Contents lists available at SciOpen

Food Science and Human Wellness

journal homepage: https://www.sciopen.com/journal/2097-0765

China Food Publishing Co.

Scytosiphon lomentaria fucoidan alleviates alcohol-induced liver injury in mice *via* the modulation of gut microbiota and bile acid-FXR/AMPK and NF-κB pathways

Yiyun Sun[a], Qiuyue Men[a], Xiaomeng Ren[a], Xiaoming Guo[b], Shuang Song[a], Chunqing Ai[a,c,*]

[a] National Engineering Research Center of Seafood, School of Food Science and Technology, Dalian Polytechnic University, Dalian 116034, China
[b] Shenzhen Key Laboratory of Food Nutrition and Health, Institute for Advanced Study, Shenzhen University, Shenzhen 518060, China
[c] National & Local Joint Engineering Laboratory for Marine Bioactive Polysaccharide Development and Application, Dalian Polytechnic University, Dalian 116034, China

ARTICLE INFO

Article history:
Received 1 January 2024
Received in revised form 29 February 2024
Accepted 19 June 2024

Keywords:
Scytosiphon lomentaria fucoidan
Alcoholic liver injury
Gut microbiota
Bile acid metabolism
FXR/AMPK pathway
NF-κB pathway
Gut-liver axis

ABSTRACT

Alcohol intake is associated with increased mortality worldwide, particularly liver diseases, making it imperative to explore innovative strategies for managing alcohol-related liver disease. In this study, the efficacy of *Scytosiphon lomentaria* fucoidan (SLF) in alleviating alcohol-induced liver injury was evaluated in a mouse model. It showed that SLF increased body weight and colon length, while reducing liver index, serum lipid, alanine aminotransferase, and aspartate aminotransferase in alcohol-treated mice. SLF inhibited inflammatory response in the liver by reducing inflammatory infiltration and the levels of pro-inflammatory cytokines. It can be associated with the alleviation of oxidative stress and the inhibition of the nuclear factor-κB pathway. SLF modulated alcohol-induced dysbiosis of gut microbiota, including a reduction in Bacteroidetes and Proteobacteria, and improved metabolites profile, primarily affecting short chain fatty acids and amino acids metabolism. In addition, SLF reduced the level of total bile acids, regulated the profile of bile acids, and increased the levels of farnesoid X receptor (FXR) and AMP-activated protein kinase (AMPK), suggesting that SLF can alleviate alcohol-induced liver injury by regulating bile acid-FXR/AMPK pathway. This study suggests that SLF holds the potential to alleviate the adverse effect of alcohol on the liver *via* the gut-liver axis.

1. Introduction

Alcohol is widely appreciated around the world for its mellifluous taste and social significance. However, the increasing global consumption of alcohol has led to a higher prevalence of alcohol-related liver diseases (ALD). ALD refers to liver injury occurring due to excessive alcohol consumption and involves various diseases, including hepatitis, liver steatosis, steatohepatitis and et al.[1]. According to the World Health Organization, alcohol is responsible for 3 million deaths worldwide and contributes to 5.1% of the overall disease burden[2]. ALD is a multi-step chronic disease process that progresses through stages of alcohol-induced steatosis, hepatitis, fibrosis and cirrhosis[3]. Alcohol abstinence and pharmacological intervention are the most widely applied strategies for treating ALD[4]. Considering the limitations of first-line treatments (e.g. drug tolerance and side effects), it is crucial to investigate innovative strategies for the management and treatment of ALD.

* Corresponding author at: School of Food Science and Technology, Dalian Polytechnic University, Dalian 116034, China.
E-mail address: acqdongying@163.com (C.Q. Ai)
Peer review under responsibility of Beijing Academy of Food Sciences.

SciOpen Publishing services by Tsinghua University Press

http://doi.org/10.26599/FSHW.2024.9250345

Accumulating evidence has shown the beneficial effects of bioactive compounds in food on host health, including obesity, liver diseases, and metabolic syndrome[5-6]. Polysaccharides have attracted a lot of attention due to their diverse bioactivities and low toxicity[7]. Polysaccharides from *Rosa rugosa* exhibited a protective effect against hepatic injury in alcohol-treated mice by decreasing inflammatory factors and oxidative stress[8]. *Auricularia cornea* polysaccharides exhibited a hepatoprotective effect by alleviating oxidative stress, improving alcohol metabolism, and reducing inflammatory factors and lipid peroxidation[9]. Farnesoid X receptor (FXR), as a bile acid receptor, plays a key role in ALD by regulating AMP-activated protein kinase (AMPK) activity to combat oxidative damage and inflammation[10]. *Crassostrea gigas* polysaccharides alleviated oxidative stress and inflammation in the liver by regulating the expression of FXR and AMPK[11]. These findings suggest that polysaccharides can be beneficial for managing ALD.

Although the precise mechanism by which polysaccharides benefit liver diseases remains unclear, changes in the gut microbiota induced by polysaccharides is believed to play a crucial role. This is because gut bacteria or their metabolites can enter the liver *via* the portal vein, affecting liver functions[12]. A reduction in Bacteroidaceae and an increase in Prevotellaceae can be observed in human alcoholics[13]. Alcohol-induced disruption of epithelial tight junction can increase intestinal permeability, resulting in a greater entry of LPS or other toxic substances into the liver[14]. Clinical data showed that some microbial factors exhibited a comparable predictive capacity to conventional risk factors for liver diseases and identified previously unknown taxa for liver diseases[15]. *R. rugosa* polysaccharides alleviated alcohol-induced liver injury in mice that associated with a reduction in Gammaproteobacteria and an increase in Firmicutes[8]. Fucoidan from different sources ameliorated alcohol-induced liver injury by reducing inflammatory mediators[16-17], indicating their potential in the management of ALD.

Scytosiphon lomentaria is popular in some Asian countries, and fucoidan is one of the most important substances. *S. lomentaria* fucoidan (SLF) has various bioactivities, which can be associated with modulation of the gut microbiota[18]. But, the effect of SLF on ALD and its underlying mechanism remain unclear. In this study, the efficacy of SLF in alleviating alcohol-induced injury was evaluated in a mouse model, involving weight loss, organ damage, lipid metabolism abnormality and inflammation. To explore the underlying mechanism of SLF on the improvement of ALD, the inhibitory effect of SLF on oxidative stress and the nuclear factor-κB (NF-κB) pathway was measured. The modulation of SLF on alcohol-induced dysbiosis of gut microbiota and bile acid profile was determined using microbiomics and metabolomics. This study suggests that SLF holds the potential to benefit the liver *via* the gut-liver axis.

2. Materials and methods

2.1 Chemical reagents

Aspartate aminotransferase (AST), alanine aminotransferase (ALT), high density lipoprotein cholesterol (HDL-C), low density lipoprotein cholesterol (LDL-C), total cholesterol (TC), superoxide dismutase (SOD), triglycerides (TG), glutathione peroxidase (GSH-Px), malondialdehyde (MDA) and total bile acid (TBA) assay kits were from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). Tumor necrosis factor-α (TNF-α) and interleukin-1β (IL-1β) ELISA kits were purchased from Shanghai Enzyme-linked Biotechnology Co., Ltd. (Shanghai, China). Anti-FXR, p65, p-p65, toll-like receptor-4 (TLR4), inhibitor of κBα (IκBα), p-IκBα, peroxisome proliferator-activated receptor-α (PPARα), PPARγ, AMPK, p-AMPK and glyceraldehyde-3-phosphate dehydrogenase (GADPH) rabbit antibodies were purchased from Abcam (Shanghai, China). Goat anti-rabbit IgG antibody was obtained from Beyotime (Beijing, China). Glycochenodeoxycholic acid-d4 (GC47408) and cholic acid-d4 (GC47086) were purchased from GlpBio (Montclair, CA, USA). All other reagents were of analytical grade and provided by Damao Chemical Reagent Co., Ltd. (Tianjin, China).

2.2 Preparation of SLF

SLF was prepared according to our previous study[18]. *S. lomentaria* powder was dissolved in water at a ratio of 1:25 (*m*/*V*) and reacted with a combination of cellulase, pectinase and papain at 50 °C for 3 h. After centrifugation at 6 000 × *g* for 10 min at 4 °C, the supernatants were precipitated with 30% of ethanol (final concentration) to remove alginate, and ethanol level was modified to 60% to collect the precipitate (SLF). SLF was dissolved in distilled water, and the above process was repeated 3 times to obtain purified SLF. The basic characteristics of SLF was consistent with our previous study[18]. SLF was composed of 2 fragments with molecular weight of 100 and 460 kDa, sulfate group level was 15.5%, and uronic acid was 8.5%. SLF was composed of mannose, galactose, fucose, galacturonic acid, glucose, xylose, glucuronic acid and rhamnose at a molar ratio of 17.1:13.6:15.9:4.7:3.3:5.7:1.1:1.0 (Fig. S1).

2.3 Animal experiment

Male BALB/c mice (SPF; 6 weeks and 18–21 g) were provided by Liaoning Changsheng Biotechnology Co., Ltd. (Shenyang, China). All mice were kept under the standard conditions and had free access to food and water. Mice experiment followed the guides for the care and use of experimental animals of National Institutes of Health, and it was approved by the Animal Ethics Committee of Dalian Polytechnic University (License number: DLPU2022012).

All mice were divided into 4 groups (n = 8/group): model group (MG), normal group (NG), low dosage of SLF group (LSLF), and high dosage of SLF group (HSLF). Mice in the NG group were given distilled water, and mice in the MG, LSLF and HSLF groups were gavaged with 56% alcohol for 60 days (6.2 to 12.4 mL/(kg·day), Fig. 1A). At the same time, mice in the LSLF and HSLF groups were gavaged with 100 and 300 mg/(kg·day) of SLF respectively, and the MG and NG groups were gavaged with distilled water as negative control.

2.4 Assessment of biochemical parameters

The contents of AST, ALT, TC, and TG in the serum were measured using the kits according to manufacturer's instructions. On the other hand, 100 mg of liver tissues was weighed and homogenized in 9 volumes of PBS solution using a glass homogenizer. After centrifugation at 2 500 × *g* for 10 min at 4 °C, LDL-C, HDL-C, MDA, GSH-Px,SOD, and TBA in the homogenates were measured using the kits, and IL-1β and TNF-α in the homogenates were measured using ELISA kits.

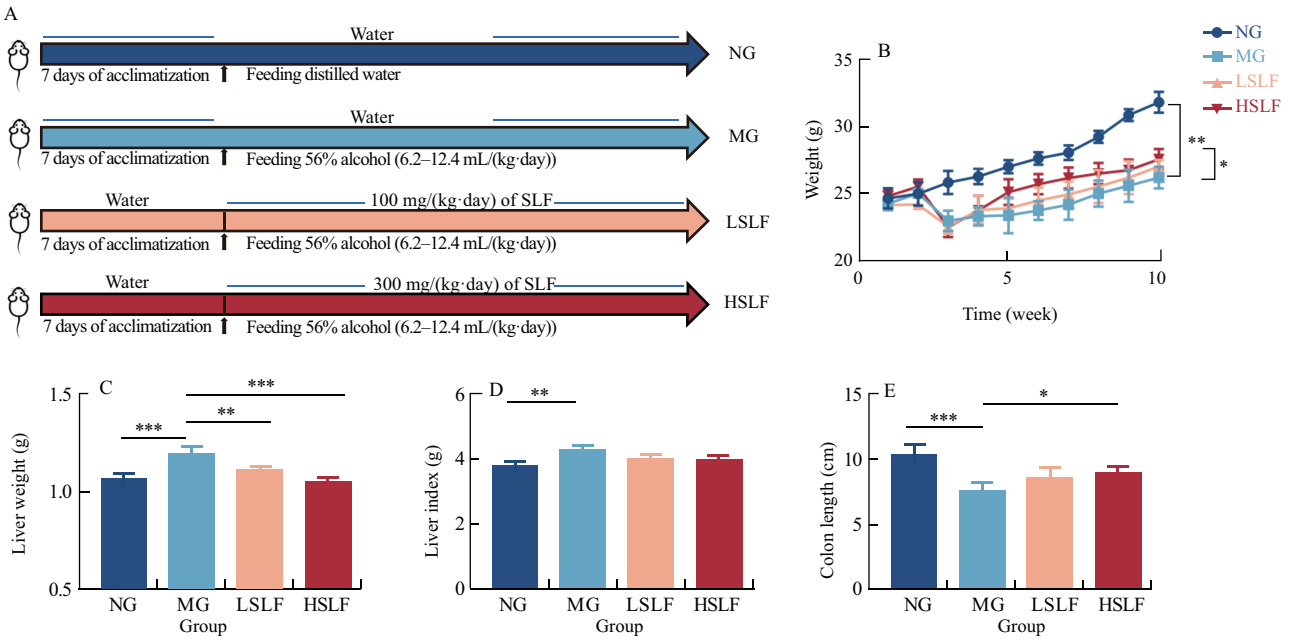

Fig. 1 Effect of SLF on alcohol-induced adverse effect on body weight and organ. (A) Flow chart of mice experiment. (B) Body weight. (C) Liver weight. (D) Liver index. (E) Colon length. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

2.5 Histological analysis

Fresh liver was fixed in paraformaldehyde, embedded in paraffin, cut into 5 μm slices, and stained with hematoxylin/eosin (HE) and Oil Red O. Histological analysis was performed using a light microscope (Leica, Weztlar, Hessen, Germany).

2.6 Analysis of the gut microbiota composition

Analysis of the gut microbiota was performed at Beijing Novogene Bioinformatics Technology Co., Ltd. (Beijing, China), as our previous study[18]. Briefly, microbial DNA were extracted using the commercial kit according to manufacturer's instructions. The quality of DNA was evaluated using a Nanodrop (Thermo; Wilmington, DE, USA) and gel electrophoresis, and the V3–V4 regions of 16S rRNA were amplified using a PCR system. The PCR products were purified and then sequenced on an Illumina HiSeq platform. The raw files were processed according to the standard protocol of Novogene Bioinformatics Technology Co., Ltd. The Shannon and Ace indexes were measured to evaluate the microbiota richness and diversity, and principal coordinate analysis (PCoA) and unweighted pair-group method with arithmetic means (UPGMA) were performed to analyze the relationships between groups. Linear discriminant analysis (LDA) was performed to identify the bacteria that differ between groups.

2.7 Targeted analysis of microbiota metabolites using HPLC-QTRAP/MS

Analysis of amino acids and organic acids were performed on a triple quadrupole tandem mass spectrometer 4000 QTRAP (ABSCIEX, Framingham, MA, USA) that coupled to a liquid chromatograph (Shimadzu, Kyoto, Japan) as our previous study[19]. Data processing and analysis were performed using previously established method[19].

2.8 Measurement of short chain fatty acids (SCFAs)

SCFAs were measured using gas chromatography (Agilent, Santa Clara, CA, USA) according to our previous study[20]. Fecal samples were dissolved in saturated NaCl solution and reacted with 10% sulfuric acid. Diethyl ether was added to extract SCFAs, and the supernatant was reacted with Na_2SO_4 to remove the residual water. The levels of SCFAs were measured using GC-2010 system with HP-INNOWAX column. The carrier gas was N_2 at a rate of 15 mL/min, and the rates of air, H_2 and N_2 were 300, 30 and 30 mL/min respectively. The initial column temperature was 100 °C, increased to 200 °C at the rate of 5 °C/min, and maintained at 250 °C for 5 min.

2.9 Western blotting analysis

Briefly, 50 mg liver was processed in RIPA lysis buffer containing proteinase and phosphatase inhibitors, and then the supernatant was collected after centrifugation. The content of protein was measured with the BCA assay kit. Equivalent amount of protein was separated by SDS-PAGE and transferred to the activated PVDF membrane. After sealing with 5% skimmed milk, the membrane was incubated with the diluted primary antibodies, followed by washing 3 times with TBST buffer, and then incubated with HRP-conjugated goat anti-rabbit IgG antibody. The intensity of each band was detected using the ChemiDoc XRS system (Bio-Rad, Hercules, CA, USA) and quantitated by ImageJ software (Java 1.8.0, NIH, Bethesda, MD, USA).

2.10 Analysis of bile acids profile

Fecal samples from each mouse were processed as previous method[21]. Briefly, 100 mg of mice feces was dissolved in 200 μL of methanol/water (1:1, *V/V*, containing glycochenodeoxycholic acid-d4 and cholic acid-d4, 50 nmol/L). After centrifugation at 13 500 × *g* for 15 min at 4 °C, the supernatant was transferred into a 1.5 mL tube.

The residue was further extracted using methanol/acetonitrile (2:8, *V/V*, containing internal standards as mentioned above), and the supernatant was mixed with the first extraction. After centrifugation at 13 500 × *g* for 15 min at 4 °C, bile acids analysis was performed on a UPLC/MS using the method established in previous study[21].

2.11 Statistical analysis

Statistical analysis was performed with GraphPad Prism 8 (GraphPad Software; San Diego, CA, USA). All data are presented as means ± standard deviation (SD). The difference between groups was analyzed using one-way ANOVA followed by post hoc Tukey test. $P < 0.05$ was considered significant. Spearman correlation analysis was used to evaluate the relation between key bacteria and biochemical parameters.

3. Results

3.1 SLF protected against weight loss and organ damage

Body weight was distinctly reduced in the MG group compared to the NG group. In contrast, high dosage of SLF decreased alcohol-induced weight loss, and low dosage of SLF had no effect on body weight (Fig. 1B). Compared to the NG group, liver weight and liver index were significantly increased in the MG group, while colon length was reduced (Figs. 1C-E). Compared to the MG group, low dosage of SLF reduced liver weight but had no significant effect on liver index and colon length, and high dosage of SLF reduced liver weight and increased colon length ($P < 0.05$).

3.2 SLF attenuated alcohol-induced lipid accumulation and liver injury

In comparison to the NG group, the contents of serum ALT, AST, TC and TG were higher in the MG group (Figs. 2A-D). Compared with the MG group, SLF distinctly decreased the levels of serum TC, AST and TG in alcohol-treated mice, while the high dosage of SLF decreased the level of ALT. Compared with NG group, the content of LDL-C was increased in the liver of the MG group, while HDL-C level was reduced; however, in contrast to the MG group, both low and high dosages of SLF had no impact on HDL-C but reduced LDL-C level, and no significant difference was found between them. Histological analysis revealed that alcohol intake induced massive formation of lipid droplet in the MG group (Fig. 2G). SLF reduced lipid droplet infiltration in the liver, resulting in a near-normal appearance, and no difference was found between the LSLF and HSLF groups.

3.3 SLF reduced inflammatory response and oxidative stress in the liver

Histological analysis showed obvious infiltration of inflammatory cells in the liver of the MG group (Fig. 3A). SLF treatments reduced inflammatory infiltration, implying that SLF can inhibit alcohol-induced inflammatory response. In addition, the levels of SOD and GSH-Px in the liver were distinctly decreased in the MG group than

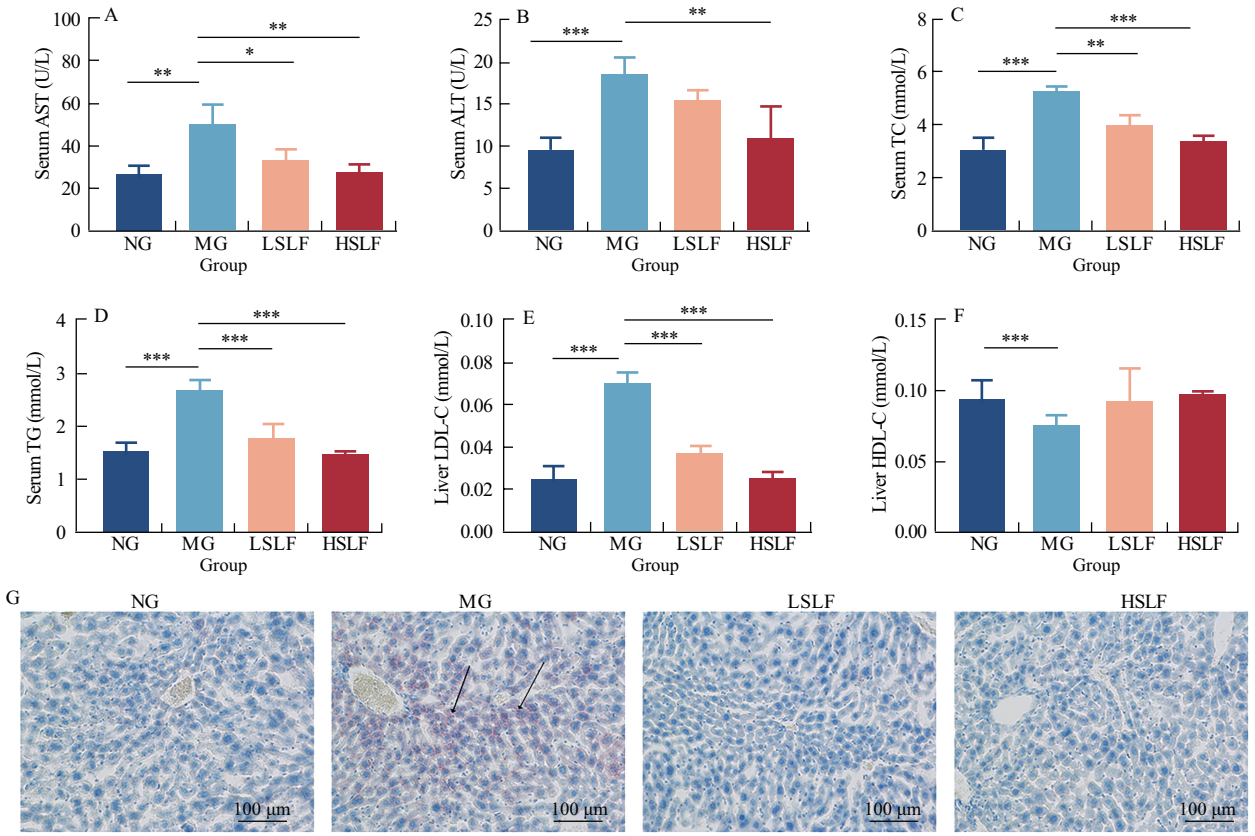

Fig. 2 Effect of SLF on lipid metabolism and liver injury. (A) AST, (B) ALT, (C) TC, and (D) TG in the serum. (E) LDL-C and (F) HDL-C in the liver. (G) Histological analysis of liver based on Oil Red O staining. Black arrow indicates lipid droplets. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

the NG group, and MDA was increased (Fig. 3B). SLF increased the levels of SOD and GSH-Px and reduced the level of MDA in alcohol-treated mice. The levels of IL-1β and TNF-α were higher in the MG group than the NG group, while SLF decreased the levels of IL-1β and TNF-α, implying its anti-inflammation activity (Fig. 3C). Compared to the NG group, the contents of TLR4 ($P > 0.05$), p65, p-p65, and IκBα were increased in the MG group. Compared to the MG group, low dosage of SLF had no effect on TLR4, p-p65, and IκBα, whereas high dosage of SLF reduced the levels of TLR4, p65, p-p65, and IκBα. It indicates that high dosage of SLF can have remarkable inhibition of the NF-κB pathway.

3.4 SLF modulated alcohol-induced alterations of the gut microbiota

As shown in Fig. 4A, approximately 65% of operational taxonomic units (OTUs) was shared between the MG and NG groups. Low and high dosages of SLF resulted in a reduction in the numbers of OTUs, with approximately 45% of OTUs being shared between these 2 groups and MG group. Ace and Shannon indexes were lower in the MG group than the NG group, while low and high dosages of SLF led to slight increase in the Ace index ($P > 0.05$, Fig. 4B). UPGMA and PCoA showed a separation between the NG and MG groups (Figs. 4C and D). SLF modulated alcohol-induced alterations of the microbiota, while the HSLF group was closer to the NG group. At the phylum level, Bacteroidetes and Proteobacteria were higher in the MG group, while Firmicutes was reduced, resulting in an increased Bacteroidetes-to-Firmicutes ratio (B/F ratio) (Fig. 4E). Compared to the MG group, Low and high dosages of SLF reduced the levels of Bacteroidetes and Proteobacteria as well as the B/F ratio, and high dosage of SLF also increased the level of Firmicutes.

LDA showed that Rikenellaceae, Marinifilaceae, *Odoribacter*, and *Alistipes* were dominant in the NG group, and Planococcaceae, Akkermansiaceae, Comamonadaceae, Aerococcaceae, Erysipelotrichaceae, *Akkermansia*, *Comamonas*, *Empedobacter* and *Kurthia* were dominant in the MG group (Fig. 5A). Fodinicurvataceae, *Novibacillus*, *Oceanobacillus*, *Planifilum*, *Parvimonas*, *Prevotella*, and *Galbibacter* were dominant in the LSLF group, while

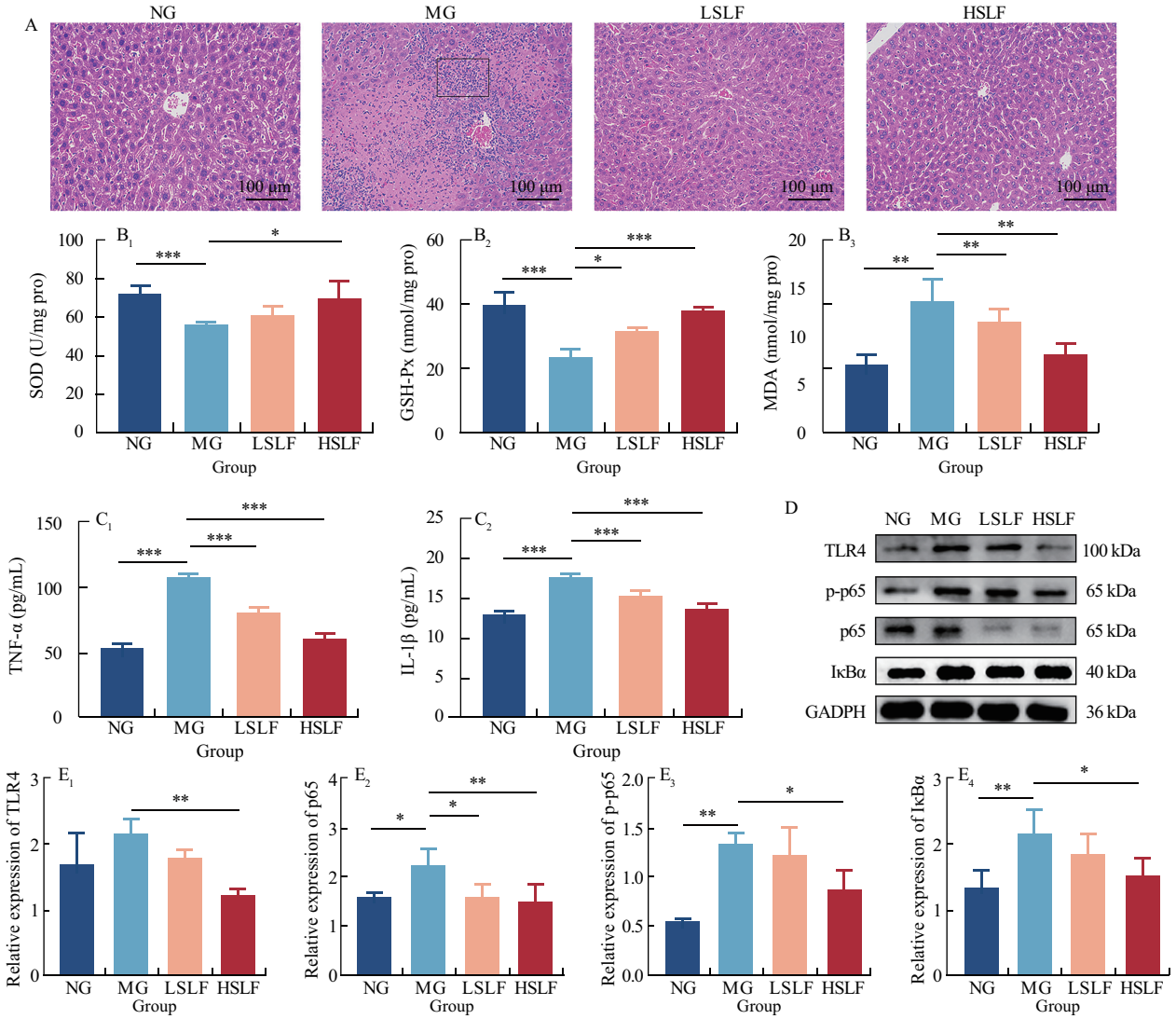

Fig. 3 Effect of SLF on alcohol-induced inflammation in the liver. (A) Histological analysis of liver based on HE staining. Inflammatory infiltration is shown in the black box. The levels of (B_{1-3}) SOD, GSH-Px, MDA, and (C_{1-2}) TNF-α and IL-1β. (D) Representative images of TLR4, p-p65, p65, IκBα and GADPH. (E_{1-4}) The relative levels of TLR4, p65, p-p65, IκBα. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

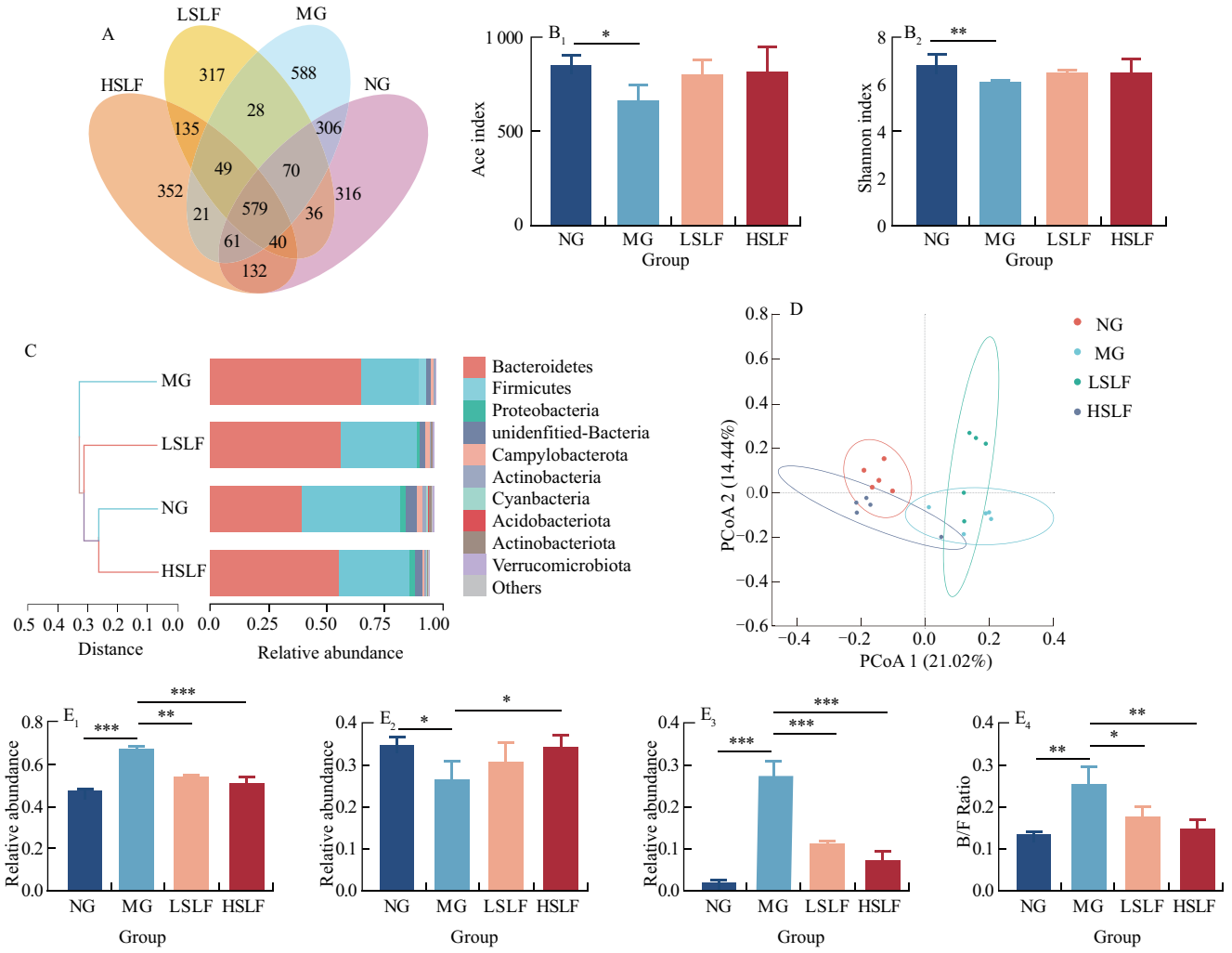

Fig. 4 Modulation effect of SLF on the gut microbiota. (A) Venn diagram. (B) Ace and Shannon indexes. (C) UPGMA and (D) PCoA based on the OTUs. (E) The abundances of Bacteroidetes (E_1), Firmicutes (E_2) and Proteobacteria (E_3) and ratio of B/F ratio (E_4). $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$.

Erwiniaceae, *Faecalibaculum*, *Enterobacter*, *Raoultella* and *Pantoea* were dominant in the HSLF group. Further study revealed that Bacteroidaceae and Erysipelotrichaceae were significantly higher in the MG group than the NG group, while Lachnospiraceae was reduced (Fig. 5B). SLF dose-dependently decreased the abundances of Bacteroidaceae and Erysipelotrichaceae and increased the level of Lachnospiraceae. At the genus level, *Alistipes* and *Faecalibaculum* were distinctly decreased in the MG group than the NG group, while *Akkermansia* was increased (Fig. 5B). Compared to MG group, High dosage of SLF significantly increased *Alistipes* and *Faecalibaculum* and reduced *Akkermansia* in mice, while low dosage of SLF only reduced the level of *Akkermansia.*

On the other hand, the contents of acetate, propionate, and butyrate were distinctly reduced in the MG group than the NG group (Fig. 5C). Low dosage of SLF had no impact on the levels of acetate, propionate, and butyrate in mice, whereas high dosage of SLF distinctly increased the contents of acetate and propionate. Spearman analysis showed that *Lactobacillus*, *Alistipes*, *Bifidobacterium*, *Helicobacter*, *Turicibacter*, and *Faecalibaculum* were positively correlated with GSH-Px, propionate and SOD, and negatively correlated with AST, ALT, MDA, TC, TG and LDL-C (Fig. 5D). *Alloprevotella* and *Akkermansia* showed opposite correlations with the aforementioned parameters. It suggests that these bacteria can play crucial roles in the improvement of ALD induced by SLF.

3.5 SLF improved alcohol-induced abnormality of microbiota metabolites

As shown in Fig. 6A, PCA revealed that alcohol affected the profile of microbiota metabolites, resulting in a significant separation between the MG and NG groups. But, no obvious difference was found between the MG and SLF groups. OPLS-DA was used to maximize the differences between groups, resulting in excellent interpretability for sample classification (Fig. 6B). A total of 200 random permutation tests showed that the OPLS-DA models were not over-fitted, which proves their stability and reliability (Fig. S2). OPLS-DA showed that obvious clustering and separation were found between the MG and NG, LSLF or HSLF groups, indicating that SLF could ameliorate alcohol-induced abnormalities of microbiota metabolite profiles.

Then, a heatmap was constructed to display the most affected metabolites between groups (Fig. 6C). Compared to the NG group, adenine and glycerophosphocholine were increased in the MG group, and homoserine, *L*-carnitine, betaine, 2-aminooctanoic acid, carnitine,

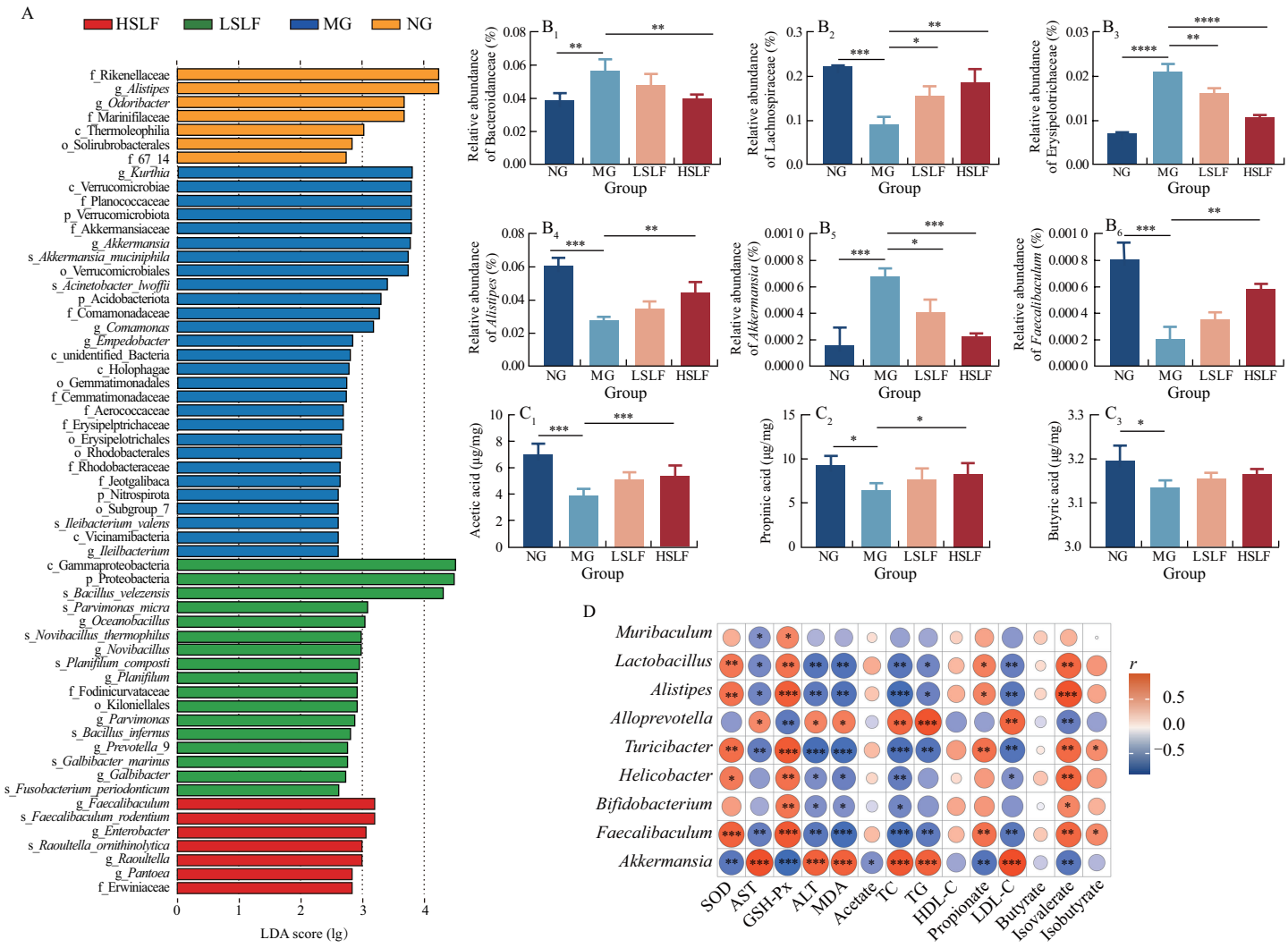

Fig. 5 Identification of the bacteria that differ between groups and their correaltion with biochemical parameters. (A) LDA. (B) Analysis of key bacteria at the family and genus levels. (C) The levels of acetate, propionate, and butyrate in mice feces. (D) Spearman correlation analysis between bacterial genera and biochemical parameters. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

cytosine, 2-methylnicotinamide, acadesine, uracil, sarcosine and alanine were reduced. Low dosage of SLF resulted in an increase in indole and a reduction in adenine, while high dosage of SLF increased the levels of homoserine, carnitine, imidazoleacetic acid and acetylcarnitine and reduced the levels of adenine, spermidine and proline. The potential metabolic pathways were analyzed by using the KEGG database (Fig. 6D). It showed that some metabolic pathways that are important for host health were affected, such as butanoate metabolism, propanoate metabolism, beta-alanine metabolism, valine, leucine and isoleucine degradation, glyoxylate and dicarboxylate metabolism, and synthesis and degradation of ketone bodies.

3.6 SLF regulated the bile acid-FXR/AMPK pathway in the liver

Bile acids circulate in the body and regulate some metabolic processes. The levels of TBA in the liver, colon, and feces were distinctly increased in the MG group than the NG group (Fig. 7A). In contrast to the MG group, SLF reduced the levels of TBA in the liver, colon and feces, while high dosage of SLF had a better effect than low dosage. To further explore the effect of SLF on bile acid, the profile of bile acids was analyzed using UPLC/MS. PCA showed a separation of bile acid profiles between the MG and NG group, and slight difference was observed between the MG and LSFL/HSLF groups (Fig. 7B). OPLS-DA revealed obvious differences between the MG and NG/LSLF/HSLF groups (Fig. 7C), implying that SLF cannot only affect the level of TBA but also modulate the profile of bile acids. A total of 200 random permutation tests showed that the OPLS-DA models were not over-fitted (Fig. S3). Alcohol intake increased the level of primary bile acids but had no effect on secondary bile acids, and SLF reduced the level of primary bile acids in alcohol-treated mice (Fig. 7D). Notably, alcohol decreased the levels of DCA and LCA and increased the levels of T-MCA and UDCA, while SLF increased the levels of DCA and LCA and reduced the contents of T-MCA and UDCA (Fig. 7E).

On the other hand, the levels of FXR, AMPK and p-AMPK were distinctly reduced in the MG group than the NG group, but no significant difference was found in PPARγ and PPARα (Fig. 8A). In contrast to the MG group, SLF increased the levels of AMPK and p-AMPK in mice, and no difference was found between the LSLF

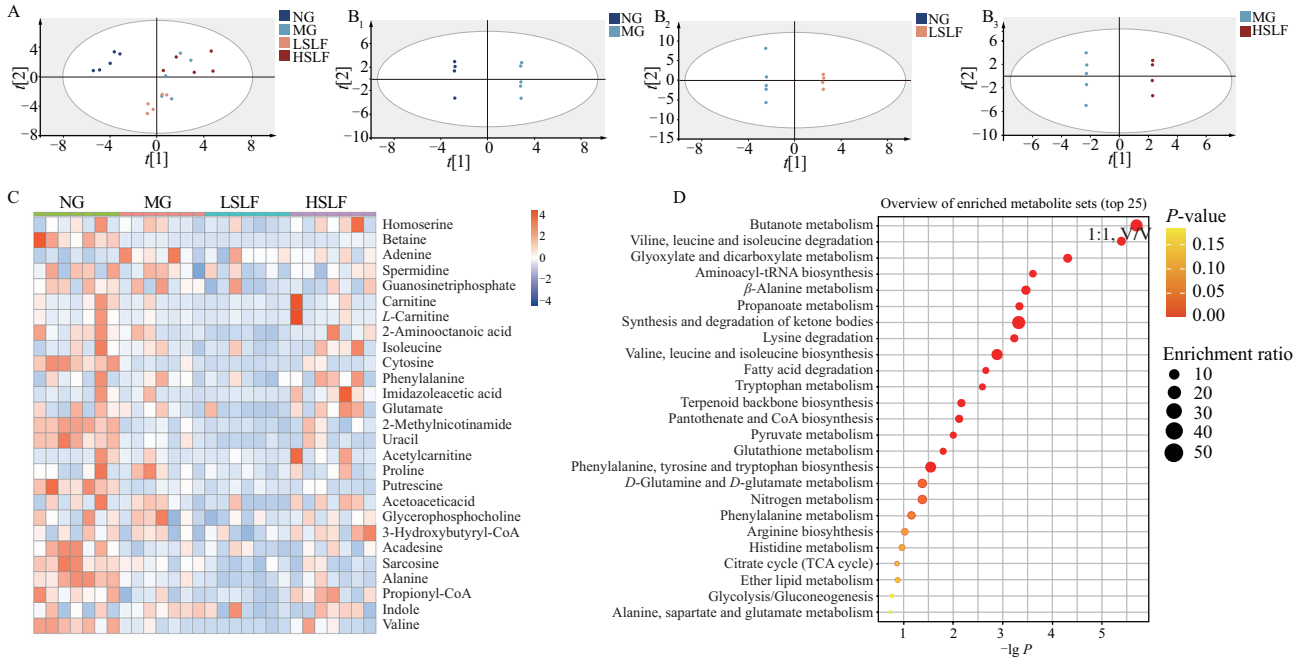

Fig. 6 Effect of SLF on microbiota metabolites. (A) PCA. (B) OPLS-DA (NG *vs.* MG, MG *vs.* LSLF, and MG *vs.* HSLF). (C) Heatmap with the most affected metabolites (VIP > 1). (D) Analysis of the metabolic pathways based on the KEGG database. **P*< 0.05, ***P* < 0.01, ****P* < 0.001.

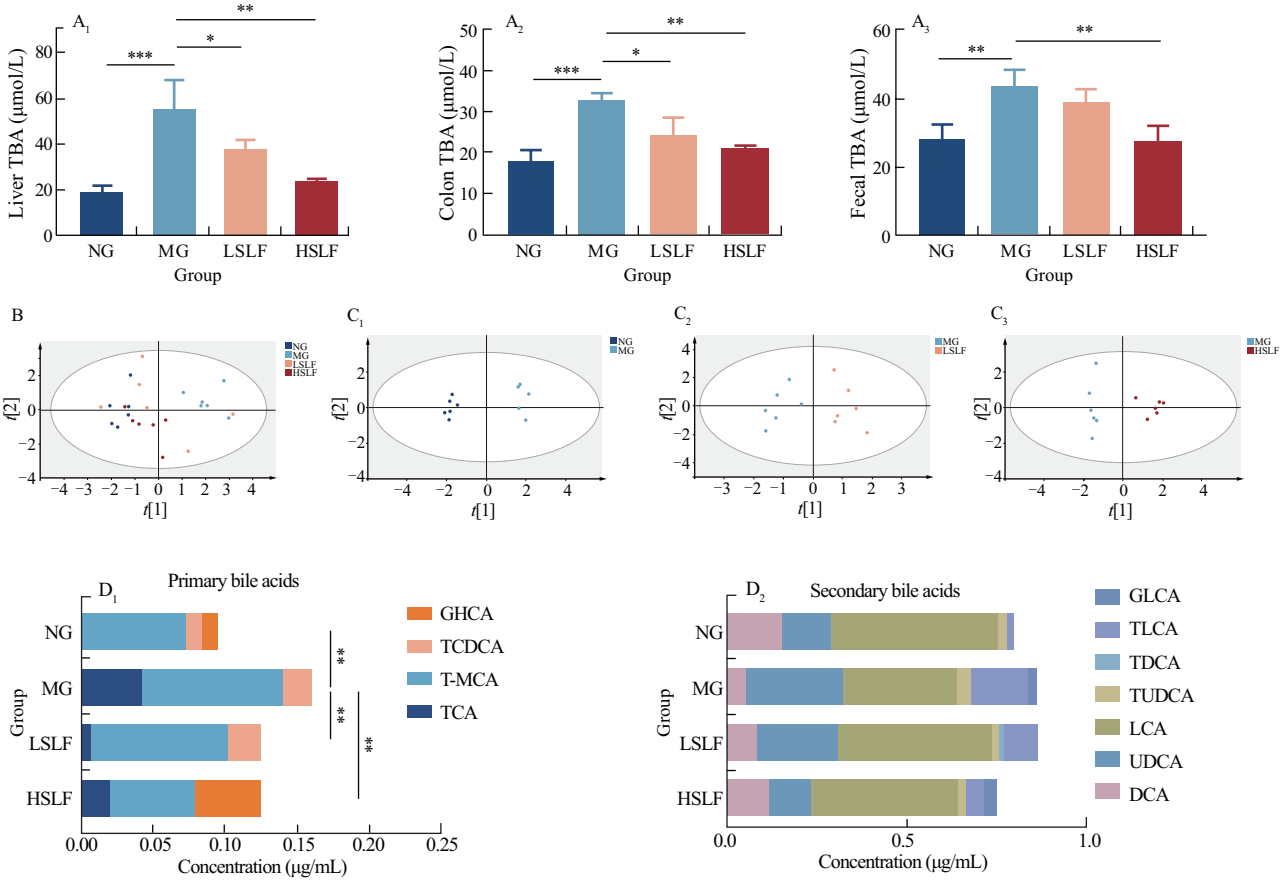

Fig. 7 Effect of SLF on bile acid in alcohol-treated mice. (A) Levels of TBA in the liver, proximal colon tissues and feces. (B) PCA that used to analyze the effect of SLF on the bile acid profile in feces. (C) OPLS-DA (NG *vs.* MG, MG *vs.* LSLF, and MG *vs.* HSLF). (D) Levels of primary bile acids and secondary bile acids. (E) Levels of T-MCA, UDCA, DCA, and LCA. **P* < 0.05, ***P* < 0.01, ****P* < 0.001.

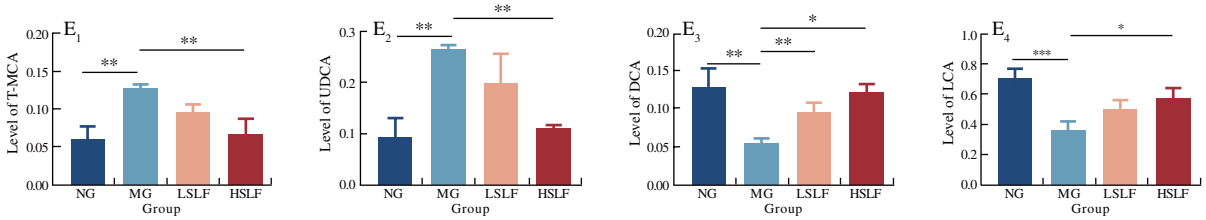

Fig. 7 (*Continued*)

and HSLF groups. It should be noted that the level of p-AMPK seemed to be higher in the HSLF group compared to the NG group. It can be considered as a short-term physiological reaction, which can potentially reach a state of equilibrium with SLF treatment. High dosage of SLF increased the level of FXR in the liver of mice, suggesting that SLF can improve ALD by regulating the bile acid-FXR/AMPK pathway.

4. Discussion

Alcohol intake has adverse effects on various aspects of the body, including body weight, organs, memory, and metabolism[22]. The liver plays a crucial role in alcohol metabolism, as it is responsible for metabolizing more than 80% of consumed alcohol[23]. AST and ALT serve as diagnostic markers for hepatic injury, whereas LDL-C, TC, HDL-C, and TG levels are indicative of lipid disorder[24]. SLF increased weight gain and colon length and reduced liver index, TG, AST, TC, ALT and LDL-C, implying that SLF can protect against alcohol-induced liver injury. It was consistent with that *Lycium barbarum* polysaccharides reduced the levels of ALT, TC, AST and LDL-C and increased HDL-C in alcohol-treated mice[25]. Polysaccharides from *Seabuckthorn* and *Astragalus* ameliorated alcohol-induced fatty liver in mice, as evidenced by a reduction in the levels of AST, ALT, TC and TG[26]. Based on these findings, SLF can potentially be a treatment option for ALD or serve as a complementary therapy to drug treatments.

Chronic inflammation is closely associated with alcohol-related medical condition, which serve as a crucial etiological factor in the onset and progression of ALD. A high level of pro-inflammatory cytokines can enhance the susceptibility of humans to ALD and tumorigenesis in mice[27-28]. SLF reduced inflammatory infiltration and levels of IL-1β and TNF-α in the liver, showing its anti-inflammatory activity. Alcohol-related inflammation inducers stem from 2 sources: substances released by alcohol-damaged cells and those produced by microbiota dysbiosis[29]. NF-κB, as a family of inducible transcription factors, regulates a wide range of genes associated with the immune and inflammatory response. Activation of the NF-κB can occur through stimuli, such as LPS or other toxic substances[30]. SLF reduced the levels of p65, IκBα and p-p65, inhibiting activation of the NF-κB pathway, leading to reduced inflammation. The metabolism of alcohol can disturb the delicate balance between antioxidants and oxidants, resulting in overproduction of reactive oxygen species (ROS) that possess the ability to trigger the activation of the NF-κB pathway[31]. SLF increased the levels of antioxidant enzymes, such us SOD and GSH-Px, thereby alleviating oxidative stress.

Alcohol can affect the composition of the gut microbiota, even prior to the onset of ALD. The alterations in the gut microbiota worsen as the disease advances and can be complicit in the progression of ALD. The gut-liver axis plays a significant role as a pathway for the development and progression of ALD[32]. Alcohol consumption can disrupt the composition and distribution of the gut microbiota, leading to a decrease in its diversity[33]. Certain bacterial overgrown may contribute to the morphological and functional abnormalities in the gut of individuals with alcohol-associated conditions. Obvious alterations in the gut microbiota were found in rats treated with alcohol, which was prevented by treatment with *Lactobacillus* or oats[34]. Previous study showed that Bacteroidetes was dominant in alcohol-fed mice, while Firmicutes was richer in normal mice[35]. SLF improved dysbiosis of the gut microbiota in mice, including a reduction in Bacteroidetes, Proteobacteria and B/F ratio, and an increase in Firmicutes. However, reduction in Bacteroidetes and Firmicutes were also found in mice treated with alcohol, while Proteobacteria and Actinobacteria were increased[36]. Recent study showed that the gut microbiota of patients with ALD was characterized by the increased presence of Proteobacteria[37]. These results suggest that Bacteroidetes and Proteobacteria can have close relationships with the development of ALD.

The levels of Bacteroidaceae, Erysipelotrichaceae, and *Akkermansia* were higher in alcohol-treated mice, whereas Lachnospiraceae, *Faecalibaculum*, and *Alistipes* were lower. Lachnospiraceae was observed at reduced level in the intestine of alcohol-related patients, while Bacteroidaceae exhibited a trend towards expansion in ALD patients[38-39]. The significance of Erysipelotrichaceae in inflammation-related disorders has been highlighted by its enrichment in colorectal cancer and intestinal bowel disease[40]. An increase in *Akkermansia* was observed in alcohol-treated mice, which may be explained by that it can degrade mucin and promote bacterial translocation[41]. Alcohol intake reduced the levels of *Faecalibaculum* and *Lactobacillus* in mice, which had negative correlations with ALT, AST and MDA levels[42]. Supplementation with *Lactobacillus* strains prevented ALD in mice by protecting the gut barrier and regulating the gut-liver axis[43]. *Faecalibaculum* strains regulated serum lipid profile and ameliorated hepatic steatosis, inflammation, and oxidative damage in mice with non-alcoholic fatty liver disease, implying its potential in the treatment of ALD[44]. *Alistipes* is a relatively new bacterial genus and may have protective effect against various diseases, including liver fibrosis, colitis, and cardiovascular disease[45]. All these bacteria can play crucial roles in the development of ALD and its alleviation by SLF.

The gut microbiota generates a range of active metabolites, which are transported to the liver *via* the portal vein. Among the most significant ones are SCFAs, bile acids, and amino acids[46]. The metabolites serve as mediators between the host and the gut microbiota, playing crucial roles in energy metabolism and modulation of the immune system[47]. Alterations in the gut microbiota can influence the profile of metabolites, particularly SCFAs, bile acids

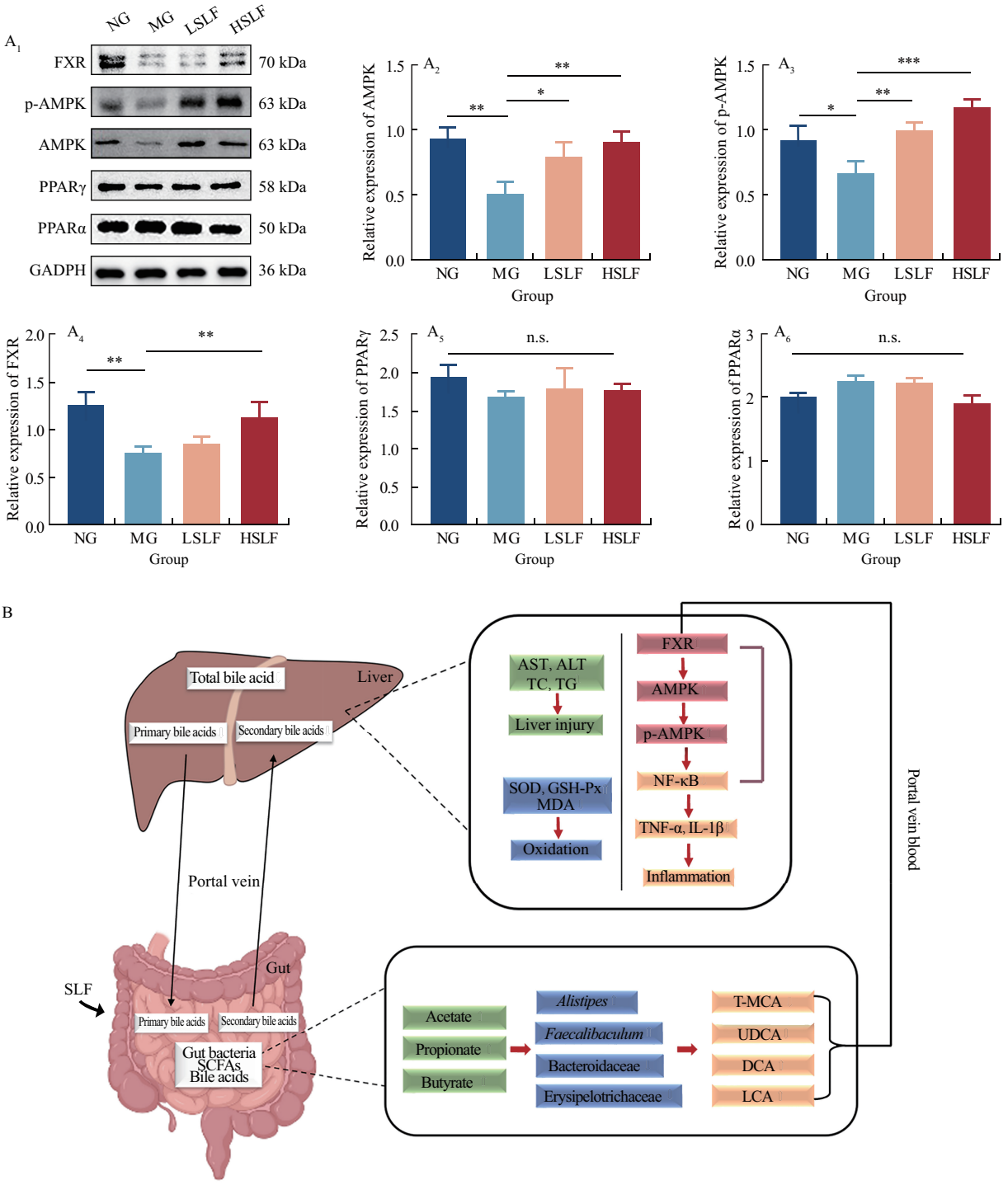

Fig. 8 Effect of SLF on the FXR/AMPK pathway. (A) Representative images and relative levels of FXR, AMPK, p-AMPK, PPARα, and PPARγ. (B) Mechanism diagram by which SLF exerts its protective effect against liver injury. $*P < 0.05$, $**P < 0.01$, $***P < 0.001$. n.s., no significance.

and amino acids, which represent key pathways that exert benefits on the host[48]. SCFAs as end products of polysaccharides fermentation play a crucial role in maintaining host physiological stability. Increased levels of SCFAs can slow or halt the progression of ALD by reducing the damage caused by LPS or other toxic substances[49]. Another study showed that inulin reduced inflammatory response in ALD through SCFAs-induced suppression of M1 macrophages and facilitation of M2 macrophages[50]. Amino acid, as intermediate metabolite can affect the biosynthesis of lipids, glutathione and other molecules, as well as cell proliferation and tricarboxylic acid cycle. Inadequate amino acid metabolism is found in ALD patients, particularly in relation to branched-chain amino acids (BCAAs), while BCAAs supplementation can prevent ALD in rodents[51]. SLF can improve alcohol-induced abnormalities of amino acids and SCFAs metabolism, thereby alleviating alcohol-induced liver injury.

Polysaccharides from *Asparagus officinalis* alleviated liver damage that associated with the modulation of bile acid metabolism[52]. Bile acid cannot only facilitate the intestinal absorption of lipids but also function

as key mediators of cellular signals by activating nuclear receptors[53]. FXR as a bile acid-sensing nuclear receptor plays a crucial role in the regulation of lipids, cholesterol, and glucose metabolism[54]. DCA and LCA as endogenous ligand agonists of FXR can activate FXR activity[55], while UDCA and T-MCA have the ability to antagonize FXR activity, resulting in an increase in bile acid synthesis[56]. SLF increased DCA and LCA levels and decreased T-MCA and UDCA levels in the liver, leading to an increase in FXR activity. FXR can regulate inflammatory response and oxidative stress, and the activation of AMPK by FXR plays a key role in pathological stress in eukaryotic cells. In addition, AMPK can also inhibit the activation of the NF-κB pathway and decrease the contents of inflammatory factors, alleviating inflammatory response[57]. Oyster polysaccharide alleviated inflammatory response in the liver by decreasing DCA and LCA levels and inhibiting the FXR/AMPK pathway[11]. These results indicate that SLF can benefit ALD *via* the regulation of the bile acid-FXR pathway.

5. Conclusion

This study provides an evidence that SLF can mitigate alcohol-induced liver injury and lipid disorder by reducing the levels of AST, ALT, TC, and TG. SLF can alleviate inflammatory response in the liver by attenuating oxidative stress and suppressing the NF-κB pathway. The underlying mechanism can be associated with modulation of the gut microbiota, such as reduction in Bacteroidetes and Proteobacteria, and improvement of metabolites profile, such us SCFAs and amino acids. In addition, SLF reduced the level of total bile acid, regulated profile of bile acid, and increased the levels of FXR and AMPK, implying that SLF can alleviate alcohol-induced liver injury by regulating the bile acid-FXR/AMPK pathway. It indicates the potential of SLF in mitigating the harmful effect of alcohol on the liver through the regulation of the gut-liver axis (Fig. 8B).

Declaration of competing interest

All 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.

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://doi.org/10.26599/FSHW.2024.9250345.

References

[1] K.J. Biddinger, C.A. Emdin, M.E. Haas, et al., Association of habitual alcohol intake with risk of cardiovascular disease, JAMA Netw. Open 5(3) (2022) e223849. https://doi.org/10.1001/jamanetworkopen.2022.3849.

[2] S.H. Park, D.J. Kim, Global and regional impacts of alcohol use on public health: emphasis on alcohol policies, Clin. Mol. Hepatol. 26(4) (2020) 652-661. https://doi.org/10.3350/cmh.2020.0160.

[3] L. Sun, S. Wen, Q. Li, et al., *L*-Theanine relieves acute alcoholic liver injury by regulating the TNF-α/NF-κB signaling pathway in C57BL/6J mice, J. Funct. Foods 86 (2021) 104699. https://doi.org/10.1016/j.jff.2021.104699.

[4] H.K. Seitz, R. Bataller, H. Cortez-Pinto, et al., Alcoholic liver disease, Nat. Rev. Dis. Primers 4(1) (2018) 16. https://doi.org/10.1038/s41572-018-0014-7.

[5] Z. Yang, H. Wang, N. Liu, et al., Algal polysaccharides and derivatives as potential therapeutics for obesity and related metabolic diseases, Food Funct. 13(22) (2022) 11387-11409. https://doi.org/10.1039/D2FO02185D.

[6] W. Wang, C. Xu, Q. Wang, et al., Protective effect of polyphenols, protein, peptides, and polysaccharides on alcoholic liver disease: a review of research status and molecular mechanisms, J. Agric. Food Chem. 71(15) (2023) 5861-5883. https://doi.org/10.1021/acs.jafc.2c07081.

[7] S.Y. Xu, X. Huang, K.L. Cheong, Recent advances in marine algae polysaccharides: isolation, structure, and activities, Mar. Drugs 15(12) (2017) 388-403. https://doi.org/10.3390/md15120388.

[8] H. Li, Z. Xie, Y. Zhang, et al., *Rosa rugosa* polysaccharide attenuates alcoholic liver disease in mice through the gut-liver axis, Food Biosci. 44 (2021) 101385. https://doi.org/10.1016/j.fbio.2021.101385.

[9] X. Wang, Y. Lan, Y. Zhu, et al., Hepatoprotective effects of *Auricularia cornea* var. *Li.* polysaccharides against the alcoholic liver diseases through different metabolic pathways, Sci. Rep. 8(1) (2018) 7574. https://doi.org/10.1038/s41598-018-25830-w.

[10] L. Zheng, L. Yin, L. Xu, et al., Protective effect of dioscin against thioacetamide-induced acute liver injury *via* FXR/AMPK signaling pathway *in vivo*, Biomed. Pharmacother. 97 (2018) 481-488. https://doi.org/10.1016/j.biopha.2017.10.153.

[11] Y. Ma, X. Liu, D. Liu, et al., Oyster (*Crassostrea gigas*) polysaccharide ameliorates high-fat-diet-induced oxidative stress and inflammation in the liver *via* the bile acid-FXR-AMPKα pathway, J. Agric. Food Chem. 70(28) (2022) 8662-8671. https://doi.org/10.1021/acs.jafc.2c02490.

[12] M. Minemura, Y. Shimizu, Gut microbiota and liver diseases, World J. Gastroenterol. 21(6) (2015) 1691-1702. https://doi.org/10.3748/wjg.v21.i6.1691.

[13] E.A. Mutlu, P.M. Gillevet, H. Rangwala, et al., Colonic microbiome is altered in alcoholism, Am. J. Physiol. Gastrointest. Liver Physiol. 302(9) (2012) G966-G978. https://doi.org/10.1152/ajpgi.00380.2011.

[14] C.A. Rivera, B.U. Bradford, V. Seabra, et al., Role of endotoxin in the hypermetabolic state after acute ethanol exposure, Am. J. Physiol. 275(6) (1998) G1252-G1258. https://doi.org/10.1152/ajpgi.1998.275.6.G1252.

[15] Y. Liu, G. Meric, A.S. Havulinna, et al., Early prediction of incident liver disease using conventional risk factors and gut-microbiome-augmented gradient boosting, Cell Metab. 34(5) (2022) 719-730. https://doi.org/10.1016/j.cmet.2022.03.002.

[16] J.D. Lim, S.R. Lee, T. Kim, et al., Fucoidan from *Fucus vesiculosus* protects against alcohol-induced liver damage by modulating inflammatory mediators in mice and HepG2 cells, Mar. Drugs 13(2) (2015) 1051-1067. https://doi.org/10.3390/md13021051.

[17] H. Zhao, S. Liu, H. Zhao, et al., Protective effects of fucoidan against ethanol-induced liver injury through maintaining mitochondrial function and mitophagy balance in rats, Food Funct. 12(9) (2021) 3842-3854. https://doi.org/10.1039/D0FO03220D.

[18] J.H. Jia, W.Y. Zheng, C.X. Zhang, et al., Fucoidan from *Scytosiphon lomentaria* protects against destruction of intestinal barrier, inflammation and lipid abnormality by modulating the gut microbiota in dietary fibers-deficient mice, Int. J. Biol. Macromol. 224 (2023) 556-567. https://doi.org/10.1016/j.ijbiomac.2022.10.144.

[19] C. Cao, L. Wang, C. Ai, et al., Impact of *Lycium barbarum* arabinogalactan on the fecal metabolome in a DSS-induced chronic colitis mouse model, Food Funct. 13(16) (2022) 8703-8716. https://doi.org/10.1039/d2fo01283a.

[20] X. Sun, Y. Liu, P. Jiang, et al., Interaction of sulfated polysaccharides with intestinal bacteroidales plays an important role in its biological activities, Int. J. Biol. Macromol. 168 (2021) 496-506. https://doi.org/10.1016/j.ijbiomac.2020.12.024.

[21] X. Zheng, F. Huang, A. Zhao, et al., Bile acid is a significant host factor shaping the gut microbiome of diet-induced obese mice, BMC Biol. 15 (2017) 1-15. https://doi.org/10.1186/s12915-017-0462-7.

[22] R. Saitz, Unhealthy alcohol use, New Engl. J. Med. 352 (2005) 596-607. https://doi.org/doi:10.1056/NEJMcp042262

[23] J. Liu, X. Wang, Z. Peng, et al., The effects of insulin pre-administration in mice exposed to ethanol: alleviating hepatic oxidative injury through anti-oxidative, anti-apoptotic activities and deteriorating hepatic steatosis through SRBEP-1c activation, Int. J. Biol. Sci. 11 (2015) 569-586. https://doi.org/10.7150/ijbs.11039.

[24] C. Liu, Y. Cui, F. Pi, et al., Torularhodin ameliorates oxidative activity *in vitro* and *D*-galactose-induced liver injury *via* the Nrf2/HO-1 signaling pathway *in vivo*, J. Agric. Food Chem. 67(36) (2019) 10059-10068. https://doi.org/10.1021/acs.jafc.9b03847.

[25] D. Cheng, H. Kong, The effect of *Lycium barbarum* polysaccharide on alcohol-induced oxidative stress in rats, Molecules 16 (2011) 2542-2550. https://doi.org/10.3390/molecules16032542.

[26] J. Liu, L. Kong, M. Shao, et al., Seabuckthorn polysaccharide combined with astragalus polysaccharide ameliorate alcoholic fatty liver by regulating intestinal flora, Front. Endocrinol. 13 (2022) 1018557. https://doi.org/10.3389/fendo.2022.1018557.

[27] N.M.W. de Alwis, C.P. Day, Genetics of alcoholic liver disease and nonalcoholic fatty liver disease, Semin. Liver Dis. 27(1) (2007) 44-54. https://doi.org/10.1055/s-2006-960170.

[28] K. Machida, H. Tsukamoto, H. Mkrtchyan, et al., Toll-like receptor 4 mediates synergism between alcohol and HCV in hepatic oncogenesis involving stem cell marker Nanog, PANS 106(5) (2009) 1548-1553. https://doi.org/10.1073/pnas.0807390106.

[29] H.J. Wang, S. Zakhari, M.K. Jung, Alcohol, inflammation, and gut-liver-brain interactions in tissue damage and disease development, World J. Gastroenterol. 16(11) (2010) 1304-1313. https://doi.org/10.3748/wjg.v16.i11.1304.

[30] P. Muriel, NF-κB in liver diseases: a target for drug therapy, J. Appl. Toxicol. 29(2) (2009) 91-100. https://doi.org/10.1002/jat.1393.

[31] A.I. Cederbaum, Y. Lu, D. Wu, Role of oxidative stress in alcohol-induced liver injury, Arch. Toxicol. 83 (2009) 519-548. https://doi.org/10.1007/s00204-009-0432-0.

[32] J.S. Bajaj, Alcohol, liver disease and the gut microbiota, Nat. Rev. Gastroenterol. Hepatol. 16(4) (2019) 235-246. https://doi.org/10.1038/s41575-018-0099-1.

[33] X. Shu, J. Wang, L. Zhao, et al., *Bifidobacterium lactis* TY-S01 protects against alcoholic liver injury in mice by regulating intestinal barrier function and gut microbiota, Heliyon 9(7) (2023) e17878. https://doi.org/10.1016/j.heliyon.2023.e17878.

[34] E. Mutlu, A. Keshavarzian, P. Engen, et al., Intestinal dysbiosis: a possible mechanism of alcohol-induced endotoxemia and alcoholic steatohepatitis in rats, Alcohol. Clin. Exp. Res. 33(10) (2009) 1836-1846. https://doi.org/10.1111/j.1530-0277.2009.01022.x.

[35] A.W. Yan, D.E. Fouts, J. Brandl, et al., Enteric dysbiosis associated with a mouse model of alcoholic liver disease, Hepatology 53(1) (2011) 96-105. https://doi.org/10.1002/hep.24018.

[36] L. Bull-Otterson, W. Feng, I. Kirpich, et al., Metagenomic analyses of alcohol induced pathogenic alterations in the intestinal microbiome and the effect of *Lactobacillus rhamnosus* GG treatment, PLoS One 8(1) (2013) e53028. https://doi.org/10.1371/journal.pone.0053028.

[37] V.B. Dubinkina, A.V. Tyakht, V.Y. Odintsova, et al., Links of gut microbiota composition with alcohol dependence syndrome and alcoholic liver disease, Microbiome 5 (2017) 1-14. https://doi.org/10.1186/s40168-017-0359-2.

[38] G. Kakiyama, W.M. Pandak, P.M. Gillevet, et al., Modulation of the fecal bile acid profile by gut microbiota in cirrhosis, J. Hepatol. 58(5) (2013) 949-955. https://doi.org/10.1016/j.jhep.2013.01.003.

[39] J.S. Bajaj, J.M. Ridlon, P.B. Hylemon, et al., Linkage of gut microbiome with cognition in hepatic encephalopathy, Am. J. Physiol. Gastrointest. Liver Physiol. 302(1) (2012) G168-G175. https://doi.org/10.1152/ajpgi.00190.2011.

[40] N.O. Kaakoush, Insights into the role of erysipelotrichaceae in the human host, Front. Cell. Infect. Mi. 5 (2015) 84. https://doi.org/10.3389/fcimb.2015.00084.

[41] A.W. Yan, B. Schnabl, Bacterial translocation and changes in the intestinal microbiome associated with alcoholic liver disease, World J. Hepatol. 4(4) (2012) 110-118. https://doi.org/10.4254/wjh.v4.i4.110.

[42] Z.W. Yi, Y.J. Xia, X.F. Liu, et al., Antrodin A from mycelium of *Antrodia camphorata* alleviates acute alcoholic liver injury and modulates intestinal flora dysbiosis in mice, J. Ethnopharmacol. 254 (2020) 112681. https://doi.org/10.1016/j.jep.2020.112681.

[43] H. Li, J. Shi, L. Zhao, et al., *Lactobacillus plantarum* KLDS1.0344 and *Lactobacillus acidophilus* KLDS1.0901 mixture prevents chronic alcoholic liver injury in mice by protecting the intestinal barrier and regulating gut microbiota and liver-related pathways, J. Agric. Food Chem. 69(1) (2021) 183-197. https://doi.org/10.1021/acs.jafc.0c06346.

[44] W. Hu, W. Gao, Z. Liu, et al., Specific strains of *Faecalibacterium prausnitzii* ameliorate nonalcoholic fatty liver disease in mice in association with gut microbiota regulation, Nutrients 14(4) (2022) 2945. https://doi.org/10.3390/nu14142945.

[45] B.J. Parker, P.A. Wearsch, A.C.M. Veloo, et al., The genus *Alistipes*: gut bacteria with emerging implications to inflammation, cancer, and mental health, Front. Immunol. 11 (2020) 906. https://doi.org/10.3389/fimmu.2020.00906.

[46] J.W. Park, S.E. Kim, N.Y. Lee, et al., Role of microbiota-derived metabolites in alcoholic and non-alcoholic fatty liver diseases, Int. J. Mol. Sci. 23(1) (2021) 426. https://doi.org/10.3390/ijms23010426.

[47] M. Levy, C.A. Thaiss, E. Elinav, Metabolites: messengers between the microbiota and the immune system, Gene. Dev. 30(14) (2016) 1589-1597. https://doi.org/10.1101/gad.284091.116.

[48] L. Wu, J. Feng, J. Li, et al., The gut microbiome-bile acid axis in hepatocarcinogenesis, Biomed. Pharmacother. 133 (2021) 111036. https://doi.org/10.1016/j.biopha.2020.111036.

[49] W. Ratajczak, A. Ryl, A. Mizerski, et al., Immunomodulatory potential of gut microbiome-derived short-chain fatty acids (SCFAs), Acta. Biochim. Pol. 66(1) (2019) 1-12. https://doi.org/10.18388/abp.2018_2648.

[50] Z. Wang, X. Zhang, L. Zhu, et al., Inulin alleviates inflammation of alcoholic liver disease *via* SCFAs-inducing suppression of M1 and facilitation of M2 macrophages in mice, Int. Immunopharmacol. 78 (2020) 106062. https://doi.org/10.1016/j.intimp.2019.106062.

[51] L. Tedesco, G. Corsetti, C. Ruocco, et al., A specific amino acid formula prevents alcoholic liver disease in rodents, Am. J. Physiol-Gastr. L. 314(5) (2018) G566-G582. https://doi.org/10.1152/ajpgi.00231.2017.

[52] Z. Zhang, S. Fan, D. Huang, et al., Polysaccharides from fermented *Asparagus officinalis* with *Lactobacillus plantarum* NCU116 alleviated liver injury *via* modulation of glutathione homeostasis, bile acid metabolism, and SCFA production, Food Funct. 11(9) (2020) 7681-7695. https://doi.org/10.1039/D0FO01435D.

[53] G. Li, L.G. Guo, Farnesoid X receptor, the bile acid sensing nuclear receptor, in liver regeneration, Acta Pharm. Sin. B 5(2) (2015) 93-98. https://doi.org/10.1016/j.apsb.2015.01.005.

[54] J.J. Eloranta, G.A. Kullak-Ublick, The role of FXR in disorders of bile acid homeostasis, Physiology 23(5) (2008) 286-295. https://doi.org/10.1152/physiol.00020.2008.

[55] H. Liu, R. Tian, H. Wang, et al., Gut microbiota from coronary artery disease patients contributes to vascular dysfunction in mice by regulating bile acid metabolism and immune activation, J. Transl. Med. 18 (2020) 1-18. https://doi.org/10.1186/s12967-020-02539-x.

[56] J.Y.L. Chiang, Bile acid metabolism and signaling in liver disease and therapy, Liver Res. 1(1) (2017) 3-9. https://doi.org/10.1016/j.livres.2017.05.001.

[57] L. Dong, L. Yin, R. Li, et al., Dioscin alleviates lung ischemia/reperfusion injury by regulating FXR-mediated oxidative stress, apoptosis, and inflammation, Eur. J. Pharmacol. 908 (2021) 174321. https://doi.org/10.1016/j.ejphar.2021.174321.