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Bifidobacterium lactis TY-S01 protects against alcoholic liver injury in mice by regulating intestinal barrier function and gut microbiota

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ABSTRACT

Alcohol-induced liver injury poses a significant threat to human health. Probiotics have been proven to prevent and treat alcohol-induced liver injury. In this study, the preventive effect of *Bifidobacterium lactis* TY-S01 on alcohol-induced liver injury in mice was investigated. TY-S01 pretreatment effectively protected mice against alcohol-induced liver injury by preserving the levels of alanine aminotransferase, aspartate aminotransferase, alkaline phosphatase, triglyceride and high-density lipoprotein-cholesterol in serum and maintaining the levels of the inflammatory cytokines tumor necrosis factor-α, interleukin-6 and interleukin-1β in liver tissue. Additionally, TY-S01 could maintain the endotoxin levels in serum, maintain the mRNA expression levels of *zonula occluden-1*, *occludin*, *claudin-1* and *claudin-3* in the gut, and prevent gut microbiota dysbiosis in mice with alcoholic liver injury. Spearman's correlation analysis revealed that there was a clear correlation among serum indicators, inflammatory cytokines and gut microbiota. In conclusion, TY-S01 attenuates alcohol-induced liver injury by protecting the integrity of the intestinal barrier and maintaining the balance of the gut microbiota.

1. Introduction

Excessive or chronic alcoholism can lead to alcoholic liver injury, characterized by steatosis, necrosis, and decreased regeneration of hepatocytes, ultimately resulting in liver fibrosis and cirrhosis [1]. Moreover, alcohol has been shown to disrupt the microbiome, impair the barrier function of the gut, and further accelerate liver disease progression [2] due to the close physical correlation between the liver and gut [3]. Routinely, a robust gut has a natural barrier function that protects the internal environment from endogenous pathogenic microorganisms and their toxins [4]. However, excessive intake of alcohol promotes the overgrowth of intestinal bacteria,

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especially gram-negative bacteria, which disturbs the balance of the intestinal microbiota. Alcohol and its metabolites have also been shown to increase intestinal permeability and break down the intestinal epithelial barrier [5]. The high permeability of the gut accelerates the transfer of excess endotoxins from the gut to portal veins and the liver, leading to the activation of immune cell responses and the release of cytokines and inflammatory mediators, culminating in hepatic inflammation and liver damage [5,6].

Intestinal barrier function is closely related to the tight junctions (TJs) of adjacent epithelial cells. TJs are composed of several protein families, including transmembrane proteins (e.g., occludin and claudins) and peripheral proteins (e.g., zonula occludens), which regulate intercellular permeability and maintain cellular polarity [7]. As previously reported, the excess expression of *zonula occludens-1 (ZO-1)*, *occludin*, and *claudin-1* reverse the disruption of intestinal barrier function in mice [8]. The upregulation of *claudin-3* and *occludin* promotes intestinal barrier integrity and ameliorates alcoholic liver injury [9].

To date, several methods for the prevention and treatment of alcoholic liver injury have been developed, including alcohol withdrawal therapy, dietary therapy, drug therapy, and surgical therapy [10]. Among them, dietary therapy is widely accepted due to its low side effects, high acceptability, and easy promotion [11]. Probiotics, including *Bifidobacterium* and *Lactobacillus*, have been proven to prevent and treat alcoholic liver injury by improving the intestinal barrier, restoring the gut microbiota balance, resisting oxidative stress, and other probiotic functions [12]. Previously, Kirpich et al. used probiotics (*Bifidobacterium* (*B.*) *bifidum* and *Lactobacillus* (*L.*) *plantarum* 8PA3) for the first time in patients for adjuvant treatment of alcoholic liver diseases through by restoring the balance of the gut microbiome [13]. Gan et al. found that *L. plantarum* HFY09 had efficacy in preventing liver injury and inflammation in mice with ethanol-induced liver injury [14]. The administration of *L. rhamnosus* GG has been shown to modulate alcohol-induced gut microbiota imbalance and elevate lipopolysaccharide (LPS) levels [15]. Additionally, *L. rhamnosus* GG culture supernatant ameliorated the increase in alcohol-induced intestinal permeability and endotoxemia in an acute alcoholism mouse model by upregulating the expression levels of *claudin-1*, *ZO-1*, and *occludin* and downregulating LPS levels [16]. Furthermore, Tian et al. found that *L. rhamnosus* CCFM1107 provided protective effects against alcoholic liver injury by restoring the intestinal flora [17].

Herein, we found that the probiotic strain *B. lactis* TY-S01 could prevent alcoholic liver injury in mice. Our experiments demonstrated that TY-S01 primarily exerted its anti-alcoholic liver injury effect by maintaining the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP), triglyceride (TG) and high density lipoprotein-cholesterol (HDL-C) in serum, as well as maintaining the levels of inflammatory cytokines tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and interleukin-1β (IL-1β) in liver tissue of alcohol intervention mice. Furthermore, we investigated the potential mechanism of anti-alcoholic liver injury, which was mainly related to the ability of TY-S01 in protecting the integrity of the intestinal barrier and maintaining the balance of gut microbiota in alcohol intervention mice.

2. Materials and methods

2.1. B. lactis strains

Fresh fecal samples of long-lived elderly individuals were collected at Bama, Guangxi, and full informed consent was obtained from all individuals. The fecal samples were diluted and spread on MRS agar with 0.05% L-cysteine and incubated at 37 °C for 48 h under an anaerobic condition. Individual milky white colonies were selected and purified using streak plate method. To prevent contamination of bacterial colonies by pathogenic bacteria such as *Escherichia coli* in feces, all purified single colonies were identified through 16S rRNA gene sequencing. The primers are 27F (5′-AGAGTTTGATCCTGGCTCAG-3′) and 1492R (5′-TACGACTTAACCCCAATCGC-3′). The results were sequenced by Shenggong Biotechnology Co., Ltd, Shanghai, China, and screened using the NCBI BLAST online database system [18]. *B. lactis* TY-S01 was derived from this fecal samples, and stored in the China General Microbiological Culture Collection Center (Beijing, China) with accession No.21255.

2.2. Animals and treatment

The animal experimental scheme was authorized by the Experimental Animal Welfare Ethics Review Committee of Chongqing Institute of Traditional Chinese Medicine (5001087226041, Chongqing, China). Six-week-old ICR male mice were housed in a standard laboratory. The mice were fed adaptively for one week before the formal experiment. The feed (SWC9101, Jiangsu Collaborative Pharmaceutical Bioengineering Co., Ltd, Jiangsu, China) for mice during the adaptation period was the same as that during the formal experimental period. The feed for mice mainly includes corn, wheat, fish meal, chicken meal, soybean meal, soybean oil, amino acids, vitamins, minerals, etc., which meets the standards for experimental animal feed [19]. After the adaptation period, the mice were randomly divided into 3 groups according to body weight (n = 8 per group), namely the control group, EtOH group, and TY-S01 group. The TY-S01 group was given 1.0×10^9 CFU/kg. bw bacterial solution (soluble in 0.9% physiological saline) by intragastric administration to each mouse at a dose of 0.2 ml/day for 28 consecutive days. The control group and EtOH group were given 0.9% physiological saline. On the 29th day, the mice in each group were fasted for 12 h before modeling. Then, the EtOH group and TY-S01 group were intragastrically administered a one-time dose of 50% ethanol (12 ml/kg. bw) to prepare the acute alcoholic liver injury model of mice, and the control group was given the same amount of normal saline. Mice were euthanized after intragastric administration of ethanol for 10 h. The design of the experimental model and the dosage of ethanol referred to the method of Han et al. [20]. Blood was collected from the eyeballs of mice and serum was obtained after cryocentrifugation at 3000 rpm for 10 min [21], while the liver, small intestine and cecum contents of all mice were collected for subsequent experiments. The liver was weighed and the liver index was calculated as follows: liver index (%) = liver weight/body weight × 100%.

2.3. Histological analysis of the liver

The same location of liver tissue in mice was taken after perfusion with PBS, fixed with 10% buffered formaldehyde solution, embedded in paraffin, sectioned, stained with hematoxylin and eosin (H&E), and observed with a DM2000 microscope (Leica, Weztlar, Germany). H&E staining and steatosis analysis of liver tissue were performed according to the method of Han et al. [20]. The scores were graded from 1 to 4, of which Level 0 = few and normal lipid droplets in hepatocytes; Level 1 = no more than 1/4 of all hepatocytes contain lipid droplets; Level 2 = no more than 1/2 of hepatocytes with lipid droplets; Level 3 = no more than 3/4 of hepatocytes with lipid droplets; Level 4 = almost all hepatocytes contain lipid droplets.

2.4. Measurement of biochemical markers in serum

According to the manufacturer's instructions, all samples were used in undiluted form, and ALT (C009-2-1), AST (C010-2-1), ALP (A059-2-2), TG (A110-1-1) and HDL-C (A112-1-1) levels in the serum of mice were measured using their respective kits (all from Nanjing Jiancheng Institute of Biotechnology, Nanjing, China), LPS levels in the serum of mice were measured using an enzyme-linked immunosorbent assay kit (CK-E20839, Shanghai Enzyme Link Biotechnology Co., Ltd., Shanghai, China).

2.5. Measurement of hepatic inflammatory cytokines

According to the manufacturer's instructions, all samples were diluted ten times with double distilled water, and TNF-α (ml002095), IL-6 (ml002293) and IL-1β (ml063132) levels in the livers of mice were measured using an enzyme-linked immunosorbent assay kit (all from Shanghai Enzyme Link Biotechnology Co., Ltd., Shanghai, China).

2.6. Measurement of mRNA expression by real-time quantitative PCR

Total RNA from ileal tissues was extracted using TRIzol reagent (15596026, Invitrogen, CA, USA) and reverse transcribed to cDNA using a RevertAid First Strand cDNA Synthesis Kit (K1622, Thermo Scientific, CA, USA). Primer sequences are shown in Table 1 (GAPDH was used as a reference gene). Real-time quantitative PCR was performed on CFX96 Touch (Bio-Rad, CA, USA) and qPCR SYBR Green Master Mix (11184ES08, YEASEN, Shanghai, China). The relative expression of mRNA was calculated by the comparative Ct method ($2^{-\Delta\Delta Ct}$).

2.7. Analysis of microbial diversity

Total microbial genomic DNA was extracted from cecal content samples. The quality and concentration of DNA were determined by a NanoDrop2000 (Thermo Scientific Inc., Wilmington, USA). Illumina MiSeq/NovaSeq high-throughput sequencing was performed by Shanghai Meiji Biomedical Technology Co., Ltd. The data were analyzed on the online platform of Majorbio Cloud Platform (www.majorbio.com). Spearman's correlation analysis was conducted to analyze the correlations among serum indicators (ALT, AST, ALP, TG, HDL-C, and LPS), inflammatory cytokines (TNF-α, IL-6, and IL-1β) and gut microbiota.

2.8. Statistical analysis

GraphPad Prism 8.0 software was used for statistical analysis. The differences between multiple groups were calculated using one-way analysis of variance and Duncan's multiple range test. In the box and whisker plot, the box represents the median and quartiles, and the whisker represents the minimum up to maximum. A P value < 0.05 was considered statistically significant.

Table 1
Primer sequences for real-time quantitative PCR.

Gene	Sequences (5′–3′)
ZO-1-F	GCTTTAGCGAACAGAAGGAGC
ZO-1-R	TTCATTTTTCCGAGACTTCACCA
Occludin-F	TTGAAAGTCCACCTCCTTACAGA
Occludin-R	CCGGATAAAAAGAGTACGCTGG
Claudin-1-F	GCCTTGATGGTAATTGGCATCC
Claudin-1-R	GGCCACTAATGTCGCCAGAC
Claudin-3-F	ACCAACTGCGTACAAGACGAG
Claudin-3-R	CGGGCACCAACGGGTTATAG
GAPDH-F	TGACCTCAACTACATGGTCTACA
GAPDH-R	CTTCCCATTCTCGGCCTTG

3. Results

3.1. The prevention of alcoholic liver injury in mice by TY-S01

Mice were administered TY-S01 for 28 days. The acute alcoholic liver injury model was established by alcohol gavage to evaluate the effect of TY-S01 on alcoholic liver injury in mice. During the experiment, the mice exhibited no significant changes in body weight

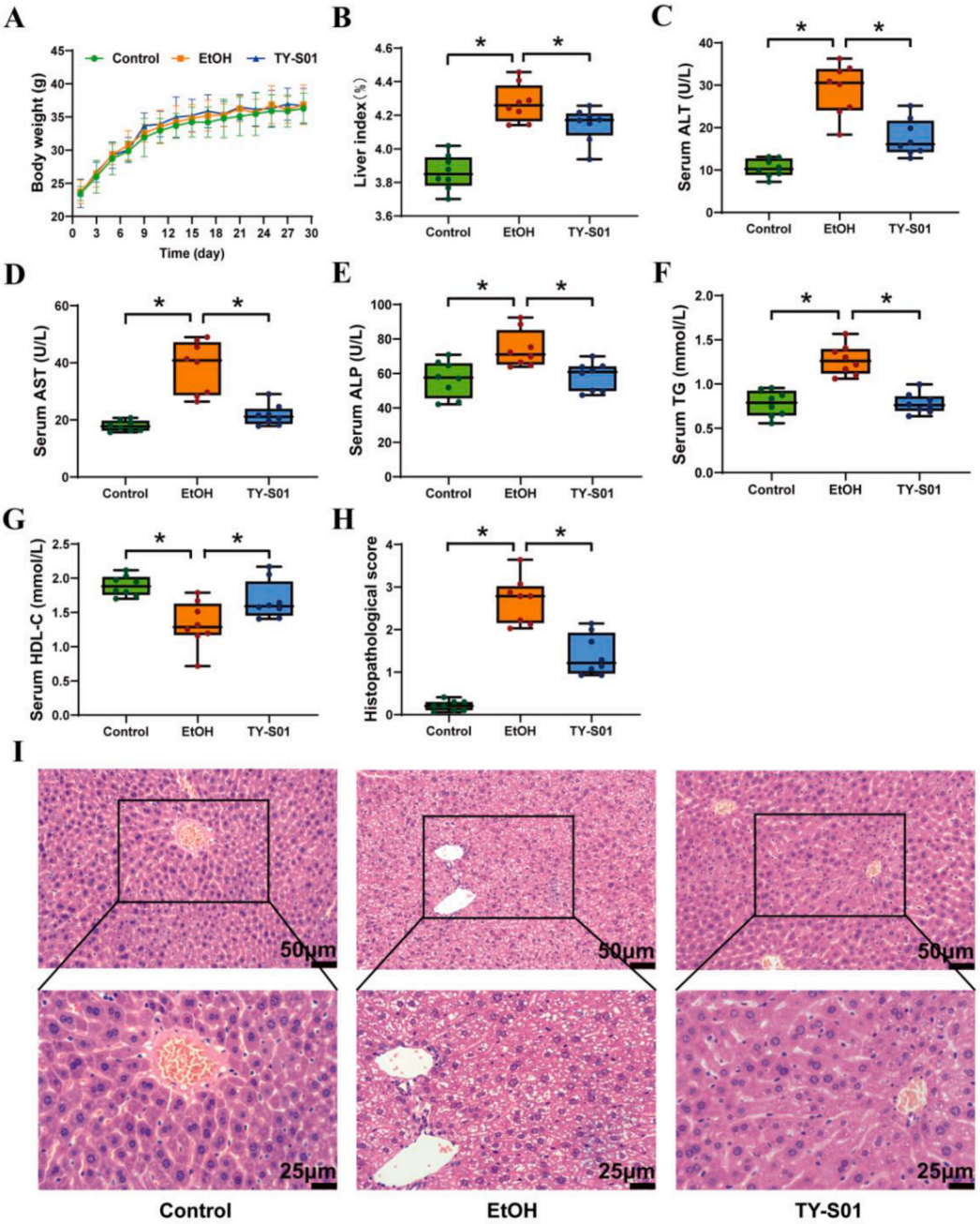

Fig. 1. The prevention of alcoholic liver injury in mice by TY-S01. Body weight (A), liver index (B), serum ALT (C), serum AST (D), serum ALP (E), serum TG (F), serum HDL-C (G), histopathological score (H) and H&E-stained liver sections (I) in the control = normal control group, EtOH = alcohol model group (mice were given 12 ml/kg. bw 50% ethanol on the 29th day), and TY-S01 = alcohol (mice were given 12 ml/kg. bw 50% ethanol on the 29th day) + TY-S01 treatment group (mice were given 1.0×10^9 CFU/kg. bw bacterial solution for 28 consecutive days). Data are representative of at least three independent experiments; n = 8 mice per group. (A) Data shown are means ± SEMs; $^*P < 0.05$, significantly different compared with the EtOH group by using one-way analysis of variance and Duncan's multiple range test.

($P < 0.05$, Fig. 1A), indicating healthy physiological status. Compared with the EtOH group, the liver index of the TY-S01 group significantly decreased ($P < 0.05$, Fig. 1B). The serum biomarkers ALT, AST, ALP, TG and HDL-C were used to evaluate liver injury. Notably, compared to the control group, the levels of ALT, AST, ALP, TG and HDL-C in the serum of the EtOH group substantially increased, but TY-S01 pretreatment markedly prevented an increase in ALT, AST, ALP, TG and HDL-C levels ($P < 0.05$, Fig. 1C–G). The histological changes in the liver observed in mice were histologically assessed using H&E-stained liver sections. In the control group, it was observed that the liver cell structure was normal, the nuclear membrane was complete, the nucleolus was clearly visible, and no lipid droplets or inflammatory cell infiltration were found. In the EtOH group, the liver cells of mice were swollen, with obvious steatosis, vacuolization of the cytoplasm, and inflammatory cell infiltration. However, TY-S01 intervention alleviated the above adverse changes. The liver tissue in the TY-S01 group showed similar morphological characteristics to the control group ($P < 0.05$, Fig. 1H–I). Collectively, these results demonstrated the ability of TY-S01 to prevent alcoholic liver injury in mice.

3.2. Effect of TY-S01 on the levels of hepatic inflammatory cytokines in mice

The inflammatory cytokines TNF-α, IL-6 and IL-1β play a crucial role in the occurrence and development of alcoholic liver injury. The effects of TY-S01 on the levels of TNF-α, IL-6 and IL-1β were evaluated in the unfiltered liver homogenates of mice perfused with PBS, and the results are depicted in Fig. 2A–C. The TNF-α, IL-6 and IL-1β levels in the EtOH group notably increased compared to those in the control group ($P < 0.05$). However, after TY-S01 treatment, TNF-α, IL-6 and IL-1β remained at levels comparable to those in the control group. The results suggested that TY-S01 could effectively inhibit the inflammatory reaction of the liver in mice caused by alcohol exposure.

3.3. Effect of TY-S01 on intestinal barrier function in mice

The integrity of TJs between intestinal epithelial cells could reflect normal intestinal barrier function. Therefore, the mRNA expression levels of *ZO-1*, *occludin*, *claudin-1*, and *claudin-3* in the ileum, as well as the LPS level in the serum, were assessed in mice treated with TY-S01 to evaluate the protection offered against alcohol-induced impairment of intestinal function. As shown in Fig. 3A–E, the mRNA expression levels of *ZO-1*, *occludin*, *claudin-1* and *claudin-3* in the EtOH group were evidently reduced compared with those in the control group. ($P < 0.05$), and the LPS level in the EtOH group significantly increased ($P < 0.05$). The LPS level and mRNA expression of *ZO-1*, *occludin*, *claudin-1* and *claudin-3* remained at normal levels after TY-S01 pretreatment ($P < 0.05$). As a consequence, TY-S01 alleviated the damage to intestinal barrier function and intestinal endotoxemia caused by alcohol exposure in mice.

3.4. Effect of TY-S01 on the Diversity of Gut Microbiota in Mice

We employed 16S rDNA sequencing technology to investigate the impact of TY-S01 on gut microbiota in alcohol-treated mice. The Shannon index, Simpson index and Chao index are α-diversity indices that can be used to evaluate the diversity of gut microbiota communities. The Shannon index and Chao index significantly decreased in the EtOH group. The Simpson index significantly increased in the EtOH group. However, the Shannon index, Chao index and Simpson index of the TY-S01 group returned to normal levels ($P < 0.05$, Fig. 4A–C). The β-diversity between microbiome samples was determined via principal coordinate analysis (PCoA), which showed that the cluster of the TY-S01 group was similar to that of the control group but relatively separated from that of the EtOH group (Fig. 4D). The results showed that TY-S01 maintained the diversity of gut microbiota in alcohol-treated mice to a balanced state.

3.5. Effect of TY-S01 on the Composition of Gut Microbiota in Mice

We explored the effect of TY-S01 on the composition of the gut microbiota in mice at the phylum and genus levels (Fig. 5A, F). At the phylum level, Firmicutes (67.30%) and Bacteroidetes (10.92%) were the major phyla in the control group. After alcohol

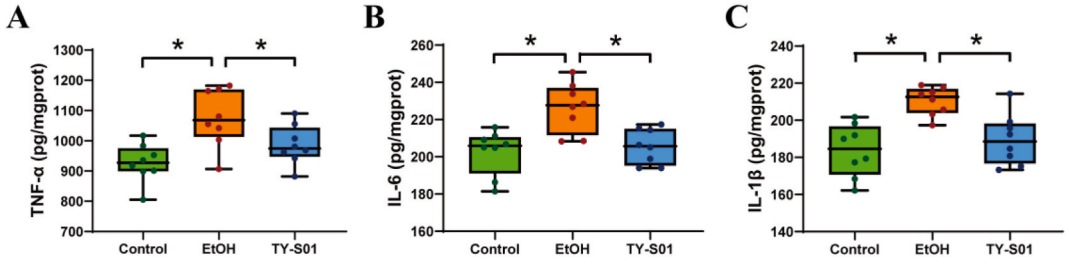

Fig. 2. Effect of TY-S01 on the levels of hepatic inflammatory cytokines in mice. The hepatic levels of TNF-α (A), IL-6 (B), and IL-1β (C) in the control = normal control group, EtOH = alcohol model group (mice were given 12 ml/kg. bw 50% ethanol on the 29th day), and TY-S01 = alcohol (mice were given 12 ml/kg. bw 50% ethanol on the 29th day) + TY-S01 treatment group (mice were given 1.0×10^9 CFU/kg. bw bacterial solution for 28 consecutive days). Data are representative of at least three independent experiments; n = 8 mice per group; *$P < 0.05$, significantly different compared with the EtOH group by using one-way analysis of variance and Duncan's multiple range test.

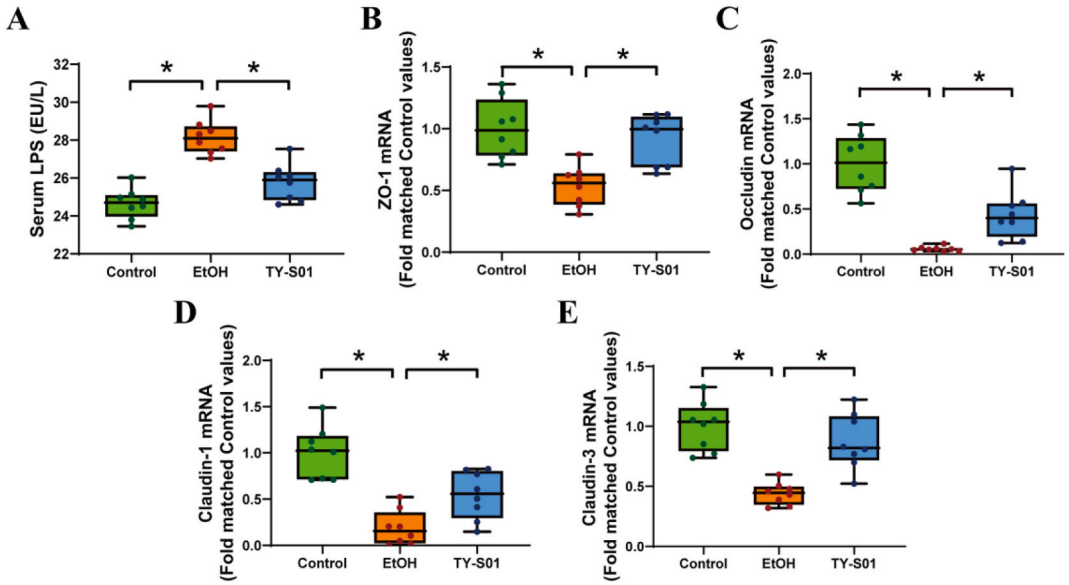

Fig. 3. Effect of TY-S01 on intestinal barrier function in mice. The serum LPS (A) and the mRNA expression levels of *ZO-1* (B), *occludin* (C), *claudin-1* (D), and *claudin-3* (E) in the control = normal control group, EtOH = alcohol model group (mice were given 12 ml/kg. bw 50% ethanol on the 29th day), TY-S01 = alcohol (mice were given 12 ml/kg. bw 50% ethanol on the 29th day) + TY-S01 treatment group (mice were given 1.0×10^9 CFU/kg. bw bacterial solution for 28 consecutive days). Data are representative of at least three independent experiments; n = 8 mice per group; $^*P < 0.05$, significantly different compared with the EtOH group by using one-way analysis of variance and Duncan's multiple range test.

intervention, compared with the control group, the abundance of Firmicutes obviously decreased, and the ratio of Firmicutes to Bacteroides was markedly reduced ($P < 0.05$, Fig. 5B–C). Meanwhile, the abundance of Proteobacteria in the EtOH group significantly increased ($P < 0.05$, Fig. 5D), but TY-S01 maintained the normal abundance of these species. At the genus level, the abundances of *Lactobacillus*, *Bacillus*, *unclassified_c_Bacilli*, *Christensenellaceae_R-7_group*, and *unclassified_o_Coriobacteriales* in the EtOH group were significantly lower than those in the control group and TY-S01 group, and the abundance of *norank_f_Christensenellaceae* in the EtOH group was significantly higher than that in the control group and TY-S01 group ($P < 0.05$, Fig. 5E).

3.6. Correlation analysis among serum indicators, inflammatory cytokines and gut microbiota

To determine whether there were potential correlations among alcohol-induced changes in the composition of the gut microbiota, serum parameters and inflammatory cytokines, we performed Spearman's correlation analysis (Fig. 6). The results illustrated that the levels of serum indicators and inflammatory cytokines were correlated with the abundance of several bacteria. For instance, ALT, AST, ALP, TG, LPS, TNF-α, IL-6, and IL-1β were negatively associated with *Lactobacillus* and *Bacillus*. HDL-C was positively associated with *Lactobacillus* and *Bacillus*. AST, TG, LPS, IL-6, and IL-1β were negatively associated with *unclassified_c_Bacilli*. Therefore, the evolution of alcoholic liver injury is closely related to the gut microbiota.

4. Discussion

In recent years, probiotics have gained popularity as dietary supplements and are consumed globally [22]. Many studies have demonstrated that probiotics possess probiotic functions such as improving body function, enhancing intestinal barrier function, and regulating gut microbiota [23]. Therefore, attention has recently been focused on the application of probiotics for alcoholic liver injury prevention and treatment [24]. Herein, we found that *B. lactis* TY-S01 could prevent alcohol-induced liver injury by regulating intestinal barrier function and gut microbiota.

ALT, AST and ALP mainly exist in various tissues, such as the liver [25]. Upon liver injury, those biomarkers are released into the blood, resulting in an increase in ALT, AST and ALP levels in serum [26]. TG can accumulate in hepatocytes and result in the development of fatty liver in the early stage of alcoholic liver injury [27]. The levels of TG and HDL-C are considered primary indicators of lipid metabolism disorders caused by alcohol [28]. Our results demonstrated that TY-S01 could maintain the levels of ALT, AST, ALP, TG and HDL-C near those of normal control mice, indicating that TY-S01 had a certain protective effect on the liver. TNF-α is a proinflammatory cytokine that is produced by immune cells and has a wide range of biological functions, such as inflammation and apoptosis [29]. In addition, IL-1β and IL-6 participate in inflammatory reactions in liver tissue [30]. As previously reported, the inflammatory cytokines TNF-α, IL-6 and IL-1β were used as typical indicators to evaluate liver inflammation [14,31]. These are the key factors involved in the regulation of alcoholic liver injury [32], and reducing their levels may decrease inflammation and apoptosis [33]. Our study confirmed that TY-S01 can significantly reduce the hepatic levels of TNF-α, IL-6 and IL-1β in alcohol-exposed mice,

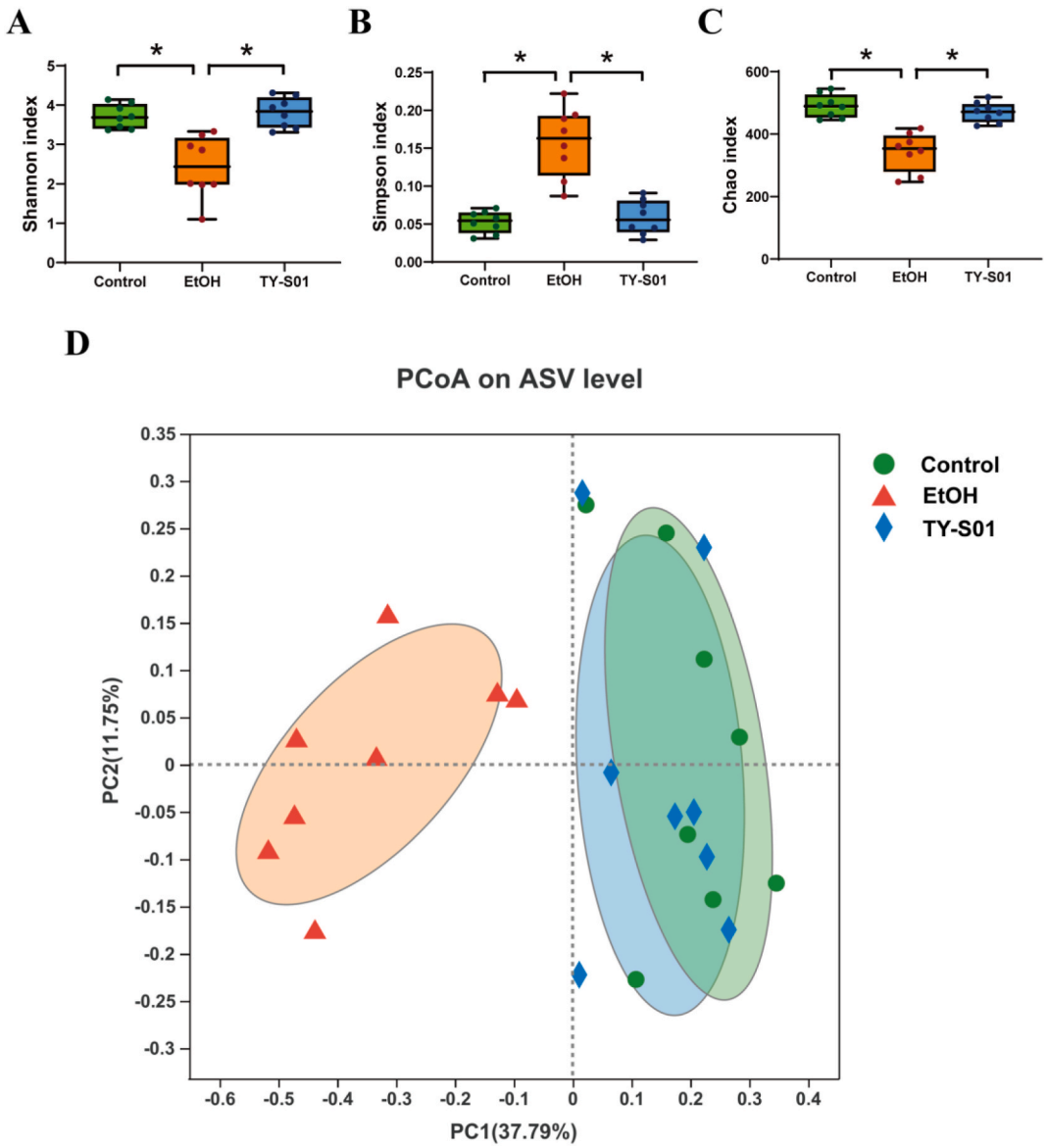

Fig. 4. Effect of TY-S01 on the Diversity of Gut Microbiota in Mice. The Shannon index (A), the Simpson index (B), the Chao index (C), and PCoA on ASV level (D) in the control = normal control group, EtOH = alcohol model group (mice were given 12 ml/kg. bw 50% ethanol on the 29th day), TY-S01 = alcohol (mice were given 12 ml/kg. bw 50% ethanol on the 29th day) + TY-S01 treatment group (mice were given 1.0×10^9 CFU/kg. bw bacterial solution for 28 consecutive days). Data are representative of at least three independent experiments; n = 8 mice per group; $^*P < 0.05$, significantly different compared with the EtOH group by using one-way analysis of variance and Duncan's multiple range test.

maintain a healthy liver morphology, and effectively prevent alcoholic liver injury.

As previously reported, alcohol causes impairment of intestinal barrier function, an increase in intestinal permeability, and rapid proliferation of intestinal gram-negative bacteria, resulting in the transfer of endotoxin from the intestines to the liver and blood; thus, activated liver immune cells trigger a series of inflammatory reactions and ultimately cause liver injury [34]. Impaired intestinal barrier function is closely associated with the loss of TJ proteins, including *ZO-1*, *occludin*, *claudin-1* and *claudin-3* [35,36]. Previous studies have indicated that the expression levels of TJ proteins are frequently decreased in alcohol-exposed mice [15] and human colon biopsies of alcoholics [37]. Our study showed that TY-S01 pretreatment significantly increased the mRNA expression levels of *ZO-1*, *occludin*, *claudin-1* and *claudin-3* in alcohol-exposed mice. Destruction of the intestinal barrier could promote endotoxin in the intestinal cavity entering the portal vein circulation and damaging the liver [38]. LPS, an endotoxin derived from gram-negative bacteria, is the primary factor in alcoholic liver diseases [39]. Excessive levels of LPS can activate excessive production of inflammatory cytokines in macrophages, which results in hepatocellular necrosis [40]. Alcohol intervention could lead to intestinal endotoxemia in mice, which was consistent with the findings of Wang et al. [15]. Additionally, HDL-C was closely related to the prevention of LPS, and

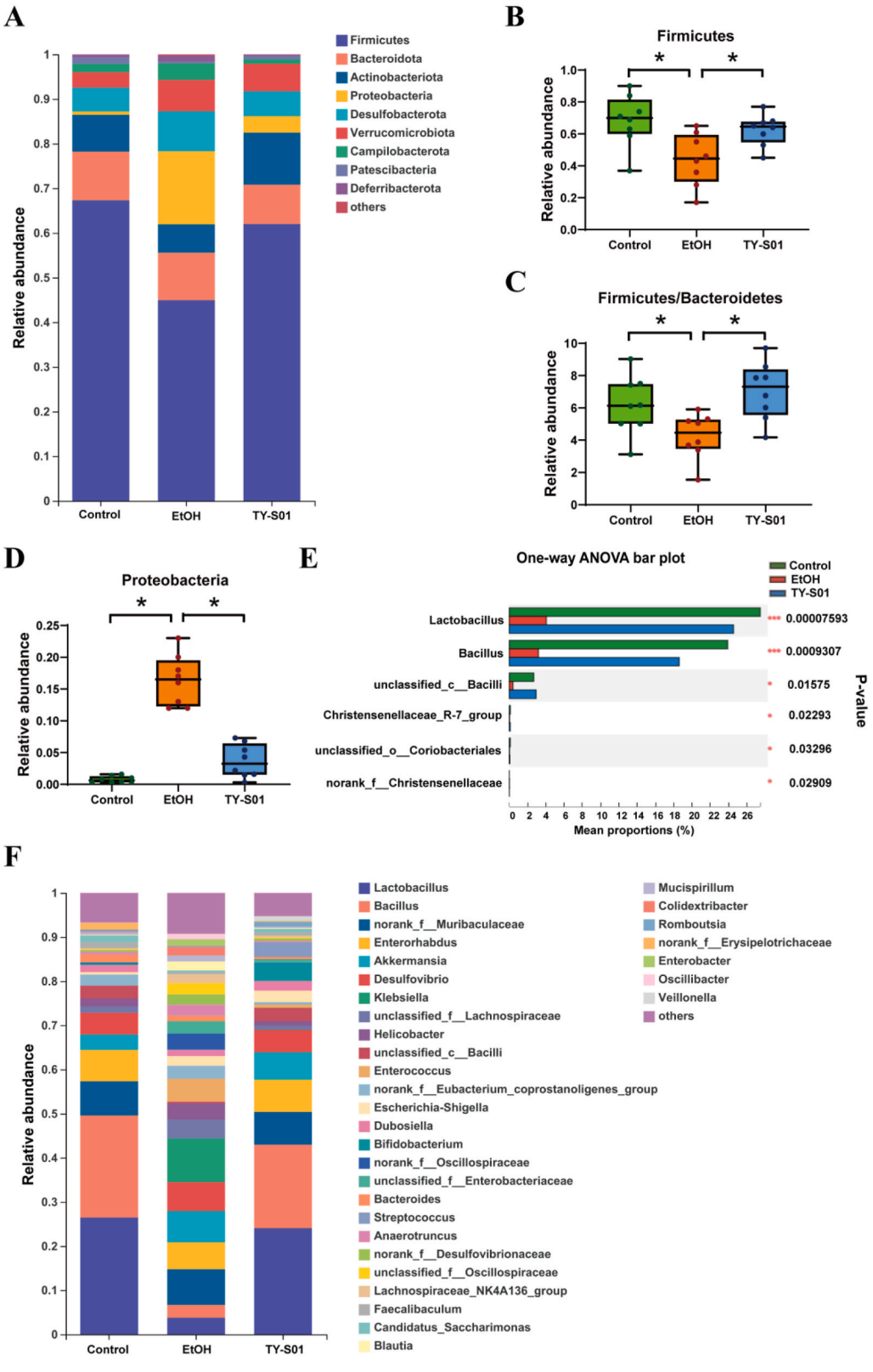

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Fig. 5. Effect of TY-S01 on the Composition of Gut Microbiota in Mice. The taxonomic distribution of bacterial communities at the phylum level (A), the relative abundance of Firmicutes (B), the ratio of Firmicutes to Bacteroidetes (C), the relative abundance of Proteobacteria (D), the species difference analysis at the genus level (E), and the taxonomic distribution of bacterial communities at the genus level (F) in the control = normal control group, EtOH = alcohol model group (mice were given 12 ml/kg. bw 50% ethanol on the 29th day), TY-S01 = alcohol (mice were given 12 ml/kg. bw 50% ethanol on the 29th day) + TY-S01 treatment group (mice were given 1.0×10^9 CFU/kg. bw bacterial solution for 28 consecutive days). Data are representative of at least three independent experiments; n = 8 mice per group; $^*P < 0.05$, significantly different compared with the EtOH group by using one-way analysis of variance and Duncan's multiple range test.

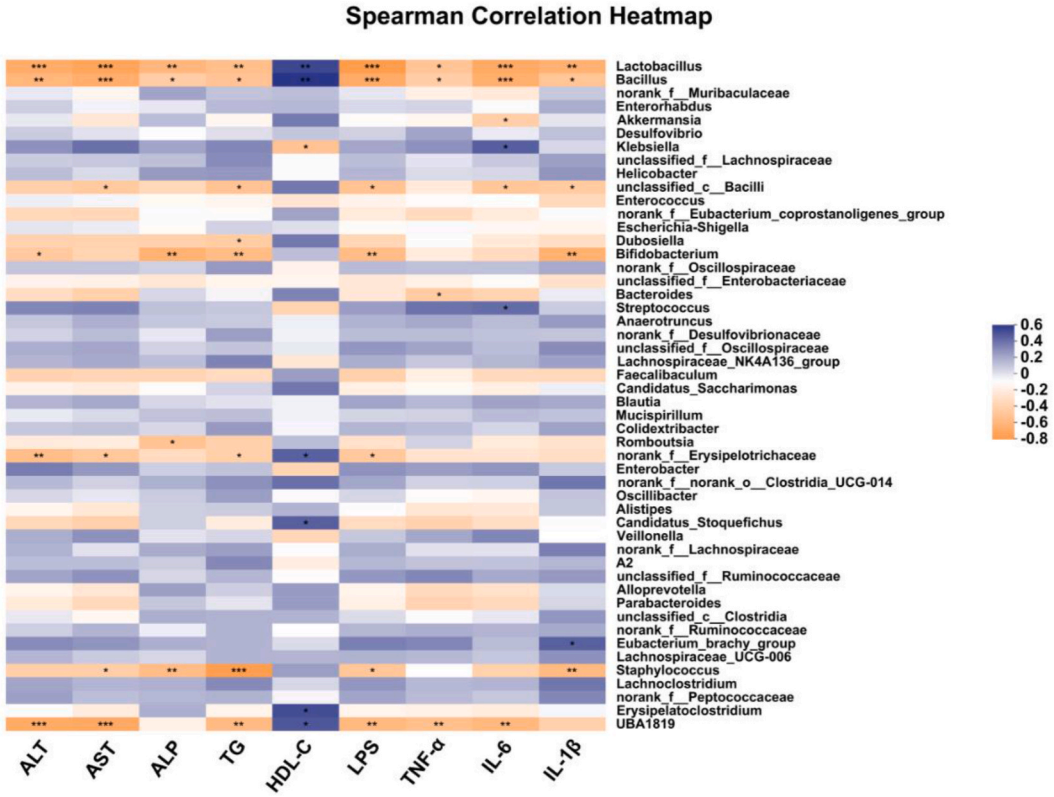

Fig. 6. Correlation analysis among serum indicators, inflammatory factors and gut microbiota at the genus level. Grids in blue indicate positive correlations, while grids in orange indicate negative correlations. The color coding scale indicates the correlation analysis value from the heatmap, and the deeper blue or orange indicates higher correlation values. Data are representative of at least three independent experiments; n = 8 mice per group; $^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$; Spearman's correlation analysis. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

mice with lower HDL-C levels exhibited greater liver injury and inflammation [41]. TY-S01 obviously reduced the LPS content and increased the HDL-C content in mice. Therefore, TY-S01 could effectively maintain intestinal barrier function in alcohol-exposed mice.

Excessive alcohol consumption disrupts the composition and distribution of gut microbiota [42]. The results of the Shannon index, Chao index and Simpson index explained that the diversity of the gut microbiota decreased after alcohol intervention, which was consistent with previous research [28]. In addition, PCoA illustrated that the EtOH group had an unequal microbiota structure compared with the control group, which was consistent with the findings of Yu et al. [43]. Therefore, TY-S01 was able to maintain the diversity of gut microbiota, which was not affected by alcohol in our studies. In addition, excessive alcohol intake can also affect the abundance of the bacterial community and lead to bacterial imbalances [44]. We further investigated the abundance of specific species in the gut with or without TY-S01 treatment. Compared with the EtOH group, TY-S01 increased the abundance of Firmicutes and the ratio of Firmicutes to Bacteroidetes (F/B ratio) while reducing the abundance of Proteobacteria, which was consistent with the results of Yi et al. [28] and Feng et al. [45]. Firmicutes was able to inhibit the invasion of opportunistic pathogens [46]. The F/B ratio serves as an indicator of intestinal microbial health [47]. Proteobacteria is considered a potential proinflammatory phylum that includes many pathogenic genera [48]. Alcohol intervention reduced the relative abundance of *Lactobacillus*, which was consistent with Ming et al. [49]. The abundance of *Lactobacillus* returned to normal levels through TY-S01 pretreatment. Previous studies have shown that the growth of the beneficial intestinal bacteria *Lactobacillus* not only reduced the proportion of harmful bacteria but also promoted body resistance to the invasion of pathogenic microorganisms [50]. TY-S01 was conducive to maintaining the abundance of *Bacillus*. *Bacillus* could retain the balance of gut microbiota and protect intestinal health by antagonizing pathogenic bacteria [51]. Meanwhile,

pretreatment with TY-S01 decreased the abundance of *unclassified_c_Bacilli, Christensenellaceae_R-7_group* and *unclassified_o_Coriobacteriales*. Therefore, TY-S01 was able to maintain the gut microbiota balance in alcohol-exposed mice, especially the stability against *Lactobacillus*. Furthermore, we assessed the relationship among gut microbiota and serum parameters and inflammatory cytokines at the genus level. Surprisingly, we found significant associations of serum parameters and inflammatory cytokines with specific species in the gut. *Lactobacillus* and *Bacillus* showed a negative correlation with ALT, AST, ALP, TG, LPS, TNF-α, IL-6, and IL-1β and a positive correlation with HDL-C. *Unclassified_c_Bacilli* showed a negative correlation with AST, TG, LPS, IL-6, and IL-1β. These three genera may be related to alcoholic liver injury, but their specific mechanisms of action still need to be further studied.

In addition, our study had several limitations. Although we demonstrated that TY-S01 could prevent liver damage caused by alcohol exposure, the specific bacterial components or metabolites that play a role in TY-S01 have not been clearly elucidated, and in-depth studies are needed to identify the key substances that TY-S01 uses to prevent alcoholic liver injury. Based on the animal experimental results of this research, further human clinical trials can also be designed to prove the regulatory effect of TY-S01 on patients with alcoholic liver injury.

5. Conclusions

In summary, *B. lactis* TY-S01 was able to prevent alcoholic liver injury. TY-S01 pretreatment protected liver tissue structure, maintained ALT, AST, ALP, TG and HDL-C levels in serum, maintained the levels of the inflammatory cytokines TNF-α, IL-6 and IL-1β in liver tissue, protected the integrity of the intestinal barrier and maintained the balance of the gut microbiota in alcohol intervention mice. Therefore, the results provided new insights for the application of probiotics in preventing or managing alcoholic liver injury.

Ethics statement

The animal experimental scheme was authorized by the Experimental Animal Welfare Ethics Review Committee of Chongqing Institute of Traditional Chinese Medicine (5001087226041), and animal procedures were strictly carried out according to the legislation for the care and use of laboratory animals of China.

Author contribution statement

Xi Shu：Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Jing Wang: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Liang Zhao: Performed the experiments; Analyzed and interpreted the data.

Jian Wang: Analyzed and interpreted the data; Wrote the paper.

Pengjie Wang: Contributed reagents, materials, analysis tools or data; Wrote the paper.

Feng Zhang：Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Ran Wang: Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Data availability statement

Data will be made available on request.

Additional information

No additional information is available for this paper.

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Declaration of competing interest

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

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