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Characterization of polysaccharides isolated from *Dendrobium loddigesii* Rolfe and study of its hepatoprotective activity in vivo

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ABSTRACT: The polysaccharides extracted from *Dendrobium loddigesii* (DLPs) possess significant nutritional and functional properties that position them as promising candidates for the development of functional foods. The rising incidence of liver diseases, particularly those induced by drugs and environmental toxins, underscores the urgent need for safer, more effective therapeutic interventions. Liver injury, characterized by its complex pathophysiology, can lead to severe outcomes, including acute liver failure, highlighting the limitations of traditional therapies, which often entail adverse effects. This study explores the potential of DLPs as hepatoprotective agents against carbon tetrachloride (CCL₄)-induced liver injury in murine models, with a focus on their health-promoting effects. DLPs were extracted and structurally characterized, followed by *in vivo* assessments of their efficacy in mitigating liver damage. Results demonstrated that DLPs reduced serum markers of liver injury (ALT, AST, AKP) and alleviated hepatocyte necrosis and inflammation. Furthermore, metabolomic analysis revealed that DLPs modulate lipid metabolism and the gut-liver axis, suggesting their role in reducing oxidative stress and inflammation. Notably, DLPs exhibited prebiotic-like effects by enhancing gut microbiota diversity, further supporting their potential as functional food ingredients. These findings provide a strong basis for the nutritional and health benefits of DLPs, highlighting their application in promoting liver health and overall wellness within the context of functional foods.

Keywords: Functional foods; *Dendrobium loddigesii* Rolfe; Polysaccharides; Hepatoprotection; Gut microbiota

1. Introduction

Liver health is a critical aspect of overall wellness, as the liver plays a central role in metabolism, detoxification, and nutrient storage [1]. Liver injury remains a significant global health concern, stemming from various causes including viral infections, alcohol abuse, and notably, drug-induced and chemically induced damage [2-4]. These forms, known as drug-induced liver injury (DILI) and chemical liver damage, are critical in clinical medicine due to their potential to precipitate acute liver failure, which carries high morbidity and mortality rates [5, 6]. DILI is challenging to predict and manage due to the vast array of

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therapeutic agents and chemicals that can cause liver injury via idiosyncratic or dose-dependent mechanisms [7, 8]. This complexity underscores the urgent need for a deeper understanding of the biochemical pathways involved in liver injury and for the development of more effective therapeutic strategies, highlighting the pressing need for interventions that can prevent liver damage while promoting long-term liver health [9].

The mechanisms of liver injury are complex, involving a cascade of events that initiate inflammatory [10, 11] and oxidative stress responses [12, 13]. Upon exposure to hepatotoxic agents, the liver typically shows an inflammatory response, marked by the activation of Kupffer cells [14, 15] and the subsequent release of cytokines and chemokines [16, 17]. These mediators facilitate the infiltration of immune cells into the liver [17], exacerbating injury through the production of reactive oxygen species (ROS) [18]. Simultaneously, oxidative stress occurs when the balance between antioxidants and pro-oxidants is disturbed, leading to lipid peroxidation [19], and damage to proteins and nucleic acids, further impairing liver function.

Amid these challenges, there is growing interest in the development of hepatoprotective agents, especially those derived from natural sources. Traditional Chinese Medicine (TCM) has long utilized various herbs and substances reputed to protect the liver [20, 21]. In recent years, the development of functional foods has garnered significant attention for their ability to provide nutritional benefits alongside health-promoting effects [22, 23]. Notably, polysaccharides extracted from medicinal and food plants are recognized for their antioxidative and anti-inflammatory properties [24], which could mitigate hepatic injuries by modulating immune responses [25, 26] and reducing oxidative stress [27]. These natural compounds are emerging as potential candidates for improving liver health, particularly through their ability to mitigate oxidative stress and inflammation while supporting gut health.

Dendrobium loddigesii Rolfe, a species within the *Dendrobium* genus, is celebrated for its medicinal virtues [28]. These plants are commonly harvested for their stems, which have been used in TCM for millennia. The stems of *Dendrobium* have been used since ancient times not only as an ingredient for juicing beverages but also as a key component in soups, offering rich nutritional value and a variety of health benefits in people's daily life. Pharmacologically, *Dendrobium* species are noted for their diverse array of bioactive compounds, including alkaloids [29], phenanthrenes [30], and polysaccharides [31–33]. These substances contribute to a broad spectrum of health benefits, such as anti-tumor, anti-inflammatory, and immune-boosting properties [29, 34]. Polysaccharides from *Dendrobium* are of particular interest due to their potential applications in functional foods, offering both nutritional value and health benefits. The inclusion of *Dendrobium* in the list of medicinal and edible plants has further positioned it as a promising ingredient for food formulations aimed at enhancing health and wellness [35]. Previous studies have demonstrated that aqueous extracts of *Dendrobium loddigesii* Rolfe can modulate cytokine expression in isolated human peripheral blood mononuclear cells (PBMCs) [36]. Specifically, ethanol extracts are known to significantly suppress the expression of inflammatory cytokines IL-1 β and TNF- α in LPS-induced PBMCs, thereby inhibiting the NF- κ B pathway. Given these foundational results, further investigation into the polysaccharides of *Dendrobium loddigesii* Rolfe (DLPs) is eagerly anticipated.

In this study, the effectiveness of DLPs in combating liver injury induced by carbon tetrachloride (CCL₄) has been thoroughly examined, revealing its comprehensive role in liver protection. In models of acute liver injury, DLPs not only lower serum levels of ALT, AST, and AKP, indicating reduced hepatocellular damage, but also strengthen the antioxidative defenses of the liver. Furthermore, DLPs exhibit significant anti-inflammatory effects. Metabolomic studies have shown that DLPs affect lipid metabolism pathways, particularly those involved in the metabolism of arachidonic acid and long-chain saturated fatty acids, which are crucial in mediating inflammatory responses and oxidative stress. By altering these metabolic pathways, DLPs not only mitigate oxidative stress but also promote cellular recovery and repair mechanisms in the liver. Moreover, we have confirmed that DLPs can regulate the PPAR signaling pathway by inhibiting the JAK2/IL-6 pathway to reduce inflammatory responses and promoting the STAT5 pathway to inhibit hepatocyte fibrosis. This action protects the liver's normal metabolic state, thereby safeguarding the liver from chemical-induced damage. Overall, in this study, we explored the hepatoprotective effects of DLPs, a novel polysaccharide extracted from *Dendrobium loddigesii* Rolfe, representing a significant advance in the natural treatment of liver diseases. By modulating biochemical pathways involved in inflammation and oxidation, and by altering liver metabolism, DLPs provide a comprehensive approach to liver protection. The findings of this study not only contribute to understanding the mechanisms underlying DLPs' health benefits but also highlight their potential as functional food ingredients aimed at improving liver health and overall wellness.

2. Materials and methods

2.1. Materials and reagents

Dendrobium loddigesii Rolfe herbs (220901) were purchased from Guangzhou Zisun Chinese Pharmaceutical Co., Ltd. Silymarin (S10165) was purchased from Beijing JM Biotechnology Co., Ltd. Carbon tetrachloride (C805332) and corn oil (C805618) were obtained from Shanghai Macklin Biochemical Technology Co., Ltd. Anhydrous ethanol, chloroform, isopropanol, and other organic reagents were procured from Sinopharm Chemical Reagent Co., Ltd. Alanine aminotransferase assay kit (C009-2-1), aspartate aminotransferase assay kit (C010-2-1), alkaline phosphatase assay kit (A059-2-2), malondialdehyde assay kit (A003-1-2), superoxide dismutase assay kit (A001-3-2), glutathione peroxidase assay kit (A005-1-2), and catalase assay kit (A007-2-1) were all purchased from Nanjing Jiancheng Bioengineering Institute. TNF- α , IL-6, and IL-1 β assay kits (Elisa) were bought from Hunan Aifang Biotech Co., Ltd.

2.2. Extract, isolate, and purify the polysaccharides from *Dendrobium loddigesii* Rolfe

The stems were first dried at 50°C for 12 hours and then pulverized using a grinder. Five hundred grams of the dried powder was soaked in 5000 mL of deionized water at room temperature for 30 minutes, maintaining a solid-to-liquid ratio of 1:10. The mixture was then subjected to two hours of reflux heating, followed by filtration and retention of the residue for a repeat of the reflux process. The filtrates from both refluxes were combined and concentrated using a rotary evaporator at 60°C until the volume was reduced to a 1:2 solid-to-liquid ratio (1000 mL). Anhydrous ethanol was added until the ethanol concentration exceeded

80%, and the solution was left to precipitate at 4°C overnight. The precipitate was filtered the next day, redissolved in deionized water to 1000 mL, and treated again with anhydrous ethanol to reach an ethanol concentration over 80%, followed by overnight precipitation at 4°C. The final precipitate was redissolved in 800 mL of deionized water. Proteins were removed using the Savage method (chloroform:butanol = 5:1). After adding 200 mL of Savage reagent and mixing thoroughly, the solution was centrifuged at 4°C at 12000 r/min for 10 minutes. The upper aqueous layer was carefully collected, and the protein and organic solvent layers were discarded. This deproteinization step was repeated until no visible protein layer remained. The residual organic solvents were removed via rotary evaporation, and the polysaccharide solution was concentrated and then freeze-dried to yield refined DLPs, weighing 12 g, with an extraction yield of 2.4%.

2.3. Structural characterization of DLPs

2.3.1. UV-Visible Spectroscopy

Following the methods as previously described [37], a 1 mg/mL DLPs solution was prepared and scanned using a UV-visible spectrophotometer across the 100-900 nm wavelength range, documenting the resultant UV-visible spectrum.

2.3.2. Fourier Transform Infrared Spectroscopy (FTIR)

DLPs was characterized using a Fourier Transform Infrared Spectrometer (Nicolet iN10), analyzing the chemical bonds and functional groups present. Prior to testing, 1 mg of the sample was thoroughly mixed with 200 mg of dry KBr powder and pressed into a pellet, then scanned over a wavelength range of 4000 to 400 cm^{-1} .

2.3.3. Monosaccharide composition analysis

The monosaccharide composition was determined using High-Performance Anion-Exchange Chromatography with Pulsed Amperometric Detection (HPAEC-PAD) following the methods as previously described [38]. A 2 mg/mL DLPs solution was acidified with 4 M trifluoroacetic acid (TFA) at 110°C for 10 hours. After drying the hydrolysate with a Termovap Sample Concentrator, methanol was used to remove TFA through three drying cycles, followed by dilution in 6 mL of deionized water. The solution was filtered through a 0.22 μm membrane and 25 μL was injected into an ICS-5000 system equipped with a Carbopac-PA10 analytical column (250 mm $\times$ 4 mm) and an electrochemical detector. The column was washed with 18 mmol/L NaOH for 15 minutes, followed by elution with 18 mmol/L NaOH in 100 mmol/L sodium acetate for 35 minutes. The flow rate was set at 1 mL/min, with data collection and analysis performed at 30°C.

2.3.4 Molecular weight calculation

5 mg of DLPs were dissolved in 5 mL of NaNO_3 solution (containing 0.02% NaN_3), filtered through a 0.45 μm filter, and prepared for chromatography. Chromatographic conditions included the use of Ohpak SB-804 HQ (300 mm $\times$ 8 mm) and Ohpak SB-806 HQ (300 mm $\times$ 7.8 mm) columns at 25°C. The mobile phase contained 0.1 M NaNO_3 with 0.02% NaN_3 , with a sample volume of 100 μL and a flow rate of 0.6 mL/min. A

uniform elution gradient was maintained for 60 minutes, detected by a multi-angle laser light scattering detector (DAWN 132 HELEOS II).

2.3.5. Rheological analysis

2 g of DLPs was dispersed in 100 mL of deionized water and stirred magnetically for 2 hours to form a 2% homogeneous gel. This mixture was then stirred at 80°C (150 r/min) for 30 minutes using a thermostatic magnetic stirrer, and left to stand at room temperature for 24 hours. The rheological properties were measured using an MCR 302 rheometer with a parallel plate sensor (gap 1 mm, diameter 24.979 mm). Constant frequency measurements were made at 1 Hz and 25°C, with stable shear testing conducted over a range of 0.01 to 0.1 s⁻¹. Strain scanning tests ranged from 0.01% to 100%, performed at a constant frequency of 1 Hz and temperature of 25°C to determine the viscoelastic range. Dynamic scanning tests were conducted at constant strain amplitudes within the linear range, with frequency settings from 0.1 to 10 Hz.

2.3.6. The detection and analysis of DLPs glycosidic bonds

In this study, we employed a synthetic approach combining partial methylation of monosaccharides and reductive end labeling, along with ultra-performance liquid chromatography-triple quadrupole mass spectrometry (UPLC-TQD), for the quantitative determination and detection of different glycosidic bonds within polysaccharides [39]. Derivatized monosaccharides were analyzed using a Waters ACQUITY UPLC H-Class system coupled with a Waters Xevo TQD triple quadrupole mass spectrometer in dynamic multiple reaction monitoring (dMRM) mode. The chromatographic column selected was a Waters ACQUITY UPLC BEH C18 column (2.1 mm×100 mm, 1.7 μm), with a column temperature of 40 °C and a sample temperature of 15°C, and an injection volume of 2 μL. The mobile phase A consisted of a 5% acetonitrile/water solution containing 10 mmol/L ammonium acetate (adjusted to pH 8.2 with ammonia solution), while mobile phase B was composed of 95% acetonitrile/water solution. The flow rate was set at 0.3 mL/min, with the following gradient: (0.0-10.0) min, (88-82)% A; (10-20) min, (82-85)% A; (20-25) min, (85-65)% A; (25-25.1) min, (65-0)% A; (25.1-27.1) min, (0-88)% A; (27.1-30.0) min, 88% A. Electrospray ionization (ESI) was used as the ion source in positive ion mode, with a capillary voltage of 3.5 kV, cone voltage of 40V, desolvation temperature of 500°C, desolvation gas flow of 1000 L/h, cone gas flow of 50 L; curtain gas and collision gas pressures were set to 30 psi and 10 psi, respectively; nebulizer gas and auxiliary gas pressures were 55 psi; collision energy was set at 30 V for collision-induced dissociation (CID). After data acquisition, analysis was performed using MassLynx™ software.

2.4. Animal experiment process

The use of carbon tetrachloride (CCl₄) to induce acute liver damage in mice provides a reliable, reproducible, and low-mortality model that closely mimics the pathophysiological aspects of human liver diseases [40, 41]. Therefore, this study employs the CCl₄ induction method. The experiment involved 45 SPF male BALB/c mice, aged six weeks (from Hangzhou Medical College), with an average weight of (22 ± 2) g. The mice were acclimatized for five days under controlled conditions at (24 ± 2)°C, with a relative humidity of

55%–65%, and a 12-hour light/dark cycle. They had free access to water and food. The animal experiment design complied with the ARRIVE guidelines and was approved by the Institutional Animal Care and Use Committee of Zhejiang University (ZJU20240268).

The BALB/c mice were randomly divided into five groups: control group, model group (induced with CCL₄, no treatment), silymarin group (50 mg/kg), DLP-low group (50 mg/kg), and DLP-high group (200 mg/kg), with nine mice per group. All groups except the control and model were administered their respective doses via oral gavage at a volume of 10 mL/kg once daily for 14 days. Three hours after the final dose, the model and treatment groups received an intraperitoneal injection of 0.3% CCL₄ solution diluted in corn oil at a volume of 10 mL/kg, while the control group received an equivalent volume of corn oil.

2.5. Sample collection and analysis of animals

2.5.1. General observations of mice

During the treatment period, observations were made regarding the fur condition and general behavior of the mice, with body weights measured every two days. Eighteen hours post-modeling, the mice were anesthetized for blood collection, and subsequently euthanized by cervical dislocation. The liver, kidneys, and spleen were harvested, rinsed with saline, dried using filter paper, and weighed to calculate the organ coefficients, which were expressed as organ weight divided by body weight.

2.5.2. Detection of liver function-related biomarkers in serum

Eighteen hours post-modeling, blood was collected from each group, allowed to settle for one hour, and then centrifuged at 3000 r/min for 10 minutes. The supernatant serum was used to measure the levels of ALT, AST, and AKP according to the reagent kit instructions.

2.5.3. Detection of inflammatory cytokines in serum

Similarly, after blood collection and centrifugation as described above, the upper layer of serum was used to determine the concentrations of IL-1 β , IL-6, and TNF- α using ELISA kits as per manufacturer's instructions.

2.5.4. Detection of related metabolic enzymes in liver tissue

Liver tissue from the left lobe was homogenized in cold saline at a 1:9 (w/v) ratio. The homogenate was centrifuged at 3500 r/min for 10 minutes at 4°C, and the supernatant was assayed for SOD, CAT, GSH-Px, and MDA levels following the manufacturer's instructions provided with the reagent kits.

2.5.5. Histological staining of liver

After weighing, liver samples were photographed for morphological examination. Right lobe liver tissues were fixed in 4% paraformaldehyde, embedded in paraffin, and sectioned for staining with hematoxylin and eosin to observe pathological changes such as hepatocyte swelling, necrosis, and inflammatory infiltration. Additionally, Masson staining was performed to assess fibroprotein expression in the tissues. All the staining was performed by Hangzhou Haoke Biotechnology Co., Ltd.

2.5.6. Metabolomics studies of serum and liver tissue

2.5.6.1. Sample collection and preparation

To prepare the serum samples, 80 μ L of serum was mixed with four times the volume of MeOH: ACN (1:1) solution, vortexed for 30 seconds, and then stored at -20°C for 1 hour. Afterwards, the samples were centrifuged at 4°C at 15000 r/min for 20 minutes to obtain the supernatant, which was then evaporated to dryness. The residue was reconstituted in an equal volume of cold ACN: H_2O (1:1) and left to equilibrate prior to analysis.

For the liver tissue sample preparation, the liver tissue (-10 mg) was homogenized in 1300 μ L of pre-chilled 80% methanol, then subjected to rapid freeze-thaw cycles in liquid nitrogen to disrupt the cells. After incubation at -20°C for 1 hour, the samples were centrifuged at 4°C at 15000 r/min for 20 minutes. The supernatant was collected, dried under vacuum, and reconstituted based on tissue weight in cold ACN: H_2O (1:1), centrifuged, and prepared for analysis.

2.5.6.2. LC-MS/MS analysis

Metabolomics analysis of the mouse serum and liver was conducted using an Agilent 6546 LC/Q-TOF system. For positive ion mode: an Agilent InfinityLab Poroshell 120 EC-C18 Column (2.1 x 50 mm, 1.9-Micron) was used at 25°C , with a sample volume of 6 μ L and a flow rate of 0.4 mL/min. The mobile phase consisted of 0.1% formic acid in water (A) and acetonitrile (B), starting at 95% A for 1 minute, reducing to 0% over 7 minutes, held for 2 minutes, then returned to 95% A within 0.1 minute, and maintained for 1.9 minutes before re-equilibration for 5 minutes. For negative ion mode: a Waters ACQUITY UPLC BEH Amide Column (2.1 x 100 mm, 1.7 μ m) was used, with the same temperature and flow rate settings but with a mobile phase of 25 mmol/L ammonium acetate with 25 mmol/L ammonia (A) and acetonitrile (B). The initial mobile phase ratio started at 5% A, increasing linearly to 35% A over 6.5 minutes, reaching 60% A in the next minute, held for another minute, then reduced to 5% A within 0.1 minutes and maintained for 2.9 minutes before re-equilibration. Both positive and negative ion modes utilized ESI source conditions set as follows: Dual ASJ ion source, drying gas temperature 320°C , flow rate 8 L/min, nebulizer pressure 35 psi, sheath gas temperature 350°C , flow rate 11 L/min, and full scan m/z range of 50–1200.

2.5.6.3. Multivariate data processing and analysis

Data acquired were subjected to chemometrics and multivariate statistical analysis to reduce dimensionality and classify data, extracting the most useful information. Raw data were converted to mzML format using ProteoWizard software, followed by retention time alignment, peak integration, and normalization using XCMS. The MS1 peak lists (.csv), MS/MS data (.mgf), and sample metadata (.csv) processed by XCMS were uploaded to the MetDNA2 Web Server for metabolite annotation and dysregulated network analysis based on a Knowledge-based Metabolic Reaction Network (KMRN). Subsequently, the knowledge-guided multi-layer metabolic networking (KGMN) approach was used to recursively annotate and analyze metabolites from known to unknown. Multifactorial statistical analysis further studied the metabolic phenotype of serum and liver to discern subtle differences between the control, model, and treatment groups. Data matrices were analyzed using MetaboAnalyst 5.0, employing principal component analysis (PCA) to

investigate differences among groups, integrating fold change and significance ($FC > 1.2$, $P\text{-value} < 0.05$) to identify differential metabolites and pathways potentially regulated by DLPs. Hierarchical clustering heatmap analysis was used to visualize the concentration of each metabolite.

2.5.7. Detection and analysis of mouse gut microbiota

2.5.7.1. Sample collection

Eighteen hours after modeling, fresh fecal samples from each group of mice were collected. Mice were fasted but allowed water access for 12 hours prior to sampling. Samples were immediately placed in sterile tubes and stored at -80°C . High-throughput sequencing was performed by Shanghai Majorbio Biopharm Technology Co., Ltd.

2.5.7.2. DNA extraction, PCR amplification, and sequencing library construction

Microbial community genomic DNA was extracted from the fecal samples of each group using the FastPure Stool DNA Isolation Kit, with 1% agarose gel electrophoresis employed to check the integrity of the extracted genomic DNA. DNA concentration and purity were assessed using a NanoDrop 2000. Libraries were constructed from the purified PCR products using the NEXTFLEX Rapid DNA-Seq Kit. Sequencing was conducted on the Illumina NextSeq 2000 PE300 platform provided by Shanghai Majorbio Biopharm Technology Co., Ltd.

2.5.7.3. High-throughput sequencing data analysis

To minimize the impact of sequencing depth on subsequent alpha and beta diversity analyses, the number of sequences from all samples was normalized to 20,000. Taxonomic annotation of OTUs was performed against the Silva 16S rRNA gene database (v138) using the RDP classifier (version 2.13) with a confidence threshold of 70%. Community composition for each sample was quantified at various taxonomic levels. All data analyses were performed on the Majorbio Cloud platform (<https://cloud.majorbio.com>).

2.5.8. Western blot

For western blotting analysis, mice liver samples were utilized. 50 mg liver tissues were used for protein extraction, working solution (1000 μL) was added to each homogenizer to homogenize centrally. The tissues were centrifuged at 4°C for 5 minutes at 14,000 g. The supernatant was collected and protein concentration was determined by the BCA method. A 40 μg protein sample was subjected to a running gel phase with 8% isolate gel, followed by transferring the protein to a PVDF membrane, after which the membrane was blocked with 5% skim milk for one hour. The information of primary antibody (1:1000) and secondary antibody (1:60000) are as follow: p-JAK2 Rabbit mAb (db14085), t-JAK2 Rabbit mAb (db14880), p-STAT5 Rabbit pAb (db14090), t-STAT5 Rabbit mAb (db12807), RXR α Rabbit mAb (db12582), PPAR α Rabbit mAb (db16114), PPAR γ Rabbit mAb (db11579) were all purchased from Diagbio Technology Co.,Ltd (China). HRP Goat Anti-Mouse IgG (H+L) (HA1006), HRP Goat Anti-Rabbit IgG (H+L) (HA1001) were all purchased from HUABIO Technology Co.,Ltd (China).

2.5.9. Immunofluorescence staining

Paraffin sections were routinely dewaxed which was placed in sodium citrate buffer for antigen repair. Standard goat serum working solution was added for blocking by incubation for 15 minutes. These slices were incubated overnight at 4°C with the following primary antibodies. PPAR α Rabbit pAb (YP-Ab-03324), PPAR γ Rabbit mAb (YP-Ab-03262) were all purchased from UpingBio Technology Co.,Ltd (China). Then, the corresponding fluorescent secondary antibody was incubated for 30 min at 37°C. The images were presented by microscope and measured using ImageJ software.

2.6. Statistical analysis

Data were statistically analyzed and graphed using GraphPad Prism 8.01 and SPSS 21.0. Experimental data were presented as mean $\pm$ standard error. Comparisons between two groups were performed using an independent samples t-test, while comparisons among multiple groups utilized one-way ANOVA with Tukey's post-hoc test for inter-group differences. A two-tailed *P*-value of less than 0.05 was considered statistically significant.

3. Results

3.1. Extraction and basic structural characterization of DLPs

Figure 1A illustrates the extraction process of refined DLPs, yielding approximately 12 g DLPs from 500 g raw material, corresponding to a yield of 2.4%. The UV-visible spectra, shown in Figure 1B, display minimal absorption peaks near 260 and 280 nm, indicating low nucleic acid and protein content, suitable for further research. The FT-IR spectrum in Figure 1C reveals characteristic peaks of polysaccharides: a strong O-H stretching vibration at 3391 cm^{-1} and weaker C-H stretching vibrations between 2900 and 2800 cm^{-1} , indicating the presence of CH, CH₂, and CH₃ groups. The absorption peak at 1602 cm^{-1} corresponds to the ester carbonyl C=O stretching, suggesting the presence of uronic acids, which align with the monosaccharide composition results. The peaks at 1083 cm^{-1} , 618 cm^{-1} , and 546 cm^{-1} are indicative of glucose, consistent with the analysis of monosaccharide components, confirming DLPs as carbohydrate compounds. Monosaccharide analysis by PMP pre-column derivatization HPLC is shown in Figure 1D, illustrating DLPs' complex composition of ten different monosaccharides. The ratio of the Glc: Man: Gal: Ara: Xyl: GlcA: Rha: Fuc is 41.09: 31.67: 11.85: 3.84: 3.83: 3.47: 2.28: 1.95. The molecular weight (MW) of DLPs is 1.116×10^5 kDa, as shown in Figure 1E, with variations reflecting the sugar composition, glycosidic linkages, and branching. Figures 1F and 1G display the viscoelastic and steady-state flow behavior of DLPs at a concentration of 2 mg/mL across shear rates from 0.01 to 100 s^{-1} , demonstrating non-Newtonian shear-thinning flow characteristics. In DLPs, the storage modulus (G') consistently exceeds the loss modulus (G''), indicating a relatively weak gel-forming ability. This observation aligns with subsequent biological tests, where DLPs form low-viscosity aqueous solutions at high doses (200 mg/kg equivalent to 20 mg/mL), demonstrating weak gel strength.

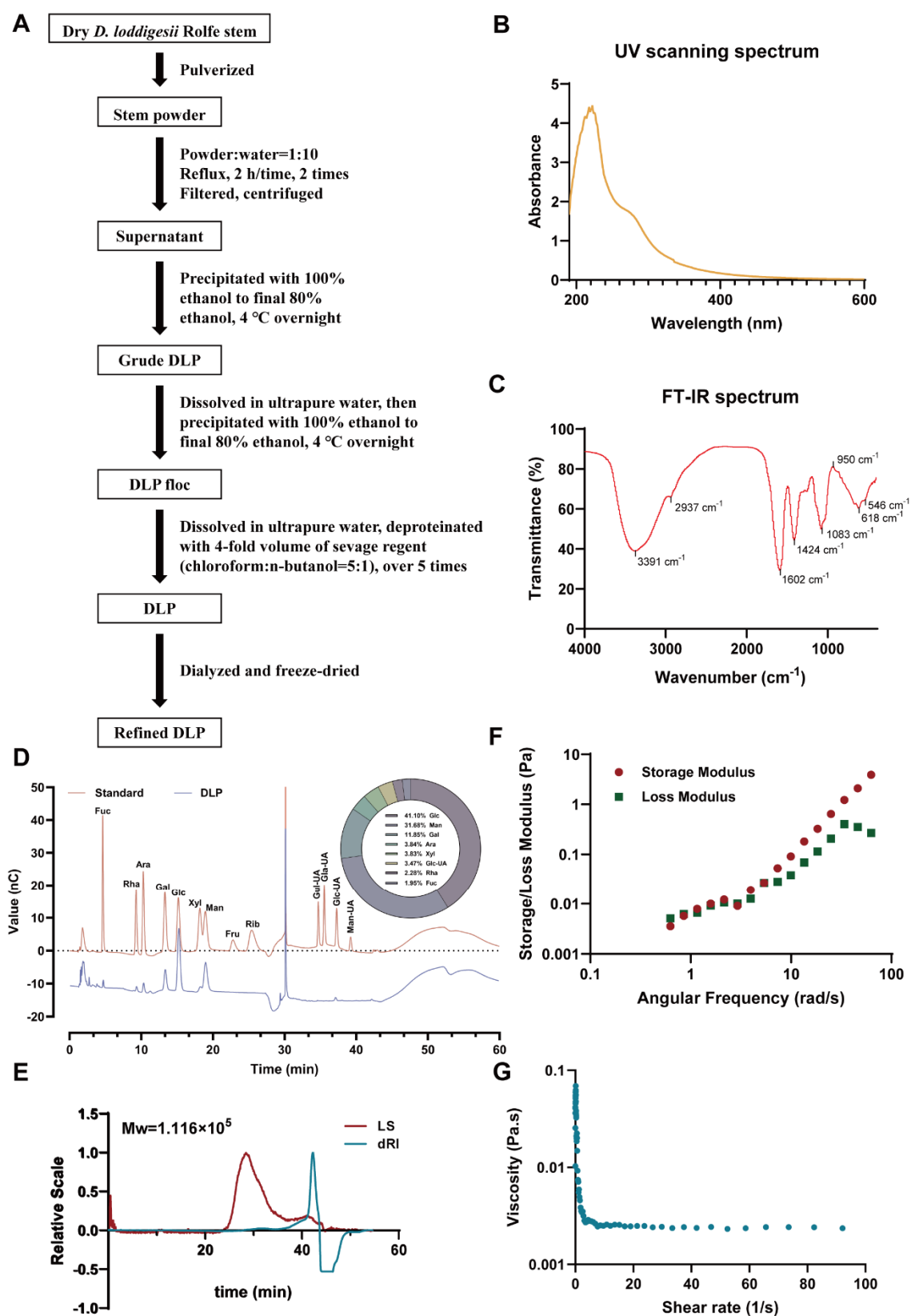


Fig. 1. Characterization of DLPs. (A) Schematic diagram of DLPs extraction, separation and purification process. (B) UV spectra of DLPs. (C) FT-IR spectra of DLPs between 4000 and 800 cm⁻¹. (D) Monosaccharide composition analysis of DLPs.

MAN, GLC, GAL, ARA, XYL, RHA, FUC, GLCA represents for Mannose, Glucose, Galactose, Arabinose, Xylose, Rhamnose, Fucose, Glucuronic Acid, respectively. (E) Molecular weight analysis of DLPs. (F) Angular frequency analysis of steady-state flow behavior of DLPs. (G) Viscosity analysis of flow behavior of DLPs.

3.2 Analysis of glycosidic bonds in DLPs via LC-MS

Under the specified liquid chromatographic conditions, the signal acquisition for multiple reaction monitoring (MRM) primarily targeted the following precursor ions based on the degree of monosaccharide content: m/z 495.2, m/z 509.2, m/z 523.2, m/z 525.2, m/z 537.2, m/z 539.2, m/z 553.3, m/z 567.6, and m/z 581.2. Through collision-induced dissociation (CID), the precursor ions generated a common fragment ion at m/z 175.1, which resulted from the fragmentation of PMP-derivatized monosaccharides at the C1 position. This ion served as the quantification ion. The secondary abundant fragment ions were related to the cleavage of C2-C3 bonds on monosaccharides. Moreover, distinctive fragments generated by certain monosaccharides were easily identifiable by the instrument.

The ions at m/z 495.2 (Figure 2A) and m/z 509.2 (Figure 2B) were attributed to the connection of arabinose or xylose, and the fragmentation of arabinose, xylose, fucose, and rhamnose derivatives, respectively, based on the formula (precursor ion = monosaccharide mass + mass of methylated group $\times$ DoPe + 2 molecules of PMP + proton ($[M+14 \times \text{DoPe}+330+H]^+$)). The origins of disaccharide residues were complex and often resulted in ions at m/z 175.1 and m/z 231.2 at positions (1 $\rightarrow$ 2,3)-, (1 $\rightarrow$ 2,4)-, or (1 $\rightarrow$ 2,6). Arabinose predominantly existed in furanose form, while xylose primarily existed in pyranose form. Consequently, potential linkages included 2,4-P-xyl and 3,5-F-ara. Similarly, m/z 523.2 (Figure 2C) arose from the connection of fucose, rhamnose, or an additional methylated group, and the connection of arabinose and xylose also yielded similar fragment ions. The glycosidic bond of fucose was commonly linked at positions 2, 3, and 4. Analysis of MRM chromatograms indicated a retention time between 15-20 minutes, with a sharp decrease in fragment channels passing through m/z 217.1 compared to m/z 231.2 and m/z 175.1, suggesting the presence of terminal linkage structures. Meanwhile, m/z 553.2 (Figure 2G) was inferred to be connected to mannose, galactose, and glucose, with a monosaccharide molecular weight of 180.2 and a methylation degree of 3. The ion at m/z 539.2 (Figure 2F) consisted of monosaccharides with a molecular formula of $C_6H_{12}O_6$ or with a reduction of one methylated group, and deoxyhexuronic acid could also form the precursor ion. The disaccharide residues at m/z 175.1 and m/z 231.2 derived from (1 $\rightarrow$ 3,4,6), thus the ion fragment at m/z 525.2 (Figure 2D) was inferred to be 3,4,6-galactose. The ion fragment at m/z 537.2 (Figure 2E) in CID only generated fragment ions at m/z 175.1 and m/z 231.2, indicating a T-rhamnose linkage due to the presence of terminal (T) residue only in isomeric hydroxyl connections. Likewise, it was deduced that the ion fragments at m/z 567.6 (Figure 2H) and m/z 581.2 (Figure 2I) corresponded to T-galacturonic acid connections, respectively. The total ion chromatogram (TIC) revealed that the two ion fragments reached their maximum values around 23 minutes.

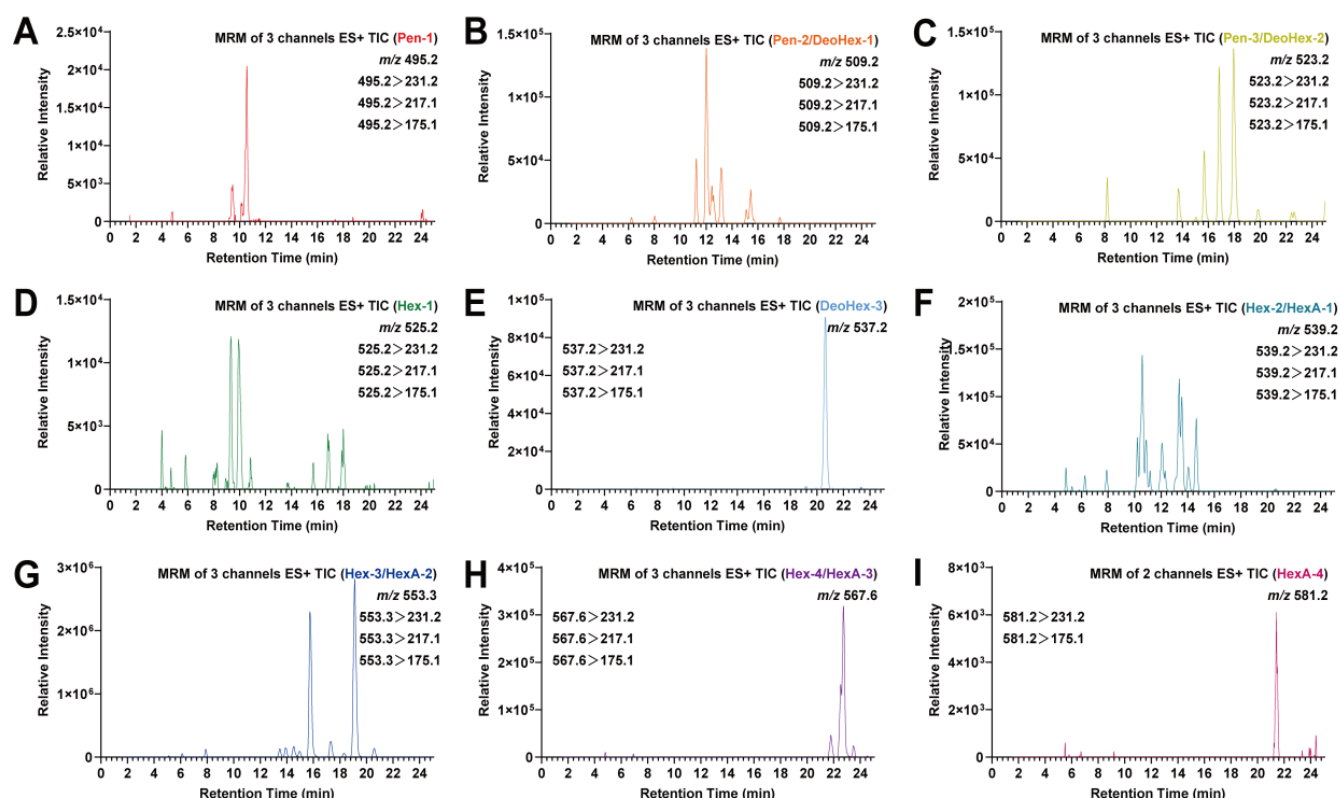


Fig. 2. Total ion chromatogram (TIC) and MRM chromatograms for the linkage analysis of DLPs. (A) Pen-1; (B) Pen-2/DeoHex-1; (C) Pen-3/DeoHex-2; (D) Hex-1; (E) DeoHex-3; (F) Hex-2/HexA-1; (G) Hex-3/HexA-2; (H) Hex-4/HexA-3; (I) HexA-4. X-axis represents retention time, while the Y-axis represents relative ion intensity.

3.3. Effects of DLPs on the general state and organs of CCL₄-induced liver injury in mice

To explore the hepatoprotective activity of DLPs, we conducted *in vivo* experiments on CCL₄-induced liver injury in mice according to the experimental design shown in Figure 3A. Before modeling, the animals in each group exhibited normal fur color, diet, and physical activity, and their body weights continued to increase without significant differences between groups (Figure 3B), indicating that various doses of DLPs and the positive control, silymarin, were not toxic to the mice. Fourteen days post-administration, a single intraperitoneal injection of CCL₄ was used to establish an acute liver injury model. Eighteen hours later, the animals were dissected, and the organ coefficients are displayed in Figure 3C-E. Compared to the control group, the liver organ index significantly increased in the model group ($P < 0.05$). Both silymarin and DLPs significantly reversed this increase ($P < 0.05$), with DLPs showing a dose-dependent effect. Moreover, the kidney organ index significantly decreased in the silymarin group compared to the model group ($P < 0.01$), suggesting potential renal toxicity of silymarin under this model, which warrants further investigation. In contrast, DLPs had no impact on kidney index at any dosage. The spleen organ index showed no differences across all groups.

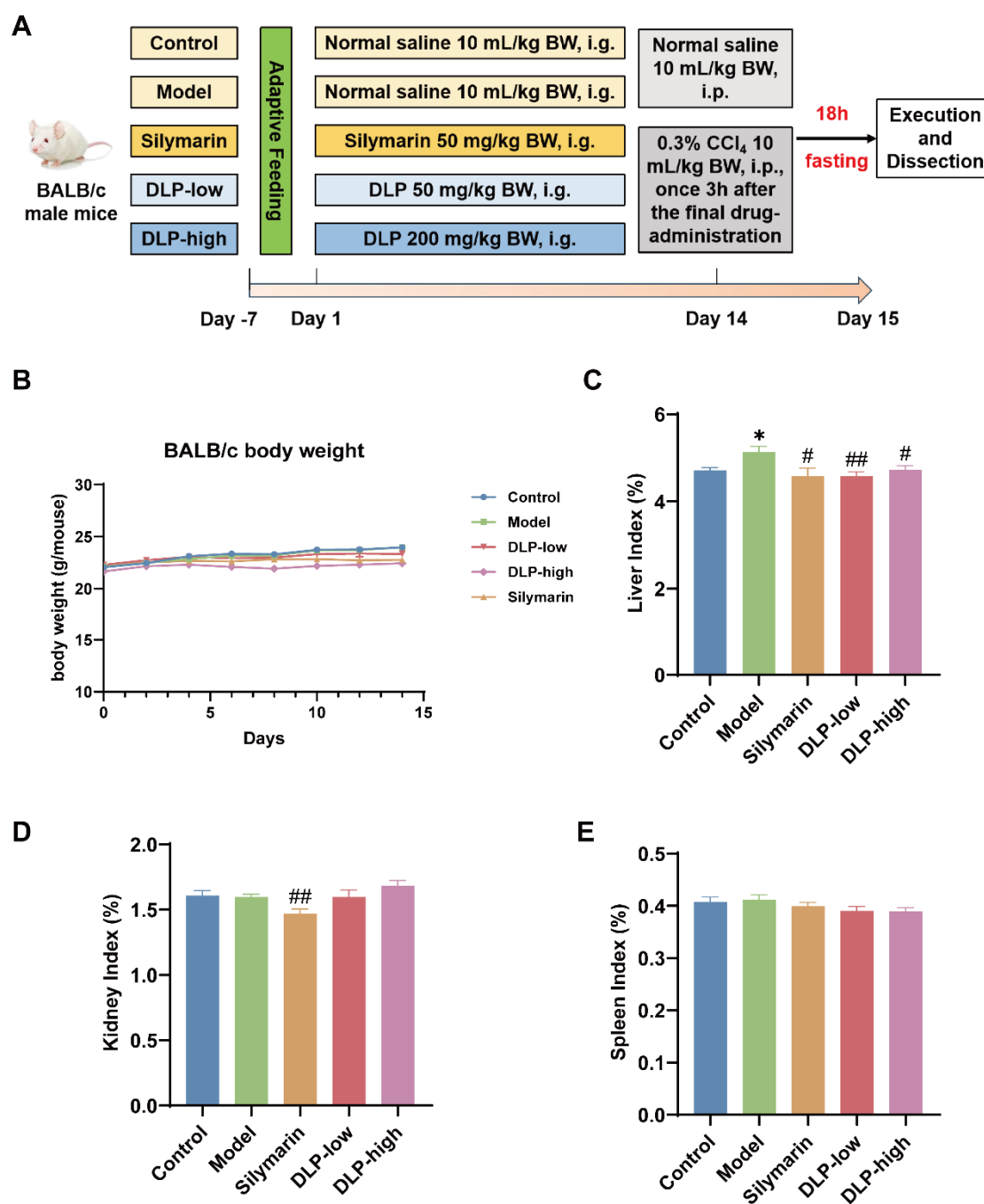
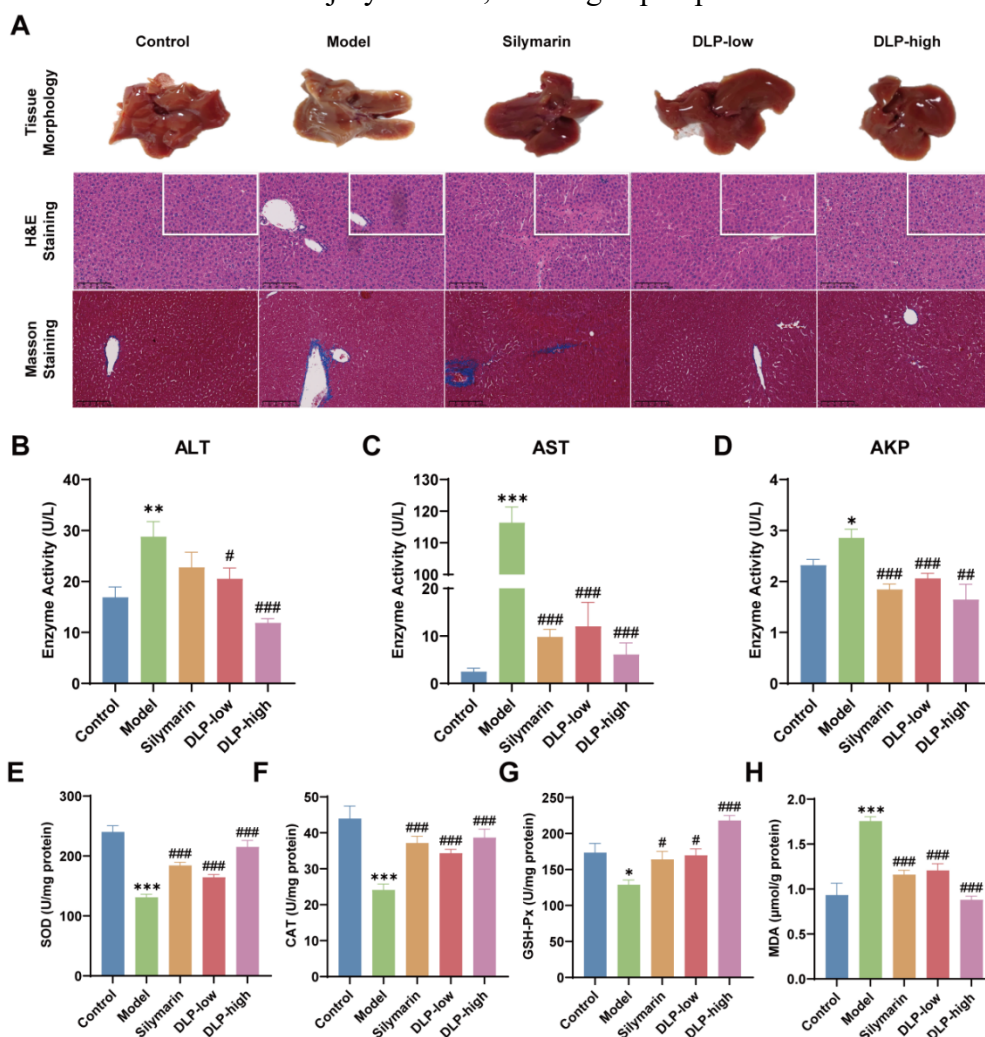


Fig. 3. Effects of DLPs on the general state and organs of CCL₄-induced liver injury in mice. (A) Schematic diagram of animal experiment design and process. (B) Body weight changes in mice. (C) Liver organ index (liver weight/body weight). (D) Kidney organ index (kidney weight/body weight). (E) Spleen organ index (spleen weight/body weight). Data were expressed as mean $\pm$ SEM from nine mice in each group. * $P < 0.05$ compared to control group individually; # $P < 0.05$ and ## $P < 0.01$ compared to model group individually.

3.4. DLPs significantly ameliorate the blood biochemical indicators and liver pathology in CCL₄-induced liver injury in mice

Liver tissue histopathology slides visibly demonstrate changes in hepatic cell morphology and inflammatory cell infiltration. As depicted in Figure 4A using H&E staining, the liver lobule structure and cellular morphology in the control group appear normal. In the model group, liver tissue sections show focal necrosis and loss of cellular structure with inflammatory cell infiltration. This infiltration is characterized by reduced nuclear size, loss of cellular structure, or abnormal cell morphology. Compared to the model group,

both silymarin and all doses of DLPs significantly mitigate inflammatory cell infiltration and decrease the extent of hepatocyte necrosis near the liver lobule drainage area, and reduce hepatocyte edema within the liver lobules. Relative to the control group, serum ALT, AST, and AKP levels in the model group significantly increased ($P < 0.01$, $P < 0.001$, $P < 0.05$, respectively), confirming the success of the acute liver injury model. When compared with the model group, both silymarin and DLPs significantly reduced serum ALT, AST, and AKP levels ($P < 0.01$ or $P < 0.001$), with DLPs showing a dose-dependent improvement (Figure 4B). As shown in Figures 4E-H, compared to the control group, the model group exhibited significantly increased liver MDA levels ($P < 0.001$) and markedly reduced activities of SOD, CAT, and GSH-Px ($P < 0.001$ or $P < 0.05$), indicating severe oxidative damage and decreased antioxidative enzyme activities. In contrast, both silymarin and all doses of DLPs significantly lowered liver MDA levels ($P < 0.001$) and increased activities of SOD, CAT, and GSH-Px ($P < 0.001$) in the model group, suggesting that DLPs enhance the expression of antioxidative enzymes in mouse liver, providing protection against CCL₄-induced liver damage through antioxidative stress. As indicated in Figures 4I-K, compared to the control group, serum IL-1 β , IL-6, and TNF- α levels significantly increased in the model group ($P < 0.01$). Treatment of DLPs reduced the serum levels of IL-1 β , IL-6, and TNF- α in a dose-dependent manner, with the DLP-high group showing the most significant effects ($P < 0.01$). Overall, DLPs markedly improves both the blood biochemical indicators and liver pathology in CCL₄-induced liver injury in mice, offering hepatoprotective effects.



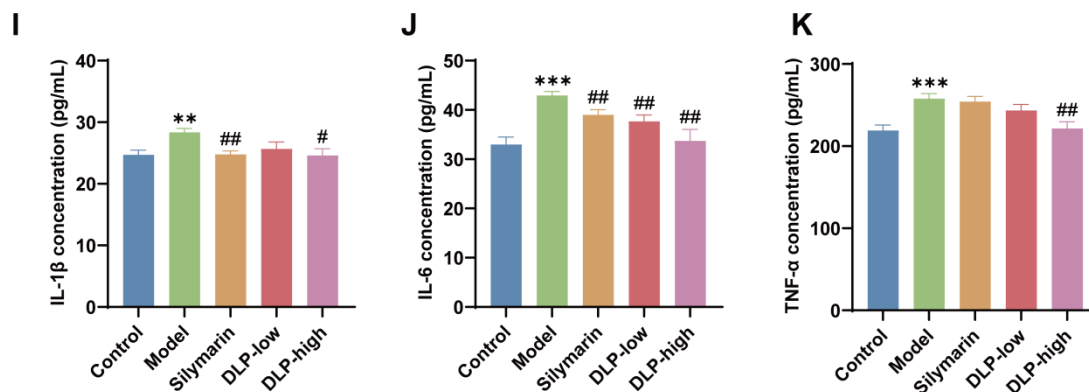


Fig. 4. DLPs can attenuate blood biochemical indicators and liver pathological changes in mice with CCL₄-induced liver injury. (A) Morphology of the liver and histopathological examination of the liver were performed using H&E staining and Masson staining (Original magnification $\times 100$ and $\times 200$, scale bar = 100 μ m). (B) Serum ALT level. (C) Serum AST level. (D) Serum AKP level. (E) Liver SOD level. (F) Liver CAT level. (G) Liver GSH-Px level. (H) Liver MDA level. (I) Serum IL-1 β level. (J) Serum IL-6 level. (K) Serum TNF- α level. Data were expressed as mean $\pm$ SEM from nine mice in each group. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ compared to control group individually; # $P < 0.05$, ## $P < 0.01$ and ### $P < 0.001$ compared to model group individually.

3.5. Serum metabolomic analysis of DLPs on CCL₄-induced liver injury in mice

Using PCA analysis, a clear separation among the control, model, and both DLPs-treated groups was observed in both positive and negative ion modes, as shown in Figures 5A and B. These groups displayed good clustering trends and all samples were within the 95% confidence interval. A univariate test (Student's t-test, with P -values < 0.05 and fold change > 1.2) revealed a significant distribution of dysregulated metabolic signatures between the model and DLP-high groups, as illustrated in the volcano plot (Figure 5C). This plot highlights significant metabolic differences, with each point representing a ion; the greater the absolute value on the coordinates, the larger the difference in expression. The enriched pathways in the control and model groups focused primarily on carbohydrate metabolism, energy metabolism, and fatty acid synthesis processes. As shown in Figure 5D, the metabolism of methyl butyrate and β -alanine, involving the production of pyruvate, plays a crucial role in gluconeogenesis and energy metabolism, with the synthesis of pantothenic acid and coenzyme A also being key. In the model and DLP-high groups, DLPs primarily mitigate liver injury by modulating lipid metabolism, including steroidogenesis, α -linolenic acid, and linoleic acid, and the mitochondrial β -oxidation of long-chain saturated fatty acids (Figure 5E). A heatmap analysis of 19 selected compounds, identified as differential metabolic products among the three groups, was completed (Figure 5F). Among these observed differential metabolites (Figure 5G), phosphatidylcholine (PC), lysophosphatidylcholine (LPC-a breakdown product of PC), and N-methyl- β -carboline-3-carboxamide significantly increased in the model group ($P < 0.001$ or $P < 0.05$) and significantly decreased in the DLP-high group ($P < 0.001$ or $P < 0.05$). Conversely, the level of linoleic acid, which has antioxidant and anti-inflammatory properties that promote liver cell regeneration and repair, significantly increased in the DLP-high group ($P < 0.001$). The damage and death of hepatocytes, leading to cell membrane rupture, cause an increase in serum PC and LPC levels. Pre-administration of DLPs controlled the damage and death of hepatocytes, improving the state of cell membrane rupture, thereby decreasing serum PC/LPC levels-a process that reflects the recovery from liver injury. Through serum metabolomic analysis, DLPs may regulate the

metabolic pathway of linoleic acid to scavenge free radicals, reducing oxidative stress on liver cells, thus protecting mice from CCL₄-induced liver injury.

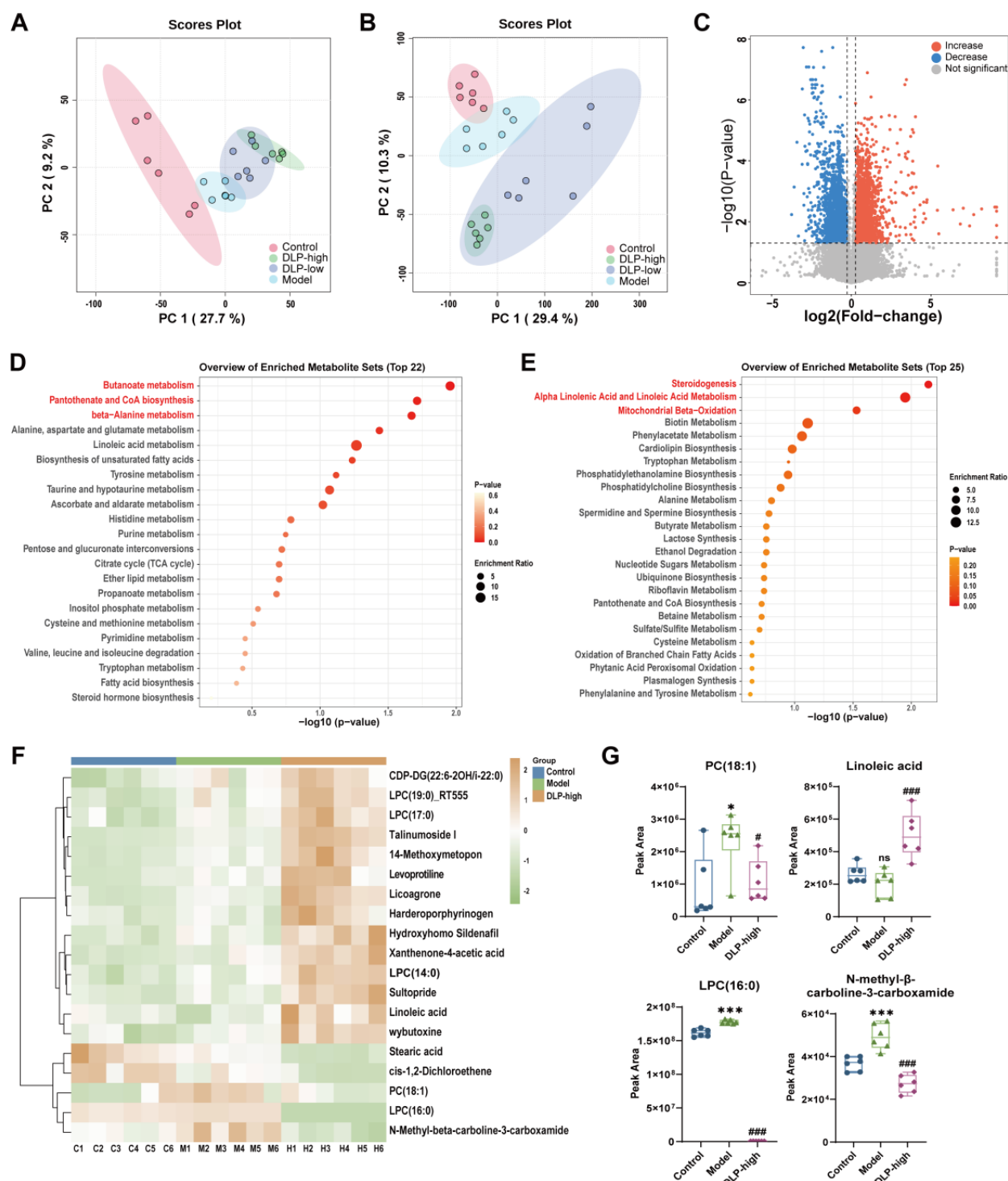
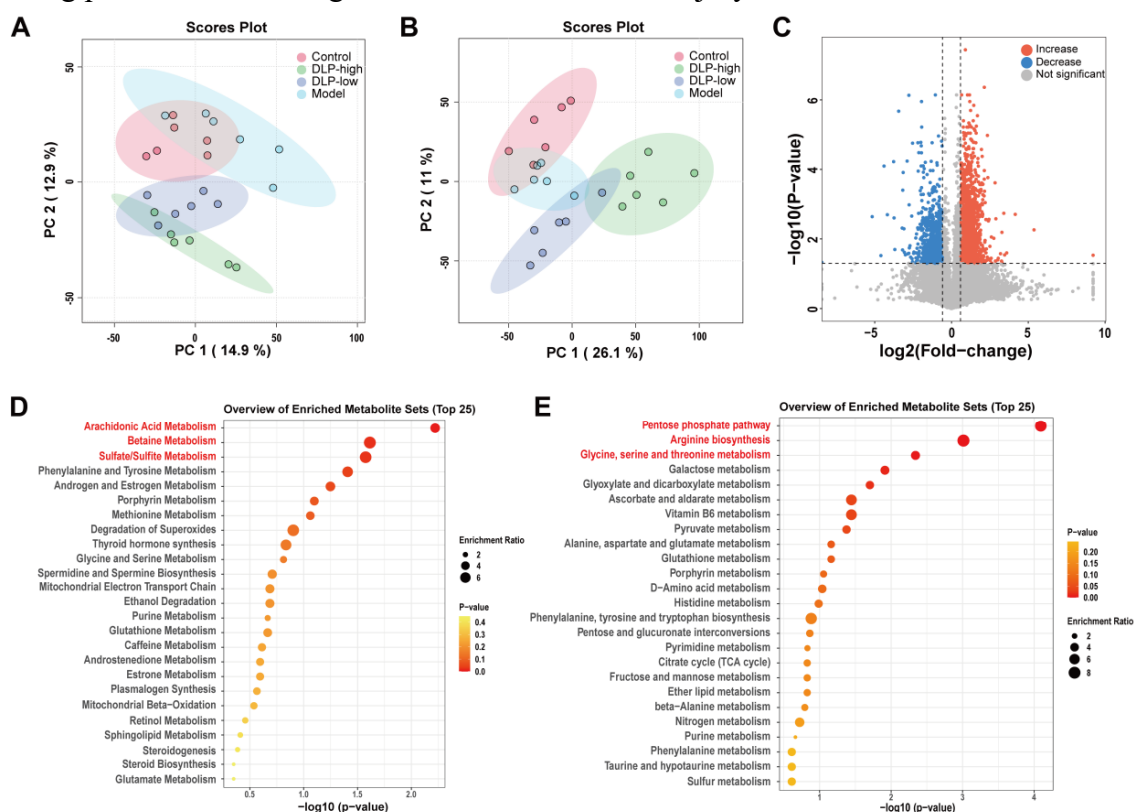


Fig. 5. Serum metabolomic analysis of the effect of DLPs in mice with CCL₄-induced liver injury. (A) PCA plots between control, model, DLP-low and DLP-high groups in positive ion mode. (B) PCA plots between control, model, DLP-low and DLP-high groups in negative ion mode. (C) Volcano plot of the differential ions in the model and DLP-high groups. Red dots represent significantly increased ions, and blue dots represent significantly decreased ions. The horizontal line represents the level of significance ($P < 0.05$), and the vertical line represents 1.2-fold changes. (D) The most relevant pathways of serum metabolic alterations between control and model groups were revealed using a bubble chart. (E) The most relevant pathways of serum metabolic alterations between model and DLP-high groups were revealed using a bubble chart. (F) A heat map with hierarchical clustering of dysregulated metabolites in the control, model and DLP-high groups. (G) Statistical analysis referring to the relative intensity of significantly altered endogenous metabolites in serum among control, model and DLP-high groups. Data were expressed as mean $\pm$ SEM from six mice in each group. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ compared to control group individually; # $P < 0.05$, ### $P < 0.01$ and #### $P < 0.001$ compared to model group individually.

3.6. Liver metabolomic analysis of DLPs on CCL₄-induced liver injury in mice.

As shown in Figures 6A and 6B, PCA analysis under both positive and negative ion modes indicates a clear segregation among the control, model, and DLPs-treated groups, with all samples fitting well within the 95% confidence interval. The volcano plot in Figure 6C illustrates a significant array of upregulated and downregulated ions between the model and DLP-high groups, which are statistically significant. The pathways enriched in the control and model groups, shown in Figure 6D, primarily involve inflammatory response-related metabolic processes, including arachidonic acid metabolism. This suggests that the mouse model of CCL₄-induced liver injury affects liver lipid synthesis and metabolism. Significant differences are observed in amino acid metabolism, carbohydrate metabolism, and cofactor metabolism between the model and DLP-high groups. The pentose phosphate pathway (PPP), as highlighted in Figure 6E, plays a crucial role in addressing oxidative stress and repairing cellular damage in the liver injury model. This pathway's activity increases due to oxidative stress, inflammatory responses, or other damaging factors, disrupting the cellular redox balance. A heatmap analysis of 30 selected differential metabolites is completed (Figure 6F), showing significant restoration in the levels of arachidonyl ethanolamide, LPC, and 11-dehydro-thromboxane B2 in the DLP-high group compared to the model group (Figures 6G). Besides, purines were highest in the DLP-high group (Figures 6G), promoting the activity of the PPP pathway and acting as hepatocyte protectors. The increase in these metabolites typically results from platelet aggregation triggered by liver cell damage. Additionally, the metabolism of arachidonyl ethanolamide produces significant amounts of free radicals and oxidative stress products, which can cause oxidative damage to liver cells. Through liver metabolomic analysis, DLPs are posited to modulate the arachidonic acid metabolic pathway, reducing oxidative stress and thereby offering protective effects against CCL₄-induced liver injury in mice.



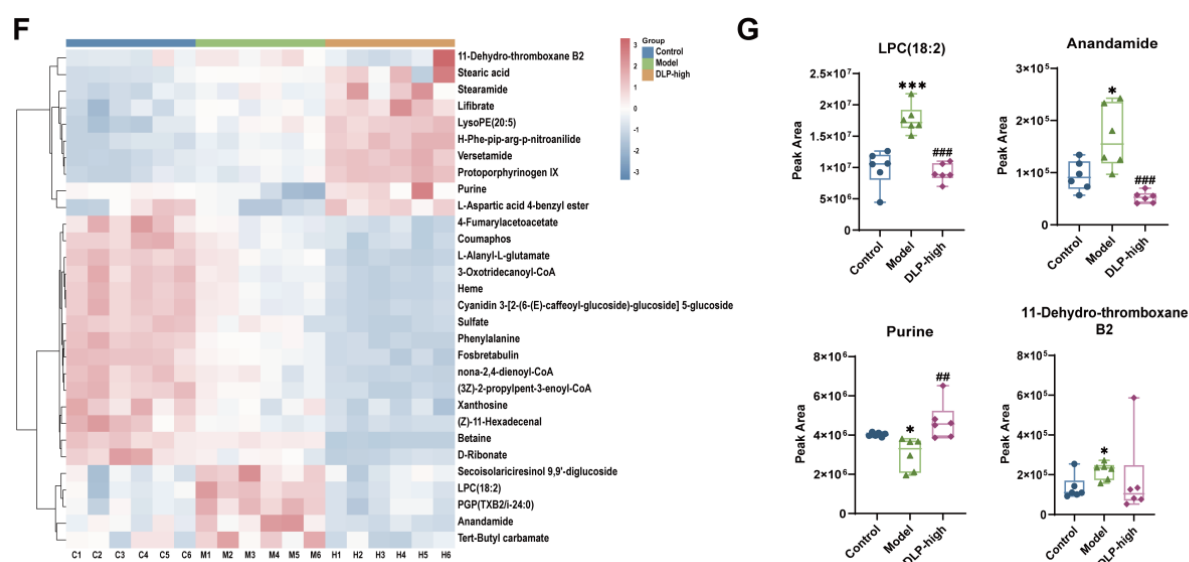
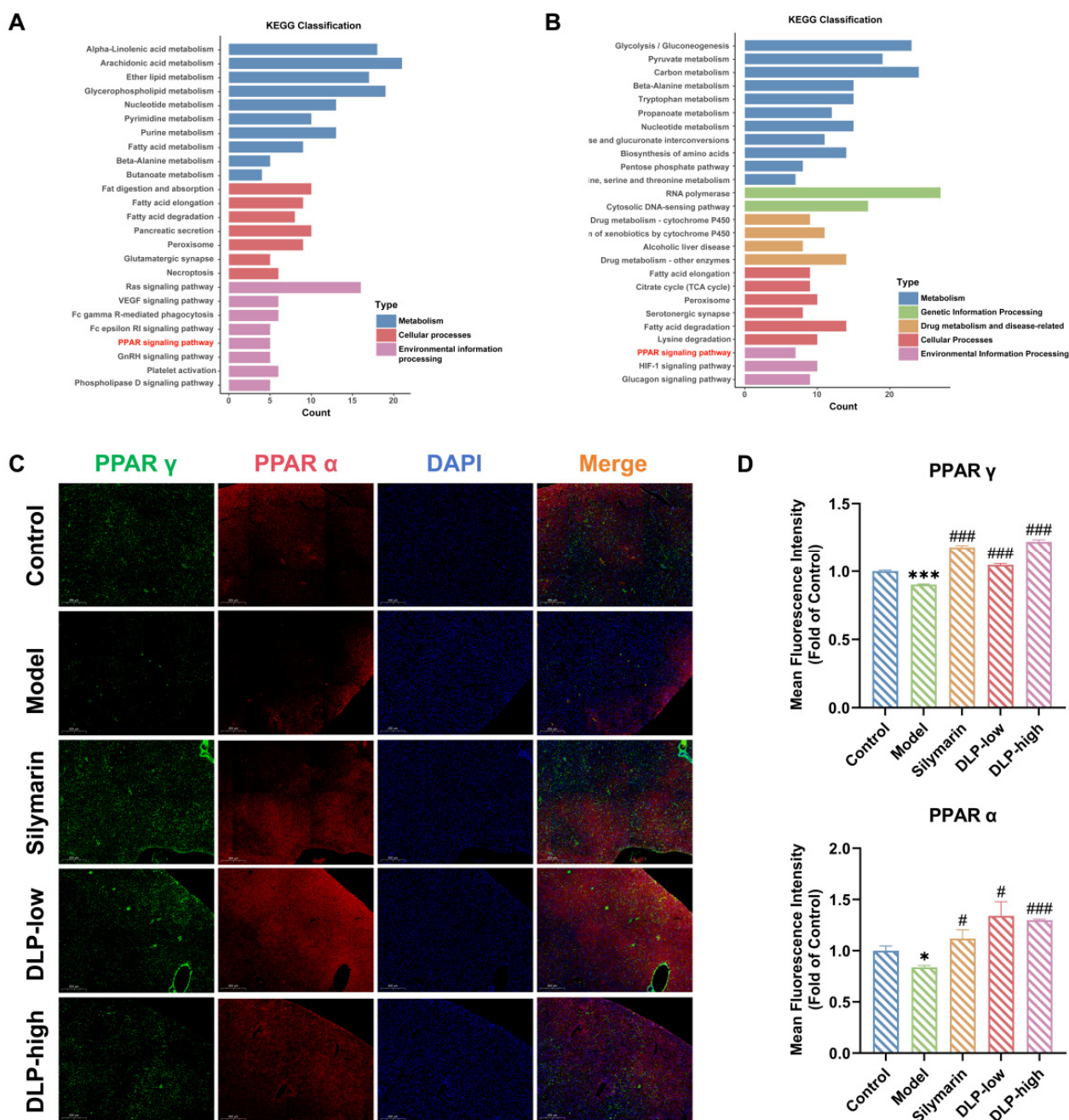


Fig. 6. Liver metabolomic analysis of the effect of DLPs in mice with CCL₄-induced liver injury. (A) PCA plots between control, model, DLP-low and DLP-high groups in positive ion mode. (B) PCA plots between control, model, DLP-low and DLP-high groups in negative ion mode. (C) Volcano plot of the differential ions in the model and DLP-high groups. Red dots represent significantly increased metabolites, and blue dots represent significantly decreased ions. The horizontal line represents the level of significance ($P < 0.05$), and the vertical line represents 1.2-fold changes. (D) The most relevant pathways of liver metabolic alterations between control and model groups were revealed using a bubble chart. (E) The most relevant pathways of liver metabolic alterations between model and DLP-high groups were revealed using a bubble chart. (F) A heat map with hierarchical clustering of differential metabolites in the control, model and DLP-high groups. (G) Statistical analysis referring to the relative intensity of significantly altered endogenous metabolites in liver among control, model and DLP-high groups. Data were expressed as mean $\pm$ SEM from six mice in each group. * $P < 0.05$, ** $P < 0.01$ and *** $P < 0.001$ compared to control group individually; # $P < 0.05$, ## $P < 0.01$ and ### $P < 0.001$ compared to model group individually.

3.7. DLPs protect against CCL₄-induced liver damage in mice by regulating the PPAR pathway

Based on liver metabolomics analysis, we imported the gene codes of differential metabolites from the liver into KEGG for pathway enrichment analysis. As shown in Figure 7A and 7B, we found that differential metabolites in both the control and model groups were enriched in the RAS and peroxisome proliferator-activated receptor (PPAR) signaling pathways. The differential metabolites in the DLP-high group and the model group were enriched in the PPAR and HIF-1 signaling pathways. The common signaling pathway between the two groups is the PPAR signaling pathway, suggesting that DLPs may exert protective effects against CCL₄-induced liver damage in mice by regulating this pathway. To verify this finding, we further investigated the relationship between liver damage and the regulation of PPAR-related signaling pathways, as well as the mechanism of DLPs' protective effect on liver damage. As shown in Figures 7C and 7D, immunofluorescence staining of PPAR α and PPAR γ proteins in liver tissue sections revealed that the expression of PPAR α and PPAR γ proteins was inhibited in the model group ($P < 0.05$ and $P < 0.001$). Compared with the model group, both silymarin and DLPs significantly increased the protein expression of PPAR α and PPAR γ ($P < 0.05$ and $P < 0.001$). Subsequently, we conducted further pathway validation using western blotting. As shown in Figures 7E and 7F, the results indicated that the protein expression of PPAR α and PPAR γ in the liver was significantly reduced in the model group compared to the control group ($P < 0.01$ and $P < 0.001$). Both the silymarin group and the DLPs group significantly enhanced the protein expression of PPAR α and PPAR γ compared to the model group ($P < 0.05$ and $P < 0.001$), consistent with the

immunofluorescence results. Meanwhile, the protein expression of RAR α was significantly increased in the model group, silymarin group, and DLP-low group ($P < 0.01$ and $P < 0.001$). Compared with the control group, the expression of p-JAK2 protein was elevated in the model group ($P < 0.05$), while the expression of p-STAT5 protein was reduced ($P < 0.01$). Both the silymarin group and the DLP group significantly reversed the changes in these proteins observed in the model group. These results validate the regulatory effect of DLPs on the PPAR signaling pathway.



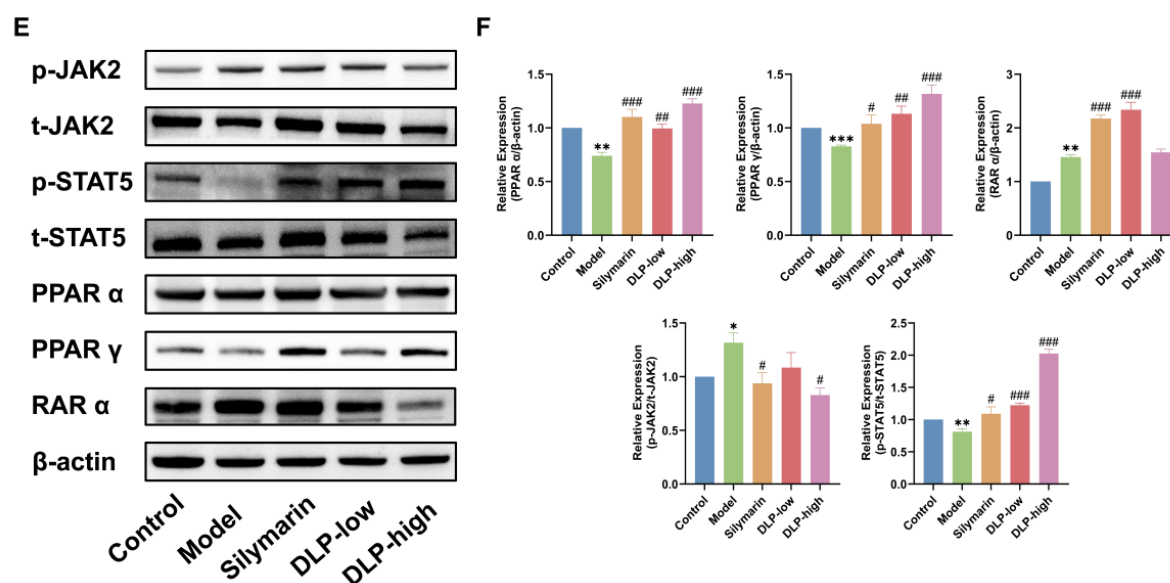


Fig. 7. DLPs exert a protective effect against CCL₄-induced liver injury in mice by modulating the PPAR pathway. (A) The most relevant pathways of liver metabolic alterations between control and model groups were revealed and classified using KEGG. (B) The most relevant pathways of liver metabolic alterations between model and DLP-high groups were revealed and classified using KEGG. (C) Expression of PPAR α and PPAR γ in the liver of each group were determined by immunofluorescence staining (Original magnification $\times 200$, scale bar = 200 μ m). (D) Relative quantification of the mean fluorescence intensity (MFI) for PPAR α and PPAR γ , obtained from immunofluorescent images of the liver slides. (E) Representative western blot images of p-JAK2, t-JAK2, p-STAT5, t-STAT5, PPAR α , PPAR γ , RAR α , and β -actin in mice liver samples from each group. (F) The western blot results were quantitatively analyzed, and fold changes of protein expression in (E) were showed in histograms. Data were expressed as mean $\pm$ SEM from three mice in each group. * P < 0.05, ** P < 0.01 and *** P < 0.001 compared to control group individually; # P < 0.05, ## P < 0.01 and ### P < 0.001 compared to model group individually.

3.8. Effect of DLPs on the intestinal microbiota of mice with CCL₄-induced liver injury

The study involved collecting fecal samples on the 15th day of the feeding period to analyze the gut microbial alteration using 16S rRNA gene sequencing. The V3-V4 region of the 16S rRNA gene was amplified using specific primers, and library preparation was performed using the Illumina MiSeq platform. Sequencing data were analyzed using QIIME2, with operational taxonomic units (OTUs) clustered at 97% similarity. As illustrated in Figure 8A, a Venn diagram showed that out of 403 operational taxonomic units (OTUs) shared among the five groups, 38 unique OTUs were specific to the model group, suggesting that CCL₄ modeling altered the gut microbiota and reduced its abundance. However, treatment with silymarin and high doses of DLPs reversed these effects, enhancing microbial richness. Beta-diversity, which reflects differences between microbial communities across different ecosystems, was analyzed using principal coordinates analysis (PCoA). Different colored points and circles represented various sample groups, with proximity indicating similar species compositions. Figures 8B and 8C indicate significant differences between the DLP-high group and both the control and model groups, distributed across three regions. These results highlight DLPs' ability to restore and modify the gut microbiota structure in mice with CCL₄-induced liver injury. Further taxonomic analysis at the genus level revealed specific microbial shifts induced by CCL₄. CCL₄ exposure reduced the proportions of *Staphylococcus* and *Alistipes*, while increasing the relative abundance of *Candidatus_Saccharimonas* and *Odoribacter*. Notably, DLPs reversed these alterations, restoring a balance in the gut microbiota composition (Figure 8D). An increase in *Alistipes* abundance after

DLPs treatment could be beneficial as SCFAs promote intestinal barrier integrity and reduce liver inflammation via the gut-liver axis [42]. This modulation of specific genera by DLPs suggests potential prebiotic-like effects, enhancing gut microbiota diversity and restoring microbial homeostasis. To identify significant biomarkers of gut microbiota between groups, we performed Linear Discriminant Analysis Effect Size (LEfSe) analysis. LEfSe results, as shown in Figure 8E, identified *Anaeroplasma* and *Lysinibacillus* as key microbial genera significantly enriched in the DLP-high group ($P < 0.05$). *Anaeroplasma* is associated with metabolic activities in the gut and may contribute to maintaining a balanced microbial environment [43]. Increased *Anaeroplasma* levels could reflect positive metabolic modulation and reduced microbial dysbiosis during DLPs treatment. *Lysinibacillus* has antimicrobial properties and may contribute to maintaining a balanced gut microbiome [44]. Its presence could help suppress opportunistic pathogens and promote liver recovery indirectly by supporting microbiota stability. These findings suggest that DLPs may promote the growth of beneficial bacteria associated with gut health and metabolic regulation. These findings highlight the potential of DLPs to treat liver injury by harnessing the gut microbiota's metabolic and immunomodulatory capabilities. Further studies, such as metabolomic profiling and immune markers analysis, would help confirm these mechanisms.

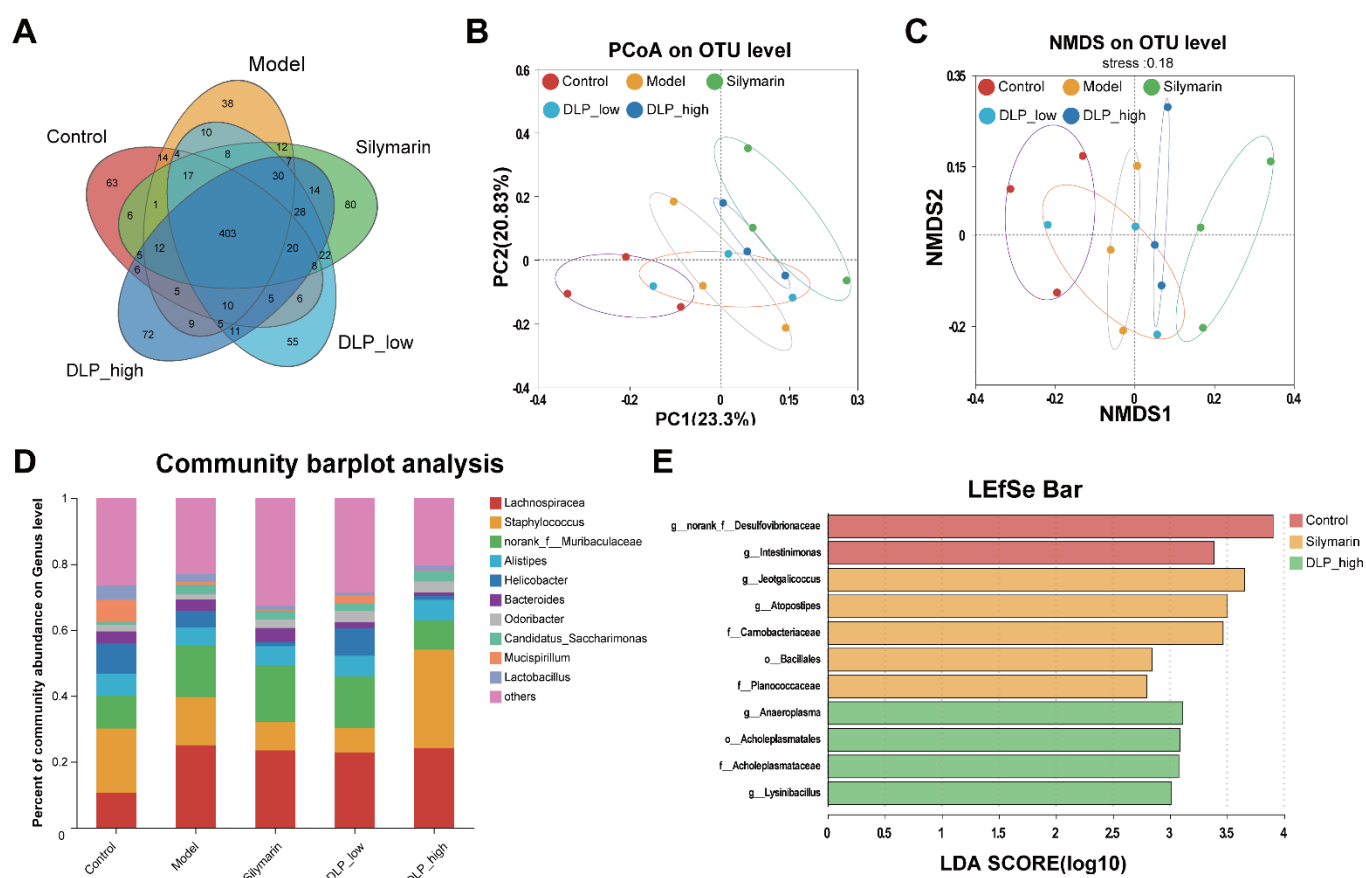


Fig. 8. Effect of DLPs on the intestinal flora in mice with CCL₄-induced liver injury. (A) Venn diagram of OTUs for five groups among control, model, silymarin, DLP-low and DLP-high groups. (B) PCoA analysis of β -diversity among control, model, silymarin, DLP-low and DLP-high groups based on OTU level. (C) NMDS analysis of β -diversity among control, model, silymarin, DLP-low and DLP-high groups based on OTU level. (D) Relative abundance of gut microbiota at the genus levels among five groups at the end of experiment. (E) LDA Effect Size (LEfSe) analysis of gut microbiota in the control, silymarin and DLP-high groups. Data were expressed as mean $\pm$ SEM from three mice in each group.

4. Discussion

The comprehensive analysis of *Dendrobium loddigesii* polysaccharides (DLPs) presented in this study highlights their structural characteristics and their hepatoprotective properties against CCL₄-induced liver injury in mice. These findings not only illuminate the structural intricacies of DLPs but also validate their therapeutic potential, marking a pioneering stride in the pharmacological exploration of *Dendrobium* species polysaccharides.

The structural analysis of DLPs revealed a complex monosaccharide composition predominantly comprising mannose and glucose, which aligns with the common structural features observed in polysaccharides known for their therapeutic activities. The advanced glycosidic bond profiling using LC-MS identified multiple linkage types, predominantly (1→3,4,6)-linked residues, which suggest a branched polysaccharide structure [45]. Such branching is often correlated with high bioactivity in polysaccharides, influencing their solubility and interaction with biological membranes [46, 47]. The molecular weight of DLPs was determined to be approximately 1.116×10^5 kDa, which is indicative of the robust macromolecular nature typical of bioactive polysaccharides that exhibit significant physiological activities through interaction with cellular receptors [48]. High molecular weight polysaccharides are known for their strong immunomodulatory and antioxidant activities, primarily through the modulation of signaling pathways and protective effects against oxidative cellular damage [49, 50].

The *in vivo* experiments highlighted the hepatoprotective efficacy of DLPs, significantly ameliorating the biochemical markers of liver injury (ALT, AST, AKP) and inflammatory cytokines (IL-1 β , IL-6, TNF- α) in serum. These biochemical results are complemented by histopathological observations, where DLPs treatment notably reduced hepatocyte necrosis and inflammatory cell infiltration. The dose-dependent effects of DLPs suggest that higher doses confer greater protective effects, potentially through enhanced modulation of biochemical pathways involved in inflammation and oxidation.

Metabolomics analyses further supported the protective role of DLPs, with significant alterations in lipid metabolism pathways, particularly those involving arachidonic acid and long-chain saturated fatty acids. These pathways are crucial for mediating inflammatory responses and oxidative stress, both of which are integral to the pathophysiology of liver injury [51-53]. The modulation of these pathways by DLPs highlights their potential mechanism of action, primarily through the mitigation of lipid peroxidation and enhancement of cellular antioxidant defences. These metabolites align with the growing interest in functional foods that target lipid homeostasis and metabolic health. DLPs could be positioned as dietary supplements or incorporated into food products aimed at promoting liver health and reducing the risk of fatty liver disease. One of the key findings of this study is the effect of DLPs on gut microbiota diversity. The increase in microbial diversity observed in DLPs-treated groups suggests a prebiotic-like effect, which is particularly relevant for their application as functional food ingredients. The gut-liver axis plays a critical role in maintaining liver health, and the modulation of gut microbiota by DLPs may contribute to their hepatoprotective effects by reducing endotoxin production and systemic inflammation [54]. This study

preliminarily explored the relationship between liver damage and the peroxisome proliferator-activated receptor (PPAR) signaling pathway, as well as its regulation of related inflammatory signaling pathways, and the mechanism by which DLPs protect against liver damage. Our research revealed that mice in the model group exhibited suppressed expression of PPAR γ and PPAR α . In contrast, DLPs activated the PPAR signaling pathway, increased the expression of RXR α protein, and reduced the protein expression of p-JAK2, which contributes to the improvement of liver damage. Many members of the JAK/STAT signaling pathway are involved in liver regeneration and liver immunity, indicating the significant role of this pathway in the liver [55, 56]. Ligand-activated PPAR γ forms heterodimers with retinoid X receptors (RXRs), recognizes its response elements, and initiates the transcription of target genes [57]. Studies have shown that PPAR γ is highly expressed in quiescent hepatic stellate cells (HSCs) and its expression decreases when HSCs are activated. Therefore, DLPs upregulate the STAT5 signaling pathway to promote the expression of PPAR γ and PPAR α , stabilize hepatic lipid metabolism, and inhibit hepatocyte fibrosis. Simultaneously, DLPs inhibit the activation of the JAK2/IL-6 pathway, suppress inflammation induced by CCL₄, and mitigate hepatocyte injury.

This study makes an important contribution to elaborate on the structural characteristics of DLPs, providing foundational data that contributes to the understanding of its complex macromolecular and compositional makeup. Furthermore, the integration of structural characterization with advanced metabolomic profiling provides a holistic view of the therapeutic implications of DLPs. By mapping the metabolic alterations induced by DLPs treatment, this study not only confirms its hepatoprotective efficacy but also identifies potential biomarkers and pathways that could be targeted in future therapeutic strategies. The findings from this study have significant implications for the development of DLPs as functional ingredients in health-promoting diets. The detailed structural and pharmacological analysis provides a strong foundation for further clinical trials and the potential formulation of DLPs-based therapeutics. Moreover, the potential for incorporating DLPs into nutraceuticals or functional beverages should also be explored, highlighting their dual role as a source of nutrition and a means of supporting metabolic and liver health.

In conclusion, this study provides valuable insights into the structural properties, gut microbiota-modulating effects, and hepatoprotective properties of DLPs. These findings highlight the potential of DLPs as natural ingredients for health-promoting diets. This research contributes to the growing field of polysaccharides as both bioactive compounds and nutraceuticals, bridging the gap between traditional medicine and modern food science.

Declaration of competing interest

Tengfei Xu reports financial support was provided by Zhejiang University-Cangnan County Joint Innovation Center of Traditional Chinese Medicine. The other authors declare that they have no competing financial interests or personal relationships that could have appeared to influence the work reported in the paper.

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