Some studies have shown that alcoholic liver injury could cause excessive accumulation of lipids in serum and liver, resulting in dyslipidemia in the body and the formation of alcoholic fatty liver [26, 27]. The current results showed that both high-dose and low-dose HPA could significantly improve the abnormalities of serum lipids and liver lipids (TC, TG, and FFA) caused by alcohol (versus MG, Figures 1(a)–1(h)). HPA could obviously reduce the content of LDL-C and increased the content of HDL-C; this result
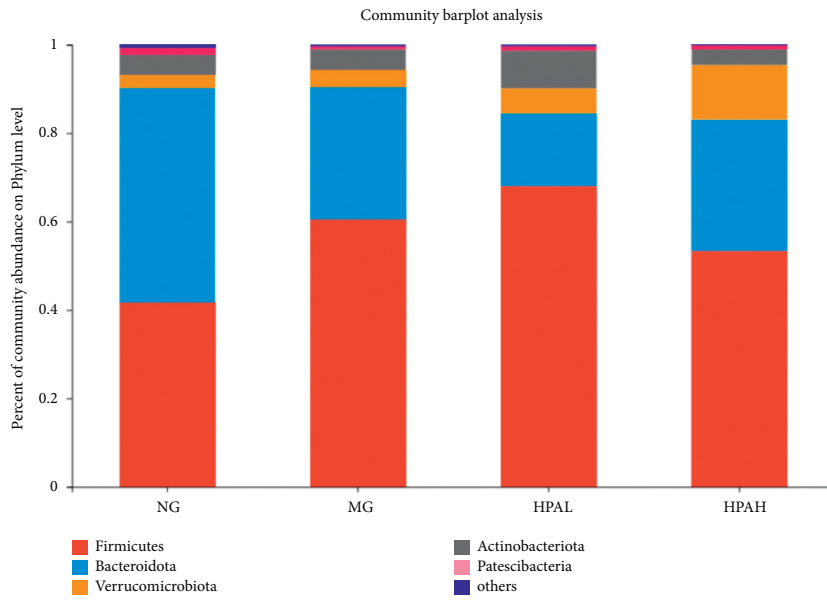
was consistent with the study of Sadeghi et al. [28]. High-dose HPA had the best effect in improving blood lipids.

The activities of AST, ALT, ALP, and γ-GT will increase with alcohol intake, thus aggravating the degree of liver injury, so they are important indicators for evaluating liver function [29, 30]. The results showed that all treatment groups could clearly reduce the levels of the above four indexes (Figure 2). Therefore, HPA could improve liver dysfunction cause by alcohol, and the improvement effect of high-dose HPA was the best.

Oxidative stress refers to the imbalance between reactive oxygen species and antioxidants, which can be exacerbated by excessive alcohol intake [25, 31]. In a normal
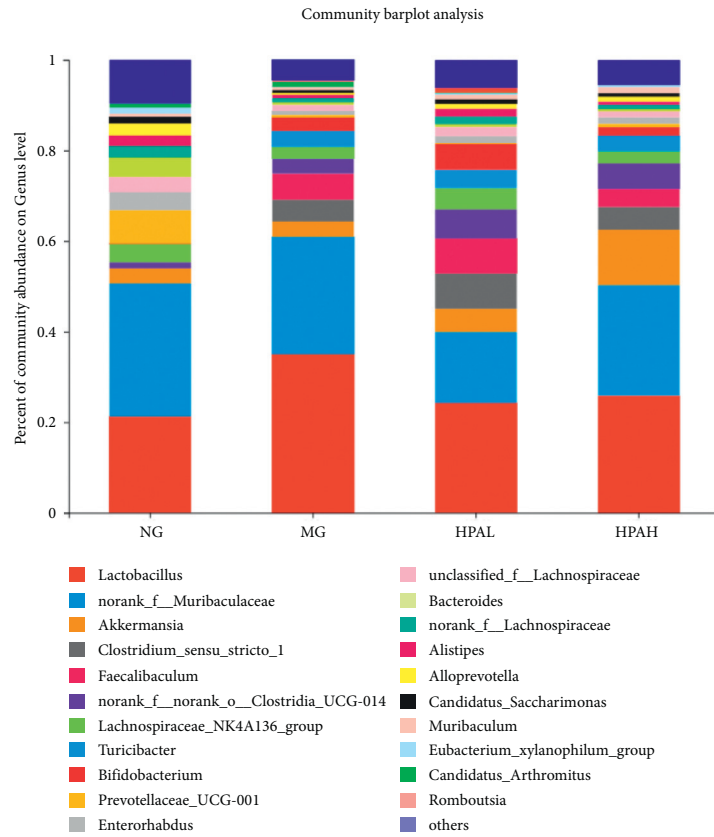
(a)

(b)

FIGURE 7: Continued.

Community barplot analysis

(c)

FIGURE 7: Continued.
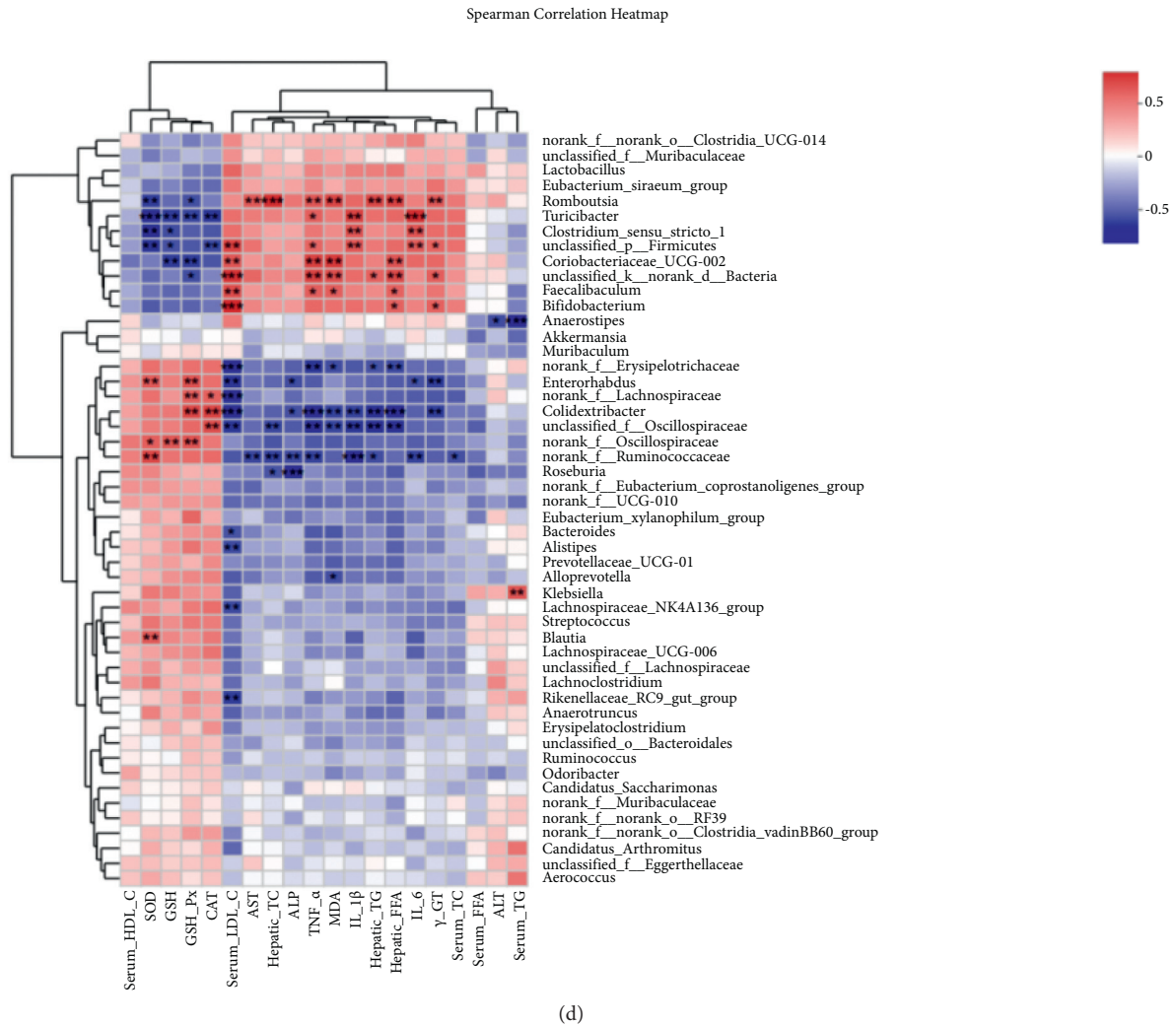
Spearman Correlation Heatmap

(d)

Figure 7: Effects of HPA on the changes of the colonic microbiota composition in ALD mice. (a) Venn diagrams of HPA; (b) percent of community abundance at phylum level; (c) percent of community abundance at genus level; (d) heatmap of spearman's correlation analysis of the biological parameters and relative abundance of colonic microbiota at species level. $***p \leq 0.001$ and $**p \leq 0.01$.

physiological environment, antioxidants are present in human cells to eliminate harmful free radicals, such as CAT, SOD, and GSH [32]. KEAP1/NRF2 is a classical oxidative stress pathway, in which NRF2 is a key transcription factor in the process of oxidative stress [33, 34]. After NFR2 is isolated into the nucleus, it binds to the ARE site to activate the expression of downstream *Hmox*-1 and *Nqo*1 gene, thus increasing the production of antioxidant enzymes and regulating the level of oxidative stress [35]. Figure 3 shows that the levels of oxidative stress markers were significantly increased in all treatment groups (versus MG). Interestingly, the low-dose HPA had a good effect on CAT and GSH levels; the high-dose HPA had the good effect on SOD and GSH-Px. In addition, HPA could obviously reduce the level of MDA in alcohol-treated mice (versus MG). At the genetic level, HPA could apparently enhance the expression of antioxidant genes (Figures 5(a)–5(d)), so as to improve the effects on oxidative stress caused by ALD.

Continuous intake of alcohol activates proinflammatory cytokines, leading to liver inflammation [36]. When NF-κB is transferred from the cytoplasm to the nucleus, it releases inflammatory mediators, including IL-1β, IL-6, and TNF-α [37]. These inflammatory mediators disrupt the balance between proinflammatory and anti-inflammatory factors, so they play an important role in the pathogenesis of ALD [38]. Excessive ethanol intake will increase the level of toxin in the body; the TLR4 was activated and then the expression level of *Myd*88 gene signal pathway was increased, leading to liver inflammation [39]. In particular, the levels of IL-1β, IL-6, and TNF-α in alcohol-treated mice decreased significantly after HPA treatment (Figures 4(a)–4(c)). In the report of Liao et al. [17], HPA also reduced the inflammatory cytokines in alcohol-induced ALD mice, which is consistent with our results. In Figures 6(a) and 6(b), the expression of genes related to inflammation was decreased in all treatment groups, and the improvement effect of high-dose HPA was the best.

It has been reported that long-term alcohol intake can lead to a disorder in the abundance of intestinal microflora [30]. In the intestinal microflora, there have been beneficial and harmful bacteria. Among them, *Faecalibaculum* is a representative genus of intestinal bacteria in alcoholic liver injury, which has the effect of promoting inflammation [40]. *norank_f_Muribaculaceae* has a great relationship with human obesity; the higher its abundance is, the thinner the person is [41]. *Akkermansia* can reduce intestinal permeability, and as its abundance increases, so does the content of lipopolysaccharide [42]. In this experiment, the results showed that the abundance of harmful bacteria (*norank_f_Muribaculaceae*, *Faecalibaculum,* and *unclassified_p_Firmicutes*) in alcohol-treated mice was increased. After treatment with HPA, the harmful bacteria abundance was decreased, and the beneficial bacteria (*Akkermansia*) was increased. The results showed that HPA could improve the intestinal microbial disorder caused by alcohol by changing the abundance of intestinal flora.

In this study, we found that HPA could reduce the indexes of serum and liver lipid in alcohol-treated mice. HPA could improve liver inflammation and oxidative stress in ALD mice by promoting or inhibiting the expression of inflammatory factors and antioxidative stress genes. After the ALD mice were treatment by HPA, the beneficial bacteria were increased and harmful bacteria were reduced.

5. Conclusion

In general, HPA could improve the lipid accumulation in serum and liver caused by alcohol and also inhibit the development of inflammatory factors and liver fibrosis. On the contrary, HPA could improve the activities of liver function and oxidation markers and regulate the imbalance of intestinal microflora. Quantitative RT-PCR results showed that HPA could alleviate oxidative stress and inflammation of ALD through two signal pathways: KEAP1/NRF2 and TLR4/MyD88/NF-κB. In addition, high-dose HPA showed a more prominent effect. This project provided a theoretical basis for HPA in the treatment of alcoholic liver disease. At the same time, it provides a certain theoretical basis for the subsequent exploration of the functions of the monomer components in HPA. It also provides new ideas for the treatment or prevention of ALD. In the future, we will study the effective monomer components in HPA.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Disclosure

Jingxuan Zhou and Nanhai Zhang share first authorship.

Conflicts of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as potential conflicts of interest.

Authors' Contributions

JZ and NZ performed the experiments and wrote the manuscript. LZhao revised the manuscript. WW and JL supervised the project. FZ and LZhan contributed to conception and design the project. FZ, JL, LZhan, and M.M. Soliman contributed to acquisition of the financial support for the project. All authors have read and agreed to the published version of the manuscript. Jingxuan Zhou and Nanhai Zhang have contributed equally to this work.

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