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Nobiletin Ameliorates Alcohol-Induced Liver Injury via Modulation of the Gut-Liver Axis

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ABSTRACT: Alcoholic liver disease (ALD) is a significant health burden with limited therapeutic options. This study aimed to elucidate the protective mechanisms of nobiletin (NOB) against alcohol-induced liver injury in mice, focusing on the gut-liver axis. After a 7-day high-dose NOB intervention, treated mice showed a significant increase in body weight ($P < 0.05$) and marked improvements in key serum parameters, including 25% reduced aspartate aminotransferase (AST), 45% decreased low-density lipoprotein cholesterol (LDL-C), 25% suppressed interleukin-6 (IL-6) and 10% reduced tumor necrosis factor- α (TNF- α) relative to the alcohol-induced model group. Untargeted metabolomics analysis revealed that NOB remodeled glycerophospholipid metabolism, specifically upregulating phosphatidylcholine (PC) and phosphatidylethanolamine (PE) while suppressing lysophospholipids (LysoPC/LysoPE). Concurrently, NOB enhanced intestinal barrier integrity via upregulation of the tight junction protein ZO-1 and Occludin. Furthermore, it modulated gut microbiota composition by enriching beneficial probiotics such as *Lactobacillus*. In liver tissue, NOB activated the Nrf2 antioxidant pathway, increasing enzymes (SOD, CAT, GSH) and reducing malondialdehyde (MDA), while simultaneously inhibiting the NF- κ B pathway and its downstream pro-inflammatory cytokines (TNF- α , IL-1 β). Spearman correlation analysis further linked specific microbial shifts (*Lactobacillus murinus*) with the observed serum metabolic changes (PC, PE). Collectively, these findings demonstrate that NOB mitigates alcoholic liver injury through multi-pathway involvement of the “intestinal flora–glycerophospholipid metabolism–hepatic inflammation” axis, presenting a novel therapeutic candidate for ALD that warrants further clinical investigation.

Keywords: Nobiletin; Gut-liver axis; Glycerophospholipid metabolism; Alcoholic liver injury

1. Introduction

Alcoholic Liver Disease (ALD) represents a major global health burden, with rising prevalence paralleling increased alcohol consumption¹. The disease spectrum progresses from steatosis to hepatitis,

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fibrosis, cirrhosis, and hepatocellular carcinoma (HCC)². Early and accurate diagnosis is critical for improving clinical outcomes in progressive liver conditions like HCC, a point underscored by recent advances in diagnostic approaches³. Current therapies-primarily abstinence, nutritional support, and limited pharmacologic options-remain unsatisfactory due to efficacy limitations and side effects^{4,5}. Liver transplantation, though effective, is constrained by donor shortage and immune complications⁶.

Recent advances highlight the critical role of the gut-liver axis in ALD pathogenesis⁷. Alcohol disrupts intestinal barrier integrity via tight junction downregulation (ZO-1/Occludin), suppression of the Wnt/ β -catenin repair pathway, and reduced mucus secretion⁸⁻¹⁰. These changes lead to dysbiosis, decreased probiotics, increased pathobionts, and translocation of microbial products such as LPS, which activate hepatic TLR4/NF- κ B signaling and drive inflammatory liver injury^{11,12}. Notably, dysbiosis also perturbs bile acid cycling and glycerophospholipid metabolism-particularly elevating lyso-phosphatidylcholines (LysoPC)-which further exacerbates hepatic inflammation and injury^{13,14}. Despite the therapeutic potential of gut-axis modulation, outcomes have been inconsistent¹⁵, underscoring the need for multifunctional agents that simultaneously target barrier function, microbial balance, and metabolic homeostasis. Natural flavonoids have emerged as promising candidates for ALD, in part through their ability to modulate gut microbiota and suppress inflammation¹⁶. Accumulating evidence has confirmed that natural compounds can alleviate liver injury by inhibiting inflammatory signaling pathways, which provides evidence for the relevance of targeting inflammation-driven hepatic damage¹⁷. For instance, recent evidence confirms that natural compounds can alleviate liver injury by inhibiting inflammatory signaling pathways central to ALD progression¹⁸. Moreover, advances in diagnostic approaches, such as those highlighted¹⁹, underscore the clinical importance of detecting progressive liver conditions like HCC, reinforcing the need for early intervention strategies.

Nobiletin (NOB), a predominant polymethoxyflavone found in citrus fruits, exhibits dual antioxidant and anti-inflammatory properties. Mechanistically, it enhances cellular antioxidant defenses by activating the Nrf2/HO-1 pathway while suppressing the expression of pro-inflammatory cytokines (such as TNF- α and IL-6) through inhibition of NF- κ B nuclear translocation^{20,21}. Importantly, flavonoids including NOB have been shown to shape gut microbial composition and function, suggesting a potential mechanistic link between their systemic effects and gut-axis modulation²². Nevertheless, how NOB mediates protection against ALD by modulating the gut-liver axis remains poorly understood. Specifically, its impact on key aspects such as intestinal barrier function, gut microbiota composition, and related metabolic dysregulation requires further investigation²³. Elucidating these mechanisms will provide an important theoretical foundation for developing gut-liver axis-targeted therapeutic strategies based on nobiletin.

Given the pivotal role of the gut-liver axis in ALD and the unmet need for effective therapies, this study hypothesizes that NOB alleviates alcohol-induced liver injury by restoring intestinal barrier function, rebalancing gut microbiota, and normalizing dysregulated host metabolism, thereby attenuating downstream inflammatory and oxidative hepatic damage. We will employ integrated in vivo and in vitro approaches to elucidate the precise targets and signaling mechanisms through which NOB modulates the gut-liver axis. Our

findings are expected to identify novel therapeutic targets and provide a natural-product-based candidate for the multifactorial management of ALD.

2. Materials and Methods

2.1 Experimental Animals and Grouping

Seventy-two male Kunming mice (20 ± 2 g), an outbred strain with genetic diversity²⁴, were randomly divided into six groups (n=12 per group): control (0.5% CMC-Na), model (CMC-Na + ethanol), and low, medium, high-dose nobiletin groups (5, 10, 20 mg/kg/day)²⁵. After 7-day acclimatization and daily gavage, acute liver injury was induced with anhydrous ethanol (1 mL/100 g) on day 7, 1 h after the final dose following a 12-h fast. Samples were collected 2 h post-ethanol. The study was approved by the Ethics Committee (DYXY141642008). All samples were stored at -80°C .

2.2 Serum Biochemical, Antioxidant, and Inflammatory Marker Analysis

Serum levels of total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and aspartate aminotransferase (AST) were measured using a BS-200 fully automatic biochemical analyzer (Mindray) with the specified commercial kits²⁶. For antioxidant capacity assessment, the activities of superoxide dismutase (SOD) and catalase (CAT), as well as the contents of glutathione (GSH) and malondialdehyde (MDA) in serum, were determined using a microplate reader in accordance with the respective kit protocols²⁷. Inflammatory cytokines including interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α) were quantified using ELISA kits. Detailed information regarding the sources and catalog numbers of all assay kits used in this section is provided in Supplementary Table 1.

2.3 Serum Non-Targeted metabolomics analysis

Serum metabolomics profiling was conducted using liquid chromatography-tandem mass spectrometry (LC-MS/MS) technology. The procedure involved adding 100 μL of serum was added to 400 μL of a methanol-acetonitrile mixture (1:1, v/v) for protein precipitation. Following centrifugation, the supernatant was collected after centrifugation, concentrated, and reconstituted prior to filtration. The analysis was performed using the Waters UPLC-Xevo TQ-S system equipped with an HSS T3 column (2.1×100 mm, 1.8 μm), employing gradient elution with 0.1% formic acid in water/acetonitrile. An ESI source was used for scanning in both positive and negative ion modes (m/z 50-1200). Data processing was carried out using Progenesis QI, and metabolites were identified through the HMDB/METLIN databases. The screening criteria applied were: $\text{VIP} > 1.0$, $P < 0.05$, or fold change (FC) < 0.67 . KEGG pathway enrichment analysis was visualized using MetaboAnalyst 5.0. All experimental procedures and data analyses were conducted by Majorbio Bio-Pharm Technology Co., Ltd²⁸. The raw sequencing data from this study have been archived in the NCBI SRA under the BioProject accession number PRJNA1256749 (<https://submit.ncbi.nlm.nih.gov/>).

2.4 Liver Tissue Histological and Molecular Pathological Examination

2.4.1 Hematoxylin and Eosin (H&E) Staining

Liver tissue samples were fixed, embedded in paraffin, sectioned, and stained with H&E staining kit (Beyotime, Cat. No.: C0105M) following the manufacturer's instructions. Stained sections were scanned using a 3DHISTECH Panoramic MIDI scanner for histological observation and morphological analysis²⁹.

2.4.2 Quantitative Real-Time PCR (qPCR) Analysis of Inflammation-Related Genes

Total RNA was extracted from liver tissue using the TRIzol reagent method. The extracted RNA was reverse-transcribed into complementary DNA (cDNA) using a reverse transcription kit. qPCR was performed to determine the mRNA expression levels of hepatic inflammation-related genes, including *NF-κB*, *TNF-α*, and *IL-1β*. *β-actin* was used as the internal reference gene. The specific primer sequences for target genes and reference gene are listed in Supplementary Table 2. The relative mRNA expression levels were calculated using the $2^{-\Delta\Delta C_t}$ method.

2.4.3 Western Blot Analysis of Signaling Pathway Proteins

Total protein was extracted from liver tissue using radioimmunoprecipitation assay (RIPA) lysis buffer containing protease inhibitors. Protein concentration was determined using the bicinchoninic acid (BCA) protein assay kit. Equal amounts of protein samples were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred onto polyvinylidene fluoride (PVDF) membranes. After blocking with blocking buffer, the membranes were incubated overnight at 4°C with primary antibodies targeting key proteins in the signaling pathways (Supplementary Table 1). On the following day, the membranes were incubated with horseradish peroxidase (HRP)-conjugated secondary antibodies at room temperature. Protein bands were visualized using a chemiluminescence imaging system. *β-actin* was used as the loading control. The grayscale values of target protein bands were semi-quantitatively analyzed using ImageJ software.

2.5 Intestinal Barrier Function Analysis via Western Blot

The expression levels of intestinal tight junction proteins ZO-1 and Occludin were assessed using the Western blot technique. The experimental procedure involved the preparation of protein samples by homogenizing jejunum tissue in RIPA lysis buffer containing protease inhibitors. The protein concentration was determined using the BCA method, after which 30 μg of protein was subjected to 12% SDS-PAGE electrophoresis (80V for stacking gel and 120V for separating gel). A wet transfer was then performed at 300 mA for 90 minutes onto a PVDF membrane. Following a 2-hour blocking step with 5% skim milk for 2 hours, the membrane was incubated overnight at 4°C with primary antibodies against ZO-1 (1:1000) and Occludin (1:1000). After washing with TBST, the membrane was incubated at room temperature for 1 hour with an HRP-labeled secondary antibody (1:5000) and subsequently developed using ECL chemiluminescence. *β-actin* (1:8000) served as an internal reference for quantitative analysis of band grayscale values, which were analyzed using ImageJ software. All procedures were conducted on ice, and three technical replicates were performed³⁰.

2.6 Gut Microbiota Analysis via 16S rRNA Gene Sequencing

Analysis of the gut microbiota was conducted using 16S rRNA gene sequencing technology. Fecal samples from mice were collected aseptically and stored immediately at -80°C . Total microbial DNA was extracted using a modified CTAB method. After passing quality control, the V4 region of the 16S rRNA gene was amplified using specific primers (515F/806R)³¹. The PCR products were purified and subjected to paired-end sequencing (2×250 bp) on the Illumina MiSeq platform. Raw data were processed for quality control using QIIME2 (v2020.6) and the DADA2 algorithm, resulting in the generation of an ASV feature table. The significance of differential abundance in microbiota between groups was assessed using the Kruskal-Wallis test followed by Benjamini-Hochberg false discovery rate (FDR) correction, with an FDR-adjusted P -value < 0.05 considered statistically significant. All experimental setups included three biological replicates, and the experiments were conducted by Majorbio Co., Ltd.

2.7 Gut-liver Axis Correlation Analysis

Based on previously obtained serum metabolomics and gut microbiota data, a multi-omics integration analysis method was employed to investigate the regulatory mechanisms of the gut-liver axis. Initially, Spearman correlation analysis was conducted on the differential metabolites ($\text{VIP} > 1.0$, $P < 0.05$) and differential microbiota ($\text{LDA} > 2.0$, FDR-adjusted $P < 0.05$) to calculate the correlation coefficients, where $|r| > 0.6$ was considered significantly correlated³². This process aimed to identify metabolite-microbiota combinations that were significantly associated with liver function indicators.

2.8 Statistical analysis

All experimental data were analyzed using SPSS 26.0 statistical software. One-way analysis of variance (ANOVA) was performed, followed by Duncan's multiple range test to determine the significant differences between groups. A $P < 0.05$ was considered statistically significant. All data are presented as the mean $\pm$ standard deviation (SD) of three biological replicates. Graphs were plotted using GraphPad Prism 9.0 software.

3. Results

3.1 Effects of NOB on body weight in mice

The body weight changes in mice treated with different doses of NOB were systematically monitored over a 7-day period (Table 1). The high-dose NOB group (H-NOB) exhibited the most significant and consistent therapeutic effects, demonstrating remarkable weight gain by Day 6 ($29.17 \pm 1.57\text{g}$ vs. $26.25\text{--}27.83\text{g}$ in other groups, $P < 0.05$), which persisted through Day 7 ($30.77 \pm 1.92\text{g}$). Based on these pronounced effects, the H-NOB group was selected for subsequent serum metabolomics analysis and further mechanistic investigations. Conversely, the medium-dose group (M-NOB) exhibited a paradoxical weight reduction on Day 7 ($25.81 \pm 0.99\text{g}$ vs. $29.83\text{--}30.77\text{g}$, $P < 0.05$), while the low-dose group (L-NOB) maintained weights comparable to the controls throughout the study period.

Table 1. Body weight assay data of NOB -treated mice after 7d (g)

Time	CON	Model	L-NOB	M-NOB	H-NOB
1d	24.14 ± 0.47	23.16 ± 0.94	24.18 ± 0.56	23.44 ± 0.55	24.14 ± 1.16
2d	25.25 ± 1.01b	25.00 ± 1.41b	26.83 ± 0.99a	24.42 ± 0.76b	25.17 ± 1.40b
3d	27.02 ± 1.06	26.18 ± 1.23	27.22 ± 0.87	26.31 ± 0.97	27.05 ± 1.47
4d	25.67 ± 0.94	25.33 ± 1.25	25.25 ± 0.83	25.25 ± 0.83	26.42 ± 1.38
5d	27.16 ± 1.04a	25.17 ± 1.25b	26.42 ± 0.94ab	27.11 ± 0.83a	26.95 ± 1.49a
6d	26.58 ± 0.60b	26.25 ± 1.36b	27.83 ± 1.07b	26.42 ± 0.95b	29.17 ± 1.57c
7d	30.50 ± 1.32a	29.83 ± 1.86a	30.56 ± 1.63a	25.81 ± 0.99c	30.77 ± 1.92ab

Note: Note: The data differences between days were compared horizontally, with different letters indicating $P < 0.05$.

3.2 NOB ameliorates serum biochemical profiles, inflammation, and oxidative stress

As depicted in Fig. 1, high-dose NOB (H-NOB) intervention exerts multi-dimensional protective effects on murine blood physiological parameters (all $P < 0.05$). Relative to the blank control (CON) group, the model (Model) group exhibited significant perturbations: 1.3-fold, 2.2-fold, and 1.2-fold elevations in AST, LDL-C, and TC, respectively; 1.5-fold and 1.1-fold increases in pro-inflammatory cytokines IL-6 and TNF- α ; a 1.1-fold rise in oxidative damage marker MDA; and 40% and 30% reductions in antioxidant mediators SOD and GSH. Following H-NOB administration, these aberrations were attenuated: AST and LDL-C decreased by 25% and 45%, with TC restoring to near-CON levels; IL-6 and TNF- α were suppressed by 25% (reverting to CON levels) and 10%, respectively; SOD and GSH activities recovered by 40% (approaching CON levels); and MDA was reduced by 10%. Collectively, NOB modulates serum biochemical profiles to mitigate hepatocellular and lipid metabolic dysfunction, suppresses inflammatory responses, and reinforces antioxidant defense, thereby exerting comprehensive protection on murine blood physiological homeostasis.

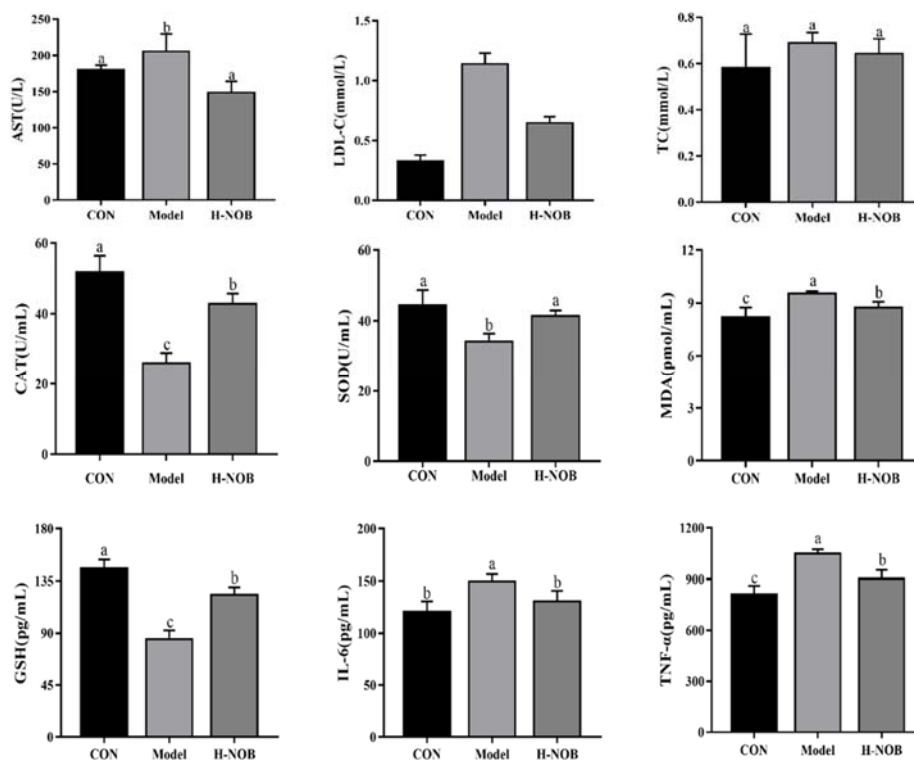


Fig.1 Biochemical indicators in the blood of mice treated with Chuan Chen Pi Su. Serum biochemistry: AST, LDL-C, TC; Inflammatory indicators: IL-6, TNF- α ; Antioxidant indicators: CAT, SOD, MDA, GSH. Different letters indicate significant differences between different groups ($P < 0.05$).

3.4 NOB enhances intestinal barrier integrity

The effect of NOB on the expression of proteins associated with the intestinal barrier function in IPEC-J2 cells was assessed using the Western blot technique in this study. As illustrated in Fig. 3a, the tight junction proteins ZO-1, Occludin, and the internal reference β -actin displayed distinct expression patterns across the various treatment groups (CON, Model, H-NOB). Quantitative analysis (Fig. 3b–c) revealed that the expression level of ZO-1 in the Model group was significantly lower than that in both the CON and H-NOB groups ($P < 0.05$). The H-NOB group showed a significant upregulation of ZO-1 by 2.48-fold compared to the Model group ($P < 0.05$), and its expression was 1.44 times higher than that of the CON group ($P < 0.05$). Similarly, the expression of Occludin in the Model group was significantly lower than that in the CON and H-NOB groups ($P < 0.05$). Compared to the Model group, the H-NOB group exhibited a marked increase in Occludin expression by 2.37-fold ($P < 0.05$), which was also 1.13 times higher than that in the CON group ($P < 0.05$). These results indicate that NOB treatment can partially upregulate ZO-1 expression and effectively restore Occludin levels, suggesting that its protective effect on intestinal barrier function may be mediated through the enhancement of tight junction integrity involving both ZO-1 and Occludin.

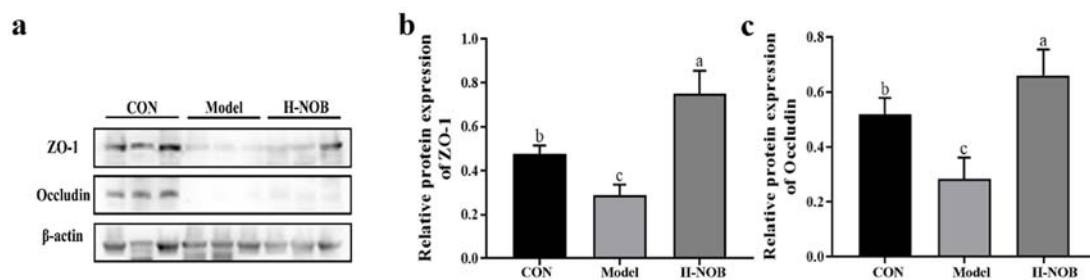


Fig. 3 Expression of intestinal barrier-related proteins in IPEC-J2 cells after treatment with NOB. (a) Western blot (WB) bands: Display the expression of tight junction proteins ZO-1, Occludin, and the internal reference β -actin after treatment with NOB. (b) Expression levels of ZO-1-related proteins in different treatment groups. (c) Expression levels of Occludin-related proteins in different treatment groups. Different letters indicate significant differences between groups ($P < 0.05$).

3.5 NOB restructures gut microbiota composition

The study demonstrates that NOB can modulate the composition and structure of the gut microbiota. The Kruskal-Wallis H test for the ACE index (Fig. 4a) reveals significant differences among the CON, Model, and H - NOB groups ($P = 0.01076$), indicating that different treatments affect microbial richness. Principal coordinates analysis (PCoA) at the OTU level (Fig. 4b) shows clear separation of samples from different groups along the PC1 and PC2 axes, with $R = 0.801$ and $P = 0.00100$ (verified by permutational test), suggesting that NOB treatment alters the structure of the gut microbiota. The Venn diagram (Fig. 4c) illustrates the uniqueness and sharing of OTUs among groups, With the H - NOB group containing 822 OTUs, some of which are shared while others are unique. Changes in the composition of the top 30 dominant bacterial species (Fig. 4d and Supplementary Table 4) indicate that the relative abundances of dominant bacteria vary among groups. For instance, *Lactobacillus johnsonii* exhibits relatively high abundance in the CON group, whereas the composition and abundance distribution of dominant bacteria differ in the Model and H - NOB groups. This suggests that NOB treatment modifies the composition of dominant bacteria in the gut

and impacts the microbial community structure. In conclusion, NOB significantly regulates the richness, structure, and composition of dominant bacteria in the gut microbiota.

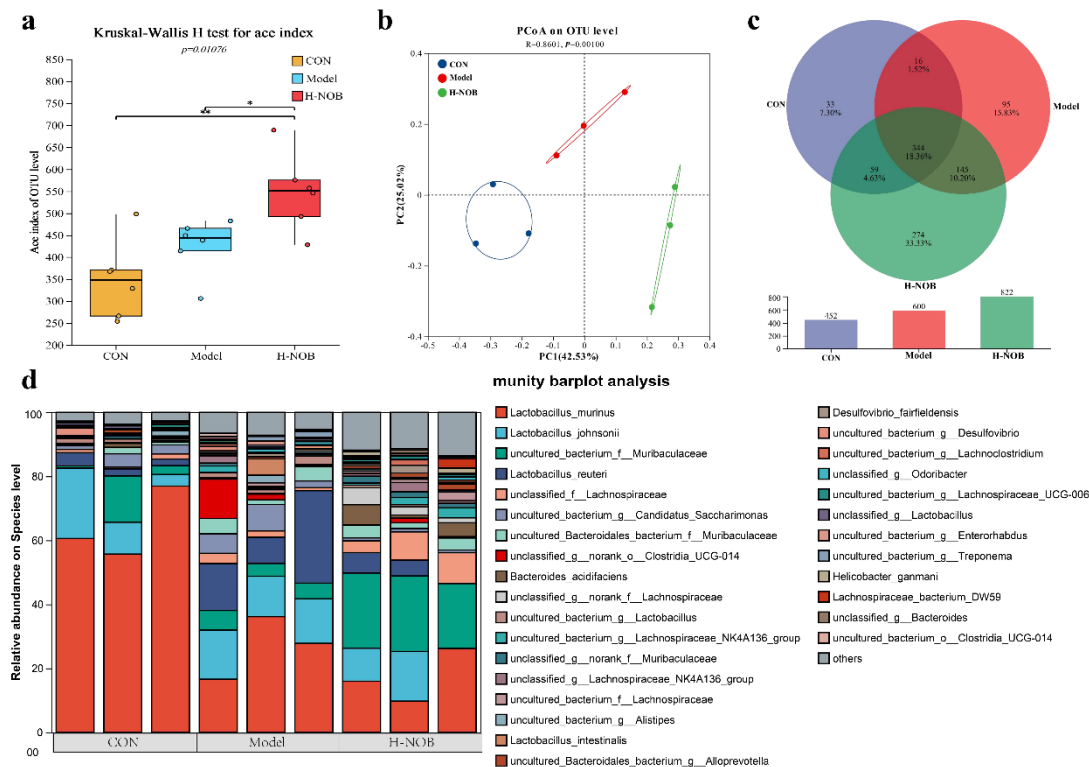


Fig. 4 NOB modulated the composition and structure of the gut microbiota. (a) Kruskal-Wallis H test for the ACE index. (b) PCoA at the OTU level. (c) Venn diagram showing the unique and shared OTUs among groups. (d) Changes in the composition of dominant bacterial species within the Top 30.

3.6 NOB alleviates hepatic oxidative stress and inflammation

This study investigated the effects of NOB on liver indices in mice. Regarding antioxidant indices (Fig. 5a), compared with the CON group, the levels of CAT, SOD, GSH, and T-AOC in the Model group were significantly decreased compared to the CON group, while the MDA level was increased. In the H-NOB group, the levels of CAT, SOD, GSH, and T-AOC were higher than those in the Model group, and the MDA level was decreased, indicating that NOB enhances liver antioxidant capacity. The mRNA expression of inflammatory genes (Fig. 5b) demonstrated that the mRNA expression levels of *NF-κB*, *TNF-α*, and *IL-1β* in the Model group were significantly higher than those in the CON group. Conversely, in the H-NOB group, the expression of these inflammatory genes was significantly lower than that in the Model group, suggesting that NOB can inhibit liver inflammatory responses. H&E staining results (Fig. 5c) showed that the liver tissue structure in the CON group was normal, while the Model group exhibited obvious pathological changes; however, and the pathological condition in the H-NOB group was improved. Analysis of key proteins in the NF-κB pathway (Fig. 5d) indicated that the expression of NF-κB and related proteins was upregulated in the Model group and downregulated in the H-NOB group. In conclusion, NOB enhances the antioxidant capacity of the mouse liver, inhibits inflammatory responses, and improves liver tissue structure, with its effects likely related to the regulation of the expression of key proteins in the NF-κB pathway.

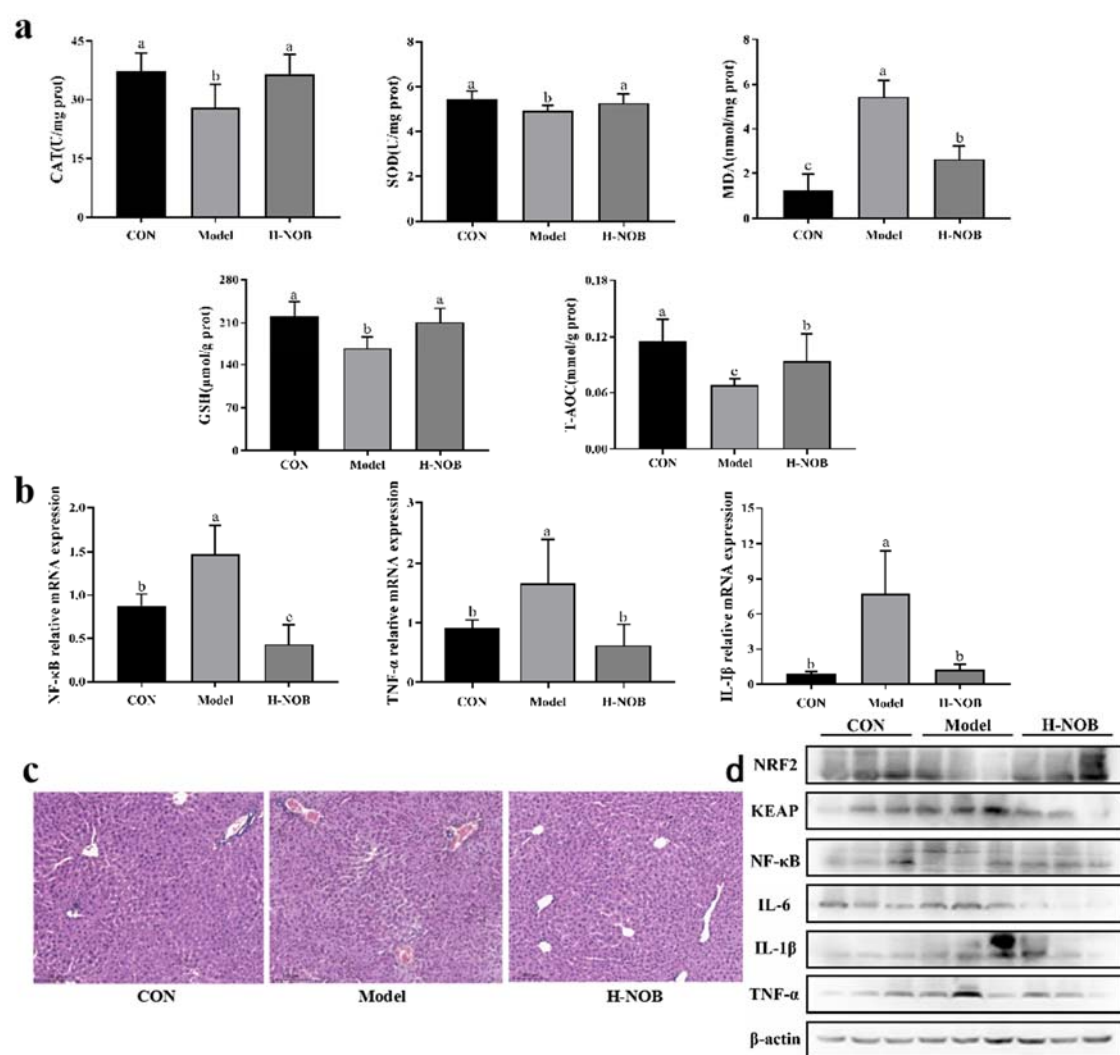


Fig.5 Effects of NOB on Liver Indices in Mice. (a) Antioxidant Indices (CAT, SOD, MDA, GSH, T-AOC); (b) mRNA Expression of Inflammatory Genes (NF-κB, TNF-α, IL-1β). (c) H&E staining; (d) key proteins of the NF-κB pathway.

3.7 Correlation between serum metabolites and gut microbiota

This study revealed a complex interaction network between gut microbiota and metabolites. Positive correlations were observed, with increased abundance of *Lactobacillus murinus* showing a significant positive association with elevated levels of PC and PE (Fig. 6). This effect is likely due to the promotion of key enzyme activity in related metabolic pathways, thereby enhancing the synthesis of PC and PE. Meanwhile, *Lactobacillus murinus* may regulate the catabolic processes of LysoPC, leading to a reduction in serum LysoPC levels. *Bacteroides acidifaciens* exhibited a more complex regulatory pattern, potentially influencing the metabolic balance of PC, PE, and LysoPC indirectly by altering intestinal pH through fermentation. Conversely, negatively correlated microbes such as *unclassified_f_Muribaculaceae* were associated with decreased levels of these metabolites. Their mechanisms may involve substrate competition, inhibition of synthase activity (reducing PC and PE synthesis), and promotion of LysoPC catabolism (lowering serum LysoPC levels). These findings suggest that gut microbiota participates in host metabolic regulation through multiple pathways, collectively forming a finely tuned metabolic network that is crucial for maintaining physiological homeostasis.

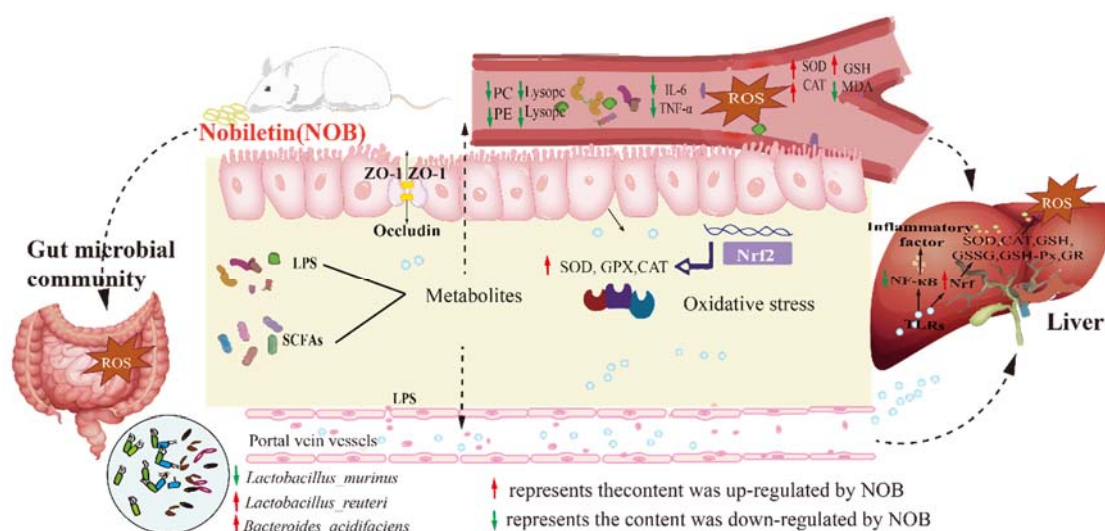


Fig.7 The working model of NOB (NOB) in alcohol-induced mice. NOB alleviates pathological damage in the intestine and liver, including inflammatory responses, oxidative stress, intestinal barrier, and lipid metabolism levels, by modulating the gut microbiota-associated gut-liver axis.

4. Discussion

4.1 Glycerophospholipid Remodeling Attenuates Liver Injury and HCC Risk

This study elucidates the multi-target mechanism by which nobiletin (NOB) ameliorates alcoholic liver injury via the gut-liver axis. Beyond confirming its efficacy in improving body weight and liver function (reduced AST), our metabolomic analysis reveals a critical intervention: NOB specifically upregulates PC and PE synthesis while inhibiting their LysoPC/LysoPE³³. This glycerophospholipid remodeling is directly linked to core ALD pathology. The restored PC/PE ratio is vital for maintaining hepatocyte membrane integrity and, importantly, mitochondrial membrane fluidity and function³⁴. Alcohol metabolism disrupts endoplasmic reticulum homeostasis, impairing PC synthesis via the PEMT pathway, leading to mitochondrial dysfunction, impaired very-low-density lipoprotein (VLDL) secretion, and subsequent hepatic steatosis³⁵. Furthermore, persistent phospholipid imbalance and the accumulation of cytotoxic lyso-phospholipids promote sustained hepatocyte apoptosis and necrosis³⁶. This repeated cycle of injury, regeneration, and inflammation creates a pro-mutagenic microenvironment, increasing the risk of DNA damage and clonal expansion of mutated hepatocytes—a foundational step towards mutation-driven hepatocellular carcinoma (HCC)³⁷. Our finding that NOB regulates LPCAT and PEMT activities positions it as a direct modulator of this pathogenic metabolic node.

4.2. Microbiota-Host Crosstalk Mediates Systemic Protection

The gut-liver axis mediation by NOB is underscored by its specific enrichment of *Lactobacillus murinus* and the strong positive correlation between this bacterium and serum PC levels ($r=0.82$, $P < 0.01$). This observation aligns with the growing paradigm that natural compounds exert hepatoprotection by reshaping gut microbiota and its metabolite profile³⁸. We propose a novel “microbiota-metabolite-host” cascade: NOB-enriched *Lactobacillus* spp. likely enhance intestinal barrier function (supported by upregulated ZO-1)³⁹, reducing systemic LPS influx. Beyond barrier fortification⁴⁰, our in vitro evidence that

L. murinus supernatant enhances hepatocyte PC synthase activity suggests microbiota-derived factors directly signal to the liver⁴¹. This concept of remote organ crosstalk is reinforced by recent evidence showing that microbiota modulation can simultaneously influence hepatic metabolism, systemic inflammation, and even extra-hepatic systems, forming a complex communication network⁴². Specifically, dietary interventions with bioactive polysaccharides have been shown to improve liver health by elevating gut-derived butyrate, a key microbial metabolite that enhances colonic tight junction function and exerts anti-inflammatory effects systemically⁴³. Our findings on NOB's effect on gut barrier proteins and microbial shifts resonate with this mechanism, suggesting shared final pathways among different nutraceuticals.

4.3 Integrative Actions and Comparative Advantages of Nobletin

The therapeutic potential of NOB stems from its integrative, multi-pathway modulation, a feature that distinguishes it from many single-target flavonoids. Unlike compounds with narrower mechanisms, NOB exerts a coordinated effect by simultaneously activating the hepatic Nrf2 pathway to enhance endogenous antioxidant defenses⁴⁴, suppressing the NF- κ B signaling cascade to mitigate inflammatory liver injury⁴⁵, and specifically remodeling dysregulated glycerophospholipid metabolism—a core pathogenic event in ALD⁴⁶. While other flavonoids such as quercetin and catechins possess documented antioxidant and anti-inflammatory properties, evidence for their targeted regulation of phospholipid homeostasis remains comparatively limited^{47,48}. Beyond its broad mechanistic scope, NOB offers practical translational advantages, including a favorable pharmacokinetic profile and established safety in preclinical models, which contrasts with the challenges of poor bioavailability seen in compounds like naringenin⁴⁹. This synergistic action across the gut barrier–microbiota–metabolism–inflammation axis enables NOB to address the multifactorial pathogenesis of ALD more comprehensively than agents with a singular focus, positioning it as a promising multi-target candidate for therapeutic development.

4.4 Clinical translation and future perspectives

The translational promise of NOB is supported by its safety and multi-target efficacy. The identified PC/PE ratio could serve as a novel pharmacodynamic biomarker for patient stratification in clinical trials. A logical development is a NOB-Lactobacillus synbiotic formulation to potentiate gut-liver axis modulation⁵⁰. Study Limitations and Future Work: While we established a correlation between *L. murinus* and PC metabolism, causal validation requires germ-free animal models or precise colonization studies. The exact molecular mediators from the microbiota remain unidentified. Future research should employ intestinal-specific LPCAT/PEMT knockout models^{51,52} to dissect NOB's local intestinal effects versus systemic impacts. Additionally, humanized organoid models and preclinical PK/PD studies will be crucial to evaluate human relevance and optimize dosing for clinical trials, not only for ALD but also for other metabolic liver diseases like NAFLD.

5. Conclusion

This study demonstrates NOB that alleviates alcohol-induced liver injury via the gut-liver axis. NOB modulates gut microbiota, upregulates ZO-1 and Occludin to enhance the intestinal barrier, and reduces LPS translocation. In the liver, NOB activates Nrf2 to boost antioxidants levels while inhibiting NF- κ B to suppress inflammation. Metabolomics reveals that NOB regulates the balance of PC/PE and LysoPC/LysoPE. Correlation analysis between microbiota and metabolites suggests that NOB exerts hepatoprotection through crosstalk among gut microbiota, metabolism, and inflammation. These findings support the potential of NOB as a functional food component for the intervention of alcoholic liver disease.

Supplementary Materials

Supplementary Table 1: Key reagents and antibodies used in this study; Supplementary Table 2. qPCR Primer Sequences; Supplementary Table 3. Differential metabolites of the glycerophospholipid metabolic pathway; Supplementary Table 4. Taxonomic composition and relative abundance of gut microbiota OTUs across experimental groups.

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Conflicts of interest

The authors declare no conflicts of interest.

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