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Research Article

Goji-Derived Exosomes-Like Nanoparticles Ameliorate Alcohol-Induced Acute Liver Injury by modulating gut microbiota and metabolites

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Abstract: Plant-derived exosome-like nanoparticles (ELNs) contain many bioactive components. Here, Goji-derived ELNs were characterized using transmission electron microscopy and Zeta potential analysis. These ELNs can improve the symptoms of liver injury in alcohol-induced acute liver disease (ALD) in mice, demonstrated by histology of liver sections and serum biomarker levels. Specifically, ELNs elevated the transcriptional level expression of hepatic antioxidant-related genes and colonic barrier-related genes. Additionally, 16S rRNA gene sequencing demonstrated that ELNs modulated gut microbiota by increasing the relative abundance of *Akkermansia*. Furthermore, metabolomics analysis shows that the protective effects of ELNs on alcohol-damaged intestinal barrier and mucus layer structure may be associated with increased L-asparagine, L-serine, D-glucuronic acid, as well as unsaturated fatty acids like docosahexaenoic acid and eicosapentaenoic acid in the mouse caecum. Overall, these findings suggest that the Goji-derived ELNs can modify gut microbiota and its metabolites, potentially emerging as a novel agent for the treatment of alcohol-induced ALD.

Keywords: alcohol-induced acute liver disease; Goji; ELNs; gut-liver axis; gut microbiota; metabolites

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1 Introduction

ELNs, originating from eukaryotic plant cells, are isolated via ultracentrifugation and typically measure between 30 and 150 nanometers in diameter. These vesicles are characterized by their lipid bilayer membranes and encapsulate a diverse array of bioactive compounds, including lipids, proteins, and RNA. They function as crucial mediators of intercellular communication and have been recognized for their influential roles in modulating the immune system, treating intestinal disorders, and enhancing cancer therapy[1]. Plant-derived ELNs are notable for their exceptional biosafety profiles and precise targeting abilities, as validated through testing on various cell lines and their demonstrated capacity to concentrate in different bodily tissues[2-5]. They exhibit a reduced risk of triggering immune responses and do not lead to heightened levels of pro-inflammatory cytokines[6]. The bioactive molecules within the ELNs, such as proteins, miRNA, and mRNA, are capable of being delivered to target cells, thereby influencing their physiological and pathological states. Emerging research indicates that ELNs are not only implicated in plant cell-to-cell interactions but also have the potential to cross the intestinal mucus barrier, suggesting a role in cross-species communication between plants and animals[7]. Given their wide availability and cost-effective production methods, plant-derived ELNs are poised to become innovative nutritional elements that could significantly contribute to human health advancements[8].

Goji berries (*Lycium barbarum* L., Wolfberry), are recognized for their dual role as both a medicinal herb and a food source, owing to their incorporation in various functional foods and dietary supplements[9]. This versatile fruit has been the subject of extensive research, revealing a range of pharmacological properties. These include its antioxidant capabilities, ability to regulate immune function, potential in cancer prevention, neuroprotective effects, and its role in safeguarding liver health.

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The fruit's bioactive components, such as polysaccharides, betaines, flavonoids, phenolic acids, and carotenoids, contribute to these beneficial effects[10-13]. Our recent studies have shed light on the link between Goji's hepatoprotective properties and its influence on the gut microbiota. This connection was elucidated using antibiotic treatments and fecal microbiota transplantation, providing evidence of a direct impact on liver health[14]. Further research is warranted to identify the specific components within Goji that are responsible for modulating the gut microbiota, which could offer insights into its therapeutic applications.

Alcohol-related liver diseases represent a prevalent category of hepatic disorders globally, with a significant contribution to mortality rates attributed to cirrhosis, estimated at around 25%[15]. A growing trend in alcohol consumption has been linked to an escalating incidence of alcohol-induced ALD. If left unchecked, ALD has the potential to progress to more severe liver pathologies, including fibrosis, cirrhosis, and hepatocellular carcinoma[16]. Over the past few decades, the scientific community has come to recognize the critical two-way relationship between the gut microbiota and liver health, collectively referred to as the gut-liver axis[17]. A substantial body of research indicates that the consumption of alcohol can disrupt the balance of the gut microbiota, a state known as dysbiosis, and can also increase the permeability of the intestinal barrier[18]. This heightened permeability can allow for the passage of harmful substances that originate from the gut, such as lipopolysaccharides (LPS), into the bloodstream. The translocation of these substances can exacerbate the condition of ALD[16]. Currently, there are limited treatment options for ALD.

In this study, we isolated and characterized Goji-derived ELNs. Then, we investigated if they have a protective role in ALD in mice by modulating gut microbiota and metabolites, using 16S rRNA gene sequencing and metabolomics analysis.

2 Material and Methods

2.1 Materials and reagents

The supply of Goji berries was sourced from Ningxia Red Power Goji Co., Ltd., located in the Ningxia Hui Autonomous Region of China. Mouse Kupffer cells were purchased from BeNa culture Collection (No. BNCC340733). *Akkermansia muciniphila* (*A. muciniphila*) was procured from the American Type Culture Collection (No. ATCC-BAA-835).

2.2 Goji-derived ELNs isolation and characterization

Goji berries (500 g) were soaked in 4.5 L of pre-cooled 0.01 M, pH 7.4 phosphate-buffered saline (PBS) for 30 min and subsequently homogenized. The collected juice was subjected to differential centrifugation at 1,000 × g (for 10 min), 2,000 ×g (for 20 min), 4,000 × g (for 30 min), and 10,000 × g (for 1 h) at 4 °C. The resulting supernatant was filtered using a 100-mesh cell strainer before being filtered again through a 0.8 μm pore-size filter. The filtrate was precipitated at 100,000 × g for 2 h by ultracentrifuged at 4 °C The Goji-derived ELNs pellet was washed with PBS and resuspended in sterile PBS[19] (Fig. 1A). Collected ELNs were stored at −80 °C until use[20]. The amount of Goji-derived ELNs was determined using the Bicinchoninic Acid (BCA) Protein Assay Kit (Thermo Scientific, Pierce™)[21]. The size and Zeta potential (ζ-potential) of the ELNs were measured using a Zetasizer (Malvern, U.K.). According to the manual, the Goji-derived ELNs were diluted in PBS and then added to the analytical cell to initiate the assay. Furthermore, the isolated ELNs (10 μL) were adsorbed to a carbon-coated grid for 1 min. Then it was washed with one drop of water and stained for 15 s with 1% uranyl acetate. The absorbed Goji-derived ELNs were observed using transmission electron microscopy (HITACH, Tokyo, Japan)[22].

2.3 Animal and treatments

Male C57BL/6J mice, aged between five and six weeks, were obtained from Shanghai SLAC Laboratory Animal Co. Ltd., situated in China. These mice were maintained under specific-pathogen-free (SPF) standards with regulated environmental parameters. This included a 12 h alternating light and dark cycle, temperatures ranging from 20 to 22 degrees Celsius, and a humidity level of 45 ± 5%. They had unrestricted access to a standard chow diet (AIN-93G) and water. Except for the control group, all mice were administered a single oral dose of ethanol (EtOH, 50% volume/volume) at a dosage of 12 mL/kg body weight on experimental day 14[14,23,24]. The control group received an equivalent volume of water, with five mice in each group. Following the ethanol challenge, all mice were sacrificed 12 h later under isoflurane anesthesia. The liver and colon tissues were then either frozen in liquid nitrogen for future analysis or fixed in 4% paraformaldehyde for histological examination. The entire experimental protocol was approved by the Ethics Committee of Animal Experiments at Jiangnan University, with permission number: JN. No20210430c1000713.

2.4 Dosage Information

The mice were administered either a low dose (ELNL) or a high dose (ELNH) of ELNs daily for 14 days. The ELNL dosage was 200 mg per kilogram of body weight[25,26], which corresponds to a human equivalent of 22 mg per kilogram, while the ELNH dosage was double that amount, equating to 44 mg per kilogram in humans. The concentration of ELNs was measured and reported in micrograms of total protein per milliliter (μg/mL) of PBS. Mice in the control group were given an equivalent volume of PBS for comparison.

2.5 Statistical analysis

The data are presented as mean ± SEM (standard error of the mean). Differences between the two groups were assessed using an unpaired two-tailed Student's t-test. Data sets encompassing more than two groups were analyzed using one-way analysis of variance (ANOVA) that was corrected for multiple comparisons using the Tukey test or the non-parametric Kruskal-Wallis test accompanied by Dunn's multiple comparison test. A *P*-value of less than 0.05 was considered statistically significant. The statistical analysis for this research was performed utilizing GraphPad Prism software (version 7.0, GraphPad Software, San Diego, California, USA).

Supporting Information Materials and Methods provides additional details on the determination of cell culture, biochemical parameters, qRT-PCR, 16S rRNA gene sequencing analysis, untargeted metabolomics analysis, and *in vitro* culture of *A. muciniphila*.

3 Results

3.1 Isolation, characterization of Goji-derived ELNs and their effects on Kupffer cells

To characterize the isolated Goji-derived ELNs (Fig. 1A),

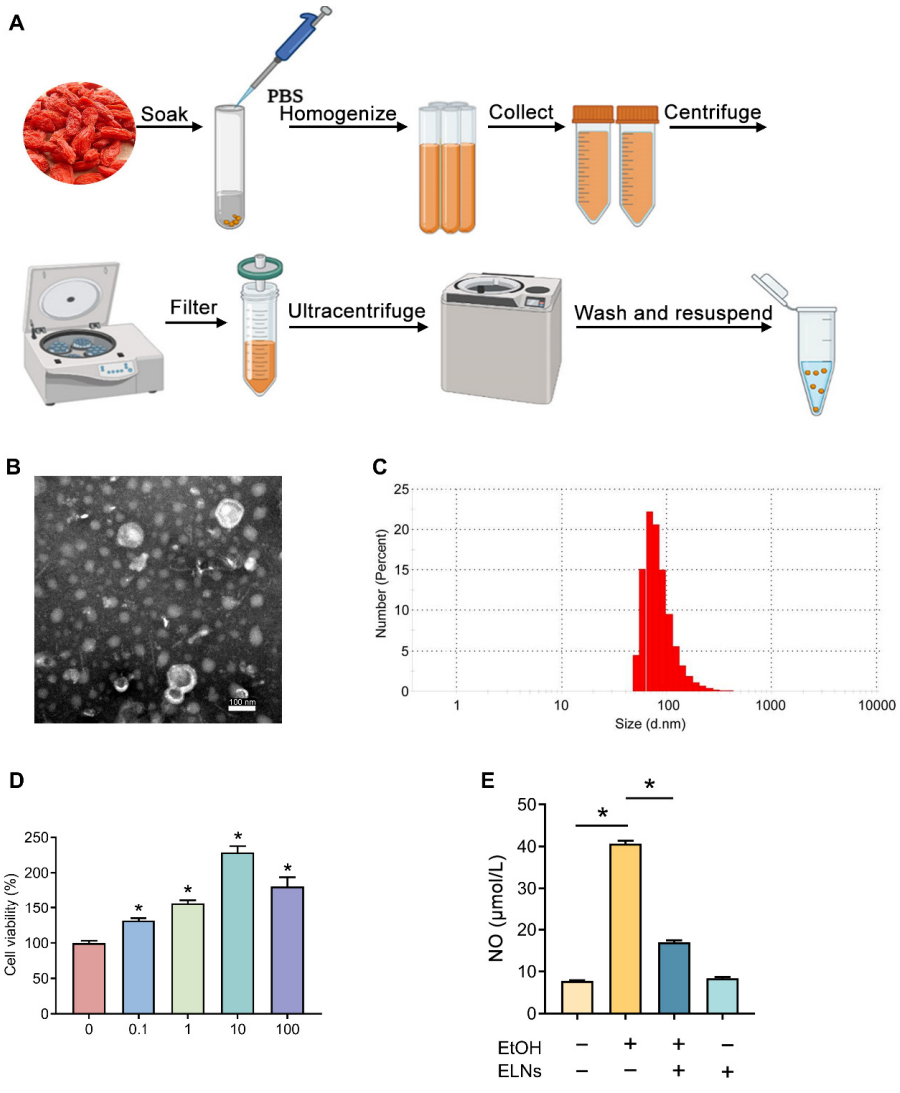

Figure 1 Preparation, characterization of Goji-derived ELNs, and their effects on Kupffer cells. A) Preparation, B) TEM image, C) Size distribution of isolated Goji-derived ELNs, D) Viability of Kupffer cells after 24 h of co-culture with different concentrations of Goji-derived ELNs, E) Effects of 10 μg/mL Goji-derived ELNs on NO production in Kupffer cells induced by EtOH and LPS. Error bars represent mean ± SEM, $^*P < 0.05$.

transmission electron microscopy (TEM) and nanoparticle tracking analysis were performed. The TEM image of the ELNs revealed that they had spherical morphologies with approximately 90-110 nm in diameter, like extracellular vesicles derived from mammalian cells or plant cells (Fig. 1B). The size under dynamic light scattering (DLS) analysis further confirmed that the average diameter of these ELNs was approximately 90-110 nm (Fig. 1C). ζ-potential refers to the shear surface potential and is also an important indicator to describe the stability of the colloidal dispersion system. The ζ-potential of Goji-derived ELNs was determined as −15.8 mV, which indicated that particles were negatively charged and in a stable state of dispersion.

To explore the bioactivity of Goji-derived ELNs, we first regarded mouse liver macrophage Kupffer cells as experimental objects to compare the effects of different concentrations of ELNs on cell viability. We found ELNs (0.1-100 μg/mL) promoted cell proliferation in Kupffer cells within 24 h (Fig. 1D). The cell proliferation in 12 h, 36 h, and 48 h was also increased by these concentrations of ELNs (Fig. S1). Among them, the concentration of 10 μg/mL had the most obvious role in promoting cell proliferation. These results suggest that Goji-derived ELNs accelerate Kupffer cell proliferation.

Then, we found ELNs (10 μg/mL) significantly reduced nitric oxide (NO) secretion in Kupffer cells with the administration of ethanol and LPS (P-value < 0.05). On Kupffer cells free of induction from EtOH, the addition of ELNs did not affect NO secretion (Fig. 1E). The results indicate that ELNs may have an anti-inflammation role on Kupffer cells.

3.2 Goji-derived ELNs ameliorate acute alcohol intoxication of the liver

To evaluate whether Goji-derived ELNs could protect against alcohol-induced liver injury, we treated mice with low- and high-dose ELNs based on acute liver injury models. Hematoxylin and eosin (H&E) staining of mouse liver tissue showed that alcohol changed mouse liver cell morphology, increased homogeneous eosinophilic bodies, accompanied by steatosis and inflammatory cell infiltration (Fig. 2B). In the ELNL group, liver inflammation reduced (Fig. 2C), while the liver of ELNH mice showed the presence of inflammatory cells in some areas of the central vein (Fig. 2D). Compared with the model group, ALT, AST, and LDH activities were significantly decreased in the ELNL group (Fig. 2E-G).

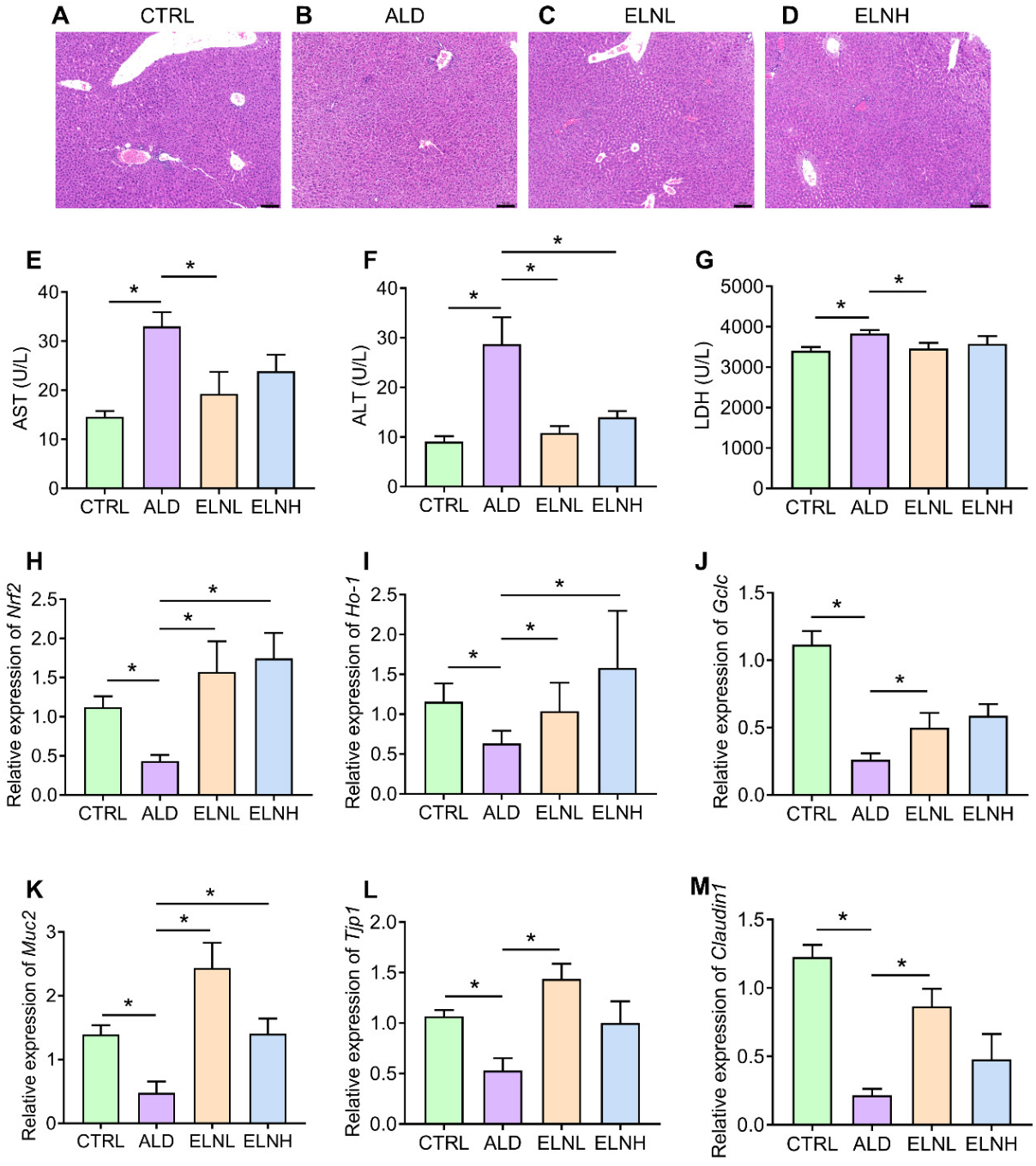

Figure 2 Goji-derived ELNs prevent acute alcohol intoxication of liver and intestinal barrier damage. A-D) The representative sections of the liver were stained with hematoxylin and eosin (scale bars, 100 μm). E) Serum AST, F) ALT, G) LDH levels in mice. H-M) Relative gene expression of *Nrf2*, *Ho-1*, *Gclc* in the liver and *Muc2*, *Tjp1*, *Claudin1* in the colon. Error bars represent mean ± SEM, **P* < 0.05.

Then we used qRT-PCR to quantify the gene transcriptional expression associated with antioxidant activity. The results showed that the gene transcriptional expression of nuclear factor (NF)-E2-related transcription factor 2 (*Nrf2*) (Fig. 2H) and heme oxygenase-1(*Ho-1*) (Fig. 2I) was significantly increased in ELNL and ELNH groups compared to the ALD group (*P*-value < 0.05). Low-dose ELNs also considerably increased the transcriptional (mRNA) level expression of the catalytic subunit (*Gclc*) gene (*P*-value < 0.05) (Fig. 2J). We also found that both ELNL and ELNH significantly increased the transcriptional (mRNA) level expression of *Muc2* gene in colon tissues compared with the ALD group (Fig. 2K). Moreover, low-dose ELNs significantly increased *Tight junction protein 1* (*Tjp1*) and *Caudin1* expression in colon tissues (Figs. 2L-M). The results suggest that Goji-derived ELNs could attenuate ALD-associated phenotypes in mice, enhance the antioxidant capacity of the liver, and reduce intestinal barrier damage.

3.3 Effects of Goji-derived ELNs on gut microbiota

To explore whether Goji-derived ELNs also affected the gut microbiota, we analyzed the cecal microbiota structure using high-throughput sequencing technology. After analyzing α diversity of gut microbiota, we found that the Chao1 index and the observed species index were decreased in the ALD group, indicating that

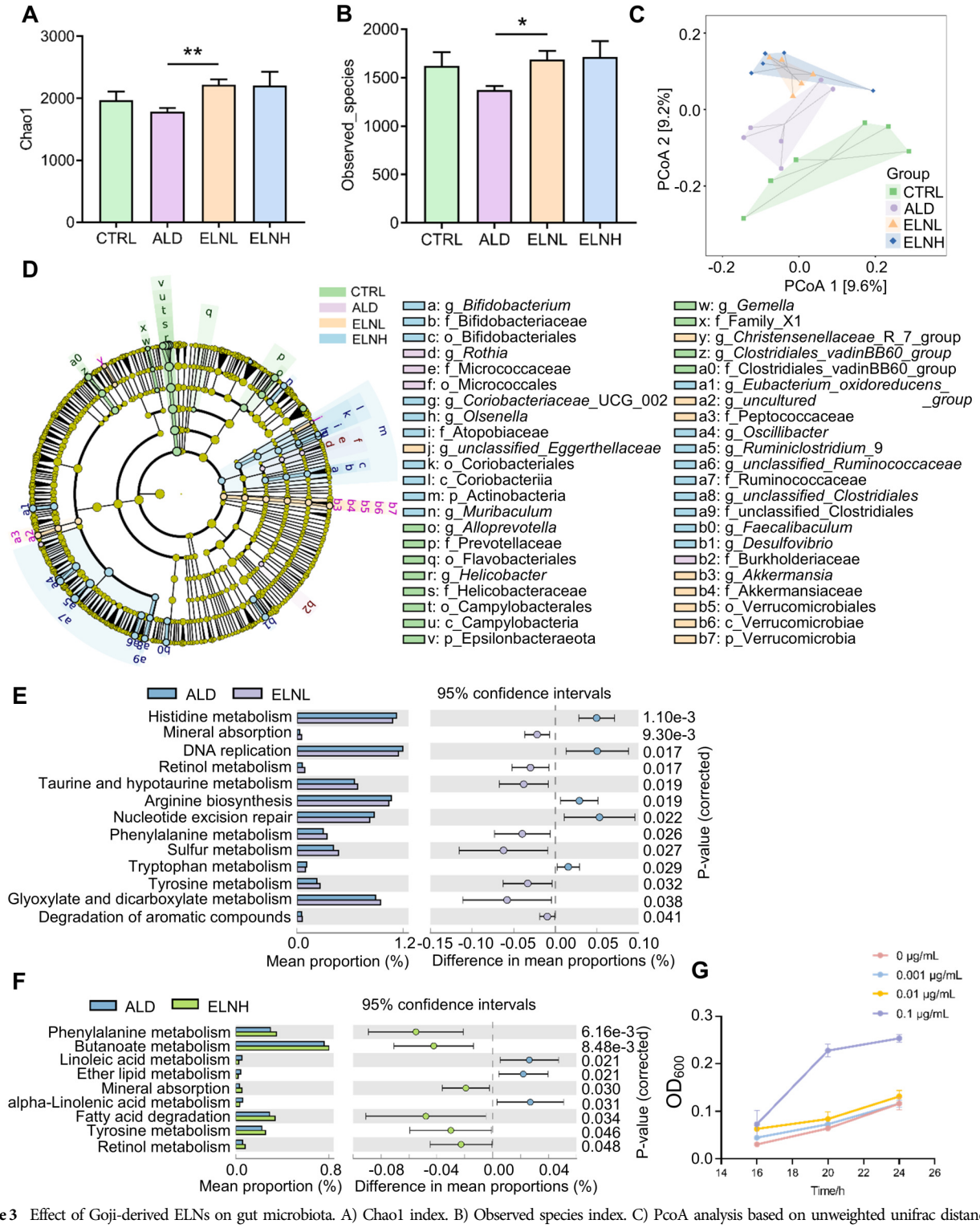

Figure 3 Effect of Goji-derived ELNs on gut microbiota. A) Chao1 index. B) Observed species index. C) PcoA analysis based on unweighted unifrac distance. D) LefSe analysis. E, F) Picrust2 predicted functional profile analysis between the ELNL and the ALD groups, the ELNH and the ALD groups, respectively. G) The effect of different concentrations of Goji-derived ELNs on the growth of *A. muciniphila* (No. ATCC-BAA-835). Error bars represent mean ± SEM, $^{*}P < 0.05$, $^{**}P < 0.01$.

the ELNL and ELNH increased the richness of the microbial community. The low-dose group showed statistically significant differences compared with the ALD group (Fig. 3A-B). The β diversity of gut microbiota analyzed by unweighted Unifrac PCoA showed that the two principal coordinate eigenvalues principal component (PC) 1 and PC2 explained was 9.6% and 9.2% of the variance, respectively (Fig. 3C). The ALD, ELNL, and ELNH groups were clearly distinguished from the CTRL group, indicating that alcohol significantly affected the structure of gut microbiota. Moreover, the ELNL and ELNH groups showed obvious separation from the ALD group, indicating a considerable modulation in gut microbiota composition after Goji-derived ELNs treatment (Fig. 3C).

To search for representative differential microbes in the gut

microbiota between different groups, we performed the linear discriminant analysis coupled with effect size measurement (LefSe) analysis. As shown in Fig. 3D, the family of Akkermansiaceae, Peptococcaceae, and the genus of *Akkermansia*, *Christensenellaceae_R_7_group* were the representative differential microbes in the ELNL group. The family of Atopobiaceae, Bifidobacteriaceae, Ruminococcaceae, and the genus of *Muribaculum*, *Olsenella*, *Coriobacteriaceae_UCG_002*, *Bifidobacterium*, *Oscillibacter*, *Ruminiclostridium_9*, *Faecalibaculum* were differed in the ELNH group from other groups.

We next performed a phylogenetic investigation of communities by reconstruction of unobserved states (PICRUSt2) analysis to predict differential microbial metabolic pathways between the ELNs and the ALD groups. Histidine metabolism, DNA replication, arginine biosynthesis, nucleotide excision repair, and tryptophan metabolism were significantly enriched in the ALD group, while mineral absorption and retinol metabolism were increased dramatically in the ELNL group (Fig. 3E). Phenylalanine metabolism, butanoate metabolism, mineral absorption, fatty acid degradation, tyrosine metabolism, and retinol metabolism were significantly increased in the ELNH group (Fig. 3F). Moreover, among the pathway above, DNA replication and butanoate metabolism pathways were significantly increased in the ELNH group, compared with the ELNL group (Fig. S2).

Moreover, we found ELNs upregulated the relative abundance of g_*Akkermansia* in the cecum of mice compared with the ALD group (Fig. S3). We further found that, with an increase in concentration, ELNs promoted the proliferation of *A. muciniphila* (No. ATCC-BAA-835) at 20 h *in vitro* (Fig. 3G). This result indicates that ELNs could modulate gut microbiota in mice.

3.4 Effects of Goji-derived ELNs on Gut Microbe Metabolites

To explore the potential metabolites and pathways regulated by Goji-derived ELNs, we performed an untargeted metabolomic analysis of mouse cecal contents. Principal component analysis (PCA) and orthogonal partial least squares discriminant analysis (OPLS-DA) score plots under positive and negative ions revealed a significant differentiation of metabolites of the four groups and good reproducibility of the samples within groups (Figs. 4A-B, S4). The greater effect on metabolites was Goji-derived ELNs administration, followed by alcohol administration. Further combined with the screening criteria (Student's -test, P-value < 0.05 and VIP ≥ 1), we compared the ELNL group and the ALD group, and the ELNH group and the ALD group to obtain 178 and 121 metabolites, respectively.

We next performed Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis of cecal differential metabolites between the ELNL and ALD groups (Fig. 4C). The most enriched pathways are ABC transporters, aminoacyl-tRNA biosynthesis, arachidonic acid metabolism, phenylalanine metabolism, tyrosine metabolism and arginine and proline metabolism. The KEGG enrichment analysis between the ELNH and the ALD groups shows that the pathways with more enrichment are ABC transporters, aminoacyl-tRNA biosynthesis, arachidonic acid metabolism, lysine degradation, glycine, serine and threonine metabolism and arginine and proline metabolism (Fig. 4D). Among these pathways, ABC transporters are related to membrane transport, while aminoacyl-tRNA biosynthesis is related to translation. Arachidonic acid metabolism is related to lipid metabolism. Pathways related to amino acid metabolism include phenylalanine metabolism, tyrosine metabolism, arginine and proline metabolism, lysine degradation, and glycine, serine and threonine metabolism. Furthermore, comparing ELNL group and ELNH group, Bile secretion is the most enriched pathway between the two groups. Among the pathways above, arachidonic acid metabolism also appears in the differential metabolite enrichment analysis between the ELNL group and ELNH group (Fig. S5).

Through the KEGG enrichment analysis above, we found that ABC transporters, aminoacyl-tRNA biosynthesis, arachidonic acid metabolism related to amino acid metabolism affected by differential metabolites both between the ELNH group and the ALD groups and between the ELNL group and the ALD group. Based on this, we performed a specific analysis of the co-regulated metabolites. The CTRL group had 236 differential metabolites compared with the ALD group, while the low- and high-dose ELNs reversed the 40 metabolites affected by alcohol (Fig. 4E). The above results indicate that Goji-derived ELNs are associated with the altered metabolism of these substances, which plays a role in protecting the colonic mucus layer and alleviating the inflammatory response.

3.5 Correlation analysis

We performed a correlation study to explore the possible connections between serum and liver biomarkers, intestinal barrier indicators, key gut microbiota, and significant gut microbial metabolites. The findings indicated that 11 distinct gut microbial metabolites and the g_*Alistipes* showed a positive correlation with serum levels of AST, ALT, and LDH. Conversely, these were negatively linked to liver antioxidant measures, the transcriptional (mRNA) level expression of genes associated with colonic barriers, and the genera g_*Ruminiclostridium_9*, g_*Bacteroides*, and g_*Akkermansia*. The correlation profile of 12 other metabolites and the genera g_*Ruminiclostridium_9*, g_*Bacteroides*, and g_*Akkermansia* was found to be in stark contrast to the aforementioned findings (Fig. 5). Among the 23 different gut microbial metabolites, D-glucuronic acid, L-serine, L-asparagine, and docosahexaenoic acid (DHA) showed a highly negative correlation with the AST ($r = -0.87$, $P < 0.001$; $r = -0.76$, $P < 0.001$; $r = -0.48$; $r = -0.81$, $P < 0.001$), ALT ($r = -0.83$, $P < 0.001$; $r = -0.80$, $P < 0.001$; $r = -0.54$, $P < 0.05$; $r = -0.70$, $P < 0.01$) levels in serum, and positive correlation with the expression level of *Gclc* in liver ($r = 0.57$, $P < 0.05$; $r = 0.88$, $P < 0.001$; $r = 0.90$, $P < 0.001$; $r = 0.51$, $P < 0.05$) and *Claudin1* in colon ($r = 0.72$, $P < 0.01$; $r = 0.78$, $P < 0.001$; $r = 0.66$, $P < 0.01$; $r = 0.70$, $P < 0.01$). In addition, genus *Bacteroides* and *Akkermansia* were remarkably positively correlated with the L-serine ($r = 0.65$, $P < 0.01$; $r = 0.51$, $P < 0.05$), sarcosine ($r = 0.66$, $P < 0.01$; $r = 0.69$, $P < 0.01$) and L-aspartic acid ($r = 0.63$, $P < 0.01$; $r = 0.66$, $P < 0.01$) in cecal content.

4 Discussion

In this study, we isolated Goji-derived ELNs and evaluated whether and how they prevented alcohol-induced acute liver injury in mice. First, ELNs improved steatosis in the liver and significantly reduced AST, ALT, and LDH levels in serum. We selected *Nrf2*[27,28], *HO-1*[29], and *Gclc*[30] as antioxidant-related genes in the liver of mice to express the extent of the acute injury in the liver. Results showed that ELNs significantly increased the transcriptional (mRNA) level expression of these genes. Moreover,

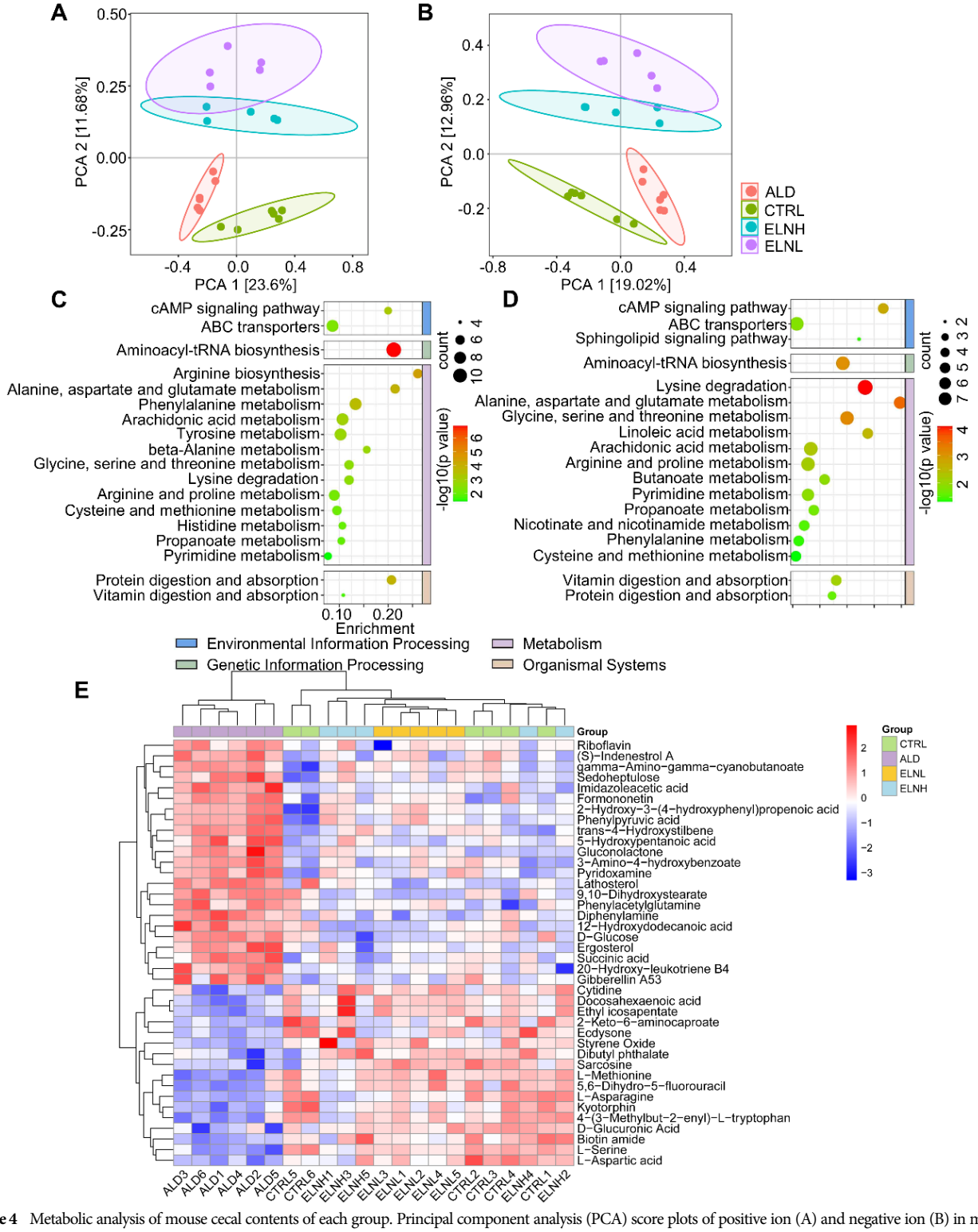

Figure 4 Metabolic analysis of mouse cecal contents of each group. Principal component analysis (PCA) score plots of positive ion (A) and negative ion (B) in mouse cecum metabolite, differential metabolite enrichment analysis between ALD vs ELNL (C) and ALD vs ELNH (D), and heatmap analysis of different metabolites in cecal contents by Goji-derived ELNs (E).

we found that ELNs prevented alcohol from damaging the intestinal barrier by enhancing the gene transcriptional expression of *Muc2*, *Tjp1*, and *Claudin1*. The expression of protein Muc2 is essential for the protective role of the intestinal mucosal barrier as an important component of the intestinal mucus layer and epithelium[31]. The expression of *Tjp1* and *Claudin1* is important for the maintenance of intestinal wall integrity as the two proteins are important functional molecules that constitute the tight junction[32,33]. These results collectively suggest that Goji-derived ELNs alleviate alcohol-induced ALD and disruption of the intestinal barrier in mice.

Our prior research has demonstrated that consuming dried Goji fruit as part of the diet can protect mice from liver damage caused by sudden alcohol consumption by altering their gut

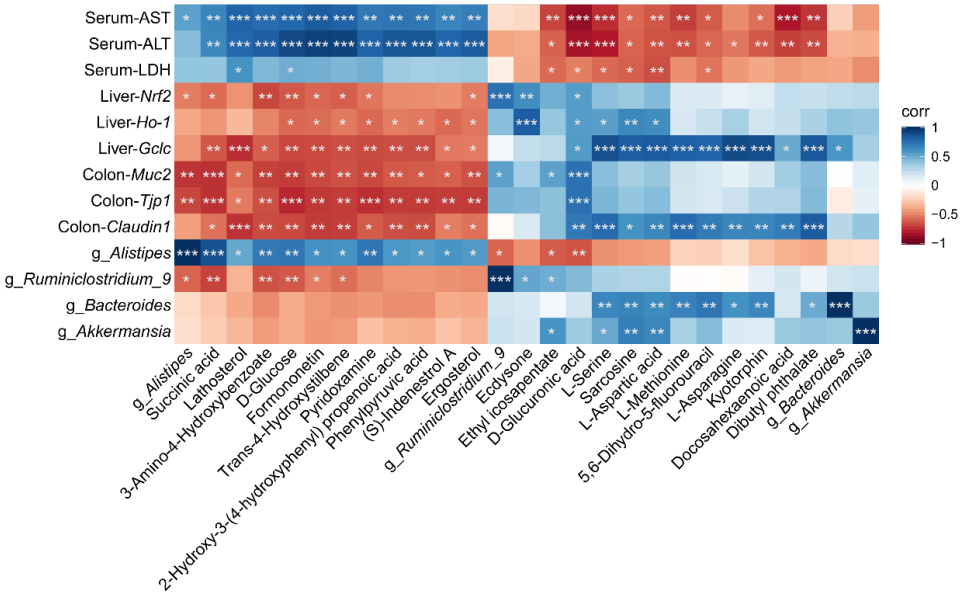

Figure 5 Spearman correlation analysis among the serum biomarkers, liver parameters, gut barrier parameters, gut microbiota, and microbial metabolites in mice with four different treatments (CTRL, ALD, ELNL, ELNH). Blue represents a positive correlation and the red represents a negative correlation. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

microbiota[14]. In recent times, the study of plant-based nanoparticles has garnered significant interest. These naturally occurring nanoparticles, enriched with bioactive substances like lipids, proteins, and RNA, exhibit unique structural and material properties that make them ideal as natural nanocarriers[6]. Zhang *et al.* have shown that ELNs derived from *taraxacum officinale* can prevent hypertension induced by intermittent hypoxia by adjusting gut metabolites[34]. Additionally, Zhao *et al.* have found that blueberry-derived ELNs can mitigate mitochondrial oxidative stress, thereby improving nonalcoholic fatty liver disease[25]. Based on these insights, we hypothesize that the oral intake of Goji-derived ELNs may alleviate acute alcohol-induced liver injury, potentially through modulating the gut microbiota.

We found that Goji-derived ELNs altered the structure of the gut microbiota, as indicated by the 16S rRNA gene sequencing method. The Chao1 and Observed Species indices showed that ELNL and ELNH could increase the microbial community richness and ASV numbers. And the representative differential microbes are distinct. The differential microbes that distinguished the model group from the other groups were Micrococcales, Micrococcaceae, Burkholderiaceae, and *Rothia*. *Rothia* is a genus of Gram-positive bacteria in the family Micrococcaceae, which may be conditionally pathogenic. Clinical studies have shown that conditions including alcohol abuse, human immunodeficiency virus or severe liver disease predispose to *Rothia* infection[35]. The differential microbes that distinguished the ELNL group from the other groups were the phylum Verrucomicrobia, the family Akkermansiaceae and Peptococcaceae, and the genus *Akkermansia* and *Christensenellaceae_R_7_group*. *Christensenellaceae_R_7_group* belongs to the family Christensenellaceae, order Clostridium, and is widely distributed in the intestine and mucus layer, where it ferments glucose to acetate and butyric acid under anaerobic conditions, thereby exerting biological activity[36]. The capacity of *A. muciniphila's* to bind to the mucus lining is regarded as a positive trait of probiotics. A reduced presence of this bacterium in the gut could cause the mucosal layer to thin, weakening the intestinal barrier. This could potentially allow toxins to more readily penetrate the host's system[37]. Moreover, many studies have focused on the effects of polyphenols and polysaccharides on *A. muciniphila* growth. However, this is the first time ELNs derived from Goji were found to influence the growth of *A. muciniphila in vitro*. The differential microbes that distinguished the ELNH group from the other groups were the phylum Actinobacteria, the family Atopobiaceae, Bifidobacteriaceae, and Ruminococcaceae, and the genus *Muribaculum*, *Oscillibacter*, and *Faecalibaculum*. *Muribaculum* belongs to the phylum Bacteroides, family Muribaculaceae with the ability to degrade and utilize complex carbohydrates and mucin monosaccharides[38]. *Oscillibacter* belongs to the family Ruminococcaceae and is widely found in the intestines of animals and humans, producing short-chain fatty acids such as butyric acid[39]. *Faecalibacterium prausnitzii* is a successfully isolated species of the genus *Faecalibacterium* that has been shown to be the main producer of butyrate in the intestine[40].

We have also analyzed the metabolic environment in the cecum using non-targeted metabolomics. Among the metabolites that were significantly downregulated by ELNs compared to ALD, phenylacetylglutamine and succinate may be associated with hepatic fat deposition. Among the metabolites significantly upregulated by ELNs compared to ALD, the levels of DHA, eicosapentaenoic acid (EPA) were all significantly higher. DHA and EPA are types of omega-3 polyunsaturated fatty acids (n-3 PUFAs). Studies have indicated that these compounds can help to maintain the integrity of the epithelial barrier and improve the condition of colitis in mice[41]. The underlying mechanisms may be strengthening of the mucus layer, specifically increasing mucin granule release, improving goblet cell differentiation, and modulating the gut microbiota, such as increasing the abundance of *A. muciniphila*[42]. L-asparagine and L-serine were found to be notably elevated in the ELNs group when contrasted with the ALD group. L-Serine has been shown to mitigate fibrotic damage in a mice with chronic liver injury[43]. Additionally, Sim *et al.* suggested that alcoholic fatty liver disease could be improved by enhancing the metabolism of homocysteine, which depends on L-serine[44]. Therefore, it was speculated that Goji-derived ELNs prevented alcohol-induced acute liver injury in mice by

modulating the metabolites in the gut.

Altogether, we found that Goji-derived ELNs could regulate gut microbiota and prevent alcohol-induced acute liver injury in mice. However, there are limitations, such as specific active components of Goji-derived ELNs and further explore their molecular mechanisms of action.

CRediT authorship contribution statement

Lin Guo: Investigation, Visualization. **Qiying Ding:** Methodology, Data curation, Writing – original draft. **Wenhui Duan and Qijie Guan:** Methodology, Validation. **Yilin Ren:** Resources, Writing – review & editing. **Yuzheng Xue:** Resources, Formal analysis. **Zheng-Hong Xu:** Supervision, Resources, Funding acquisition; **Yan Geng:** Conceptualization, Supervision, Writing – review & editing, Funding acquisition.

Conflicts of interest

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

Data availability

Data will be made available on request. The raw data were uploaded to the NCBI database (SRP: PRJNA1091424).

Electronic Supplementary Material (ESM)

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