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RESEARCH ARTICLE

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# Inulin alleviates acute alcoholism in mice by enhancing ethanol metabolism and regulating the intestinal flora

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## Abstract

Inulin, a recognized prebiotic with diverse biological activities, has not yet been reported for its potential in treating alcoholism. Therefore, this study investigated the antialcoholic effects of short, long, and phosphorylated long-chain inulin and the possible mechanisms. An acute alcoholic intoxication mice model was established, with the water extract of *Hovenia dulcis Thunb* serving as a positive control. The different inulins were used for intervention, and the drunkenness of the mice was observed by an animal behavior experiment. The serum liver function indexes (TP, ALB, GLB, ALT, and ALP), as well as the activity of ethanol metabolizing enzymes (ADH and ALDH) in the liver were measured, and the changes in intestinal flora were analyzed. The results showed that short-chain inulin could alleviate acute alcoholism by activating ethanol metabolizing enzyme activity and improving intestinal flora, and it had a potential protective effect on acute alcoholic liver injury in mice.

**KEYWORDS**

acute alcoholism, antialcohol effect, ethanol metabolism, *Hovenia dulcis Thunb*, intestinal flora, inulin

## 1 | INTRODUCTION

Alcohol holds a significant place in human culture, yet it has led to the emergence of alcoholism as a prominent global health concern. The consumption of excessive alcohol can result in both acute and chronic alcoholism, which causes numerous detrimental effects on the body. These effects include dizziness, nausea, vomiting, slowed movement, memory loss, coma, and other symptoms. Moreover, excessive alcohol intake places a heavy burden on the heart, damages liver cells, and negatively impacts multiple bodily systems such as digestion, the cardiovascular and cerebrovascular systems, and the nervous system. In severe cases, alcohol abuse can even lead to death (Wu et al., 2020). The liver plays a crucial role in alcohol metabolism, and excessive consumption inflicts severe harm on liver tissue. The most common manifestation of such damage is liver fat deformation, which progresses to steatohepatitis, liver fibrosis, cirrhosis, and potentially liver cancer. However, it is worth noting that the damage caused by alcoholic liver disease in its acute stage is mostly reversible. By providing timely and targeted treatment, the progression of this disease into irreversible lesions can be prevented. Nowadays, the number of people who drink alcohol is extremely large, and the health and social problems caused by alcohol are becoming more and more serious. At present, although various studies have been conducted on the development of drugs for treating alcoholic liver diseases, such as disulfide, naltrexone,

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and acamprosate, progress in this field has been limited. The morbidity and mortality rates associated with the disease remain high, with no satisfactory treatment strategy available other than alcohol withdrawal and nutritional support. Nowadays, natural drugs have garnered significant attention due to their unique advantages, such as high pharmacological activity and minimal toxic side effects. Research has indicated that compound turmeric recipes (CTR) (composed of *Curcuma longa*, *Panax ginseng*, *Pueraria lobata*, *Pueraria flower*, and *Hovenia dulcis Thunb*) and apple have the potential to mitigate acute alcoholism, promote wakefulness, and alleviate alcoholic liver damage. These effects are attributed to the acceleration of ethanol metabolism, enhancement of antioxidant capabilities, and suppression of inflammatory mediator production (Liang et al., 2021; Wang et al., 2023). Furthermore, the extract of Semen Hoveniae extracts demonstrate a protective function against alcoholic liver injury primarily by boosting antioxidant properties and optimizing the composition of intestinal flora (Du et al., 2010; Qiu et al., 2019; Wang et al., 2012). Therefore, the search for natural compounds with antialcoholic and hepatoprotective properties holds immense significance in preventing or slowing down the progression of alcoholic liver injury. And it is also a new direction for the research and development of antialcoholic products.

Inulin is a natural fructan that is abundant in plants and has been deemed "Generally Recognized as Safe" (GRAS) for use in food since 2002. Usually, its degree of polymerization (DP) ranges from 2 to 60, of which the average $DP \leq 10$ is called short-chain inulin and $10 < DP < 60$ is called long-chain inulin. With the different degree of polymerization, the corresponding physical and chemical properties and biological activities will also change significantly. Notably, inulin is resistant to hydrolysis by digestive enzymes in the human body and can only be fermented by bacteria in the colon. This imparts dietary fiber and prebiotic properties to inulin (Ni et al., 2019). As a prebiotic, it has demonstrated the ability to enhance the intestinal microbial environment, regulate blood sugar levels, regulate blood lipids, exhibit antioxidant and anticancer effects, and modulate immunity (Hughes et al., 2022; Qin et al., 2023; Wan et al., 2020). Furthermore, studies have revealed promising properties and potential applications of inulin-derived products through biosynthesis or chemical modification (Stevens et al., 2001). For instance, chemical modifications of inulin have shown improved antifungal activity (Chen et al., 2018). Due to its favorable biocompatibility and broad biological activity, it has found widespread usage in the fields of food, medicine, and beyond. However, despite its extensive use, there is currently a lack of published reports on inulin's antialcohol effects and its underlying mechanisms, thus rendering it a realm of considerable scholarly worth.

**Highlights**

- Short-chain inulin can reduce the rate of drunkenness, mortality, and shorten the sober time.
- Short-chain inulin can improve acute alcoholism and liver injury.
- Short-chain inulin activated ethanol metabolizing enzyme activity.
- Short-chain inulin could improve the intestinal flora structure of mice with acute alcoholism.

During the initial phase of this study, it has been ascertained that inulin possesses a discernible antialcohol effect. However, the exploration of its antialcohol effect remains limited to surface-level observations, and the precise mechanism of action remains unknown. As a traditional Chinese medicine, *Hovenia dulcis Thunb* is widely used to treat alcoholism and alcohol-related liver diseases (Fang et al., 2007; Qiu et al., 2019; Wang et al., 2012). Therefore, on the basis of previous studies, an acute alcoholic intoxication mice model was established, with *Hovenia dulcis Thunb* water extract as a positive control. Subsequently, the effects of inulin on its antialcohol properties, as well as the potential mechanisms involved, were thoroughly investigated. The aim of this investigation was to offer insights and references for the development of natural products and medications for the treatment of alcoholic liver injury.

## 2 | MATERIALS AND METHODS

### 2.1 | Materials

Short-chain inulin Fibrulose® (FS, average DP <10, and inulin content ≥87%) and long-chain inulin Fibruline® (FXL, average DP ≥23, and inulin content ≥98%) were acquired from Cosucra (Belgium). Phosphorylated long-chain inulin (PFXL, substitution degree of phosphorylated inulin was 0.01) was obtained by laboratory preparation. *Hovenia dulcis Thunb* (HDT) was procured from a local Traditional Chinese Medicine (TCM) Pharmacy. The Chinese Baijiu, which contains 56% (V/V) alcohol, was obtained from Hongxing Limited by Share Ltd. (Beijing).

### 2.2 | Establishment of an acute alcoholic intoxication mice model

Male KM mice, aged 6 weeks and weighing approximately $38 \pm 2$ g, were procured from the Beijing SPF Biotechnology Co., Ltd., China. The experimental

procedures adhered to the institutional guidelines governing the care and use of laboratory animals and were duly approved by Spife (Beijing) Biotechnology Co., Ltd. [SCXK (Jing) 2019-0010]. The mice were housed under controlled conditions, maintaining a temperature of 22 ± 2°C, a humidity of 55 ± 5%, and a 12-h light/dark cycle. They were provided with unrestricted access to deionized water and a standard diet.

Following a 1-week adaptation period, the mice were randomly assigned to six groups, each containing eight individuals. The groups were designated as follows: (1) control group (CON); (2) alcohol model (Alcohol) group (MOD); (3) positive (*Hovenia dulcis Thunb* water extract, HDTs) group (PC); (4) short-chain inulin group (FS); (5) long-chain inulin group (FXL); (6) phosphorylated long-chain inulin group (PFXL). The PC, FS, FXL, and PFXL groups received a single intragastric dose of 400 mg/kg·BW, while the control and alcohol model groups were administered the same volume of distilled water. Half an hour after administration, the mice in all groups except the control group received 14 mL per kg of body weight (Previous studies have shown that 14 mL/kg·BW is the optimal gavage dose.) of 56% edible alcohol intragastrically to induce acute alcoholism.

## 2.3 | Assessment of the antialcoholic effect of inulin

The determination of drunkenness in the study was based on the disappearance of the "righting reflex." The presence of this reflex was assessed by observing whether the test animals were able to return to the prone position with all four paws within 30 s. Once an acute alcoholic intoxication mice model was established, various parameters were recorded, including the drunkenness rate, mortality rate, tolerance time (time from drinking to the disappearance of the righting reflex), sleeping time (duration from when the righting reflex disappears until it recovers again), and sober time (time from drinking to the recovery of the righting reflex).

## 2.4 | Effects of inulin on liver and blood biochemical indexes in mice

An additional 48 male KM mice were randomly assigned to six groups and subjected to treatment with inulin and HDTs as previously described. Following the intragastric administration of liquor for a duration of 30 min, all the mice were then killed under anesthesia induced by diethyl ether asphyxiation. Both liver tissue and blood samples were collected for the subsequent determination of biochemical indexes. Serum samples were obtained from the blood samples and subsequently centrifuged at a rate of 3000 r/min for a duration of 15 min to acquire the serum, which was then stored at a temperature of −80°C after being frozen in liquid nitrogen. The contents of total protein (TP), albumin (ALB), globulin (GLB), alanine aminotransferase (ALT), alkaline phosphatase (ALP), triglycerides (TG), and total cholesterol (TC) in serum were measured by an automatic biochemical analyzer. The activity or content of alcohol dehydrogenase (ADH) and aldehyde dehydrogenase (ALDH) in the liver was determined using kits sourced from Nanjing Jiancheng Bioengineering Institute, in accordance with the instructions provided by the manufacturer.

## 2.5 | Gut microbe analysis

Fecal samples were submitted to Shanghai Personal Biotechnology Co., Ltd. for analysis of the intestinal flora. The DNA samples were obtained from the excrement of mice, which were subsequently subjected to PCR amplification targeting the V3 and V4 hypervariable regions of the 16S rRNA sequences. The forward primer was V3-V4F (5′-ACTCCTACGGGAGG CAGCA-3′) and the reverse primer was V3-V4R (5′-GG ACTACHVGGGTWTCTAAT-3′). Subsequently, the purity of PCR products was detected, and fluorescence quantitative analysis was performed. The Illumina's TruSeq Nano DNA LT Library Prep Kit was used to prepare sequencing libraries. 16S rRNA sequencing was performed on the Illumina Miseq platform to obtain high-quality sequences and perform operational taxonomic units (OTU) cluster analysis based on 97% sequence similarity.

## 2.6 | Statistical analysis

The data were expressed as the mean ± standard deviation. The data underwent processing and mapping using SPSS 26.0 and Origin 2023 software. To compare multiple groups, an analysis of variance was conducted with a 95% confidence interval, where a significance level of $p < 0.05$ was considered.

# 3 | RESULTS

## 3.1 | Effects of inulin on behavioral indexes in mice with acute alcoholism

The natural tendency of an organism to return to its regular position after being placed in an abnormal posture is known as the righting reflex. At the same time, the loss of righting reflex test has also been widely acknowledged as a reliable method to evaluate drunkenness. By utilizing the disappearance of the righting reflex as an indicator, one can determine if mice have become intoxicated. Upon consuming alcohol, the mice

were gently placed in a supine position, and if they remained in this position for more than 30 s, indicating the disappearance of the righting reflex, they were deemed intoxicated. Following intoxication, if the righting reflex was restored after a certain period, it was considered a state of sobriety (Lai et al., 2022; Ornelas et al., 2015). The drunkenness of mice in each group was observed after the administration of liquor, and the drunkenness rate, mortality rate, tolerance time, sleeping time, and sober time were recorded. Figure 1 displays the outcomes of behavioral indexes conducted on intoxicated mice. In comparison to the model group, the positive group exhibited a 50% reduction in drunkenness rate, an 80% decrease in mortality rate, and a 23% decrease in sober time. It can be concluded that the water extract of HDT possesses a favorable capability to alleviate hangover symptoms. Furthermore, when compared with the model group, FS, FXL, and PFXL all demonstrated a 33% decrease in drunkenness. FS exhibited a 40% decrease in mortality rate, while FXL and PFXL displayed an 80% decrease in mortality rate. At the same time, the sober time of FS was shortened by 44%, which was significantly lower than that of the model group ($p < 0.05$). Together with these results, FS, FXL, and PFXL could improve the behavioral indexes of intoxicated mice and have certain antialcohol function. Among these, FS exhibited a superior antialcohol effect compared with FXL and PFXL, albeit with a slightly weaker hangover effect than that of the HDT water extract.

## 3.2 | Effects of inulin on serum indexes of mice with acute alcoholism

The determination of serum protein content can reflect the synthesis function of the liver, and when the liver is damaged, its synthesis function will be affected to some extent. Figure 2A demonstrates that, in the alcohol model group, the levels of TP, ALB, and GLB in the serum were lower compared with the control group, with reductions of 9.8%, 9.4%, and 10.3%, respectively. Compared with the control group, the levels of TP and ALB in the FXL and PFXL groups were significantly decreased ($p < 0.05$), while there was no significant change in the FS group. Furthermore, the TP and ALB levels in the FXL and PFXL groups decreased by 6.7% and 8.1%, respectively, compared with the model group, while there was also little change in the FS group. GLB levels in the FS group exhibited a slight increase of 1.1% compared with the alcohol model group. These results indicated that FS could alleviate the tendency for serum protein to decrease.

Alcohol possesses both fat-soluble and water-soluble properties, thereby leading to potential damage to cell membranes. This can result in alterations to the structure, dielectric constant, fluidity, permeability, and other physical characteristics of the cell membrane, as well as the peroxidation of lipids within the membrane (Cederbaum, 2012; Zakhari, 2006). In biological liver cells, ALT is located in the cytoplasm, and its concentration in serum is typically very low under

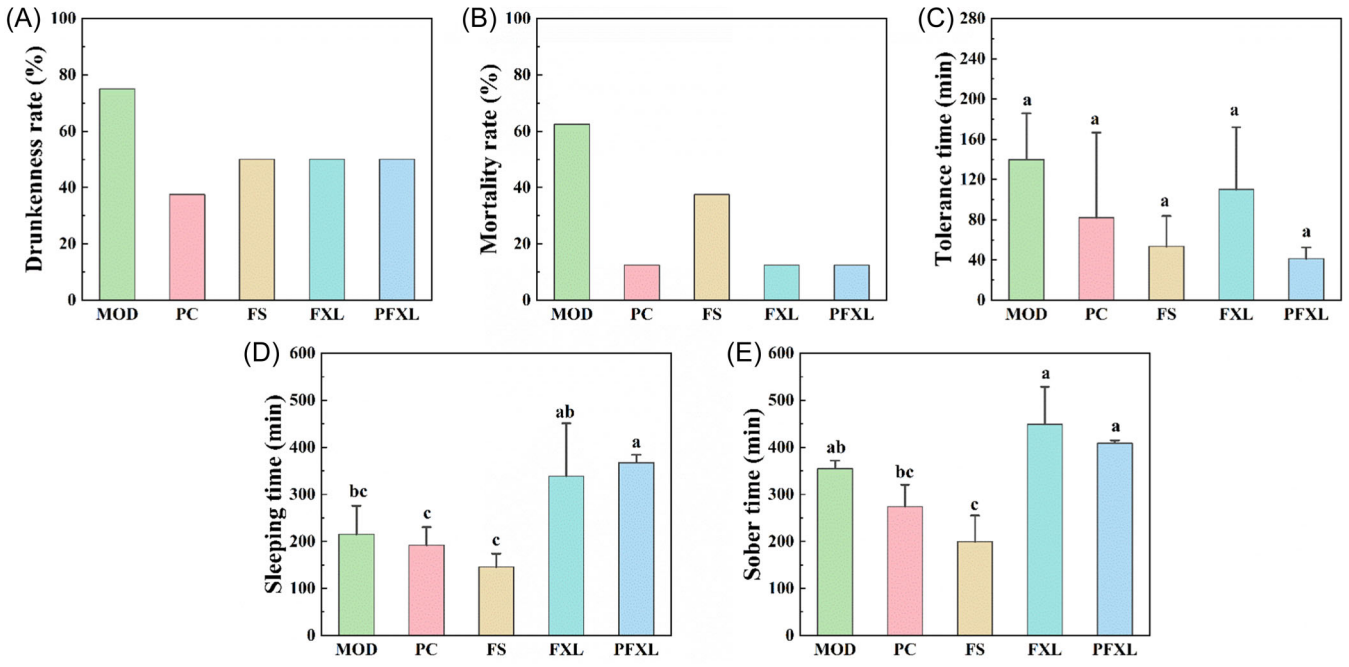



**FIGURE 1** Ameliorating effects of inulin on acute alcoholism in a mice model ($n = 8$). The drunkenness rate (A), mortality rate (B), tolerance time (C), sleeping time (D), and sober time (E) of mice. Each value represents the mean ± SEM. Different lowercase letters represent significant differences between groups ($p < 0.05$). MOD, alcohol model group; PC, positive group; FS, short-chain inulin group; FXL, long-chain inulin group; PFXL, phosphorylated long-chain inulin group.

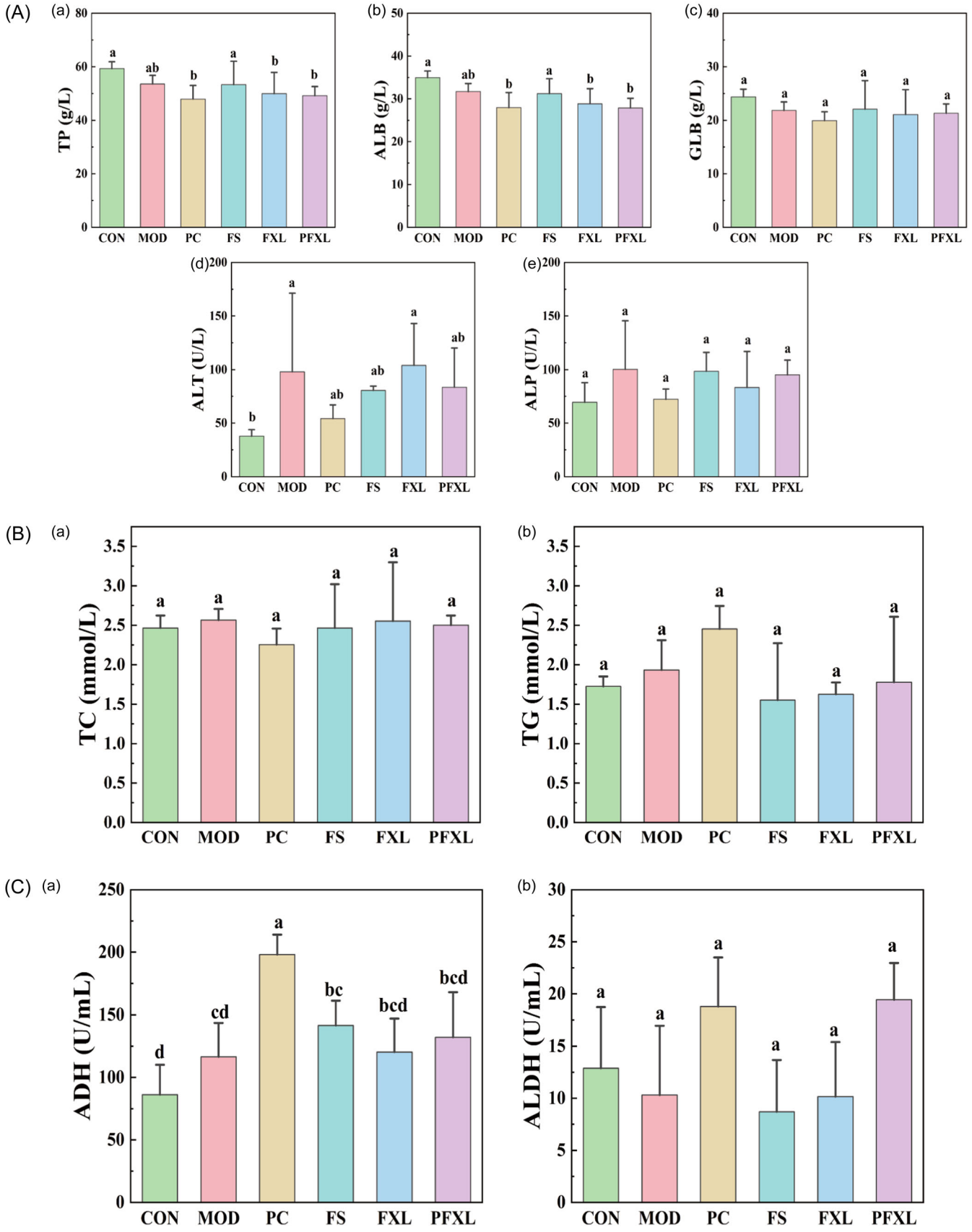



**FIGURE 2** (See caption on next page).

normal physiological conditions. However, when alcohol-induced damage occurs to liver cells, the cell membrane becomes compromised, leading to an increase in cell permeability. Consequently, ALT from the liver cells can enter the bloodstream, causing an elevation in serum ALT activity. Therefore, the level of ALT is frequently employed as a crucial indicator to determine whether there is abnormal liver function. At the same time, ALP serves as an essential marker for cholestasis, as it can harm liver cells when bile production or discharge is obstructed. Moreover, ALP can be used to assess the severity of liver injury. The data presented in Figure 2A demonstrates that, compared with the control group, the model group exhibited a 159.6% increase in ALT levels ($p < 0.05$) and a 44.2% increase in ALP levels. ALT levels were significantly increased, indicating that alcohol intake caused liver cell damage. The PC group displayed a 44.6% decrease in ALT levels, the FS group exhibited a 17.9% decrease, and the PFXL group showcased a 14.8% decrease compared with the model group. The ALP level in the PC group displayed a 27.9% decrease relative to the model group. Additionally, the FS, FXL, and PFXL groups demonstrated decreases of 1.7%, 17.0%, and 5.2% in ALP levels, respectively, when compared with the model group. The results showed that the intake of three kinds of inulin had a certain protective effect on the liver in different degrees.

A disturbance of lipid metabolism is the earliest reaction to heavy drinking. During alcohol metabolism, steatosis can be induced, resulting in fat deposition in liver cells. Meanwhile, alcohol affects the transport of blood lipids, leading to abnormal levels of serum TG and TC (Qi et al., 2023; Zhao et al., 2023). Research conducted on ALD mice has shown that alcohol significantly elevates TC, TG, and LDL-C levels in serum while decreasing HDL-C levels, thereby causing lipid accumulation in the liver (Shen et al., 2014). The effects of different types of inulin on serum TC and TG levels in mice with acute alcoholism are depicted in Figure 2B. In comparison to the control group, the alcohol model group exhibited a 4.0% increase in TC levels and a 10.4% increase in TG levels, indicating that alcohol intake raises the levels of TC and TG in the serum, potentially leading to lipid metabolism disorders, which aligns with previous research findings. Following the inulin intervention, serum TC and TG levels were lower than those in the model group. The FS group exhibited a decrease of 4.0% and 20.0% in TC and TG levels, respectively. The FXL group displayed a decrease of 0.8% and 15.5%, respectively. The PFXL group demonstrated a decrease of 3.0% and 8.0%, respectively. These outcomes showed that inulin intervention alleviated acute alcohol-induced lipid metabolism disorders, with the FS group exhibiting the most favorable effect.

### 3.3 | Effect of inulin on alcohol metabolizing enzyme activity in liver tissue of mice with acute alcoholism

The ethanol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH) play a pivotal role in the metabolic process of ethanol within the human body (Edenberg & McClintick, 2018). To some extent, the velocity of alcohol metabolism can be inferred by assessing the activity of these enzymes in the liver. As shown in Figure 2C, the ADH activity increased by 35.1% and the ALDH activity decreased by 20.0% in the alcohol model group compared with the control group. This indicates that alcohol intake has a certain effect on the activity of ethanol metabolizing enzymes. The activity of ADH in the PC group was significantly higher than that in the control group and the alcohol model group ($p < 0.05$). The activity of ADH in the FS group was significantly higher than that in the control group by 84.2% ($p < 0.05$) and a 21.5% increase compared with the alcohol model group. Additionally, both the FXL and PFXL groups demonstrated higher ADH activity than the control and model groups. The ALDH activity in the FS and FXL groups did not significantly differ from the control and model groups, while the PC and PFXL groups exhibited an 82.3% and 88.6% increase in ALDH activity, respectively. These results indicated that different types of inulin and HDT water extract could activate the activities of ADH and ALDH to a certain extent, promote the metabolism of ethanol and acetaldehyde, and alleviate the injury caused by acute alcohol poisoning. This is consistent with the results of previous studies (Gao et al., 2023; Yang et al., 2023)

### 3.4 | Effects of inulin on α and β diversity of intestinal flora

Alpha diversity is a widely utilized metric used to describe the richness and diversity of the gut microbiota. The Chao1 index and Observed_species index serve as

**FIGURE 2** (A) Serum liver function indexes of mice with acute alcoholism ($n = 5$). The total protein (a), albumin (b), globulin (c), alanine aminotransferase (d), and alkaline phosphatase (e) of mice. (B) Serum lipid metabolism indexes of mice with acute alcoholism. The total cholesterol (a) and triglyceride (b). (C) Liver alcohol metabolizing enzyme activity index in mice with acute alcoholism. The ethanol dehydrogenase (a) and acetaldehyde dehydrogenase (b). Each value represents the mean ± SEM. Different lowercase letters represent significant differences between groups ($p < 0.05$). CON, control group; FS, short-chain inulin group; FXL, long-chain inulin group; MOD, alcohol model group; PC, positive group; PFXL, phosphorylated long-chain inulin group.

indicators of species richness within a community, with higher values indicating greater richness. The Shannon index serves as a reliable predictor of diversity magnitude and is more sensitive to rare species. Conversely, the Simpson index is susceptible to significant influence from the dominant species within a community, and as the values of both indices increase, so does the level of species diversity (Tian et al., 2020; Yi et al., 2021). As depicted in Figure 3A, the model group exhibited an 18.5% increase in the Observed_species index and a 16.0% increase in the Chao1 index compared with the normal group, suggesting that alcohol consumption enhances species richness in the gut. After the intervention of inulin, both indices displayed a decreasing trend. Specifically, the FS, FXL, and PFXL groups exhibited decreases of 6.4%, 11.4%, and 30.8% in the Observed_species index, respectively, and decreases of 5.0%, 11.1%, and 28.4% in the Chao1 index, respectively. When compared with the model group, there was no significant difference in the Shannon and Simpson indices between the control group and the model group, nor were any significant changes noted in these indices within the inulin group. In conclusion, excessive alcohol consumption in mice can elevate species richness within the intestinal flora, while inulin intervention can mitigate this phenomenon, albeit with a limited impact on overall intestinal flora diversity.

The principal coordinate analysis (PCoA) based on Jaccard distance is utilized to assess the resemblance of microflora composition among distinct samples, enabling

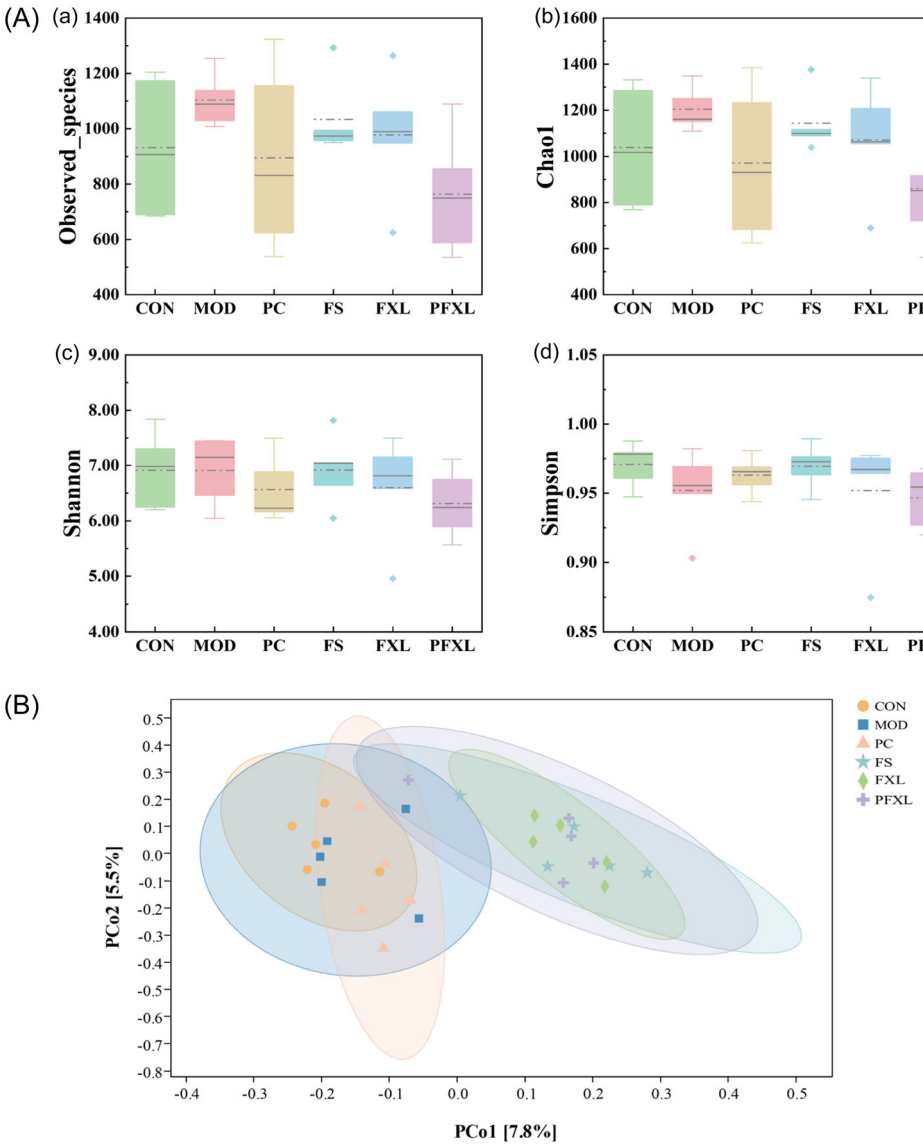



**FIGURE 3** (A) Alpha diversity analysis of the gut microbiota of mice in each group ($n = 5$). The Observed_species index (a), Chao1 index (b), Shannon index (c), and Simpson index (d). (B) Beta diversity analysis of the gut microbiota of mice in each group ($n = 5$). Principal coordinate analysis of PCoA based on Jaccard distance.

an evaluation of the overall alterations in intestinal microbial community structure across different sample groups. The proximity of two samples on the PCoA plot denotes a higher similarity in species composition. As can be seen from Figure 3B, following alcohol induction, the intestinal flora structure of mice in the model group, control group, and positive group exhibited similarity, with no significant modifications observed. The intestinal flora structure of the three inulin groups also demonstrated resemblances, although evident disparities were evident when compared with the model and control groups. These outcomes indicate that inulin exerts a discernible impact on the composition of intestinal flora in mice.

### 3.5 | Effects of inulin on the species distribution of intestinal flora in mice with acute alcoholism

At the gate classification level, it is shown in Figure 4A. The microflora in each group was mainly composed of Bacteroides, Firmicutes, Proteobacteria, Verrucomicrobia, and Actinobacteria. Compared with the control group, alcohol consumption altered the composition of intestinal flora in mice, mainly reflected in the fact that alcohol decreased the relative abundance of Bacteroides (17.2%) and increased the relative abundance of Firmicutes (25.2%) and Proteobacteria (77.2%). Furthermore, compared with the model group, the positive group and inulin group exhibited an upward trend in the abundance of Bacteroides in the intestinal flora of mice and a downward trend in the abundance of Firmicutes and Proteobacteria ($p < 0.05$). Additionally, alcohol reduced the abundance of Verrucomicrobia (51.8%) and Actinobacteria (62.8%), but this phenomenon was reversed in the positive group and inulin group, which exhibited similar levels to the control group.

The family classification level, as illustrated in Figure 4B, demonstrates several noteworthy findings. First, in comparison to the control group, the alcohol model group exhibited a decrease in the relative abundance of S247 by 19.1%, Bacteroidaceae by 26.6%, and a significant increase in the relative abundance of Lachnospiraceae by two times ($p < 0.05$). The introduction of inulin intervention effectively mitigated these aforementioned phenomena, particularly by significantly reducing the relative abundance of Lachnospiraceae ($p < 0.05$). Furthermore, alcohol consumption increased the relative abundance of Ruminococcaceae (19.5%) and Helicobacteraceae (43.0%) in the intestinal tract of mice, while inulin administration effectively suppressed the excessive growth of Helicobacteraceae and Ruminococcaceae. Compared with the MOD group, the inulin group could increase the relative abundance of Lactobacillaceae and Prevotellaceae.

At the level of generic classification, Figure 4C demonstrates that the alcohol model group exhibited a decrease in the relative abundance of *Bacteroides* (26.6%), *Allobaculum* (98.7%), *Parabacteroides* (66.7%), and *Akkermansia* (52.7%) compared with the control group. Conversely, the relative abundance of *Oscillospira* (54.7%) and *Helicobacter* (83.0%) increased. However, the introduction of inulin reversed this change. Furthermore, the relative abundance of *Lactobacillus* and *Prevotella* increased (55.5%) in the inulin group when compared with the model group.

### 3.6 | Critical differential microbiological analysis

As shown in Figure 5, the identification of different strains among the groups was accomplished through the utilization of LEfSe (LDA Effect Size) analysis. Linear discriminant analysis (LDA) was employed to assess the species exhibiting significant differences among the groups as well as to determine the influence on the variation of species richness between the groups. According to the petal plot, there were 428 species shared by the six groups, and alcohol consumption increased the abundance of species, which was consistent with the α diversity result analysis above. The bar chart generated from the LEfSe statistical difference analysis revealed the presence of three distinct levels of taxa, primarily observed in the control group and the model group. Specifically, the microorganisms g-*Arthrobacte* and g-*Enterobact* were identified as the differentiating factors in the CON group, while in the case of acute alcohol intake, g-*Gluconacetobac* emerged as a significant distinguishing microorganism in the MOD group when compared with the other groups.

### 3.7 | Correlation analysis between intestinal flora and biochemical indexes in acute alcoholism mice

The correlation between the intestinal microbiota (at the phylum, family, and genus levels) and nine biochemical indexes of acute alcoholism mice in each group was analyzed (Figure 6). It was observed that TP and ALB showed a significant negative correlation with changes in the abundance of *Bacteroides* in the intestinal flora, while they exhibited a significant positive correlation with *Oscillospira*. The ALT and ALP were negatively correlated with the abundance of *[Prevotella]* and *Parabacteroides*, respectively ($p < 0.05$). Moreover, TG was negatively correlated with the abundance of *Allobaculum* ($p < 0.01$). These results suggest that an increase in the abundance of *Oscillospira*, *[Prevotella]*, *Parabacteroides*, and *Allobaculum*, along with a decrease in the abundance of Bacteroides following inulin intervention, might have a beneficial effect on the remission of acute alcoholism.

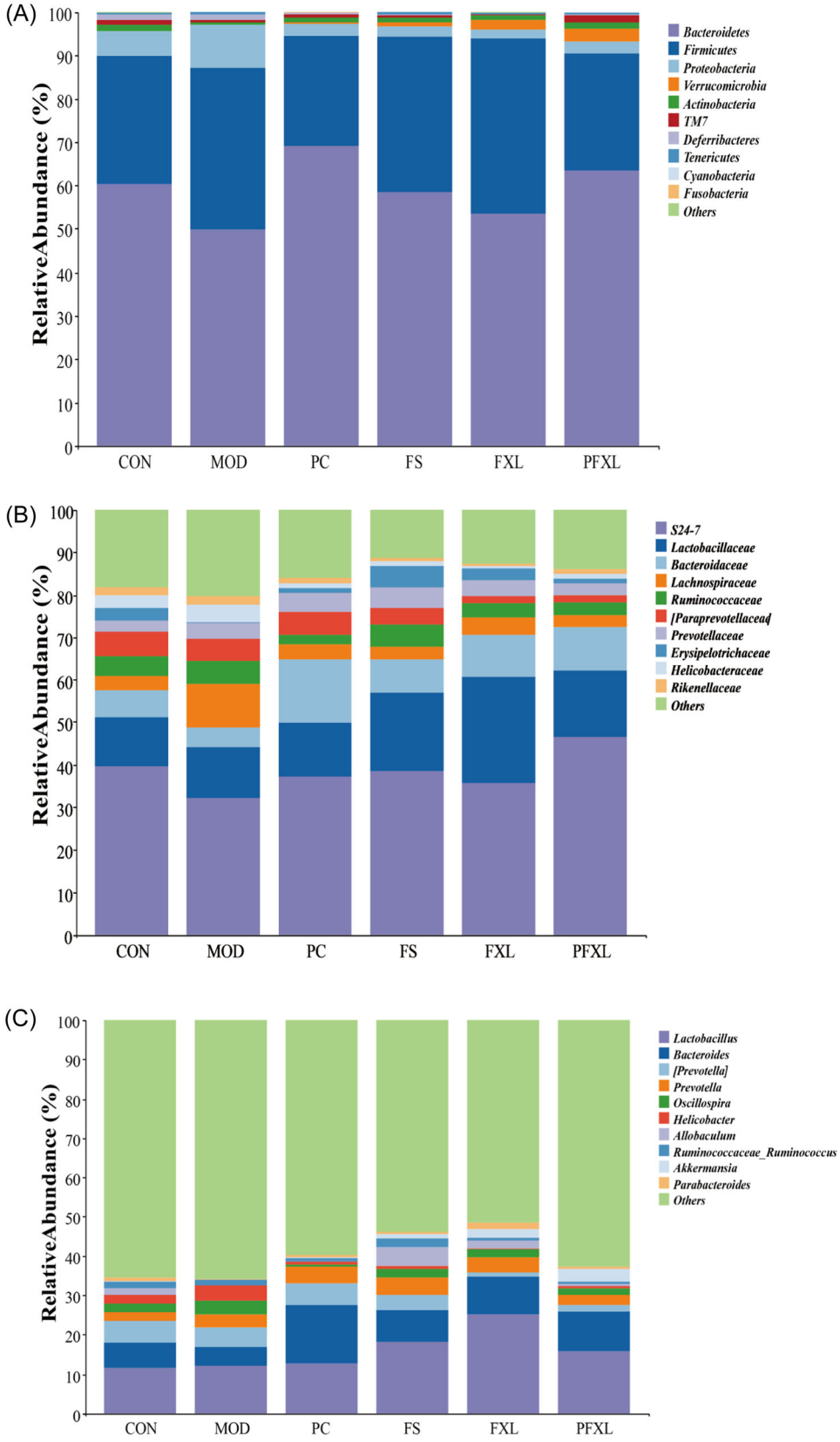



**FIGURE 4** Species distribution map of gut microbiota between groups (A) at the phylum level; (B) at the family level and (C) at the genus level ($n = 5$).

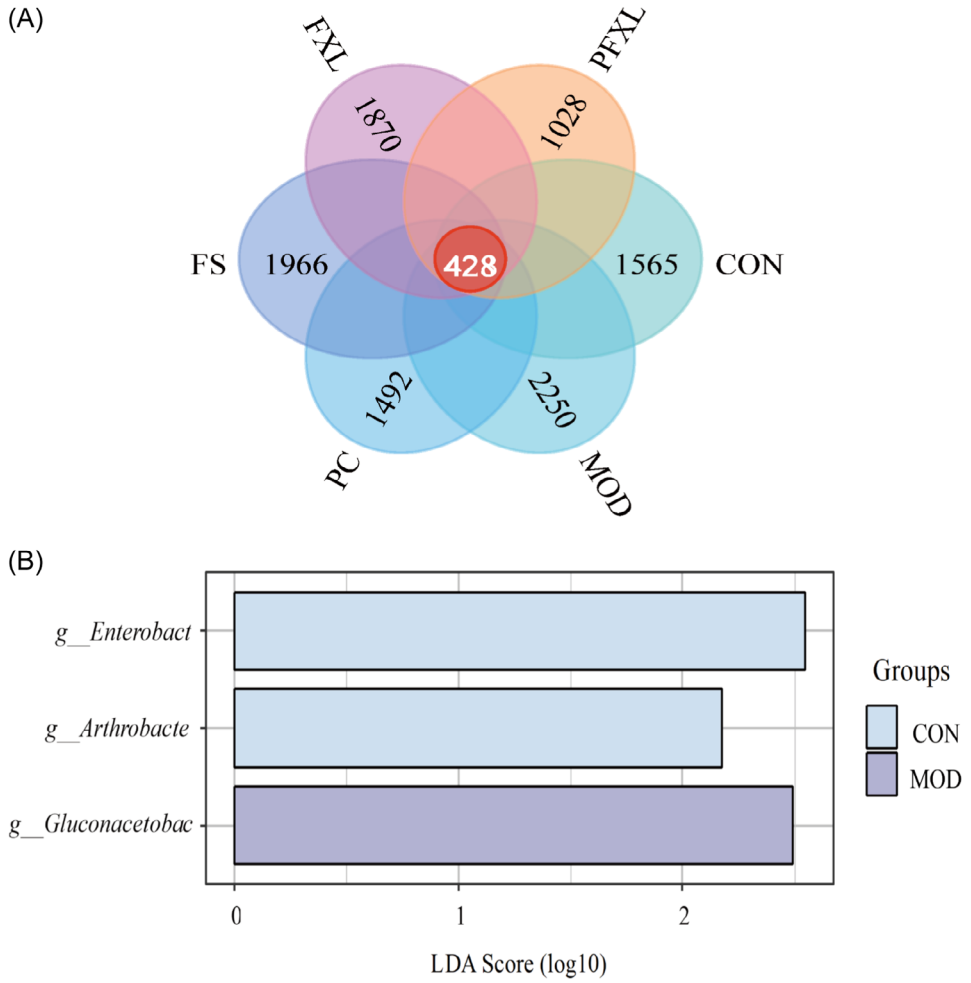



**FIGURE 5** Structural analysis of different species between groups (A) Petal map of intestinal microbiome composition in each group, (B) LEfSe statistical difference analysis histogram.

## 4 | DISCUSSION

Alcohol can exert its effects on the central nervous system, resulting in various behavioral issues, such as the loss of the righting reflex. This study aimed to observe the behavioral changes in mice drinking alcohol by assessing the righting reflex. The findings revealed that the administration of inulin effectively reduced the rate of drunkenness and mortality, and FS can shorten the sober time, which has the best hangover effect. Previous research indicated a significant positive correlation between the rate of drunkenness and the total sugar content of inulin. However, no correlation was found between biochemical components and the other observed indexes. It was speculated that the effect of inulin on the behavior of mice with acute alcoholism might be the result of the comprehensive action of various biochemical components. Researchers investigated the effects of the respective water extracts of six kinds of tea preparations (WEATs) on the time of drunkenness and the time of sleep in mice with acute alcoholism, and six kinds of tea showed different degrees of hangover effect. In addition, no correlation was found between the time of drunkenness and sleep and the biochemical composition of WEATs (Lai et al., 2022). This is consistent with the results of this experiment. In addition, Liang et al. have identified that the CTR, consisting of *Curcuma longa*, *Panax ginseng*, *Pueraria lobata*, *Pueraria flower*, and *Hovenia dulcis Thunb*, effectively extends the alcohol tolerance time in mice afflicted with acute alcoholism. It also reduces the time required for sobriety and enhances the 24-h survival rate of the mice. The mechanism is likely attributed to the decrease in alcohol concentration in the bloodstream, facilitated by the heightened activity of ADH, ALDH, and cytochrome P450 enzymes in the liver due to the administration of compound turmeric, thereby expediting alcohol metabolism (Liang et al., 2021).

The assessment of liver damage can be accomplished by measuring serum proteins (TP, ALB, and GLB), as well as ALT and ALP levels. The research findings indicate that alcohol consumption does indeed cause liver

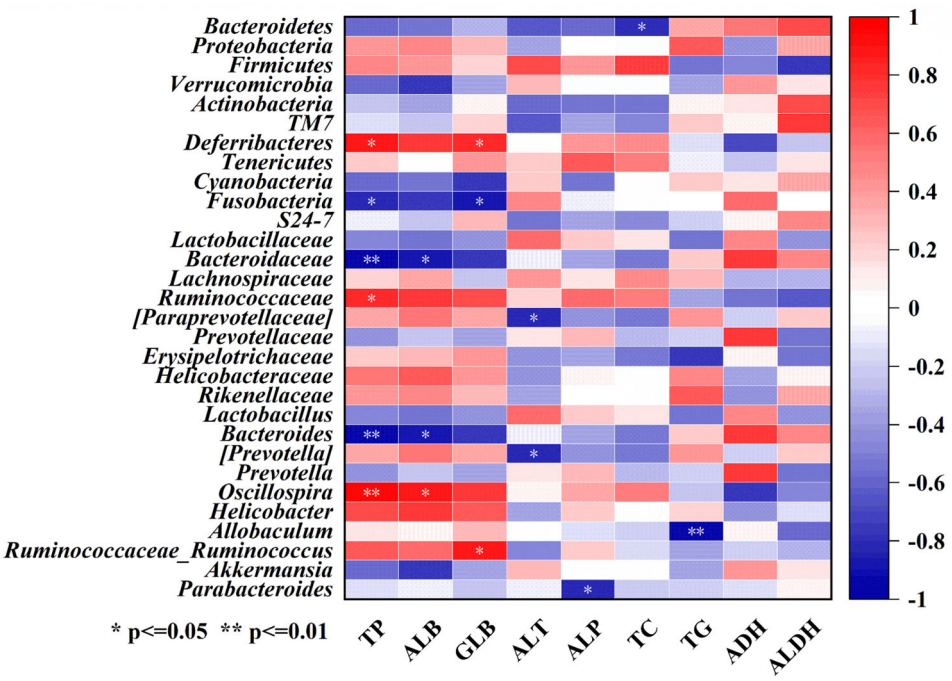



**FIGURE 6** The Spearman correlation between nine biochemical indexes and gut microbiota in mice with acute alcoholism. Blue and red represent negative correlation and positive correlation, respectively.

damage, resulting in decreased serum protein levels and increased ALT and ALP levels. However, inulin intake has been shown to have a significant protective effect against alcohol-induced liver damage. In addition, the levels of TC and TG are also reduced, and the lipid metabolism disorders induced by alcohol are alleviated. The combination mixture (MSH) comprising fermented Schizandrae Fructus pomace and Hoveniae Semen cum Fructus hot water extracts in a 1:1 (w:w) proportion demonstrated a noteworthy decrease in the levels of serum ALT, AST, ALP, and GGT, along with a reduction in hepatic TG accumulation. This formulation exhibited a potential synergistic protective impact against ethanol-induced hepatotoxicity by impeding steatosis (Jegal et al., 2023). It is generally understood that over 90% of ingested alcohol is absorbed by the gastrointestinal tract, with only a small portion being excreted through breath, sweat, and urine. The absorbed alcohol is primarily metabolized by various enzymes in the liver, with ADH and ALDH being the key enzymes responsible for ethanol metabolism. These enzymes are predominantly present in the cytoplasm and mitochondria of liver cells. ADH converts ethanol to acetaldehyde, which is then further oxidized to acetic acid by ALDH. The acetic acid is subsequently metabolized into water and carbon dioxide and discharged from the body, allowing for a significant reduction in alcohol content in the bloodstream. Accelerating the rate of alcohol metabolism through the augmentation of alcohol metabolism enzymes stands as a prevalent mechanism of alcohol detoxication. It is evident that inulin holds the capability to expedite ethanol metabolism by augmenting the activity of ADH and ALDH, consequently contributing to its potential in combating alcoholism. This finding aligns with the results of a study conducted by Kaviarasan et al. (2008), which investigated the mechanism of fenugreek polyphenol hangover. Furthermore, scholars have discovered that the prenylated flavonoids derived from the rhizomes of Echinosophora koreensis can enhance the activity of ADH and ALDH at micromolar concentrations, which possess the capacity to mitigate the effects commonly referred to as "hangovers" following the consumption of alcohol (Choi et al., 2009).

Intestinal flora normally distributes in the intestine according to certain rules and forms a defense barrier together with intestinal mucosa, which plays a key role in maintaining intestinal homeostasis and promoting body health. In recent years, many studies (Bjorkhaug et al., 2019; Bluemel et al., 2020; Wang et al., 2018) have shown that the consumption of alcohol disrupts the composition and distribution of the intestinal microbiota, resulting in a decrease in the number of beneficial bacteria in the intestine and an increase in the proliferation of harmful bacteria. This alteration prompts the secretion of endotoxin, which not only damages the intestinal mucosa but also enters the bloodstream, inducing endotoxemia and ultimately leading to liver impairment. These findings highlight the intricate connection between intestinal flora and alcohol-induced liver damage. Through the examination of the intestinal microbiota in mice, this study revealed that the α

diversity of the intestinal microbiota in the model group exhibited an increase following alcohol induction which was similar to the results of Xia et al. (2021). Alcohol consumption resulted in significant changes in the structure of the intestinal flora in mice. Specifically, there was an increase in the abundance of pro-inflammatory microorganisms like Proteobacteria, while the abundance of normal symbiotic bacteria like Bacteroidetes decreased. The ratio of Firmicutes/Bacteroidetes in the gut also increased, but this change was mitigated by the intervention of inulin. Bacteroidetes can produce propionic acid, promote liver gluconeogenesis, and regulate alcohol-induced hypoglycemia (Li et al., 2020). Proteobacteria include many pathogenic bacteria, such as *Escherichia coli*, *Salmonella*, and *Helicobacter*, which multiply in the gut and cause microbial community imbalance. Additionally, Verrucomicrobia is believed to be a probiotic that has been inversely associated with liver inflammation and oxidative stress. It also produces short-chain fatty acids like propionic and butyric acid, which play a crucial role in maintaining gut health and regulating the immune system. In addition, *Allobaculum* can also use carbohydrates to produce butyric acid. Notably, studies have found that intervention with polysaccharide from the fruits of *Lycium barbarum* L. can significantly affect the intestinal microbial composition of diabetic mice, especially increasing the relative abundance of *Allobaculum* by 3.3 times (Zhou et al., 2022). Simultaneously, *Akkermansia*, a well-researched strain within the Verrucomicrobia, has the ability to counteract weight gain resulting from a high-fat diet. Furthermore, it can restore the integrity of the damaged intestinal epithelial barrier, decrease levels of blood endotoxins, and enhance insulin resistance (Fujisaka et al., 2020; Ou et al., 2020). It is worth noting that inulin intake can effectively modulate adverse changes in the abundance of Verrucomicrobia, *Allobaculum*, and Akkermansia due to alcohol. The abundance of *Allobaculum* in mice subjected to short-chain inulin treatment exhibited a higher level compared with the other experimental groups. Lactobacillaceae have the capacity to regulate intestinal flora, enhance immune function, promote intestinal health, and reduce inflammation (Kirpich et al., 2008). In comparison to the alcohol model group, the abundance of Lactobacillaceae in each inulin group increased to different degrees. Similar changes were observed at the genus level in *Lactobacillus* as well. In addition, inulin can inhibit an increase in the abundance of Helicobacteraceae in the intestine caused by alcohol. Studies have proven that the imbalance of intestinal flora is caused by the overgrowth of facultative anaerobic bacteria such as Helicobacteraceae, which subsequently triggers intestinal inflammation (Ying et al., 2020). This experiment proved that inulin intervention could improve the intestinal flora disturbance caused by alcohol, enhance the abundance of intestinal probiotics (such as *Bacteroides*, Verrucomicrobia, Lactobacillaceae, etc.), reduce the abundance of harmful bacteria (such as Proteobacteria and Helicobacteraceae, etc.), and then improve liver injury. Currently, the specific mechanism by which alcohol intake leads to structural alterations in the gut microbiota is not clear, necessitating further investigations for elucidation.

## 5 | CONCLUSION

Inulin has exhibited the potential to mitigate the effects of acute alcoholism in mice, leading to a reduction in drunkenness rate, mortality, and sober time. It has also shown promise in alleviating the abnormal levels of liver function indexes induced by alcohol, effectively ameliorating both alcohol-induced intoxication and liver damage. Notably, short-chain inulin has displayed the most favorable efficacy in relieving alcohol-related symptoms and protecting the liver. Its mechanism may be related to enhancing the activity of ethanol-metabolizing enzymes (ADH and ALDH) and improving the structure of intestinal flora (increasing the abundance of beneficial bacteria such as Bacteroidetes, Lactobacillaceae, and *Allobaculum* while decreasing the abundance of harmful bacteria such as Proteobacteria and Helicobacteraceae). Nevertheless, this proposition remains speculative, requiring rigorous investigation for substantiation. Therefore, inulin can lay the foundation for the development of natural plant health products and drugs with high efficiency, low toxicity, and antialcohol and liver protection effects. Additionally, further research is required to confirm the mechanism.

## AUTHOR CONTRIBUTIONS

**Zhouya Bai**: Experiment design; data analysis; funding acquisition; writing—review and editing; project administration. **Honglin Lan**: Experiment design and operation; formal analysis; paper writing-original draft. **Denglin Luo**: Conceptualization; validation; supervision; paper reviewing; funding acquisition. **Xingguo Li**: Validation; supervision. **Yunhui Zhang**: Validation; supervision. **Mengyuan Geng**: Validation; supervision. **Shaohe Chang**: Validation; supervision.

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## CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

## ETHICS STATEMENT

The animal study was reviewed and approved by Spife (Beijing) Biotechnology Co., Ltd. [SCXK (Jing) 2019-0010].

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## REFERENCES

Bjørkhaug, S. T., Aanes, H., Neupane, S. P., Bramness, J. G., Malvik, S., Henriksen, C., Skar, V., Medhus, A. W., & Valeur, J. (2019). Characterization of gut microbiota composition and functions in patients with chronic alcohol overconsumption. *Gut Microbes*, *10*(6), 663–675.

Bluemel, S., Wang, L., Kuelbs, C., Moncera, K., Torralba, M., Singh, H., Fouts, D. E., & Schnabl, B. (2020). Intestinal and hepatic microbiota changes associated with chronic ethanol administration in mice. *Gut Microbes*, *11*(3), 265–275.

Cederbaum, A. I. (2012). Alcohol metabolism. *Clinics in Liver Disease*, *16*(4), 667–685.

Chen, Y., Tan, W., Li, Q., Dong, F., Gu, G., & Guo, Z. (2018). Synthesis of inulin derivatives with quaternary phosphonium salts and their antifungal activity. *International Journal of Biological Macromolecules*, *113*, 1273–1278.

Choi, E. J., Kwon, H. C., Sohn, Y. C., Nam, C. W., Park, H. B., Kim, C. Y., & Yang, H. O. (2009). Four flavonoids from Echinosophora koreensis and their effects on alcohol metabolizing enzymes. *Archives of Pharmacal Research*, *32*(6), 851–855.

Du, J., He, D., Sun, L.-N., Han, T., Zhang, H., Qin, L.-P., & Rahman, K. (2010). Semen Hoveniae extract protects against acute alcohol-induced liver injury in mice. *Pharmaceutical Biology*, *48*(8), 953–958.

Edenberg, H. J., & McClintick, J. N. (2018). Alcohol dehydrogenases, aldehyde dehydrogenases, and alcohol use disorders: A critical review. *Alcoholism: Clinical and Experimental Research*, *42*(12), 2281–2297.

Fang, H. L., Lin, H. Y., Chan, M. C., Lin, W. L., & Lin, W. C. (2007). Treatment of chronic liver injuries in mice by oral administration of ethanolic extract of the fruit of *Hovenia dulcis*. *The American Journal of Chinese Medicine*, *35*(4), 693–703.

Fujisaka, S., Usui, I., Nawaz, A., Igarashi, Y., Okabe, K., Furusawa, Y., Watanabe, S., Yamamoto, S., Sasahara, M., Watanabe, Y., Nagai, Y., Yagi, K., Nakagawa, T., & Tobe, K. (2020). Bofutsushosan improves gut barrier function with a bloom of Akkermansia muciniphila and improves glucose metabolism in mice with diet-induced obesity. *Scientific Reports*, *10*(1), 5544.

Gao, Y., Zong, Z., Xia, W., Fang, X., Liu, R., Wu, W., Mu, H., Han, Y., Xiao, S., Gao, H., & Chen, H. (2023). Hepatoprotective effect of water bamboo shoot (*Zizania latifolia*) extracts against acute alcoholic liver injury in a mice model and screening of bioactive phytochemicals. *Food Frontiers*, *4*(3), 1362–1371.

Hughes, R. L., Alvarado, D. A., Swanson, K. S., & Holscher, H. D. (2022). The prebiotic potential of inulin-type fructans: A systematic review. *Advances in Nutrition*, *13*(2), 492–529.

Jegal, K. H., Park, H. R., Choi, B. R., Kim, J. K., & Ku, S. K. (2023). Synergistic protective effect of fermented Schizandrae Fructus Pomace and Hoveniae Semen cum Fructus extracts mixture in the ethanol-induced hepatotoxicity. *Antioxidants*, *12*(8), 1602.

Kaviarasan, S., Sundarapandiyan, R., & Anuradha, C. V. (2008). Protective action of fenugreek (Trigonella foenum graecum) seed polyphenols against alcohol-induced protein and lipid damage in rat liver. *Cell Biology and Toxicology*, *24*(5), 391–400.

Kirpich, I. A., Solovieva, N. V., Leikhter, S. N., Shidakova, N. A., Lebedeva, O. V., Sidorov, P. I., Bazhukova, T. A., Soloviev, A. G., Barve, S. S., McClain, C. J., & Cave, M. (2008). Probiotics restore bowel flora and improve liver enzymes in human alcohol-induced liver injury: A pilot study. *Alcohol*, *42*(8), 675–682.

Lai, X., Wang, X., Wen, S., Sun, L., Chen, R., Zhang, Z., Li, Q., Cao, J., Lai, Z., Li, Z., Sun, S., & Liu, X. (2022). Six types of tea reduce acute alcoholism in mice by enhancing ethanol metabolism, suppressing oxidative stress and inflammation. *Frontiers in Nutrition*, *9*, 848918.

Li, W., Wang, Y., Sun, M., Liang, Y., Cai, X., Qi, D., Zhang, Y., & Han, C. (2020). The prebiotic-like effects of *Coprinus comatus* polysaccharides on gut microbiota in normal mice and those with acute alcoholic liver injury: A comparative study. *Evidence-Based Complementary and Alternative Medicine*, *2020*, 2027570.

Liang, X., Wang, Y., Hu, X., & Wang, S. (2021). The protective effects of water extracts of compound turmeric recipe on acute alcoholism: An experimental research using a mouse model. *Evidence-Based Complementary and Alternative Medicine*, *2021*, 6641919.

Ni, D., Xu, W., Zhu, Y., Zhang, W., Zhang, T., Guang, C., & Mu, W. (2019). Inulin and its enzymatic production by inulosucrase: Characteristics, structural features, molecular modifications and applications. *Biotechnology Advances*, *37*(2), 306–318.

Ornelas, L. C., Novier, A., Van Skike, C. E., Diaz-Granados, J. L., & Matthews, D. B. (2015). The effects of acute alcohol on motor impairments in adolescent, adult, and aged rats. *Alcohol*, *49*(2), 121–126.

Ou, Z., Deng, L., Lu, Z., Wu, F., Liu, W., Huang, D., & Peng, Y. (2020). Protective effects of Akkermansia muciniphila on cognitive deficits and amyloid pathology in a mouse model of Alzheimer's disease. *Nutrition & Diabetes*, *10*(1), 12.

Qi, X., Sun, X., Wang, M., Wang, M., Qi, Z., & Cui, C. (2023). Ginseng polysaccharides ameliorate abnormal lipid metabolism caused by acute alcoholic liver injury by promoting autophagy. *Food Frontiers*, *4*(1), 394–406.

Qin, Y.-Q., Wang, L.-Y., Yang, X.-Y., Xu, Y.-J., Fan, G., Fan, Y.-G., Ren, J. N., An, Q., & Li, X. (2023). Inulin: properties and health benefits. *Food & Function*, *14*(7), 2948–2968.

Qiu, P., Dong, Y., Zhu, T., Luo, Y., Kang, X., Pang, M., Li, H., Xu, H., Gu, C., Pan, S., Du, W., & Ge, W. (2019). Semen hoveniae extract ameliorates alcohol-induced chronic liver damage in rats via modulation of the abnormalities of gut-liver axis. *Phytomedicine*, *52*, 40–50.

Shen, Z., Munker, S., Wang, C., Xu, L., Ye, H., Chen, H., Xu, G., Zhang, H., Chen, L., Yu, C., & Li, Y. (2014). Association between alcohol intake, overweight, and serum lipid levels and the risk analysis associated with the development of dyslipidemia. *Journal of Clinical Lipidology*, *8*(3), 273–278.

Stevens, C. V., Meriggi, A., & Booten, K. (2001). Chemical modification of inulin, a valuable renewable resource, and its industrial applications. *Biomacromolecules*, *2*(1), 1–16.

Tian, X., Li, R., Jiang, Y., Zhao, F., Yu, Z., Wang, Y., Dong, Z., Liu, P., & Li, X. (2020). Bifidobacterium breve ATCC15700 pretreatment prevents alcoholic liver disease through modulating gut microbiota in mice exposed to chronic alcohol intake. *Journal of Functional Foods*, *72*, 104045.

Wan, X., Guo, H., Liang, Y., Zhou, C., Liu, Z., Li, K., Niu, F., Zhai, X., & Wang, L. (2020). The physiological functions and pharmaceutical applications of inulin: A review. *Carbohydrate Polymers*, *246*, 116589.

Wang, C., Ma, C. N., Liu, X. L., Sun, Q., Zhang, Q., Lin, Y. Y., Yan, C. Y., & Hu, D. G. (2023). Apple consumption protects against acute ethanol-induced liver injury in rats. *Applied sciences*, *13*(8), 5112.

Wang, G., Liu, Q., Guo, L., Zeng, H., Ding, C., Zhang, W., Xu, D., Wang, X., Qiu, J., Dong, Q., Fan, Z., Zhang, Q., & Pan, J. (2018). Gut microbiota and relevant metabolites analysis in alcohol dependent mice. *Frontiers in Microbiology*, *9*, 1874.

Wang, M., Zhu, P., Jiang, C., Ma, L., Zhang, Z., & Zeng, X. (2012). Preliminary characterization, antioxidant activity in vitro and hepatoprotective effect on acute alcohol-induced liver injury in mice of polysaccharides from the peduncles of *Hovenia dulcis*. *Food and Chemical Toxicology*, *50*(9), 2964–2970.

Wu, X., Yang, L., Gao, P., Qiao, Z., Xu, D., & Zhang, F. (2020). Effect of Xingnaojing injection for the treatment of acute alcoholism: A protocol of systematic review and meta-analysis. *Medicine*, *99*(26), e20785.

Xia, T., Duan, W., Zhang, Z., Li, S., Zhao, Y., Geng, B., Zheng, Y., Yu, J., & Wang, M. (2021). Polyphenol-rich vinegar extract regulates intestinal microbiota and immunity and prevents alcohol-induced inflammation in mice. *Food Research International*, *140*, 110064.

Yang, Z., Gao, Y., Wu, W., Mu, H., Liu, R., Fang, X., Gao, H., & Chen, H. (2023). The mitigative effect of lotus root (*Nelumbo nucifera* Gaertn) extract on acute alcoholism through activation of alcohol catabolic enzyme, reduction of oxidative stress, and protection of liver function. *Frontiers in Nutrition*, *9*, 1111283.

Yi, Z., Liu, X., Liang, L., Wang, G., Xiong, Z., Zhang, H., Song, X., Ai, L., & Xia, Y. (2021). Antrodin A from Antrodia camphorata modulates the gut microbiome and liver metabolome in mice exposed to acute alcohol intake. *Food & Function*, *12*(7), 2925–2937.

Ying, M., Yu, Q., Zheng, B., Wang, H., Wang, J., Chen, S., Nie, S., & Xie, M. (2020). Cultured Cordyceps sinensis polysaccharides modulate intestinal mucosal immunity and gut microbiota in cyclophosphamide-treated mice. *Carbohydrate Polymers*, *235*, 115957.

Zakhari, S. (2006). Overview: How is alcohol metabolized by the body? *Alcohol Research & Health*, *29*(4), 245–254.

Zhao, Y., Xia, T., Qiang, X., Wang, Y., Kang, C., Meng, Y., Cheng, Y., Wang, M., & Xiao, J. (2023). Zhenjiang aromatic vinegar prevents the alcohol liver disease in mice via autophagy. *eFood*, *4*(6), e118.

Zhou, W., Yang, T., Xu, W., Huang, Y., Ran, L., Yan, Y., Mi, J., Lu, L., Sun, Y., Zeng, X., & Cao, Y. (2022). The polysaccharides from the fruits of *Lycium barbarum* L. confer anti-diabetic effect by regulating gut microbiota and intestinal barrier. *Carbohydrate Polymers*, *291*, 119626.

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