



Research Article

Hepatoprotective effects of sweet tea and trilobatin against alcohol-induced acute liver injury in mice

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Abstract: *Lithocarpus polystachyus* Rehd. or *Lithocarpus litseifolius* (Hance) Chun., a natural functional food highly rich in trilobatin and phlorizin, is named “sweet tea” (ST) in the Chinese folk. The present study aims to explore the protective effects of ST and trilobatin against alcoholic liver injury and its potential mechanisms. Firstly, KM mice were given alcohol to establish an alcohol exposure model, and ST and trilobatin were administered for 3 weeks. The results of the animal experiment manifest that ST and trilobatin probably enhanced alcohol metabolism and inhibited oxidative stress activation to exhibit their hepatoprotective properties. Besides, the outcomes of 16S rRNA Gene Sequencing Analysis provided the first evidence that trilobatin enriched the beneficial microbiota abundance and decreased the bacterial community diversity and potential detrimental bacteria, thus modulating the gut microbiota and maintaining the integrity of barrier function. Overall, ST and trilobatin could alleviate alcohol-induced acute liver damage in mice via modulating gut microbiome. These findings laid the foundation for the further the development and application of ST and trilobatin in functional foods and nutraceutical supplements for preventing liver injury.

Keywords: sweet tea; trilobatin; liver injury; oxidative stress; gut microbiome

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1 Introduction

Social activities, psychosocial factors, as well as genetic and neurobiological factors contribute to the abuse of alcohol products worldwide^[1]. Alcohol consumption is associated with a wide range of health conditions related to injury, accounting for about 5% of global deaths^[2]. As the most frequent consequence of excessive drinking, alcoholic liver disease (ALD) is one of the primary health issues worldwide^[3]. Apart from pharmacotherapy, dietary intervention is also perceived as a reliable strategy for the management of hepatic injury^[4].

Sweet tea (ST) is derived from the leaves of *Lithocarpus polystachyus* Rehd. or *Lithocarpus litseifolius* (Hance) Chun., as a natural tea drink with over 1600 years of consumption history and tradition, was approved as a new resource food in China in 2017^[5]. ST is a valuable and important functional food, which is rich in flavonoids, and has been proven to exert antioxidant, anti-inflammatory, antimicrobial, antidiabetic, anticancer, hepatoprotection, cardioprotection, antiobesity, neuroprotection, and antiallergic activities^[6]. Our previous evidence-based studies have shown that in animal models, dihydrochalcones in sweet tea (DST) could treat diabetes in animal models by regulation blood

glucose/lipid metabolism, oxidative/carbonyl stress, inflammatory response etc^[7]. Trilobatin and phlorizin were identified as the main bioactive components in ST. Our previous studies showed that trilobatin alleviated non-alcoholic fatty liver disease in high-fat diet plus streptozotocin-induced diabetic mice by suppressing NLRP3 inflammasome activation^[8], but no study has reported that ST and trilobatin exert a hepatoprotective effect against ALD *in vivo*.

Although ALD has been a hot topic for years, the pathogenesis of ALD is not yet clear. Oxidative stress, inflammation or immune disturbance, alterations of intestinal permeability, hepatocyte destruction caused by alcohol and its toxic metabolites and other factors are involved in the pathogenesis of ALD^[9]. Among them, modifying intestinal homeostasis and oxidative stress plays a crucial role in the underlying target for the treatment of ALD^[10]. Reactive oxygen species (ROS) engaged in ethanol metabolism can contribute to free radical-mediated liver cell damage^[11]. Additionally, alcohol is well-established to be a disruptor of the homeostasis of the intestinal microbiota. Dysregulated gut microbiota has also been shown to be a significant consideration responsible for the onset and maintenance of ALD^[12]. Furthermore, it still remains confusing whether consumption of

ST and trilobatin may modify the gut microbiome and thereby ameliorate alcoholic liver injury.

Therefore, the purpose of the present study was to explore the beneficial effects of ST and the major component trilobatin against ALD and investigate the underlying mechanisms. Firstly, an animal model of ALD was established by alcohol treatment and ST or trilobatin were administered to determine their protective effects, and special focus was paid to the contribution of gut microbiome manipulation in meantime. The detailed experimental illustration is shown in Fig. 1.

2 Methods

2.1 Materials and reagents

Sweet tea was obtained from Jiangshan Keduosui Agricultural Science and Technology Co., Ltd, China and identified as *Lithocarpus polystachyus* Rehd. by Professor Hong-Yan Ma, Guangdong Pharmaceutical University. Trilobatin (purity $\geq 98\%$) was purchased from Chengdu Pryfa Technology Development Co., Ltd. (Sichuan, China). Biphenyl diester was provided by Beijing Xiehe Pharmaceutical Factory (Beijing, China). The white spirit (56% vol) was obtained from Red-star Co., Ltd. (Beijing, China). The assay kits for malondialdehyde (MDA), superoxide dismutase (SOD), alcohol dehydrogenase (ADH) and acetaldehyde dehydrogenase (ALDH) were obtained from Nanjing Jiancheng Bioengineering Institute (Nanjing, China).

2.2 Animals and treatment design

56 four-week-old SPF-grade male KM mice (20 ± 2 g) were obtained from Zhuhai Baishitong Biotechnology Co., Ltd. (SCXK (Yue) 2020-0051) and housed in the Laboratory Animal Center of Guangdong Pharmaceutical University. Mice were enabled to acclimatize under SPF laboratory conditions for 7 days prior to the start of the experiment and were placed under a 12 h light and 12 h dark cycle at 24 ± 2 °C with 50–70% humidity and were provided with sterile filtered water. All animal experimental procedures and animal welfare were admitted by the Animal

Research Ethics Committee of Guangdong Pharmaceutical University.

All mice were divided into 7 groups randomly ($n = 8$): control group (Con), ALD group (Mod), low/high-dosage group of ST (STL, 300 mg/kg/d; STH, 900 mg/kg/d), low/high-dosage group of trilobatin (TRL, 20 mg/kg/d; TRH, 60 mg/kg/d) and positive treatment group (Ben, bifendate, 150 mg/kg/d). During the experimental period, the ST-treated mice, trilobatin-treated mice and positive medicine-treated mice were administered ST extract or trilobatin or bifendate, respectively, by oral gavage for 21 consecutive days in the morning, while the mice control group were administered an equal volume of physiological saline solution. After consulting literature^[13], liver injury in mice was induced by administered 56% (v/v) alcohol solution for 21 consecutive days in the afternoon apart from the Con group, and the amount of the alcohol solution was gradually raised from 0.10 mL/10 g BW to 0.15 mL/10 g BW within 7 days.

At the 21-day end, all animals were anesthetized with isoflurane, blood was obtained by cardiac puncture and centrifuged to obtain plasma. A portion of liver tissues was then cut and placed in 4% paraformaldehyde, and the rest were preserved at -80 °C for in-depth analysis.

2.3 Measurement of plasma biochemical indexes

Blood samples were centrifuged at 4,000 rpm for 10 min at 4 °C, and then plasma was obtained. The levels of plasma aspartate aminotransferase (AST), alanine aminotransferase (ALT) and glutamyl transferase (GGT) were detected by an automated hematology analyzer (Guilin URIT Medical Electronic Co., Ltd., China).

2.4 Measurement of hepatic biochemical indexes

The liver samples (100 mg) were homogenized with nine-fold (w/v) pre-cooled physiological saline and centrifuged (10,000 rpm, 10 min) to obtain the liver homogenates used for biochemical assays. Based on the manufacturer's instructions, the levels of MDA, SOD, ADH and ALDH activities in liver homogenate were

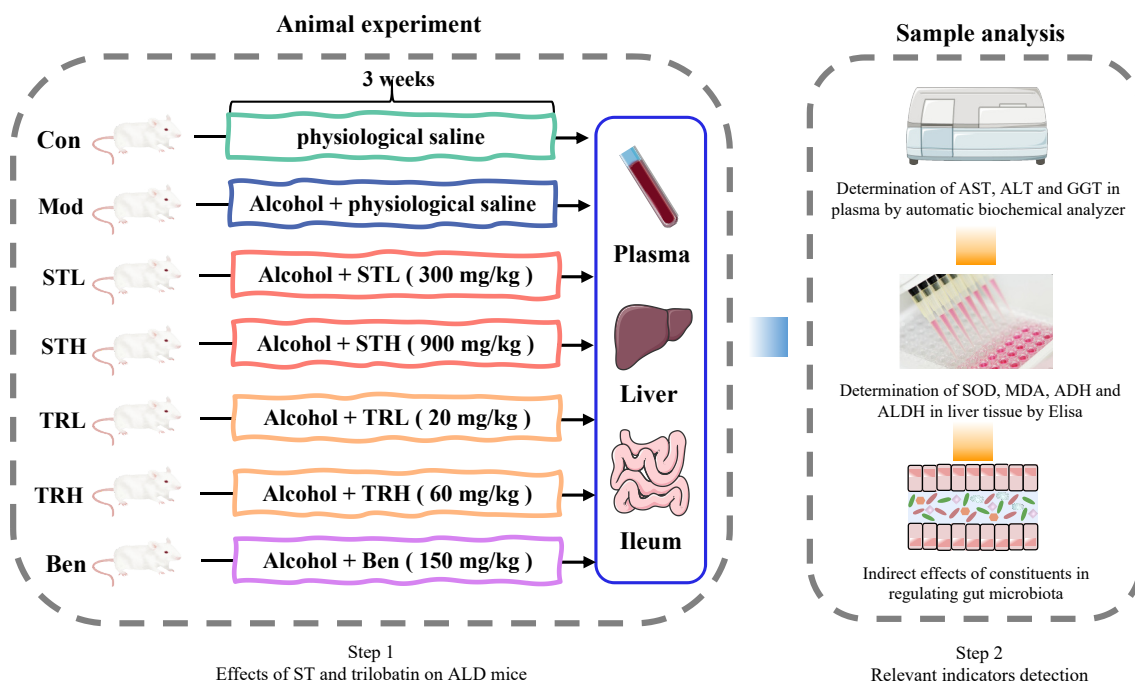


Figure 1 Schematic diagram of overall experiment.

measured by assay kit.

2.5 Histopathological evaluation of liver, ileum and colon

Liver, ileum and colon fragments were fixed in 10% formalin for 48 h, dehydrated, embedded in paraffin, sectioned (4 μ m thick), then stained with H&E and Masson, respectively, based on standard protocols. Then the observations were conducted under a light microscope to evaluate the degree of tissue damage. Eventually, the slices were photographed at 400 $\times$ magnification.

2.6 Determination of faecal microbial composition by 16S rRNA Gene Sequencing Analysis

Complete genomic DNA was derived from caecum content samples based on the manufacturer's instructions using an HiPure Stool DNA kit (Magen Biotechnology Co., Ltd., Guangzhou, China). The hypervariable V3–V4 region of the bacterial 16S rRNA gene was amplified using a thermocycler PCR system (ETC811, Eastwin Scientific Equipments Inc., China) and sequencing was accomplished using the Illumina MiSeq platform at GENEDENOVO Biological Technology Co. Ltd. (Guangzhou, China). Omicsmart online platform (<http://www.omicsmart.com>) was used to present the results.

2.7 Data processing and statistical analysis

The data were displayed as the mean $\pm$ SEM. Statistical analysis was accomplished using one-way ANOVA followed by the LSD test with SPSS software (25.0 version, Inc., Chicago, IL, USA). The

significance test chose a $P < 0.05$ level. All the figures were processed using GraphPad Prism 8 software.

3 Results

3.1 ST and trilobatin alleviated the alcohol-induced liver injury by promoting alcohol metabolism and enhancing antioxidant capacity

3.1.1 Effects of ST and trilobatin on liver injury indicators in alcohol-fed mice

Plasma ALT, AST and GGT levels are significant indicators for evaluating ALD. Excessive alcohol exposure will lead to liver damage, which will lead to considerable enhancement in plasma ALT, AST and GGT levels. The results are shown in Figs. 2A–2C. In contrast to the Con group, the levels of ALT, AST and GGT in the plasma samples of the Mod group mice were remarkably enhanced ($P < 0.001$). In contrast to the Mod group, the levels of ALT, AST and GGT in plasma of mice in the low and high dose groups of ST and trilobatin showed a considerable decreasing trend ($P < 0.01$). The findings displayed that ST and trilobatin could inhibit the deterioration of liver function in ALD mice.

3.1.2 Effects of ST and trilobatin on liver oxidative stress in alcohol-fed mice

To determine the effects of ST and trilobatin on antioxidant capacity in ALD mice liver, the hepatic levels of SOD and MDA were measured (Figs. 2D–2E). Acute ethanol exposure caused a

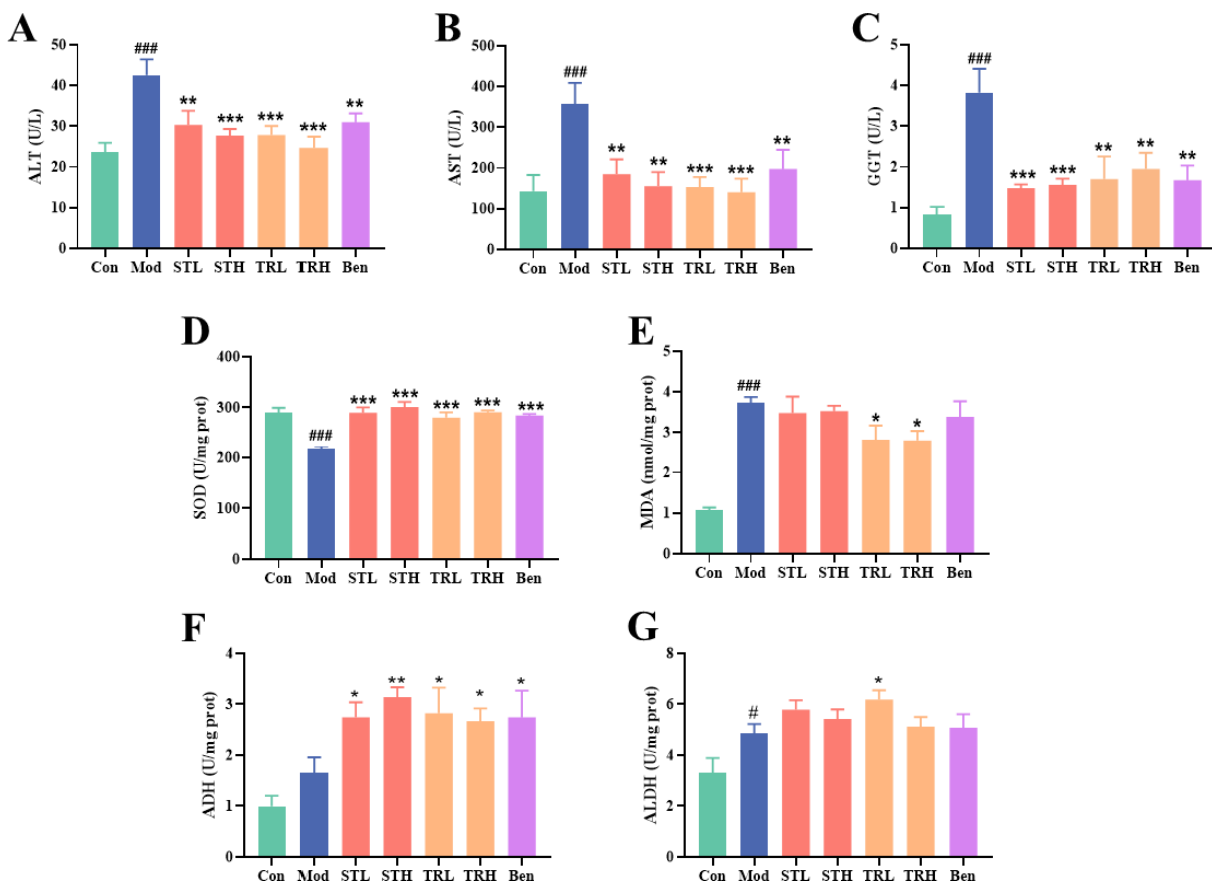


Figure 2 Effects of ST and trilobatin on alcoholic liver injury, oxidative stress and alcohol metabolism in KM mice. (A) ALT, (B) AST, (C) GGT in the plasma. (D) SOD, (E) MDA, (F) ADH, (G) ALDH in the liver. * $P < 0.05$, *** $P < 0.001$ in comparison with the Con group. # $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ in comparison with the alcohol-treated group.

considerable enhancement in MDA level ($P < 0.001$) and a decrease in SOD ($P < 0.001$), while trilobatin intervention was elevated in SOD ($P < 0.001$) and lowered in MDA ($P < 0.05$) compared with the Mod group. However, ST intervention could only increase SOD ($P < 0.001$) and had no effect on MDA compared with the Mod group. The findings revealed that ST and trilobatin could reverse alcohol-induced oxidative stress damage in liver.

3.1.3 Effects of ST and trilobatin on alcohol metabolism in alcohol-fed mice

It is generally accepted that the main pathway to eliminate ethanol is to oxidize it to acetaldehyde by ADH. In the reaction catalyzed by ALDH, acetaldehyde is converted into acetate. Hepatic ADH and ALDH levels in ST- and trilobatin-treated mice were remarkably enhanced compared to those in the Mod group (Figs. 2F-2G). ST and trilobatin could remarkably increase alcohol metabolism by increasing the hepatic level of ADH ($P < 0.05$) and ALDH.

3.1.4 Effects of ST and trilobatin on liver pathological damage in alcohol-fed mice

To investigate the effects of ST on ALD, we further conducted the histopathological observation of the liver, and the results are displayed in Figs. 3A-3B. H&E staining results displayed, the structure of the liver tissue was clearly visible in the Con group. Uniform liver cells, no edema and inflammatory cells were noticed without obvious histological abnormalities. By comparison, alcohol caused pronounced histopathological alterations: disordered liver cords, swollen hepatocytes, and severe inflammatory cell infiltration. Compared with the Mod group, ST and trilobatin administration ameliorated pathological changes caused by alcohol. Masson staining showed that the liver section of acute alcohol-fed mice displayed that the liver tissue was partially fibrotic in contrast to the Con mice, while ST and trilobatin treatment could improve liver fibrosis in Mod mice. In general, the histological observation revealed that ST and trilobatin might have a protective effect against ALD.

3.2 Trilobatin alleviated the alcohol-induced intestinal injury and gut microbial dysbiosis

3.2.1 Effects of ST and trilobatin on intestinal barrier in alcohol-fed mice

H&E staining of the ileum specimens indicated an integrated villi and a compactly arrayed epithelium in the Con groups. Mice in the alcohol-fed group displayed remarkable ileum injury characterized by disorganized, shortened and exfoliated villi, as well as edema of the intestinal mucosa (Fig. 3C). The ileum injury and edema in the high and low dose groups of ST and trilobatin were significantly improved.

H&E staining of colon specimens showed a tightly organized arrangement of colonic epithelial cells in the Con group, abundant cup cells, and a large number of well-structured crypts. Mice in the alcohol-fed group exhibited significant colonic damage characterized by impaired crypt structure, shortened and irregular villi (Fig. 3D). The destruction of crypts in the colonic tissues of mice in both the high and low dose groups of ST and trilobatin was somewhat attenuated, and the cellular structure was arranged in an orderly manner.

ST and trilobatin could effectively alleviate alcohol-induced intestinal injury and significantly restore the intestinal integrity.

3.2.2 Effects of trilobatin on gut microbiota diversity in alcohol-fed mice

Species evenness and richness can usually be assessed as the species diversity in sequencing. The abundance curve and dilution curve can visualize the uniformity and diversity of the intestinal flora for each sample at different sequencing depths. As seen in Fig. 4A, with the increase of experimental sequencing depth, the dilution curve of each sample gradually tends to flatten out, suggesting that the increased sequencing depth has been saturated and the sequencing volume is sufficient, revealing that the current sequencing data of each sample can meet the sequencing depth. The Rank abundance curve and α -diversity index (e.g., Sob, Simpson and Chao1) reveal good evenness and richness for each

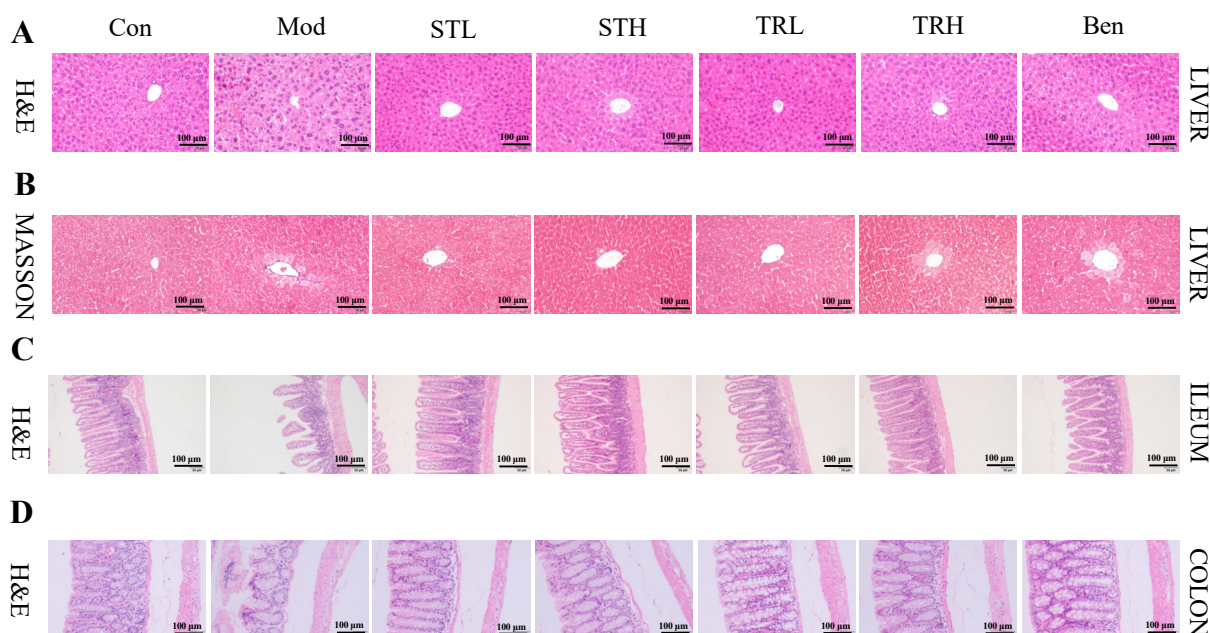


Figure 3 Effect of ST and trilobatin on pathologic changes in the liver, ileum and colon of KM mice. (A) H&E staining of liver tissue section. (B) Masson staining of liver tissue section. (C) H&E staining of ileum tissue section. (D) H&E staining of colon tissue section. All images have a magnification of $400\times$ and a scale of $100\mu\text{m}$.

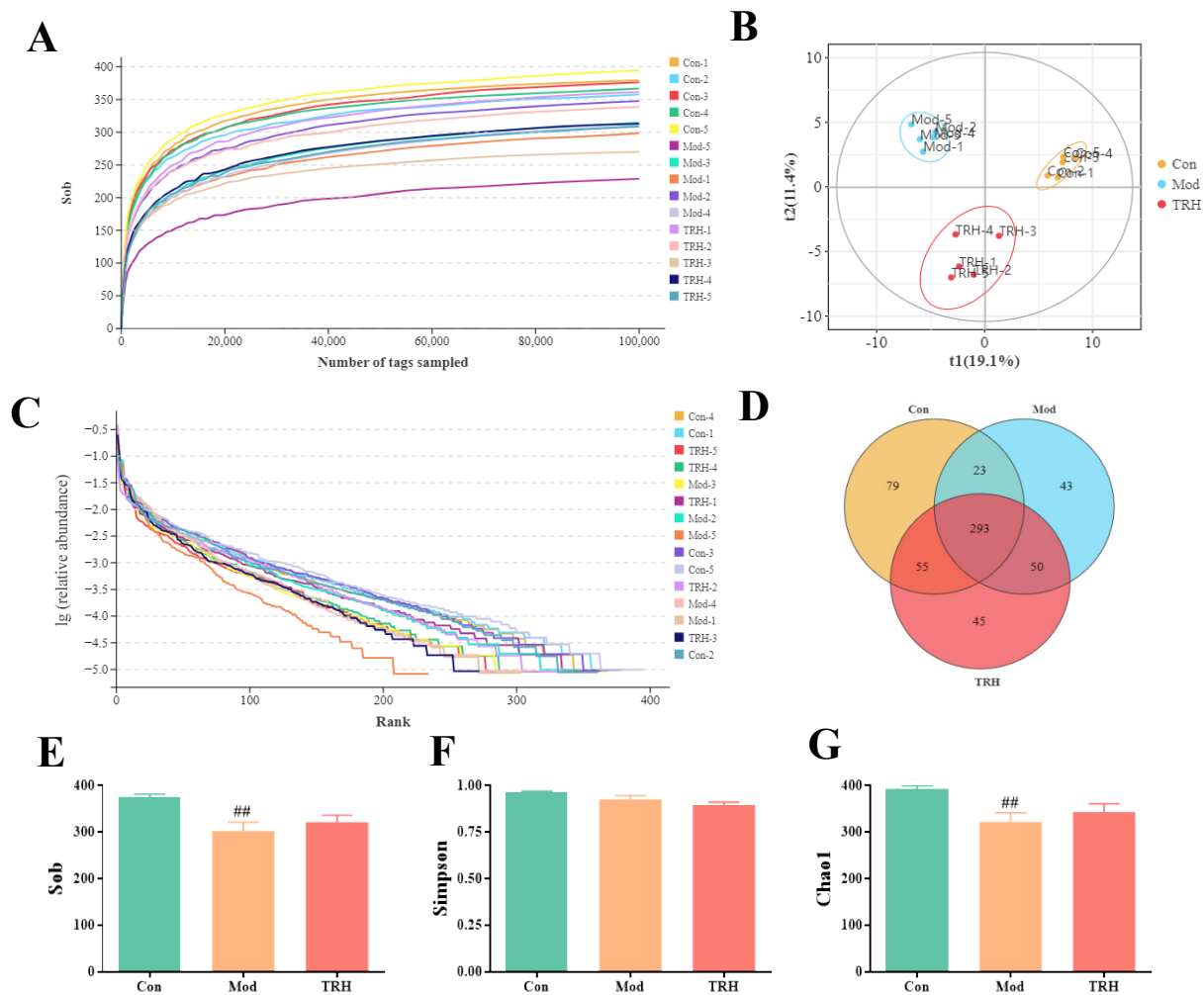


Figure 4 Effects of trilobatin on the gut microbiota diversity of the ALD mice. (A) Shannon dilution curve; (B) Rank abundance curve; (C) Supervised partial least squares-discriminant analysis (PLS-DA); (D) Venn diagram; (E) Sob index; (F) Shannon index and (G) Chao1 index. ## $P < 0.01$ in comparison with the Con group.

sample (Figs. 4C, 4E-4G), proving that all sequences present in the samples have been tested. More than that, the α -diversity index of the Mod group was remarkably lower than that of the Con group ($P < 0.01$), except for Simpson, demonstrating that the intestinal flora of the Mod group had been out of balance. After the administration of trilobatin, each index could be reversed to some extent.

Operational Taxonomic Unit (OTU) refers to all sequences with resemblance between sequences greater than 97% (species level), and each OTU manifests one species. As seen in the OUT-PLS-DA analysis, the Con and Mod groups were clearly distanced, and the TRH group was also moved farther from the Mod group and moved nearer to the Con group (Fig. 4B). From the total number of OTUs, the total number of OUT in the Mod group was decreased compared to the Con group, and it was up-regulated after the administration of trilobatin (Fig. 4D). The Venn diagram of OTUs observed that there are 588 OTUs in the three groups, 293 OTUs are common to all three groups, 343 OTUs are common to the Mod and TRH groups, and 316 OTUs are common to the Con and Mod groups (Fig. 4D).

Collectively, the apparent clustering of microbiota between the Mod and the Con groups revealed the appearance of gut microbiota dysbiosis and trilobatin supplementation were partially reversed alcohol-induced changes in the diversity.

3.2.3 Effects of trilobatin on gut microbiota difference in alcohol-fed mice

We analyzed the distinctions in the composition of the flora of the Con, Mod and TRH groups using LEfSe, where the threshold of LDA was set to 3. As shown in Figs. 5A-5B, distinctions in the structures of the bacterial community were obvious between Con, Mod and TRH group, with 32 species differing in the Con group, 31 species differing in the Mod group and 30 species differing in the TRH groups. In detail, Lachnospiraceae_NK4A136_group, *Lactobacillus*, *Enterococcus_gasseri*, *Turicibacter*, *Prevotellaceae_UGG_001*, etc., were the differential species in the Con group; *Proteobacteria*, *Desulfovibrio*, *Actinobacteria*, *Deltaproteobacteria*, *Desulfovibrionaceae*, etc., were the differential species in the Mod group; *Clostridiales*, *Lachnospiraceae*, *Facklamia*, *Roseburia*, *Eubacterium_xylanophilum_group*, etc., were the differential species in the TRH group.

3.2.4 Effects of trilobatin on gut microbiota composition in alcohol-fed mice

We subsequently further explored the 16S rRNA Gene Sequencing outcomes to elucidate the gut microbiota ecology of mice belonging to various groups.

At the phylum level, it appeared that the Firmicutes and Bacteroidetes comprised the majority of the total sequences

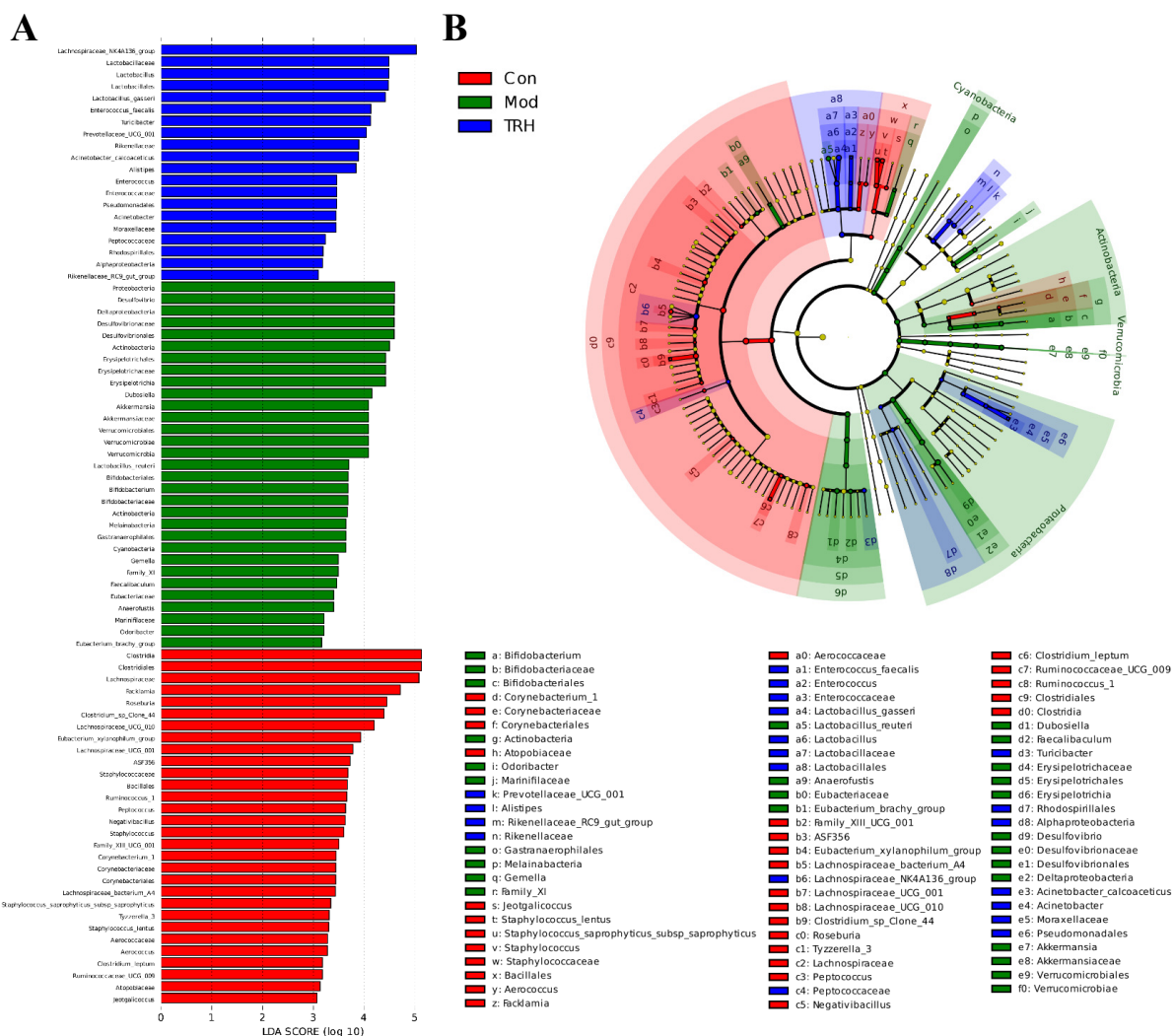


Figure 5 Effects of trilobatin on the gut microbiota disorder of the ALD mice. (A and B) LEfSe analysis of microbiota.

(Fig. 6A). Acute alcohol exposure induced a remarkable increase in the abundance of Proteobacteria, Patensibacteria, Bacteroidetes and Actinobacteria, reduce in the abundance of Firmicutes in contrast with the Con group. Overall, trilobatin administration inverted these abundance trends (Fig. 6C).

At the genus level, it appeared that the *Lachnospiraceae*-NK4A136 and *Desulfovibrio* represented the majority of the total sequences (Fig. 6B). The abundances of *Enterorhabdus*, *Desulfovibrio* and *Ruminiclostridium*-5 increased, while the sections of *Lachnospiraceae*-NK4A136 and *Roseburia* decreased in the Mod group. Trilobatin administrations were shown to invert these trends (Fig. 6D).

3.2.5 Correlation analysis of intestinal flora and ALD

To explore the correlation between intestinal flora and ALD, Pearson correlation coefficient was applied to correlation analysis and visualized by correlation heat map. In the heat map, the more it tends to red, the more the two factors are positively correlated, while blue is the opposite. As shown in Fig. 7, the intestinal flora showed significant correlations with ALD indicators. The outcomes suggested that the imbalance of intestinal flora in ALD mice affected the release of liver injury, antioxidant and alcohol metabolism indicators, which in turn affected the course of disease.

4 Discussion

ALD is a common clinical liver disease^[14]. Unfortunately, the incidence of ALD continues to rise each year due to a widespread increase in the frequency of human drinking and unhealthy changes in dietary structure^[15]. At present, acute or chronic drinking above metabolic capacity can disrupt the liver metabolism and gut microbiota balance, causing a range of ALD^[16]. Dietary interventions that preserve the liver and recover intestinal homeostasis are efficacious strategies against alcoholic liver damage^[17,18]. Recently, with a better understanding of the development of ALD, more researchers have committed themselves to searching for some natural plants including antioxidants and hepatoprotective components, for example flavonoids^[19].

Notably, flavonoids are widely present in common plant-based functional ingredients, which have recently attracted significant attention as potential agents for the prevention of ALD^[20]. Dihydrochalcones, the primary flavonoids and antioxidants in ST-including trilobatin, phlorizin, and phloretin-can remarkably scavenge DPPH and ABTS free radicals *in vitro*^[21,22]. Besides, Xiao *et al.* revealed the effect of structural differences between dihydrochalcones on antioxidant activity^[23]. There is increasing evidence that oxidative damage is a primary factor of ALD, caused by an imbalance between the ROS production system and the free

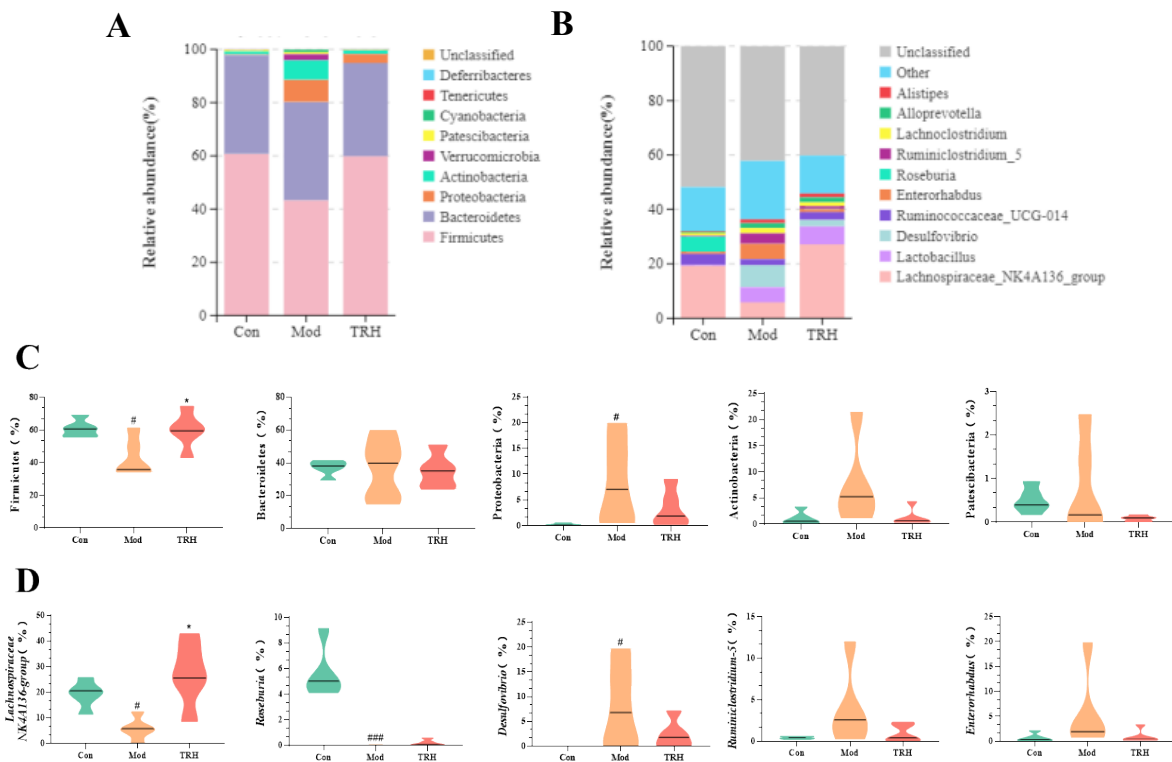


Figure 6 Effects of trilobatin on the gut microbiota in ALD mice. Gut bacterial composition at the phylum level (A) and genus level (B); The relative abundance comparison of some bacteria at the phylum level (C) and genus level (D). # $P < 0.05$ in comparison with the Con group. * $P < 0.05$ in comparison with the alcohol-treated group.

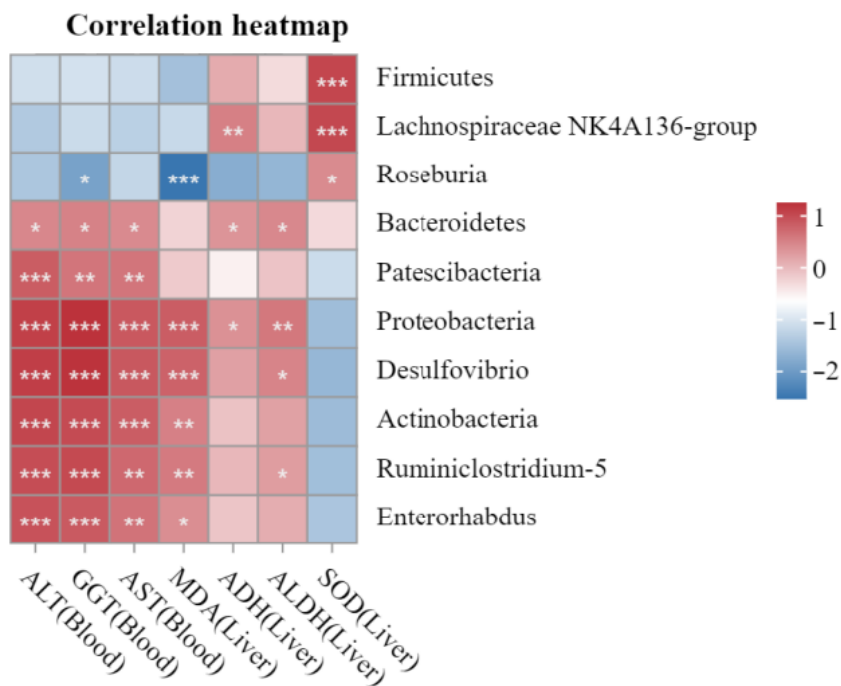


Figure 7 Correlation analysis of trilobatin on intestinal flora and alcoholic liver injury in ALD mice.

radical scavenging system^[24]. First, as one of the essential peroxidation products, MDA can cross-link with phosphatidylethanolamine and proteins to generate inactive lipofuscin in hepatocytes, thus disrupting the cell membrane structure^[25]. The hepatic MDA content was remarkably increased after alcohol exposure in the present study, while treatment with ST and trilobatin decreased the MDA content. In addition, SOD is

a pivotal antioxidant enzyme that eliminate ROS *in vivo*^[26]. As a result, SOD is the vital component of the antioxidant system. However, ST and trilobatin could decrease those unexpected results by enhancing the SOD activity in our research. In this study, two antioxidant enzymes were detected, but the antioxidant signaling pathway such as Nrf2 was not studied in depth. We will make improvements in subsequent experiments. Eventually, the

ADH and ALDH system are a well-established pathway involved in ethanol metabolism in the liver^[27]. Ethanol can be oxidized by ADH to generate acetaldehyde, the product is oxidized by ALDH to form acetic acid^[28]. The outcomes of ADH and ALDH activity revealed that compared to the Mod group, ST and trilobatin could have a more positive effect on ethanol metabolism. ST and trilobatin can preserve hepatocytes from alcohol-induced oxidative stress not only by scavenging intracellular MDA and reinforcing endogenous antioxidant enzyme systems, but also by enhancing ethanol metabolism. In the research, the *in vivo* antioxidant effect of ST and trilobatin in ALD was confirmed for the first time to provide more dependable evidence for the development of natural antioxidant-related functional foods.

Acute or chronic overconsumption of alcohol not only induces damage to the liver but also disrupts the intestinal barrier^[29]. In both preclinical and clinical studies, alcohol consumption appears to influence the amount and composition of the gut microbiome^[30]. Microbiota dysbiosis is a vital contributor in the progression of ALD. Nowadays, natural flavonoids can attenuate intestinal damage by improving antioxidant activity, reinforcing intestinal barrier function, and modifying intestinal microbiota composition^[31]. However, so far, the gut-derived regulatory mechanisms of trilobatin in attenuation of ALD have not been reported thus far. Consistent with previous studies^[1,10,32], acute ethanol stimulation leads to notable changes in the composition and abundance of bacterial communities, which was recovered after the supplementation of trilobatin. Furthermore, it appeared that trilobatin normalized Bacteroidetes and Firmicutes after alcohol intake. Bacteroidetes and Firmicutes are two of the main intestinal microflorae, and they have a symbiotic relationship, which can work together to facilitate the absorption or storage of energy by the host^[33]. In addition, we also discovered that the supplementation of trilobatin reduced the relative abundance of phylum Proteobacteria and Patescibacteria. Moreover, it was found that Proteobacteria and Patescibacteria were negatively correlated with tight junction protein expression in the intestine and positively correlated with inflammatory and oxidative stress parameters in the liver^[34]. Subsequently, we observed that trilobatin administration reversed the increase in Actinobacteria. Although the phylum Actinobacteria constitutes a small proportion of intestinal microorganisms, it is one of the major phyla that maintain intestinal barrier homeostasis, and its change may indicate the increase of intestinal permeability and disruption of intestinal barrier homeostasis^[35].

Notably, we also observed that the growth in the abundance of opportunistic pathogens such as *Enterorhabdus* (phylum Actinobacteria) and Gram-negative bacteria such as *Desulfovibrio* (phylum Proteobacteria) was owing to prolonged alcohol stimulation. *Enterorhabdus* increased progressively with deterioration of liver function and showed a significant positive correlation with inflammation and cirrhosis^[36]. It has been found that *Ruminiclostridium 5* is mainly related to chronic diseases such as intestine and liver and has the effect of promoting inflammation^[37]. *Desulfovibrio* produces high concentrations of hydrogen sulfide from methionine, which can inhibit cytochrome C oxidase, weaken cellular respiration, destroy intestinal mucosal epithelial cells and so on^[38]. Nevertheless, trilobatin supplementation adjusted these alterations of the bacterial communities. In addition, the administration of trilobatin also increased the following two beneficial bacteria. *Lachnospiraceae* participates in the metabolism of various carbohydrates, providing energy for the intestinal mucosal epithelial cells and the host^[39].

Roseburia is part of the commensal bacteria that produce short-chain fatty acids, especially butyric acid, which have anti-inflammatory properties^[40]. It is worth noting that, so far, gut microbiota is subject to multifactorial regulation and remains unpredictable under multiple disease conditions. Therefore, it is necessary to clarify the complex interplay between commensal microbiota and host from human and other animal experiments.

5 Conclusion

In this study, we demonstrated that ST and trilobatin could reduce the levels of liver injury cytokines in plasma and enhanced the activities of antioxidant and alcohol metabolism enzymes in the liver, thereby ameliorating alcoholic liver injury. The outcomes of 16S rRNA gene sequencing analysis were the first to prove that trilobatin increased the abundance of beneficial microbiota and decreased bacterial community diversity and potential detrimental bacteria, thus reverting gut dysbiosis, modulating the gut microbiota, and maintaining the integrity of the barrier function. The outcomes indicate that trilobatin could improve alcohol-induced acute liver damage in mice by modulating the gut microbiome. In conclusion, sweet tea and trilobatin ameliorate acute alcoholic liver injury in mice by inhibiting oxidative stress, enhancing ethanol metabolism, and modulating the gut microbiota for protective effects.

Conflicts of interest

The authors declare that there are no conflicts of interest in this work.

Acknowledgments

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