Microbial treatment of alcoholic liver disease: A systematic review and meta-analysis

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Background and aims: Alcoholic liver disease (ALD) is characterized by impaired liver function due to chronic alcohol consumption, even fatal in severe cases. We performed a meta-analysis to determine whether microbial agents have therapeutic potential for ALD and elucidate the underlying mechanisms.

Methods and results: Forty-one studies were eligible for this meta-analysis after searching the PubMed, Cochrane, and Embase databases. The combined analysis showed that microbial therapy significantly decreased hepatic enzymatic parameters, including alanine transaminase (SMD: –2.70, 95% confidence interval (CI): –3.33 to –2.07), aspartate aminotransferase (SMD: –3.37, 95% CI: –4.25 to –2.49), γ-glutamyl transpeptidase (SMD: -2.07, 95% CI: –3.01 to –1.12), and alkaline phosphatase (SMD: –2.12, 95% CI: –3.32 to –0.92). Microbial agents endotoxin to enter the portal circulation and increasing reduced total cholesterol (SMD = -2.75, 95%CI -4.03 to -1.46) and triglycerides (SMD = –2.64, 95% CI: –3.22 to –2.06). Microbial agents increased amounts of the beneficial flora Lactobacillus (SMD: 4.40, 95% CI: 0.97–7.84) and Bifidobacteria (SMD: 3.84, 95% CI: 0.22–7.45), Bacteroidetes (SMD: 2.51, 95% CI: 0.29–4.72) and decreased harmful Proteobacteria (SMD: –4.18, 95% CI: –6.60 to –1.77), protecting the integrity of the intestinal epithelium and relieving endotoxin (SMD: –2.70, 95% CI: -3.52 to –2.17) into the portal vein, thereby reducing the production of inflammatory factors such as tumor necrosis factor-α (SMD: –3.35, 95% CI: –4.31 to –2.38), interleukin-6 (SMD: –4.28, 95% CI: –6.13 to –2.43), and interleukin-1β (SMD: –4.28, 95% CI: –6.37 to –2.19). Oxidative stress was also relieved, as evidenced by decreased malondialdehyde levels (SMD: –4.70, 95% CI: –6.21 to –3.20). Superoxide dismutase (SMD: 2.65, 95% CI: 2.16–3.15) and glutathione levels (SMD: 3.80, 95% CI: 0.95–6.66) were elevated.

Conclusion: Microbial agents can reverse dysbiosis in ALD, thus significantly
Interfering with lipid metabolism, relieving inflammatory response and inhibiting oxidative stress to improve liver function.

KEYWORDS
alcoholic liver disease, microbial agents, probiotics, prebiotics, gut-liver axis, meta-analysis

Introduction

Alcoholic liver disease (ALD) is a spectrum of diseases, including steatohepatitis, alcoholic hepatitis, cirrhosis, and associated complications, which in severe cases can progress to liver failure and lead to multi-system dysfunction (1). The dynamics of ALD involve interactions between the direct toxicity of alcohol and its metabolites, oxidative stress, inflammatory cascades, and other complex alcohol-related consequences (2). The optimal management strategy for ALD is still debated due to the limited efficacy of current treatments (3).

Several lines of evidence suggested a link between intestinal flora and liver diseases. The overgrowth of gram-negative bacteria and subsequently elevated gut-derived endotoxin were found in patients with ALD, participating in the pathogenesis of ALD (4). Chronic heavy alcohol consumption damages the intestinal epithelial barrier. Consequently, it increases intestinal permeability, allowing intestinal endotoxin to enter the portal circulation and increasing the production of pro-inflammatory cytokine IL-1β, TNF-α, and IL-6 through the activated toll-like receptor (TLR) 4-nuclear factor kappa B (NF-κ B) pathway (5).

Probiotics are living microorganisms that offer health benefits to the host, whereas prebiotics are non-digestible food ingredients that selectively stimulate the growth or activity of probiotics (6, 7). Lactobacillus rhamnoses GG helped prevent chronic alcohol exposure-induced hepatic steatosis by increasing hepatic AMPK phosphorylation and Bax-regulated apoptosis (8). In a mouse model of ALD, the probiotic Akkermansia muciniphilak reinforces the gut vascular barrier (GVB) and thus protects against alcohol-induced liver damage (9). Most probiotics targeting ALD attenuate the barrier disruption caused by ethanol exposure (as seen by a reduction in intestinal leakiness) and restore tight junction protein expression as well as the thickness of the mucus layer (10). Bifidobacterium bifidum and Lactobacillus plantarum 8PA3 restore the normal intestinal flora in ALD, significantly enhancing liver function by mitigating liver-specific bio-enzymatic values (11). Prebiotic fructo-oligosaccharides increased the abundance of beneficial bacteria such as Lactobacillus and Bifidobacterium and improved alcoholic steatohepatitis (12). Pectin, as a prebiotic, restored intestinal homeostasis in mice with ALD, increasing the number of cupped cells and the expression of defensins Reg3β and Reg3γ (13). One study found that treatment by fecal transplantation from prebiotic (pectin)-fed mice prevented ALD (14).

Nevertheless, due to the diversity of microbial agents, the therapeutic effects of microbes on ALD have not been comprehensively described. In the present study, we collected data from published clinical research and preclinical studies and systematically assessed serum biochemical parameters, serum inflammatory parameters, and blood lipids to determine the effectiveness of microbial agents in ALD treatment. We also screened studies for oxidative stress parameters and intestinal barrier function to elucidate the underlying mechanisms.

Materials and methods

Literature search

Meta-analysis was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) guidelines (15). PubMed, the Cochrane Library, and Embase were searched for studies in English up to November 2021. The search strategy was devised using medical subject headings and synonyms, as follows: ("prebiotics" OR "yogurt" OR "inulin" OR "oligosaccharide" OR "galactose oligosaccharide" OR "fructose oligosaccharide" OR "probiotics" OR "Lactobacillus" OR "Bifidobacterium" OR "Enterococcus" OR "Streptococcus" OR "Saccharomyces" AND disease "liver injury" OR "alcohol-induced liver injury" OR "Alcoholic liver disease"). After filtering the titles, abstracts, full texts and eliminating duplicates, appropriate studies were retained if they matched the inclusion criteria. Two reviewers carried out these screenings independently, and disagreements were resolved by discussion and consensus.

Study selection

The literature included basic animal experiments and clinical trials. Animal experiments followed the PICOS principles (i.e., participants, interventions, comparisons, outcomes, and study design). Participants (P): Animal models of ALD, usually shaped by feeding with large amounts of alcohol. Intervention (I): The intervention group received...
microbial preparations, including probiotics and prebiotics. Comparison (C): the comparison group uses no microbial agents. Outcome (O): outcome indicators include several key components: (1) liver enzymes, alkaline phosphatase, γ-glutamyl transpeptidase (GGT); (2) blood lipids like triglyceride (TG), total cholesterol (TC); (3) inflammatory indicators such as TNF-α, IL-6, IL-10, endotoxin, malondialdehyde (MDA), superoxide dismutase (SOD). Study design (S): randomized controlled studies. The inclusion criteria for clinical trials were similar to those for animal trials Cohort studies, case-control studies, and cross-sectional studies were excluded during the selection process.

Quality evaluation and data extraction

We included preclinical and clinical studies. For preclinical studies, methodological quality was assessed according to the Systematic Risk of Bias Evaluation Center for Laboratory Animal Experiments (SYRCLE) tool (16). Clinical studies were assessed using the Cochrane Risk Assessment Scale in the Cochrane Handbook. All experimental data were extracted independently and cross-checked by two authors. Graphical data were generated using Get Data software. The following information was extracted: (1) first author and publication year; (2) participant characteristics. It should be noted that, for animal experiments, we recorded animal species, modeling approach, and sample size. We recorded the age, the number of participants, and nationality for clinical experiments. (3) route of administration, dosage, and duration of treatment; (4) outcome variables. Discrepant opinions were resolved through third-party discussions.

Statistical analysis

The 95% confidence interval (CI) and standard deviation of the combined mean difference were used to determine differences in continuous variables. We also used the Cochran Q-test and I² statistic ($I^2 < 25\%$, low heterogeneity; $25\%–50\%$, moderate heterogeneity; $I^2 > 50\%$, high heterogeneity). Because animal studies and clinical trials are exploratory, a random effects model was used. A statistically significant difference was defined as $p < 0.05$. Seventeen indicators were analyzed in subgroups to explore the sources of heterogeneity based on the type of microbes, strains, animal models, and modeling approaches. A meta-regression was conducted to determine potential heterogeneity origins. A sensitivity analysis was conducted to identify studies that significantly influenced the results by eliminating them one by one. Publication bias was estimated quantitatively using Egger’s test. Contour-enhanced funnel plots obtained using the trim-and-fill method help to distinguish asymmetries caused by publication bias or heterogeneity, among other factors (17). If the missing study is in a non-significant region, the asymmetry is attributable to publication bias. Alternatively, the observed asymmetry could be attributed to factors other than publication bias. The statistical analysis was performed using R and R Studio software.

Results

Identification of relevant studies

The flow diagram of this meta-analysis is displayed in Figure 1. A total of 9,858 records were initially obtained from the three databases, of which 3,484 were removed due to duplication. The initial screening of titles and abstracts yielded 87 articles after excluding 6,287 studies. A further 41 articles were rejected based on a detailed full-text evaluation. There were 41 studies (including 37 animal studies and four clinical studies) that met the inclusion criteria after a thorough screening of the full text (4, 11, 18–56).

Study characteristics and quality assessment

Tables 1, 2 display the characteristics of the 41 studies. All animal experiments were carried out using rodent models, mainly C57BL/6N mice and Wistar rats, the microbial agents in the intervention group were primarily probiotics (mostly Lactobacillus), and placebos were usually used in the control groups. In the clinical studies, subjects were patients of various nationalities with alcoholic hepatitis and cirrhosis caused by chronic heavy alcohol consumption.

Two reviewers independently assessed method quality using the SYRCLE tool. Blinding caused most biases. The items were judged as low, unclear, or high risks. In most studies, blinding and allocation bias were unclear because no specific details of relevant information were provided. All studies were rated as having a low risk of reporting bias. Overall, the studies had similar high-quality evaluations with a low risk of bias (Supplementary Tables 1, 2). Differences in the quality evaluation process were addressed through discussion.

Effect of microbial agents on lipid control

Microbial therapy has a moderating effect on hyperlipemia, as indicated by a more dampened level of TC (SMD = −2.75, 95% CI −4.03 to −1.46, $I^2 = 81\%$) and TG (SMD = −2.64, 95% CI −3.22 to −2.06, $I^2 = 69\%$) (Figure 2). Subgroup analysis showed that biopsy tissue and mouse species are responsible for
high TG heterogeneity (Supplementary Table 3). The contour-enhanced funnel plot with the trim-and-fill method indicates that publication bias was not the leading cause of asymmetry (Figure 3). Sensitivity analysis confirmed the robustness of the study. There were no clinical studies. About the effect of microbial agents on lipid control.

**Effect of microbial agents on liver biochemical indicators**

After pooled analysis of the data, there were significant differences in ALT (SMD: –2.70, 95% CI: –3.33 to –2.07, $I^2 = 78\%$), AST (SMD: –3.37, 95% CI: –4.25 to –2.49, $I^2 = 82\%$) and alkaline phosphatase (SMD: –2.12, 95% CI: –3.32 to –0.92, $I^2 = 66\%$), between the experimental and control groups (Figure 4). In addition, GGT (SMD: –1.8, 95% CI: –2.39 to –1.24, $I^2 = 68\%$), which is more specific to ALD, has also been addressed (Figure 2). Because of significant heterogeneity, the reasons for these differences were investigated by conducting subgroup analyses (Supplementary Table 4). The heterogeneity of ALT was slightly altered after considering probiotics and prebiotics separately; however, the heterogeneity changed more significantly when the variables were controlled for the animal model, flora type, and feeding pattern. This finding suggested that the more significant heterogeneity may be due to these factors. There was an inconspicuous asymmetry in the contour-enhanced funnel plot. The trim-and-fill method demonstrated that the asymmetry was caused by factors other than publication bias (Figure 3).

There were many reports investigating the role of probiotics in experimental ALD. Clinical studies were rare, but we have conducted a careful analysis, according to the clinical study data we have obtained: ALT (SMD: –0.95, 95% CI: –0.40 to –1.1, $I^2 = 69\%$), AST (SMD: –1.4, 95% CI: –3.2 to –0.4, $I^2 = 97\%$), and GGT (SMD: –0.63, 95% CI: –1.07 to –0.20, $I^2 = 70\%$) decreased significantly compared to the control group (Supplementary Figure 2).

**Effect of microbial agents on inflammation mediators**

In animal studies, TNF-\(\alpha\), IL-6, and IL-1\(\beta\) were used to assess inflammatory infiltration due to ALD (Figure 5). There were lower levels of TNF-\(\alpha\) (SMD: –3.35, 95% CI: –4.31 to –2.38, $I^2 = 81\%$), IL-6 (SMD: –4.28, 95% CI: –6.13 to –2.43, $I^2 = 84\%$), and IL-1\(\beta\) (SMD: –4.28, 95% CI: –6.37 to –2.19, $I^2 = 87\%$).
TABLE 1 Characteristics of included studies investigating the effects of probiotics and prebiotics on animal ALD.

| References        | Animal Sample | Sample size | Modeling methods | Dosage | Route and duration | Comparison | Outcome indicators                      |
|-------------------|---------------|-------------|------------------|--------|--------------------|------------|-----------------------------------------|
| Page et al. (15)  | Adult Wistar rat's female, 180 – 200 g | 25          | Orally treated with alcohol for 5 days | Probiotics: 14 × 10^10 CFU/mouse of Lactobacillus acidophilus 2 Bioferment. 2 Bifidobacterium longum | Orally, for 2.5 days | Normal saline | AST↓, ALT↓ and Endotoxin↓ |
| Hooijmans et al. (16) | Male C57BL/6N mice 7-week-old | 24          | Orally treated with alcohol for 5 weeks | Probiotics: 500 mg/kg of heat-killed L.brevis8803 | Orally, for 5 weeks | Distilled water | AST↓, ALT↓, TG↓, TC↓ and TNF-a↓ |
| Qing and Wang (18) | Male C57BL/6N mice | 24          | Orally treated with alcohol for 5 weeks | Probiotics: Lactobacillus rhamnosus GG (1 × 10^10 CFU/mouse per day) | Orally, for 2 weeks | Isocaloric maltose-dextrin | TG↓, ALT↓, Endotoxin↓ |
| Segawa et al. (19) | Male C57BL/6N mice 8-week-old | 20          | Orally Lieber-DeCarli liquid diet for 2 weeks and 5% (v/v) alcohol diet for weeks | Probiotics: Lactobacillus acidophilus (1 × 10^10 CFU/mouse per day) | Orally, for 2 weeks | Isocaloric maltose-dextrin | TG↓, ALT↓, LPS↓ and microbial transform |
| Stadlbauer et al. (20) | Female Wistar rats (200–250 g) | 60          | Oral gavage 10 g/kg/day of 35% (v/v) ethanol orally for 2 weeks. 14 g/kg/day for next 10 weeks | Probiotics: 1 L. plantarum (10^10 CFU/mL). 2 AL-CA L. plantarumbeads (Equivalent to 10^12 CFU/mL) | Oral gavage, for 10 weeks | Distilled water | AST↓, ALT↓, ALP↓, Endotoxin↓ and TNF-a↓ |
| Wang et al. (21)   | Male mice (20 ± 2 g) | 50          | Orally gavage 6.25 mL of ethanol/kg per day for 5 weeks | Probiotics: Lactobacillus Whey fermented liquid | Oral gavage, for 5 weeks | Distilled water | AST↓, ALT↓, TG↓, GSH↓, SOD↓ and MDA↓ |
| Bull-Otterson et al. (22) | Male C57BL/6N mice 8-week-old | 20          | Intragastric alcohol diets for 3 weeks | Probiotics: 1. Unsaturated fatty acid; 2. Saturated fatty acid | Intragastric, for 3 weeks | Isocaloric diet | TG↓, ALT↓ and microbial transform |
| Arora et al. (23)  | Male Wistar rats 8-week-old | 32          | Orally treated with ethanol liquid diet for 12 weeks | Probiotics: Symbiotic supplementation | Orally, for 12 weeks | Normal liquid diet | ALT↓, TG↓, AST↓, TNF-a↓, IL-1β↓, Endotoxin↓ and microbial transform |
| Chen et al. (25)   | Male C57BL/6 mice 8-week-old | 100         | Intra-gastric ethanol (5 g/kg/day twice/week for 9 weeks) | Probiotics: Lactobacillus rhamnosus R0011 and acidophilus R0052 Prebiotics: KRG (Korea red ginseng), urushiol (Rhus verniciflua Stokes) | Intra-gastric, for last 2 weeks (1 mg/mL/day). | Normal Chow diet | ALT↓, TNF-a↓, IL-1β↓ |
| Hong et al. (28)   | Male C57BL/6 mice 8–10 week-old | 16 – 40     | Orally treated with alcohol for 4 weeks | Probiotics: LGGs at a dose equivalent to 10^9 CFU/day/mouse | Orally, for 12 weeks | Isocaloric maltose dextrin | TNF-a↓, Endotoxin↓ |
| Chiu et al. (26)   | Male Kunming mice (19 ± 1 g) | 60          | Orally treated with alcohol for 3 months | Probiotics: 1. L. rhamnosus CCFM1107, 2 LGG; 3. L. plantarum CCFM1112 | Orally, for 3 months | Skimmed milk | AST↓, ALT↓, TG↓, TC↓, GGT↓, GSH↓, SOD↓, MDA↓ and microbial transform |
| Han et al. (27)    | Male C57BL/6N mice | 16 – 40     | Orally treated with 5% alcohol for 4 weeks | Probiotics: LGGs at a dose at equivalent to 10^9 CFU/day/mouse | Orally, for 12 weeks | Isocaloric maltose dextrin | AST↓, ALT↓, TG↓ |
| Tian et al. (29)   | Female 12-month-old mice | 48          | Orally treated with alcohol for 12 weeks | Probiotics: Lactobacillus fermentum | Orally, for 12 weeks | Normal Chow diet | ALT↓, ALT↓ |
| Zhang et al. (30)  | Male C57BL/6 mice 10 weeks of age | 18          | Orally treated with Lieber-DeCarli diet containing 5% EtOH (w/v) for 10 days, and a bolus of EtOH (5 g/kg) was gavaged | Probiotics: Lactobacillus rhamnosus GG | Orally, for 10 days | Isocaloric diet | AST↓, ALT↓ |
| Zhao et al. (31)   | Male Spraque-Dawley 6-week-old | 45          | Orally gavaged with a single dose of alcohol | Probiotics: 1. Lactobacillus salivarius; 2. Lactobacillus johnsonii | Orally gavaged, for 10 days | Gavaged with normal saline | ALT↓, AST↓, GGT↓, MDA↓, TG↓ and TC↓ |

(Continued)
| References         | Animal          | Sample size | Modeling methods                                      | Dosage                                                                 | Route and duration | Comparison                          | Outcome indicators                |
|--------------------|-----------------|-------------|------------------------------------------------------|------------------------------------------------------------------------|--------------------|-------------------------------------|-----------------------------------|
| Barone et al.      | Male albino     | 36          | Orally treated with 30% ethanol (equivalent to 6 g/kg b.w. p.o. for 60 days) | Prebiotics: Zingerone in different concentrations                      | Orally, for 60 days | Isocaloric glucose and dimethyl sulfoxide (DMSO) | AST↓, ALT↓, GGT↑ and ALP↓         |
| Chen et al.        | Female wistar rats (200–250 g) | 60          | Orally gavaged with alcohol                          | Lactobacillus plantarum MTCC 2621                                     | Orally gavaged, for 8 weeks | Distilled water                     | ALT↓, AST↓, ALP↑, TNF-α↓           |
| Chuang et al.      | Male ICR mice 7 weeks old | 56          | Orally gavaged with alcohol                          | 1. Lactobacillus plantarum LC27, 2. Bifidobacterium longum LC07, 3. LC27 + LC07 | Orally gavaged, for 16 days | Vehicle (1% dextrose)               | ALT↓, AST↑, TG↑, TNF-α↓, MDA↑ and microbial transform |
| Mani et al.        | Female C57BL/6 mice 8-10-week-old | 48          | Orally treated Lieber-DeCarli liquid                | Lactobacillus plantarum                                               | Orally, for 4 weeks | Isocalicloric maltodextrin          | AST↓, ALT↑, TNF-α↓ and TG↓        |
| Rishi et al.       | Male C57BL/6 mice (22–25 g) | 48          | Orally treated with alcohol for 6 weeks             | Lactobacillus plantarum                                               | Orally, for 6 weeks | Normal saline                       | ALT↓, AST↑, TG↑, TNF-α↓, IL-10↓, GSH↑, SOD↑, Endotoxin↓,ZO-1↑, MDA↑ and microbial transform |
| Kim et al.         | C57BL/6 mice (8-12 weeks) | 33          | Orally treated with Lieber-DeCarli liquid for 15 days | Prebiotics: Indole-3-acetic acid (IAA)                                 | Orally gavaged, for 16 days | Normal saline                       | ALT↓, TG↑                         |
| Shukla et al.      | Sprague-Dawley rats 8-10-week-old | 60          | Orally gavaged normal chow diet and intra gastric ethanol | Probiotics: Lactobacillus acidophil Plus 1. 1 ml/kg/day Golden Bifid 2. 1 ml/kg/day Medialac-S 5% suspension 3. Golden Bifido suspension + glutamine | Orally gavaged, for 8 weeks | Chow diet and 1 ml/kg/day saline    | BW↑, AST↑, ALT↑, TG↑, TNF-α↑, IL-10↓, GSH↑, SOD↑, Endotoxin↓,ZO-1↑, MDA↑ and microbial transform |
| Fang et al.        | Sprague-Dawley rats 8-10-week-old | 30          | Orally gavaged with 50% alcohol at 4 g/kg BW daily. | Lactococcus bulgaricus                                                | Orally gavaged, for 8 weeks | Phosphate buffer saline              | BW↑, AST↑, ALT↑, TG↑, TNF-α↑, IL-10↓, GSH↑, SOD↑, Endotoxin↓,ZO-1↑, MDA↑ and microbial transform |
| Hendriks et al.    | Female C57BL/6 mice | 60          | Orally treated with Lieber-DeCarli liquid with alcohol for 6 weeks | Prebiotics: Inulin                                                    | Orally, for 6 weeks | Lieber-DeCarli liquid               | BW↑, AST↑, ALT↑, IL-10↑, TNF-α↑, GSH↑, SOD↑, Endotoxin↓,ZO-1↑, MDA↑ and microbial transform |
| Huang et al.       | Male Kunming mice 6-week old | 60          | Orally treated with 50% alcohol (v/v) at the concentration of 0.1 mL/10 g per day. | Probiotics: Lactobacillus plantarum HSY 85; 2. Lactobacillus delbrueckii subsp. Bulgaricus | Orally, for 8 weeks | Normal saline                       | ALT↓, AST↓, ALP↑, TNF-α↓, MDA↓, IL-6↑ and microbial transform |
| Yang et al.        | Male C57BL/6 mice | 48          | Orally treated with 10% alcohol plus HFD            | Bifidobacterium longum                                              | Orally, for 6 weeks | Normal diet                         | ALT↓, AST↑, TG↑, GGT↑, TNF-α↑, IL-10↑, SOD↑, MDA↑, IL-6↑ and microbial transform |
| Yi et al.          | Male C57BL/6 mice | 40          | Orally treated with alcohol for 8 weeks             | Lactobacillus fermentum                                              | Orally, for 8 weeks | Isocalicloric maltose dextrin       | ALT↑, AST↑, TC↑, TG↑, Endotoxin↑, TNF-α↑, and GSH↑ |
| Jiang et al.       | Male C57BL/6N mice 8-week-old | 80          | Orally gavaged ethanol, 5 g/kg of BW                 | Lactobacillus reuteri                                               | Orally gavaged, for 8 weeks | Normal chow diet                    | BW↑, AST↑, ALT↑, TC↑, TG↑, Endotoxin↑, and TNF-α↑ |
| Yi et al.          | Male C57BL/6N mice 8-week-old | 56          | Via stomach injection ethanol                       | Probiotics: Dried probiotic tablets containing Bifidobacterium infantis, B. animalis, and Lactobacillus acidophilis  | Orally gavaged, for 10 days | Isocalicloric maltose dextrin       | MDA↓, GSH↑, SOD↑                   |

(Continued)
TABLE 1 (Continued)

| References          | Animal Sample size | Modeling methods | Dosage | Route and duration | Comparison | Outcome indicators |
|---------------------|--------------------|------------------|--------|--------------------|------------|-------------------|
| Peters et al. (17)  | C57BL/6 mice 18 and 30–65 years | Prebiotics: *Lactobacillus plantarum*, *Lactobacillus fermentum*, *Lactobacillus reuteri* | Orally gavaged, for 28 days | Normal chow diet | AST↓, ALT↑, GSH↑, IL-6↓, and TNF-α↓ |
| Zhao et al. (24)    | USA 117 52.7 ± 11.3 (year) | Prebiotics: *Lactobacillus casei* | Orally gavaged, for 14 days | Isocaloric maltose-dextrin | ALT↑, AST↑, SOD↑, MDA↑, and TNF-α↓, Endotoxin↑, TG↑, and microbial transform |
| Nam et al. (42)     | C57BL/6 mice 8–6-week-old | Prebiotics: Polysaccharides from *crassostrea gigas* (RPS); Polysaccharides from steamed oyster (SPS) | Orally gavaged, for 25 days | Isocaloric maltose-dextrin | ALT↑, AST↑, ALP↑, TC↑, TG↑, TNF-α↓, IL-1β↓, TLR-4↓ |
| Jiang et al. (52)   | Young male (4–6-week-old) Wistar rats | Prebiotics: *Lactobacillus acidophilius*, *Lactobacillus rhamnosus* | Orally gavaged, for 12 weeks | Normal saline | ALT↑, GGT↑, TG↑, Endotoxin↑, and TNF-α↓ |
| Li et al. (53)      | Male C57BL/6N mice 60 days | Prebiotics: Lactobacillus casei | Orally gavaged, for 11 days | Isocaloric maltose-dextrin | ALT↑, AST↑, SOD↑, MDA↑, GSH↑, IL-6↓, and TNF-α↓ |
| You et al. (47)     | Male Kunming mice 40 | Prebiotics: 1. *Lactobacillus plantarum* HY09; 2. *Lactobacillus delbrueckii* subsp. Bulgaricus | Orally gavaged, for 7 days | Normal saline | ALT↑, AST↑, IL-6↑, TNF-α↑, IL-1β↑, SOD↑, MDA↑, GSH↑, and TNF-α↑ |
| Gu et al. (54)      | Male C57BL/6 mice 117 | Prebiotics: *Lactobacillus subtilis* | Orally gavaged, for 16 days | Isocaloric maltose-dextrin | ALT↑, AST↑, and Endotoxin↑, IL-6↑ |

† and ↓ represent increased or decreased outcome indicators in the treatment group compared with control group, respectively. ALP, alanine aminotransferase; AST, aspartate transaminase; ALP, alkaline phosphatase; GGT, gamma-glutamyl transferase; TNA-α, tumor necrosis factor-α; IL-6, interleukin-6; IL-1β, interleukin –1β; SOD, superoxide dismutase; MDA, malondialdehyde; GSH, glutathione; TG, triglyceride; TC, total cholesterol; TB, total bilirubin; CPU, colony-forming unit.

TABLE 2 Characteristics of included studies investigating the effects of probiotics and prebiotics on human ALD.

| References          | Country | Sample size | Age              | Intervention of experimental group | Route and duration | Comparison | Outcome indicators |
|---------------------|---------|-------------|------------------|-----------------------------------|--------------------|------------|-------------------|
| Grander et al. (10) | Russian| 66          | 18 years or older| *Lactobacilli* and *Bifidobacteria* | 12 weeks           | Vitamin B1 and B6 | ALT↑, AST↑, and TB↓ |
| Peters et al. (17)  | European| 20          | 18 and 75 years old | *Lactobacillus casei* Shirotu       | 4 weeks            | Placebo | TB↑, ALT↑, and TNF-α↓ |
| Zhao et al. (24)    | USA     | 117         | 52.7 ± 11.3 (year) | *Lactobacillus subtilis* or *Streptococcus faecium* | 7 days             | Placebo | AST↑, ALT↑, TB↑, and TNF-α↓ |
| Hsieh et al. (51)   | China   | 158         | 30–65 years old  | *Lactobacillus casei* strain       | 60 days            | Placebo | ALT↑, AST↑, and TNF-α↓ |

with evident heterogeneity. The analysis of subgroups was based on three items including animal models, tissues, and routes. The heterogeneity changed markedly among the animal models, suggesting that differences in animal species may be responsible for heterogeneity (Supplementary Table 5). The pooled analysis of TNF-α in serum and liver suggests that different tissues might not be the source of the heterogeneity. Egger's test indicated publication bias and profile-enhanced funnel plots (drawn using the trim-and-fill method; Figure 3) showed that publication bias was not the leading cause of asymmetry. Sensitivity analysis
revealed that no studies interfered significantly with the meta-analysis, implying good stability.

Due to the paucity of clinical literature on indicators of inflammation, we only performed a pooled analysis of TNF-α (SMD: –1.7, 95% CI: –4.39 to 0.9, \(I^2 = 89\%\)). This finding suggests that probiotics moderate inflammation (Supplementary Figure 2).

Effect of microbial agents on floral translocation and endotoxin

A comprehensive study of intestinal flora translocation and endotoxin was conducted to evaluate the changes of each in patients with ALD. Intestinal flora underwent dramatic changes in response to alcohol (Supplementary Figure 1), with most flora showing an upward trend, including *Lactobacillus* (SMD: 4.40, 95% CI: 0.97–7.84, \(I^2 = 85\%\)), *Bifidobacteria* (SMD: 3.84, 95% CI: 0.22–7.45, \(I^2 = 88\%\)), and *Bacteroidetes* (SMD: 2.51, 95% CI: 0.29–4.72, \(I^2 = 80\%\)). Most proliferating bacteria were beneficial to the intestinal tract (e.g., *Lactobacillus*); however, this finding could be because the microbial preparations administered in the animal models were *Lactobacillus*. We also explored the variation in endotoxin (SMD: –2.70, 95% CI: –3.52 to –1.88, \(I^2 = 79\%\)). Contour-enhanced funnel plots using the trim-and-fill method showed that publication bias was not the primary cause of asymmetry (Figure 3). The robustness of the results was demonstrated by sensitivity analysis. There were no clinical studies investigating floral translocation and endotoxin.

![FIGURE 2](http://example.com/figure2.png)

**FIGURE 2**

Effectiveness of microbial agents on lipid index. (A) The effect of microbial agents on TG. (B) TC. (C) The effect of microbial agents on GGT. SMD, Standardized mean difference; CI, Confidence interval.

Effect of microbial agents on oxidative stress

To evaluate the free radical-mediated lipid peroxidation damage and the antioxidant status of tissues, we measured levels of glutathione (GSH), SOD, and MDA (Figure 6). Microbial agent treatment contributed to increased levels of SOD (SMD: 2.65, 95% CI: 2.16–3.15, \(I^2 = 44\%\)) and GSH (SMD: 3.80, 95% CI: 0.95–6.66, \(I^2 = 87\%\)), while there was a significant decrease.

| Study                  | SMD   | 95% CI          | Weight (random) | Standardised Mean Difference |
|------------------------|-------|-----------------|-----------------|------------------------------|
| Microbial agents & probiotics  |       |                 |                 |                              |
| Cheng-Hung 2016        | –2.25 | [–3.46; –1.01]  | 10.9%           |                              |
| Farhin Palai 2021      | –4.09 | [–7.22; –1.96]  | 8.9%            |                              |
| Fengwen Tian 2015      | –1.79 | [–2.86; –0.72]  | 11.2%           |                              |
| Huanan Fan 2020        | –3.30 | [–4.16; –2.44]  | 7.2%            |                              |
| HUIPING HANG 2019      | –1.19 | [–2.52; 0.19]   | 2.1%            |                              |
| MEIDCI ZHAO 2021       | –1.47 | [–2.85; –0.08]  | 5.0%            |                              |
| Min Zhang 2015         | –3.17 | [–4.49; –1.85]  | 5.0%            |                              |
| Padawak K, Shukla 2018 | –2.82 | [–5.11; 0.47]   | 1.5%            |                              |
| Rukun Yi 2019          | –1.37 | [–1.94; –0.80]  | 5.3%            |                              |
| Shuich Segawa 2016     | –2.84 | [–5.11; 0.47]   | 1.5%            |                              |
| Tan-xiang Zhang 2019   | –0.71 | [–1.82; 0.40]   | 11.4%           |                              |
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| Tony J. Fang 2019      | –0.45 | [–0.93; –0.07]  | 9.6%            |                              |
| Wain-Gyeyong Kim 2018  | –0.21 | [–0.47; –0.05]  | 10.7%           |                              |
| Yi Hong-Whe 2019       | –1.60 | [–2.28; –0.92]  | 10.7%           |                              |
| Ying You 2020          | 0.00  | [–0.15; 0.15]   | 11.5%           |                              |

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FIGURE 3
Contour-enhanced funnel plot with trim-and-fill method. (A) ALT, (B) TG, (C) TNF-α, (D) Endotoxin. If the missing studies were in the non-significant area, the asymmetry was due to publication bias. Otherwise, the observed asymmetry could be attributed to factors other than publication bias.

in MDA (SMD: –4.70, 95% CI: –6.21 to –3.20, \(I^2 = 83\%\)) with considerable heterogeneity. We performed a subgroup analysis of MDA according to mice models and feeding practices and obtained no meaningful results. Asymmetry was present in the contour-enhanced funnel plot (Figure 3), demonstrating that publication bias was not the leading cause of asymmetry. There were no clinical studies.

Discussion

We identified convincing evidence for the use of microbial treatment of ALD through careful analysis of clinical trials and animal studies. To our knowledge, this is the first meta-analysis of such therapies. Although alcohol is primarily metabolized in the liver, alcohol consumption causes ecological dysregulation of bacteria in the intestine, damage to the intestinal mucosa, and increased intestinal permeability, resulting in increased transport of bacteria and their products (e.g., endotoxins) into the portal circulation. The increased inflammatory cytokine levels during liver injury reach the intestine through the circulation to damage the intestinal mucosal barrier. This phenomenon disrupts the balance of the intestinal flora, creating a vicious cycle (57). Once the dynamic balance is disturbed under the onslaught of pathogenic factors, intestinal and hepatic dysfunction is triggered. Fortunately, probiotics and prebiotics can effectively maintain intestinal homeostasis (58).

Lactobacillus and Bifidobacterium are the most predominant antimicrobial genera among probiotics. Most studies chose probiotics containing Lactobacillus, Bifidobacterium, or
a mixture of the two. These beneficial bacteria compete with pathogenic bacteria for binding sites in the intestinal epithelium, and they effectively reduce the pathogenic microorganisms by releasing antibacterial substances such as lactic acid and hydrogen peroxide (59). We found that, after the probiotic intervention, the dysbiosis in ALD mice improved with increased abundance of *Bifidobacterium*, decreased *Proteobacteria*, and a corresponding decrease in the rate of intestinal infections was also observed. *Bifidobacteria* and *Lactobacilli* are thought to promote mucosal immunity through the intestinal microbiota (60). Other potentially beneficial flora such as *Lachnospiraceae* might promote intestinal mucosal integrity through the metabolite butyrate, a short-chain fatty acid (61). Our findings demonstrated that the intestinal epithelial barrier (IEB) was more consolidated than the model groups, suggesting that probiotics and prebiotics can reinforce each other and work together to maintain intestinal immunity (62).

In addition to active modification of the intestinal flora, studies reported that probiotics protect the intestine and liver from alcohol stimulation by regulating the synthesis, catabolism and lipid transport, mitigating oxidative stress, and reinforcing the IEB (63). Sterol regulatory element binding proteins (SREBPs) are transcriptional mediators of lipid homeostasis.
that are upregulated in response to alcohol abuse, raising hepatic steatosis and plasma TG levels. SREBP-1c is the primary regulator of hepatic fatty acid and TG synthesis, and SREBP-2 regulates cholesterol synthesis (64). Alcohol reduces the expression of PPAR-α and MTP, critical participants in the transfer of TG and TC in the liver, leading to increased lipid accumulation (65). Probiotics inhibit weight gain, epiphyseal adipose tissue expansion, and partially reverse fructose (FRD)-induced adipocyte hypertrophy (66). Probiotics prevent the elevation of plasma triglycerides, leptin, and hepatic TG levels (67). Prebiotic fermentation products increased the production of hepatic mucin and modulated the action of hepatic lipogenic enzymes. Our findings suggest a significant decrease in TG and TC in the liver and serum compared to the model group (68).

Alcohol-induced inflammatory reactions should also be considered in the liver and throughout the body. The oxidative pathway of alcohol metabolism mediated by ethanol dehydrogenase and acetaldehyde dehydrogenase produces large amounts of acetaldehyde, which is thought to be the primary mediator of alcohol toxicity in the liver (69). Excess acetaldehyde displaces the intestinal flora and damages the intestinal mucosal barrier (70). Intestinal bacteria-derived endotoxins function through pattern recognition receptors such as TLRs, expressed in hepatic cells such as Kupffer cells. Lipopolysaccharide-induced inflammation boosts inflammatory cytokines, including TNF-α and IL-1β, which stimulate NF-κB activation through the MAPK (mitogen-activated protein kinase) pathway, and the activated NF-κB enters the nucleus, forming a cytokine-NF-κB loop and causing a series of inflammatory responses in the cells (71). Consistent with these findings, we found that human trials and animal studies showed a considerable increase in inflammatory parameters in ALD model compared to the control group.

In the inflammatory cascade, unsaturated fatty acids are driven by reactive oxygen species (ROS) to produce lipid peroxidases, which trigger fatty acid side chain reactions. Oxidative metabolites of ethanol, such as acetaldehyde and...
ROS, play essential roles in the clinical and pathological spectra ALD’s. At the same time, excess acetaldehyde entering the bloodstream is converted to superoxide by p-xanthine oxidase, producing MDA, the end product of free radical-mediated lipid peroxidation, which is used as a marker of oxidative stress (72). The antioxidants SOD and GSH reflect antioxidant levels. Ethanol-induced oxidative stress damage was confirmed by the decreased levels of SOD and GSH and the high content of MDA in alcohol-fed mice (73). The presence of microorganisms not only ameliorates oxidative stress by suppressing ROS and significantly reducing cytokine levels by inhibiting TLR-mediated endotoxins (21). Our findings showed that probiotics or prebiotics could reduce MDA levels by inhibiting the inflammatory response and the oxidative effect of alcohol. Meanwhile, there were increased concentrations of the antioxidants SOD and GSH.

The toxic effects of alcohol on the liver are mediated by interfering with lipid metabolism, disrupting the mucosal barrier, enhancing the inflammatory response and promoting oxidative stress. In contrast, microbial treatment can lead to significant changes in liver-specific biological enzymes. In clinical trials, we could observe a greater decrease in AST, ALT, and GGT compared to the control group. Interestingly, in patients with alcoholic liver disease, elevations in AST were more pronounced than in ALT and serum AST concentrations are usually more than twice as high as ALT because alcohol induces mitochondrial dysfunction through activation of the CYP2E1 enzyme and because of the massive release of AST from the mitochondrial matrix (74). And in animal experiments we obtained the same results, which fully illustrates the incredible effect of microbial treatment for ALD.

Our meta-analysis of 41 studies showed that microbial agents could help to treat ALD; nevertheless, there were still some limitations. First, due to the exploratory nature of this study, heterogeneity was inevitable when combining specific indicators, even when using random effects models and subgroup analysis. Nevertheless, sensitivity analysis supported the robustness of our results. Asymmetry appeared in the funnel plot and Egger’s test, indicating publication bias. Contour-enhanced funnel plots using the trim-and-fill method demonstrated that heterogeneity was the primary cause of asymmetry. In addition, although the number of clinical studies is limited and the data used for statistical analysis is small, we have conducted a careful analysis to summaries while hoping...
that more clinical studies will be available to support our conclusion. In this light, the conclusions we drew from 41 articles remain valid.

**Conclusion**

Prebiotics and probiotics exert hepatoprotective effects by regulating intestinal flora, maintaining the integrity of the intestinal mucosa, reducing the entry of endotoxins released by pathogenic microorganisms into the portal system, and inhibiting oxidative stress as well as pro-inflammatory factors. Our study provides new insights into the management of ALD. Nevertheless, clinical studies are still needed to translate microbial therapy into practical clinical applications.

**Data availability statement**

The original contributions presented in this study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author/s.

**Author contributions**

ZX conceived the idea and designed the study strategy. QW, JS, and MZ conducted reference search. QW and CX summarized the data. QW, GR, YX, and OY drafted the manuscript. QW conducted data acquisition and statistical analyses. FW provided critical revisions of the manuscript for important intellectual content, administrative and funding support, and supervision. All authors contributed to the article and approved the submitted version.

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**Conflict of interest**

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

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**Supplementary material**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fnut.2022.1054265/full#supplementary-material

**References**

1. Singal, AK, Bataller R, Ahn J, Kamath PS, Shah VH. ACG clinical guideline: alcoholic liver disease. *Am J Gastroenterol.* (2018) 113:175–94. doi: 10.1038/ajg.2017.469

2. Nibourg GA, Chamuleau RA, van der Hoeven TV, Maas MA, Ruiter AF, Lammers WH, et al. Liver progenitor cell line HepaRG differentiated in a biaортal liver effectively supplies liver support to rats with acute liver failure. *PLoS One.* (2012) 7:e38778. doi: 10.1371/journal.pone.0038778

3. Ki SH, Park O, Zheng M, Morales-Ibanez O, Kolls JK, Bataller R, et al. Interleukin-22 treatment ameliorates alcoholic liver injury in a murine model of chronic-binge ethanol feeding: role of signal transducer and activator of transcription 3. *Hepatology.* (2010) 52:1291–300. doi: 10.1002/hep.23837

4. Lu X, Wang F. *Lactobacillus acidophilus* and vitamin C attenuate ethanol-induced intestinal and liver injury in mice. *Exp Therap Med.* (2021) 22:1005. doi: 10.3892/etm.2021.10438

5. Chen P, Stärkel P, Turner JR, Ho SB, Schnabl B. Dysbiosis-induced intestinal inflammation activates tumor necrosis factor receptor 1 and mediates alcoholic liver disease in mice. *Hepatology.* (2015) 61:883–94. doi: 10.1002/hep.27489

6. Milesevic I, Vujovic A, Barac A, Djelic M, Korac M, Radovonic Spurnic A, et al. Gut-liver axis, gut microbiota, and its modulation in the management of liver diseases: a review of the literature. *Int J Mol Sci.* (2019) 20:395. doi: 10.3390/ijms20020395

7. Catry E, Bindels LB, Tailleux A, Lestavel S, Neyrinck AM, Goossens FJ, et al. Targeting the gut microbiota with inulin-type fructans: preclinical demonstration of a novel approach in the management of endothelial dysfunction. *Gut.* (2018) 67:271–83. doi: 10.1136/gutjnl-2016-313316

8. Forsyth CB, Farhari A, Jakate SM, Tang Y, Shaikh M, Keshavarzian A. *Lactobacillus* GG treatment ameliorates alcohol-induced intestinal oxidative stress, gut leakiness, and liver injury in a rat model of alcoholic steatohepatitis. *Alcohol.* (2009) 43:163–72. doi: 10.1016/j.alcohol.2008.12.009

9. Grander C, Grabherr F, Spadoni I, Einrich B, Oberhuber G, Rescigno M, et al. The role of gut vascular barrier in experimental alcoholic liver disease and *A. muciniphila* supplementation. *Gut Microbes.* (2020) 12:1851986. doi: 10.1080/19490976.2020.1851986

10. Grander C, Adolph TE, Wieser V, Lowe P, Wronsek L, Gyorgyosi R, et al. Recovery of ethanol-induced *Akkermansia muciniphila* depletion ameliorates alcoholic liver disease. *Gut.* (2018) 67:891–901. doi: 10.1136/gutjnl-2016-313432

11. Kirpich IA, Solovieva NV, Leikhter SN, Shidakova NA, Lebedeva OV, Sidorenko PI, et al. Probiotics restore bowel flora and improve liver enzymes in human...
Lactobacillus rhamnosus GG culture supernatant increases intestinal occludin expression and protects mice from alcoholic liver disease. Toxicol. Lett. (2021) 35:194–200. doi: 10.1016/j.toxlet.2021.03.002

22. Barone R, Rappa F, Macaluso I, Caruso Barisotto C, Sangiorgi C, Di Paola G, et al. Alcohol-induced liver disease: a mouse model reveals protection by Lactobacillus fermentum. Clin Transl Gastroenterol. (2016) 7:e138. doi: 10.1038/ctg.2015.66

23. Chen RC, Xu LM, Du SJ, Huang SS, Wu H, Dong JH, et al. Lactobacillus rhamnosus GG supernatant promotes intestinal barrier function, balances Treg and TH17 cells and ameliorates hepatic injury in a mouse model of chronic binge alcohol feeding. Toxicol Lett. (2016) 241:103–10. doi: 10.1016/j.toxlet.2015.11.019

24. Chuang CH, Tsai CC, Lin ES, Huang CS, Lin YY, Lan CC, et al. Heat-killed Lactobacillus salivarius and Lactobacillus johnsonii reduce liver injury induced by alcohol in vitro and in vivo. Molecules. (2021) 26:1456. doi: 10.3390/molecules26111456

25. Miani V, Siddique AI, Atirvalagan S, Thomas NS, Namvasiamaya N. Zingerone ameliorates hepatic and renal damage in alcohol-induced toxicity in experimental rats. Int J Nutr Pharmacol Neurol Dis. (2016) 6:125–32. doi: 10.4103/2319-0738.184585

26. Rishi P, Arora S, Kaur UJ, Chopra K, Kaur IJ. Better management of alcohol liver disease using a ‘microstructured box’ system comprising L. plantarum and EGCG. PLoS One. (2017) 12:e0168459. doi: 10.1371/journal.pone.0168459

27. Kim WG, Kim KL, Kwon EH, Han MJ, Kim DH. Lactobacillus plantarum LC27 and Bifidobacterium longum LC67 mitigate alcoholic steatitis in mice by inhibiting LPS-mediated NF-kB activation through restoration of the disturbed gut microbiota. Food Funct. (2019) 10:6425–65. doi: 10.1039/C9FO00202H

28. Shukla PK, Meena AS, Manda B, Gomez-Soleica M, Dietrich P, Dragatis I, et al. Lactobacillus plantarum prevents and mitigates alcohol-induced disruption of colonic epithelial tight junctions, endotoxemia, and liver damage by an EGF receptor-dependent mechanism. FASEB J. (2018) 32:E201800351R. doi: 10.1096/00351R

29. Fang TJ, Guo JT, Lin MK, Lee MS, Chen YL, Lin WH. Protective effects of Lactobacillus plantarum against chronic alcohol-induced liver injury in the murine model. Appl Microbiol Biotechnol. (2013) 97:4062–71. doi: 10.3168/jds.2014-7954

30. Zhao ZW, Pan DD, Wu Z, Sun YY, Guo YX, Zeng XQ. Antialcoholic liver disease via suppressing LPS-TLR4-M ψ expression. Molecules. (2021) 26:6224–40. doi: 10.3748/wjg.v26.i40.6224

31. Wang Q, Li Y, Lv L, Jiang H, Yan R, Wang S, et al. Identification of a protective Bacillus strains of alcoholic liver disease and its synergistic effect with pectin. Toxicol In Vitro. (2020) 26:891–7. doi: 10.1016/j.tiv.2019.09.018

32. Yang M, Wang J, Lactic acid bacteria prevent alcohol-induced steatohepatitis in rats by acting on the pathways of alcohol metabolism. Clin Exp Med. (2012) 12:371–7. doi: 10.14357/cem.2012.371

33. Chen RC, Torralba M, Tan J, Embree M, Zengler K, Stärkel P, et al. Metagenomic analyses of alcohol induced pathogenic alterations in the intestinal microbiome and the effect of Lactobacillus rhamnosus GG treatment. PLoS One. (2011) 6:e25028. doi: 10.1371/journal.pone.0025028

34. Arora S, Kaur IJ, Chopra K, Rishi P. Efficiency of double layered microencapsulated probiotic to modulate proinflammatory molecular markers for the management of alcoholic liver disease. Mediators Inflamm. (2014) 2014:715130. doi: 10.1155/2014/715130

35. Mani V, Siddique AI, Atirvalagan S, Thomas NS, Namvasiamaya N. Zingerone ameliorates hepatic and renal damage in alcohol-induced toxicity in experimental rats. Int J Nutr Pharmacol Neurol Dis. (2016) 6:125–32. doi: 10.4103/2319-0738.184585

36. Rishi P, Arora S, Kaur UJ, Chopra K, Kaur IJ. Better management of alcohol liver disease using a ‘microstructured box’ system comprising L. plantarum and EGCG. PLoS One. (2017) 12:e0168459. doi: 10.1371/journal.pone.0168459

37. Kim WG, Kim KL, Kwon EH, Han MJ, Kim DH. Lactobacillus plantarum LC27 and Bifidobacterium longum LC67 mitigate alcoholic steatitis in mice by inhibiting LPS-mediated NF-kB activation through restoration of the disturbed gut microbiota. Food Funct. (2019) 10:6425–65. doi: 10.1039/C9FO00202H

38. Shukla PK, Meena AS, Manda B, Gomez-Soleica M, Dietrich P, Dragatis I, et al. Lactobacillus plantarum prevents and mitigates alcohol-induced disruption of colonic epithelial tight junctions, endotoxemia, and liver damage by an EGF receptor-dependent mechanism. FASEB J. (2018) 32:E201800351R. doi: 10.1096/00351R

39. Fang TJ, Guo JT, Lin MK, Lee MS, Chen YL, Lin WH. Protective effects of Lactobacillus plantarum against chronic alcohol-induced liver injury in the murine model. Appl Microbiol Biotechnol. (2013) 97:4062–71. doi: 10.3168/jds.2014-7954

40. Hendrick T, Duan Y, Wang Y, Oh JH, Alexander LM, Huang W, et al. Bacteria engineered to produce IL-22 in intestine induce expression of REGEG to reduce ethanol-induced liver disease in mice. Gut. (2019) 68:1504–15. doi: 10.1136/gutjnl-2018-317322

41. Huang H, Lin Z, Zeng Y, Lin X, Zhang Y. Probiotic and glutamine treatments attenuate alcoholic liver disease in a rat model. Exp Therap Med. (2019) 18:4733–9. doi: 10.3892/etm.2019.8123

42. Nam Y, Kim KH, Konkit M, Kim W. Hepatoprotective effects of Lactococcus changensis CAU 1447 in alcoholic liver disease. J Dairy Sci. (2012) 102:10737–47. doi: 10.3168/jds.2012-61691

43. Yang X, He F, Zhang Y, Yue J, Li K, Zhang X, et al. Inulin ameliorates alcoholic liver disease via suppressing LPS-TLR4-M ψ and modulating gut microbiota in mice. Alcohol Clin Exp Res. (2019) 43:411–24. doi: 110.1111/acer.13950

44. Yi R, Tan F, Liao W, Wang Q, Ma J, Zhou X, et al. Isolation and identification of Lactobacillus plantarum HFY05 from natural fermented yak yogurt and its effect on alcoholic liver injury in mice. Microorganisms. (2019) 7:530. doi: 10.3390/ microorganisms7110530

45. Jiang XW, Li YT, Ye JZ, Xu LX, Yang LY, Bian XY, et al. New strain of Pediosum pentecosteus alleviates ethanol-induced liver injury by modulating the gut microbiota and short-chain fatty acid metabolism. World J Gastroenterol. (2020) 26:6522–40. doi: 10.3748/wjg.v26.i40.6224

46. Yi HW, Zhu XX, Huang XL, Lai YZ, Tang Y. Selenium-enriched Bifidobacterium longum protected alcohol and high fat diet induced hepatic injury in mice. Chin J Nat Med. (2020) 18:1689–77. doi: 10.3186/cjnm.0153.180418

47. You Y, Liu YL, Ai ZY, Wang YS, Liu JM, Piao CH, et al. Lactobacillus fermentum KP-3-fermented ginseng ameliorates alcoholic liver disease in C57BL/6N mice through the AMPK and MAPK pathways. Food Funct. (2020) 11:9801–9. doi: 10.1039/D0FO0236E

48. Zheng TX, Pu SL, Tan P, Du YC, Qian BL, Chen H, et al. Liver metabolomics reveals the effect of Lactobacillus reuteri on alcoholic liver disease. Front Physiol. (2020) 11:595382. doi: 10.3389/fphys.2020.595382

49. Fan H, Shen Y, Ren Y, Mou Q, Lin T, Zhu L, et al. Combined intake of blueberry juice and probiotics ameliorate mitochondrial dysfunction by activating SIRT1 in alcoholic fatty liver disease. Nutri Metab (2021) 18:50. doi: 10.1186/s12986-021-00554-3

50. Gan Y, Tong J, Zhou X, Long X, Pan Y, Liu W, et al. Hepatoprotective effect of Lactobacillus plantarum HFY09 on ethanol-induced liver injury in mice. Front Nutri. (2021) 8:684588. doi: 10.3389/fnut.2021.684588
51. Hsieh, P.S., Chen, C.W., Kuo, Y.W., Ho, H.H. *Lactobacillus* spp. reduces ethanol-induced liver oxidative stress and inflammation in a model of alcoholic steatohepatitis. *Exp Therap Med.* (2021) 21:188. doi: 10.3892/etm.2021.9619
52. Jiang, S., Ma, Y., Li, Y., Liu, R., Zeng, M. Mediation of the microbiome-gut axis by oyster (*Crassostrea gigas*) polysaccharides: a possible protective role in alcoholic liver injury. *Int J Biol Macromol.* (2021) 182:968–76. doi: 10.1016/j.ijbiomac.2021.04.050
53. Li, X., Han, J., Liu, Y., Liang, H. *Lactobacillus casei* relieves liver injury by regulating immunity and suppression of the enterogenic endotoxin-induced inflammatory response in rats cotreated with alcohol and iron. *Food Sci Nutr.* (2021) 9:5391–401. doi: 10.1002/fsn3.2486
54. Li, X., Liu, Y., Guo, X., Ma, Y., Zhang, H., Liang, H. Effect of *Lactobacillus casei* on lipid metabolism and intestinal microflora in patients with alcoholic liver injury. *Eur J Clin Nutr.* (2021) 75:1227–36. doi: 10.1038/s41430-020-00852-8
55. Patel, E., Parwani, K., Patel, D., Mandal, P. Metformin and probiotics interplay in amelioration of ethanol-induced oxidative stress and inflammatory response in an in vitro and in vivo model of hepatic injury. *Mediators Inflamm.* (2021) 2021:6636152. doi: 10.1155/2021/6636152
56. Zhao, M., Chen, C., Yuan, Z., Li, W., Zhang, M., Cui, N., et al. Dietary *Bacillus subtilis* supplementation alleviates alcohol-induced liver injury by maintaining intestinal integrity and gut microbiota homeostasis in mice. *Exp Therap Med.* (2021) 22:1312. doi: 10.3892/etm.2021.10747
57. Thurman, R.G., Bradford, B.U., Iimuro, Y., Knecht, K.T., Arteel, G.E., Yin, M., et al. The role of gut-derived bacterial toxins and free radicals in alcohol-induced liver injury. *J Gastroenterol Hepatol.* (1998) 13(Suppl.) S39–50. doi: 10.1111/j.1523-7313.1998.tb0039
58. Bajaj, J.S. Alcohol, liver disease and the gut microbiota. *Nat Rev Gastroenterol Hepatol.* (2019) 16:235–46. doi: 10.1038/s41575-018-0099-1
59. Roodpeshi, M., Dabiri, N. Effects of probiotic and prebiotic on average daily gain, fecal shedding of *Escherichia coli*, and immune system status in newborn female calves. *Asian Australas J Anim Sci.* (2012) 25:1255–61. doi: 10.5713/ajas.2011.11312
60. Vitetta, L., Saltzman, E.T., Thomsen, M., Nikolov, T., Hall, S. Adjuvant probiotics and the intestinal microbiome: enhancing vaccines and immunotherapy outcomes. *Vaccines.* (2017) 5:30. doi: 10.3390/vaccines5040050
61. Xiao, X., Nakatsu, G., Jin, Y., Wong, S., Yu, J., Lau, J.Y. Gut microbiota mediates protection against enteropathy induced by indomethacin. *Sci Rep.* (2017) 7:40317. doi: 10.1038/srep40317
62. Markowiak, P., Sliwowska, K. Effects of probiotics, prebiotics, and symbiotics on human health. *Nutrients.* (2017) 9:1021. doi: 10.3390/nu9091021
63. Wang, Y., Liu, L., Moore, D.J., Shen, X., Peek, R.M., Acra, S.A., et al. An LGG-derived protein promotes IgA production through upregulation of AFRIL expression in intestinal epithelial cells. *Microb Immunol.* (2017) 10.375–84. doi: 10.1038/mi.2016.57
64. Wei, Q., Zhou, B., Yang, G., Hu, W., Zhang, L., Liu, R., et al. JAZF1ameliorates age and diet-associated hepatic steatosis through SREBP-1c–dependent mechanism. *Cell Death Dis.* (2018) 9:859. doi: 10.1038/s41419-018-0923-8
65. Tang, C.C., Huang, H.P., Lee, Y.J., Tang, Y.H., Wang, C.J. Hepatoprotective effect of mulberry water extracts on ethanol-induced liver injury via anti-inflammation and inhibition of lipogenesis in C57BL/6J mice. *Food Chem Toxicol.* (2013) 62:786–96. doi: 10.1016/j.fct.2013.10.011
66. Nakamura, M., Yudell, B.E., Loor, J.J. Regulation of energy metabolism by long-chain fatty acids. *Prog Lipid Res.* (2014) 53:123–44. doi: 10.1016/j.plipres.2013.12.001
67. Kumar, M., Nagpal, R., Kumar, R., Hemalatha, R., Verma, V., Kumar, A., et al. Cholesterol-lowering probiotics as potential biotherapeutics for metabolic diseases. *Exp Diabetes Res.* (2012) 2012:902917. doi: 10.1155/2012/902917
68. Sekiya, M., Yahagi, N., Matsuzaka, T., Najima, Y., Nakakuki, M., Nagai, R., et al. Polyunsaturated fatty acids ameliorate hepatic steatosis in obese mice by SREBP-1 suppression. *Hepatology.* (2003) 38:1529–39. doi: 10.1001/hep.2003.9.028
69. Libes, C.S. Role of oxidative stress and antioxidant therapy in alcoholic and nonalcoholic liver diseases. *Adv Pharmacol.* (1997) 38:601–28. doi: 10.1016/S1054-3890(08)61001-7
70. Seitz, H.K., Bataller, R., Cortez-Pinto, H., Gao, B., Gual, A., Lackner, C., et al. Polyunsaturated fatty acids ameliorate steatohepatitis in mice by reducing CYP2E1-dependent oxidative stress. *Biomed Pharmacother.* (2014) 68:786–96. doi: 10.1016/j.biopha.2013.12.001
71. Inokuchi, S., Tsukamoto, H., Park, E., Liu, Z.X., Brenner, D.A., Seki, E. Toll-like receptor 4 mediates alcohol-induced steatohepatitis through bone marrow-derived and endogenous liver cells in mice. *Alcohol Clin Exp Res.* (2011) 35:1509–18. doi: 10.1111/j.1530-0277.2011.01487.x
72. Na, H.K., Lee, J.Y. Molecular basis of alcohol-related gastric and colon cancer. *Int J Mol Sci.* (2017) 18:1116. doi: 10.3390/ijms18061116
73. Wang, M., Ma, L.J., Yang, Y., Xiao, Z., Wan, J.B. n-3 polyunsaturated fatty acids for the management of alcoholic liver disease: a critical review. *Crit Rev Food Sci Nutr.* (2019) 59:S116–29. doi: 10.1080/10408398.2018.1544542
74. Yuan, R., Tao, X., Liang, S., Pan, Y., He, L., Sun, J., et al. Protective effect of acidic polysaccharide from *Schisandra chinensis* on acute ethanol-induced liver injury through reducing CYP2E1-dependent oxidative stress. *Biomed Pharmacother.* (2018) 99:537–42. doi: 10.1016/j.biopha.2018.01.079