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RESEARCH ARTICLE

Prunella vulgaris L. Polysaccharide Alleviated Liver Injury in Carbon Tetrachloride-Induced Mice Via Regulating Oxidation Resistance, Inflammatory Response and Microbiota

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ABSTRACT

Prunella vulgaris L. (*P. vulgaris* L.) is a traditional medicinal herb renowned for its various pharmacological properties through antioxidant and anti-inflammatory effects. The present study investigated the hepatoprotection of *P. vulgaris* L. polysaccharide against carbon tetrachloride-induced liver injury in mice. Thirty (n= 30) ICR mice were divided into a control group (AC), a CCl4-induced liver injury model (AM), and a *P. vulgaris* L. polysaccharide treatment group (AXK). The AM and AXK groups received intraperitoneal injections of 10% CCl4 every two days, while the AXK group was also intragastrically administered *P. vulgaris* L. polysaccharide @150 mg/kg/day. The present research indicated that CCl4 remarkably decreased liver weight ($P<0.05$) and liver index ($P<0.001$) in mice, while treatment with *P. vulgaris* L. reversed these changes. Histopathological observation indicated that structural injury of the liver and the infiltration of inflammatory cells were alleviated, and Sirius Red-positive collagen deposition was qualitatively reduced in the AXK group compared to the CCl4 model group. Moreover, *P. vulgaris* L. significantly decreased liver function markers such as ALT and AST ($P<0.001$), inflammation factors like IL-6 ($P<0.01$), IL-1β ($P<0.01$), TNF-α ($P<0.05$), and oxidative stress marker MDA ($P<0.01$), while increasing GSH-Px ($P<0.001$), T-AOC ($P<0.01$), SOD ($P<0.01$) and IL-10 ($P<0.01$) levels. Analysis of gut microbiota suggested that *P. vulgaris* L. improved intestinal flora by reducing harmful bacteria such as *Colidextribacter* and *Mammaliicoccus*, while increasing *Lachnospiraceae UCG-006, Coriobacteriales Incertae Sedis,* and *Streptomyces*. This study provides a complete profile of the influence of *Prunella vulgaris* L. on the morphology of the liver, inflammation factors, oxidative stress parameters, and intestinal microbiota in CCl4-challenged mice and suggests that *Prunella vulgaris* L. polysaccharides may exert hepatoprotection in relation to favorable modulation of the gut–liver axis.

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INTRODUCTION

The liver is the largest solid organ in the body, which regulates and balances primary energy metabolism, the immune system, and overall physiological homeostasis (Karabekir *et al.,* 2024). Both the liver and gut microbiota participate in the dynamic communication for metabolic, immune, and detoxification functions, which is referred to as the 'gut-liver axis (Xie *et al.,* 2024). Approximately 70% of the liver's blood supply comes from the portal blood from the portal vein, which drains the intestines and contains a lot of associated microbial byproducts, endotoxins, and nutrient components. Because of this closely intertwined relationship, the liver has some effects in the body and is the recipient of the consequences of the dysregulated intestinal microbiota, which refers to 'gut dysbiosis carcinoma (Anand and Mande, 2022). This leads to inflammation, oxidative injury, and metabolic disruption in the liver that is the precursor to myriad hepatobiliary diseases, including, but not limited to, fibrosis, cirrhosis, and hepatocellular carcinoma (Anand and Mande, 2022; Fuchs and Trauner, 2022).

Lipopolysaccharides in the liver's immune system as a result of the intestinal barrier being compromised by infection, toxins, and diet (Brescia and Rescigno, 2021). This generates a self-perpetuating cycle of worsening liver damage and decreased healing (Duan *et al.,* 2022; Pellegrini *et al.,* 2023). Thus, methods aimed at modulating the gut microbiome and improving the intestinal barrier are emerging as novel approaches to slow or prevent the progression of liver disease (Saleem *et al.,* 2025; Yang *et al.,* 2025). Among them, plant-derived polysaccharides emerge as multi-purpose molecules that can address both oxidative stress and immune dysregulation and simultaneously target the gut microbiome. An extensive review explains how such polysaccharides function in the treatment of liver diseases by modulating the following pathways: ROS scavenging, regulating inflammatory mediators NF-κB and Nrf2, communicating with bile acid and lipid pathways, and rebalancing the gut microbiota (Wei *et al.,* 2024).

The carbon tetrachloride (CCl4) model is also an established method for simulating human liver damage (Hafeez *et al.,* 2024). It can simulate the major processes involved in liver damage, which also include fibrosis and the disruption of the gut microbiome and the intestinal barrier, as the CCl4 model also affects the gut microbiome and the intestinal barrier (Unsal *et al.,* 2021). The liver is more important in the process of detoxification, which makes it more susceptible to toxic damage, and this is where reliable models for the simulation of hepatoprotection come into play. Although previous studies have established that extracts of *P. vulgaris* L. attenuate acute CCl_4-induced liver injury, largely by antioxidative and anti-inflammatory mechanisms (Hong *et al.,* 2023), the influence of its polysaccharide fraction in the context of chronic injury is still poorly characterized.

Traditional hepatoprotective approaches have focused on antioxidants, anti-inflammatory agents, and modulators of fibrosis. Very recently, attention has been given to natural products with prebiotic, antioxidant, and immunomodulatory properties in their potential to coordinate the gut microbiota and protect liver tissue simultaneously (Zhang, 2023). *Prunella vulgaris* L., used as a medicinal herb in traditional medicine, contains a variety of bioactive compounds such as polysaccharides, flavonoids, and triterpenoids that exert antioxidant, anti-inflammatory, and immunoregulatory effects (Kaufmann *et al.,* 2012; Nag *et al.,* 2017). More recently, evidence has emerged that *P. vulgaris* L. extracts may ameliorate liver inflammation by inhibiting pro-inflammatory pathways like TLR4/MyD88 and improve gut microbiota composition (Rao *et al.,* 2024). Although flavonoids and triterpenoids of *P. vulgaris* L. have been investigated for hepatoprotection, the exact contribution of polysaccharides, especially regarding the modulation of oxidative stress, inflammation, and restoration of gut microbiota, is under-investigated. This knowledge gap formed the basis for our focus on *P. vulgaris* L. polysaccharides in CCl_4-induced liver injury.

We chose the chronic model of CCl_4 characterized by the repeated administration of low doses over several weeks to better recapitulate the persistent hepatocellular injury, progressive fibrotic remodelling, and sustained gut microbiota dysbiosis that more closely resembles human chronic liver disease pathophysiology. Such an approach now contrasts with acute single-dose CCl_4 models, representing the early necroinflammatory events primarily. By integrating hepatic biochemical, histological, and microbiota analyses along a protracted course of injury, the present study is designed to explore the longer-term modulatory effects of *P. vulgaris* L. on the gut-liver axis. *P. vulgaris* L. polysaccharides exert hepatoprotective effects against CCl_4-induced liver injury by restoring gut microbiota regularity, enhancing intestinal barrier function, reducing oxidative stress, and suppressing hepatic inflammation. Current research aims to investigate the protective role of *P. vulgaris* L. polysaccharides on liver injury, oxidant stress, inflammatory response, and gut microbiota dysbiosis in CCl_4-induced chronic liver injury of mice. Current integrated hepatic and intestinal analyses will attempt to describe the mechanistic basis for how *P. vulgaris* L. polysaccharides mitigate chronic liver injury by gut–liver axis modulation and thus could represent a promising multi-target therapeutic approach.

MATERIALS AND METHODS

Characterization of P. vulgaris L.: *P. vulgaris* L. polysaccharide extract (purity≥95% by phenol-sulfuric acid assay and HPLC) was sourced from Shanghai Yuanye Bio-Technology Co., Ltd. According to the manufacturer's specification sheet, *P. vulgaris* L. is obtained from dried aerial spikes of *P. vulgaris* by hot-water extraction followed by ethanol precipitation and deproteinization, yielding a polysaccharide-enriched fraction that is quality-controlled for total carbohydrate content (mannose, galactose, and glucose) and residual solvent levels. No additional purification or chemical modification was carried out in our laboratory.

Experimental design: All procedures performed in this research were approved by the Laboratory Animal Welfare and Ethics Committee of the Yunnan Agricultural University (SQ202503087) and Nanjing Agricultural University (NJAU.No20240910164). Thirty male ICR mice (6-7 weeks old, 25±1.9 g) were purchased from the Qinglongshan Experimental Animal Centre in Nanjing. After three days of adaptive feeding, those animals were randomly divided into a control group (AC), model group (AM), and PVL treatment group (AXK) (n=10). The AXK group was given 150 mg/kg of *P. vulgaris* L. extract by gavage daily, whereas the AC and AM groups were given equal amounts of distilled water. The AM group was given 2ml/kg of 10% CCl_4 (Shanghai Macklin Biochemical Co., Ltd) by intraperitoneal injection every 2 days, diluted in olive oil every two days. In contrast, mice in other groups were administered an equivalent volume of olive oil via the same intraperitoneal route. On day 32 of the experiment, all animals were weighed and euthanised via inhalant anaesthesia of Ketamine and Tolazoline (3 min) for strangulation, and later their serum, rectal contents, and liver tissues were taken for further analysis. All animals contributed to body weight and organ index measurements. Both the liver and kidney indices (%) were calculated using the formula: Organ index (%) = (organ weight (g) / body weight (g)) × 100.4.

Biochemical, oxidative stress, and inflammatory marker analysis: Blood specimens were collected and kept at 4°C for one hour, followed by centrifugation at 1200 rpm for 15 minutes to collect the serum. Serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) activities, as well as oxidative stress indicators including superoxide dismutase (SOD), total antioxidant capacity (T-AOC), glutathione peroxidase (GSH-Px), and malondialdehyde (MDA), were evaluated utilizing commercially available kits (Nanjing Jiancheng Bioengineering Institute). Interleukin-6 (IL-6), IL-10, IL-1β, and tumour necrosis factor-alpha (TNF-α) in serum samples were quantified using enzyme-linked immunosorbent assay (ELISA) kits (Beijing Solarbio Science & Technology Co., Ltd., Beijing, China), following the manufacturers' protocols.

Histopathological analysis: Liver specimens underwent fixation in 4% paraformaldehyde, paraffin embedding, sectioning, and were then sent to stain with Hematoxylin and Eosin (H&E), and Sirius Red (Pinuofei Biological Technology, Wuhan, China). Morphological and fibrotic features were evaluated microscopically by piloting an GX41 system (Olympus Co., Japan). All histological assessments were performed by a veterinary pathologist who was blinded to the group allocation and treatment codes to minimize observer bias.

Intestinal microbiota sequencing: Genomic DNA extraction from rectal contents was performed on fecal samples from 5 randomly selected mice per group (AC, AM, AXK; total n = 15) (Table 1) using DNA extraction kits (Tiangen Biotech, China). The quality of extracted products was evaluated using 1.5% agarose gel electrophoresis, while their concentration and purity were examined with a NanoDrop 2000 UV-Vis spectrophotometer (Thermo Scientific, USA). The 16S rRNA V3-V4 hypervariable region was amplified via primers 338F (5'-ACTCCTACGGGAGGCAGCA-3') and 806R (5'-GGACTACHVGGGTWTCTAA-3') (Xu *et al.,* 2025). Before sequencing, purification, quantification, and normalization were completed. Sequencing was performed on the Illumina NovaSeq 6000 platform. Clustering of acquired sequences into operational taxonomic units was performed using USEARCH (v10.0). 70% confidence cut-off was used for taxonomic assignment through the SILVA database (v138.1). Within-sample species richness (alpha diversity) was calculated using QIIME2. Before diversity analysis, sequencing data were rarefied to the minimum read depth per sample to standardize sampling effort across groups. Beta diversity differences among groups were assessed using permutational multivariate analysis of variance (PERMANOVA) based on Bray–Curtis distances. Inter-group community composition (beta diversity) was compared through principal coordinate analysis (PCoA) and Nonmetric Multidimensional scaling (NMDS) with Bray-Curtis distances (Chen *et al.,* 2023). The significant abundance differences were highlighted using linear discriminant analysis, effect size, and T-test (Chen *et al.,* 2023; Xu *et al.,* 2025). The functional analysis of KEGG pathways of mice was performed by using PICRUSt2 (Xu *et al.,* 2025).

Table 1: Summary of sequencing data processing metrics

Sample ID	Raw Reads	Clean Reads	Denoised Reads	Merged Reads	Non-chimeric Reads
AC1R	80,169	73,265	73,036	71,737	57,888
AC2R	80,108	73,406	73,173	70,816	54,784
AC3R	64,203	58,840	58,780	58,625	57,555
AC4R	61,458	56,412	56,315	55,218	50,537
AC5R	79,965	73,272	73,130	71,922	63,843
AM1R	80,025	73,193	73,028	71,696	63,751
AM2R	79,956	73,289	73,163	72,055	70,153
AM3R	79,921	72,974	72,628	68,344	44,537
AM4R	44,974	41,177	41,165	41,073	33,206
AM5R	77,889	71,306	70,967	67,596	44,938
AXK1R	80,039	73,131	72,962	71,076	51,224
AXK2R	80,185	73,417	73,324	71,787	67,597
AXK3R	71,559	65,733	65,565	63,242	53,425
AXK4R	78,088	71,656	71,527	70,321	56,692
AXK5R	73,831	68,118	67,951	66,598	54,900

Statistical analysis: Analyses were carried out in SPSS (v22.0, IBM Corp., USA). Results are shown as mean ± standard error of the mean (SEM). Mice comparisons employed Student's t-tests and one-way ANOVA, with statistical significance defined as $^*P<0.05$.

RESULTS

Body weight, liver weight, and organ indexes: During the initial 16 days of the experiment, the body weight of animals in different groups gradually increased. The control group exhibited the highest weight gain, followed by the AM group, while the PVL treatment group displayed the lowest weight gain. However, between days 16 and 32, the model group exhibited a decline in body weight, while the treatment group continued to gain body. By the final day of the study, the body weight of the treatment group was obviously higher than AM group ($P<0.05$) (Fig. 1a). Following CCl4 exposure, a noteworthy reduction in liver weight ($P<0.05$) and liver index ($P<0.001$) was noticed (Fig. 1b&c), while the kidney index significantly increased ($P<0.001$) (Fig. 1d).

Biochemical, oxidative stress, and inflammatory marker assessment: Significant elevation of serum markers relative to controls was caused by CCl4-induced hepatic injury: ALT ($P<0.001$), AST ($P<0.001$), IL-6 ($P<0.01$), IL-1β ($P<0.001$), TNF-α ($P<0.001$), and MDA ($P<0.001$). Conversely, IL-10 levels were markedly decreased ($P<0.001$). Antioxidant capacity was markedly decreased as evidenced by significant decreases in GSH-Px ($P<0.001$), SOD ($P<0.05$), and T-AOC ($P<0.001$). *P. vulgaris* L. treatment provided some level of hepatoprotection against CCl4-induced injury in comparison to untreated model animals (Fig. 2a-j).

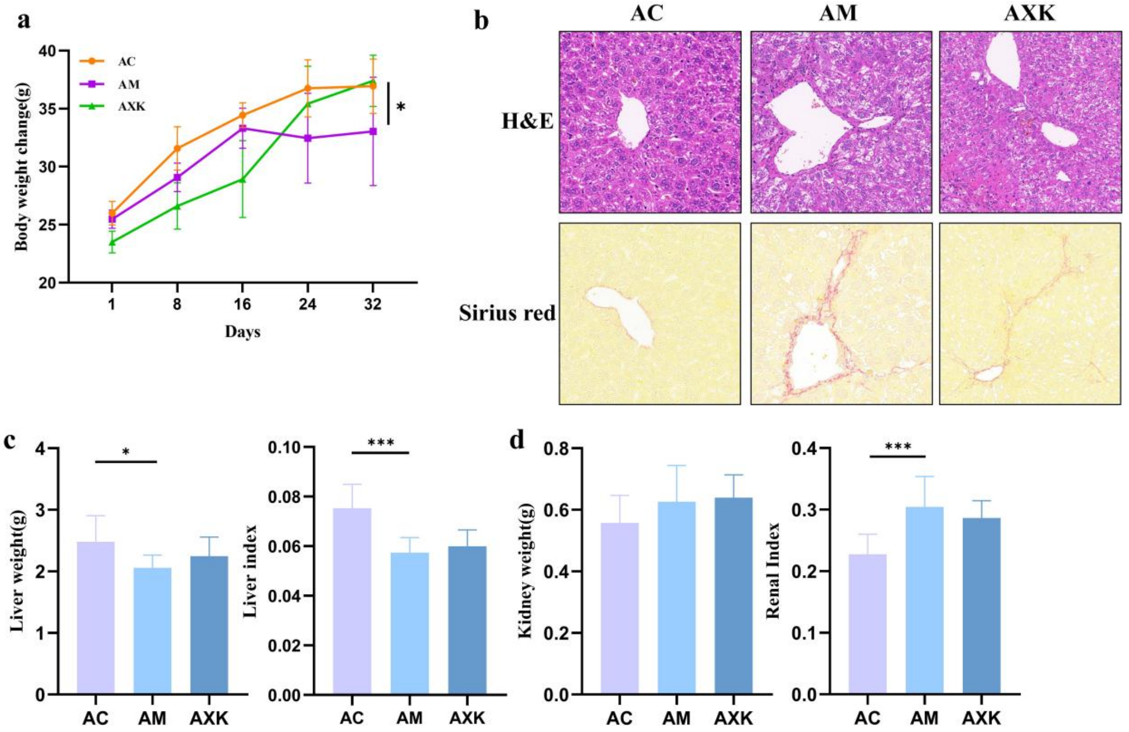


Fig. 1: Effects of *Prunella vulgaris* L. on body weight and organ morphology in CCl_4-treated mice. (a) Changes in body weight across different groups. (b) Representative H&E and Sirius Red-stained liver sections. (c) Liver weight and liver index. (d) Kidney weight and renal index. $^*P<0.05$, $^{***}P<0.001$; data presented as mean ± SEM.

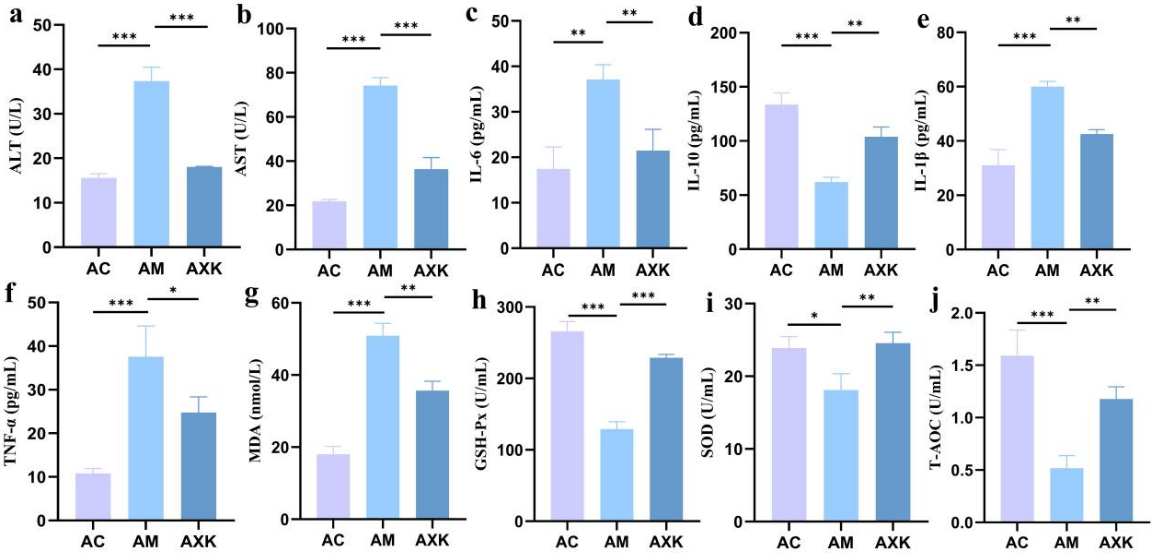


Fig. 2: Effects of *Prunella vulgaris* L. on biochemical markers, inflammatory cytokines, and oxidative stress in mice. (a) ALT, (b) AST, (c) IL-6, (d) IL-10, (e) IL-1β, (f) TNF-α, (g) MDA, (h) GSH-Px, (i) SOD, and (j) T-AOC levels. $^*P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$; data presented as mean ± SEM.

Histopathology: To further evaluate the hepatoprotective effects of the *P. vulgaris* L. polysaccharides, hepatic tissues were analyzed histopathologically, including analyses of H&E and Sirius Red staining. H&E images from the control group showed normal hepatic histoarchitecture, including radially arranged hepatocyte cords with centrally located nuclei and preserved lobular organization. In the model group, widespread vacuolar necrosis around the portal tracts, prominent inflammatory cell infiltration, disordered hepatocyte arrangement, and disrupted lobular architecture could be seen. However, in the treatment group, hepatic architecture was relatively preserved with a remarkable reduction in hepatocyte swelling, necrosis, and inflammatory infiltration (Fig. 1b).

***Prunella vulgaris L.* modulates gut microbiota in mice:** Sirius Red staining revealed very minor collagen deposition in the control group, consistent with an intact lobular structure. The model group exhibited pronounced expansion of collagenous septa and lobular distortion, indicative of early fibrotic remodelling. By contrast, *P. vulgaris* L.-treated livers displayed reduced Sirius Red–

positive collagen and better preservation of lobular architecture compared with the model group, indicating the attenuation of CCl4-induced fibrotic changes at a histological level (Fig. 1b). *P. vulgaris* L. affects gut microbiota in mice. High-throughput sequencing by Illumina NovaSeq produced 1,112,370 raw sequences from the 15 fecal samples. After strict quality control, the high-quality sequences remaining totaled 825,030, representing 5,263 ASVs. A total of 242 ASVs were identified across the three groups (Fig. 7a). The Shannon index and rank-abundance curves reached a plateau, suggesting that the sequencing depth is appropriate to capture relatively homogenous species (Fig. 7b, c).

Microbial diversity and richness were measured with PD_whole_tree, ACE, Shannon, and Simpson indices. All Shannon and Simpson indices presented an upward trend in the control and treatment groups; however, such changes did not attain statistical significance. By contrast, the PD_whole_tree index significantly increased in the treatment group with a P-value of 0.029 (Fig. 8a). PCoA presented clear group clustering. NMDS analysis revealed that samples from the AC and AXK groups clustered together, whereas AM samples were highly separated. In summary, all the results above indicated that CCl4 disrupted gut microbiota composition, while *P. vulgaris* L. administration partially resumed gut microbial balance (Fig. 8b).

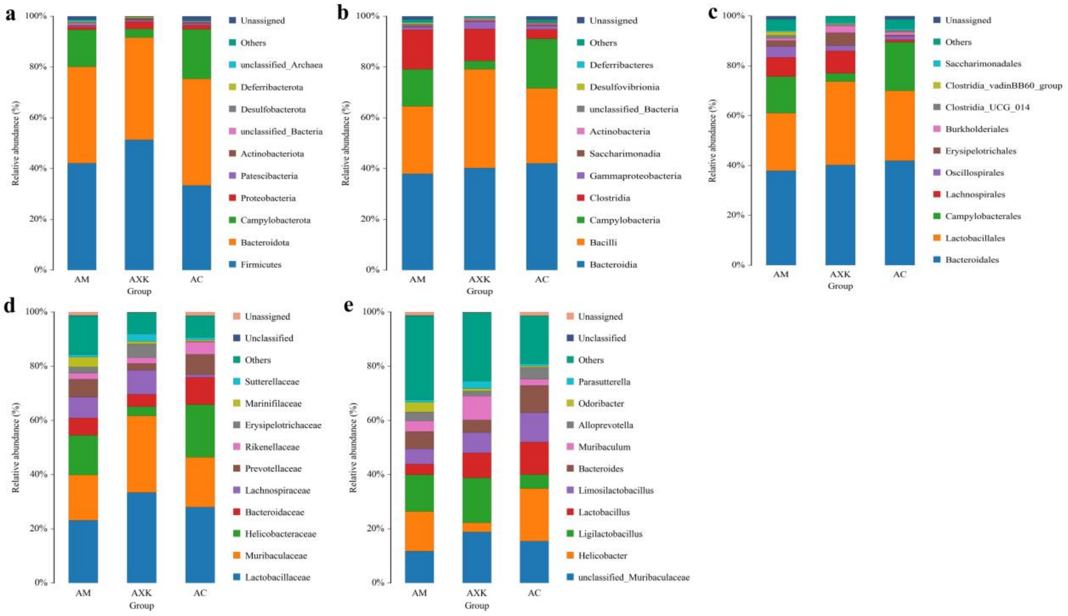


Fig. 3: Taxonomic composition of intestinal microbiota. Bar chart displaying bacterial composition at different taxonomic levels: (a) Phylum, (b) Class, (c) Order, (d) Family, and (e) Genus.

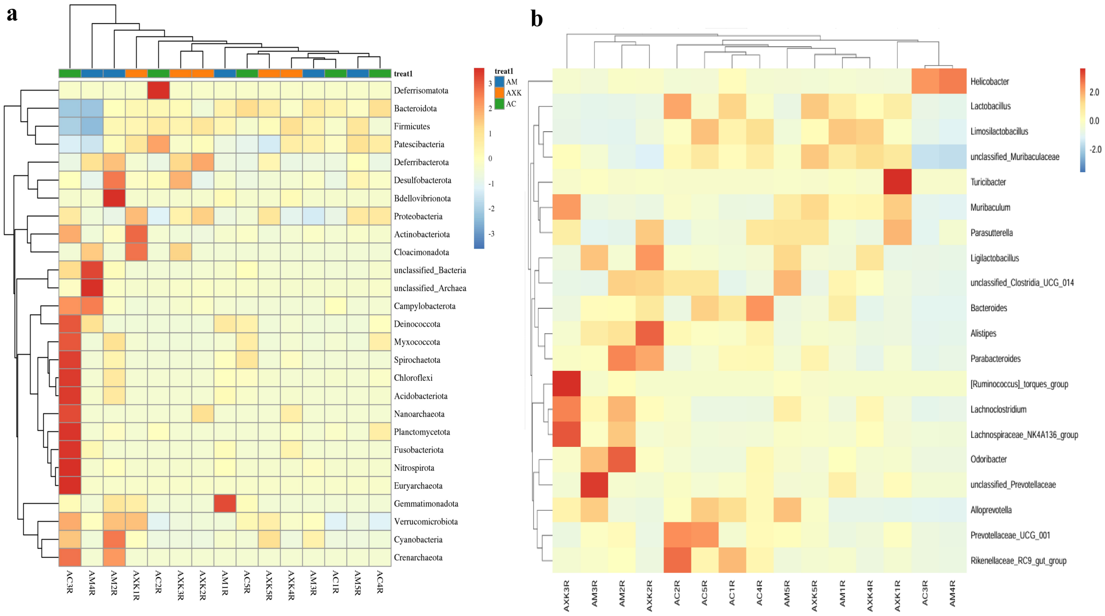


Fig. 4: Correlation heat maps of intestinal microbiota. Correlation heat maps of horizontal abundance of phylum and genus in the intestinal flora of mice in each group. (a) phylum (b) genus.

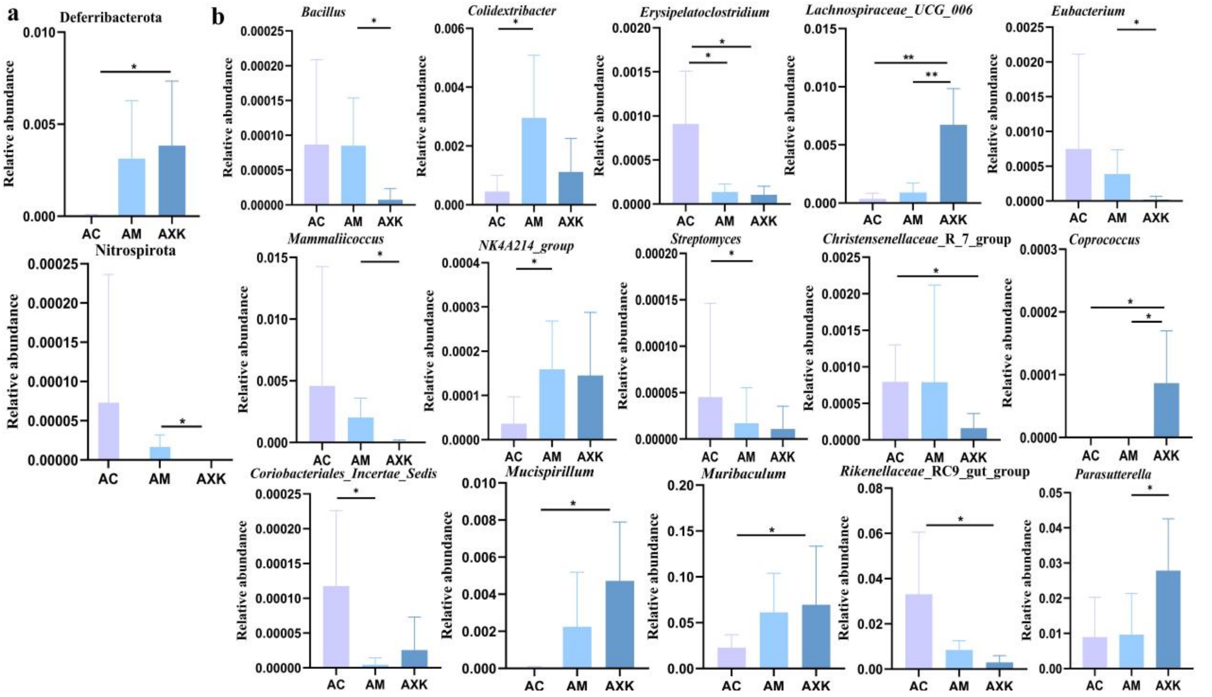


Fig. 5: Differentially abundant bacteria at the (a) phylum and (b) genus Level. Bacteria showing significant differences in abundance at the genus level. *P<0.05, **P<0.01; data presented as mean ± SEM.

Fig. 6: Analysis of KEGG metabolic pathway differences between groups.

Taxonomic analysis at various levels revealed that Firmicutes (AC=33.32%; AM=42.11%; AXK=51.37%) and Bacteroidota (AC=42.07%; AM=37.94%; AXK = 40.25%) were the predominant phyla across groups (Fig. 3a). At the class level, Bacteroidia (AC=42.05%; AM=37.94%; AXK=40.25%), Bacilli (AC=29.57%; AM= 26.56%; AXK=38.84%), and Clostridia (AM=15.52%; AXK = 12.52%) were the dominant classes (Fig. 3b). At the order level, *Bacteroidales* (AC=41.99%; AM=37.91%; AXK=40.24%) and *Lactobacillales* (AC=28.04%; AM=23.12%; AXK=33.42%) were predominant (Fig. 3c). The most abundant families included *Lactobacillaceae* (AC=28.00%; AM=23.11%; AXK=33.40%) and *Muribaculaceae* (AC=16.77%; AM=28.30%; AXK=18.40%) (Fig. 3d). At the genus level, *Muribaculaceae* (AC=15.42%; AM=11.78%; AXK=18.82%) was consistently dominant, while Lactobacillus (AC=11.98%; AXK=9.25%) was more enrichment in control and treatment mice compared to the model mice (Fig. 3e).

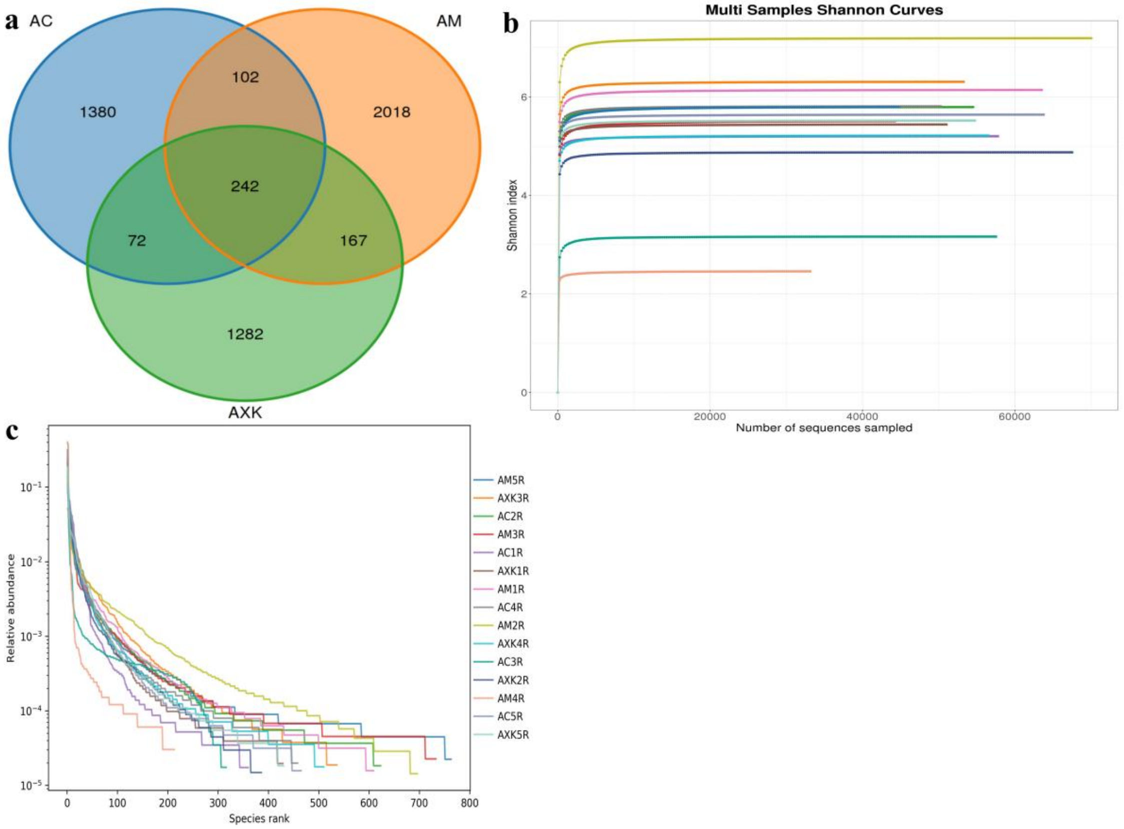


Fig. 7: Sequencing quality and depth analysis. (a) Venn diagram showing shared and unique features. (b) Rarefaction curve depicting sequencing depth. (c) Rank abundance curve illustrating species distribution.

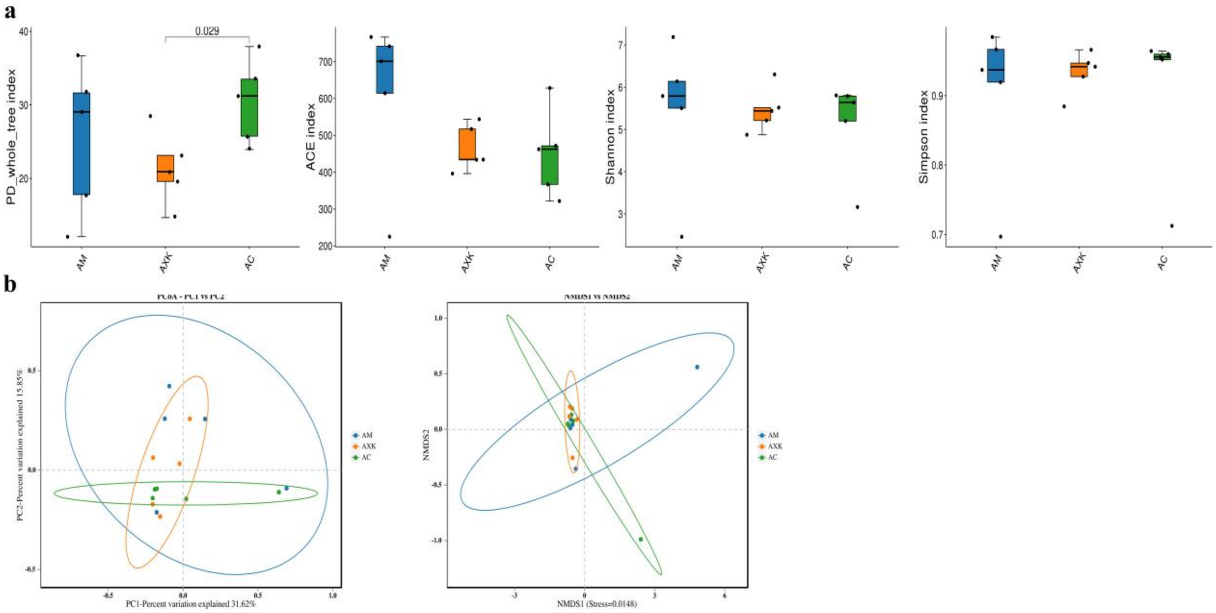
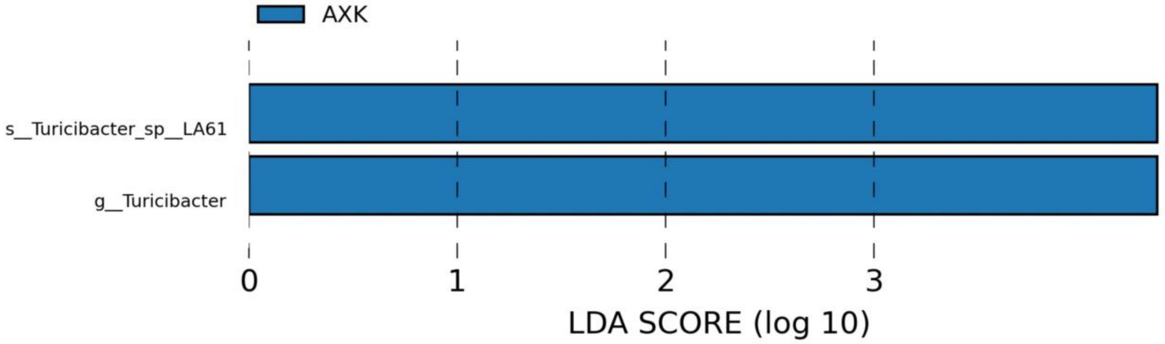


Fig. 8: Species diversity analysis of intestinal microbiota. (a) Alpha diversity analysis. (b) Beta diversity analysis.

Heatmap clustering analysis showed that CCl4 induced a decline in the abundance of phyla of Myxococcota, Spirochaetota, Planctomycetia, and Nitrospirota. The abundance of some microorganisms has reversed (Fig. 4a). At the genus level, the abundances of Mucispirillum and Alistipes in the CCL4-induced group increased, and after the intervention of Prunellia sinensis, the abundances of Mucispirillum and Alistipes in the treated animals were reduced to close to the level of the control group (Fig. 4b). LefSe analysis pinpointed notable marker species exclusive to the treatment group, specifically observing differential abundance of *Turicibacter* at the genus level and Turicibacter_sp_LA61 at the species level (Fig. 9).



Cladogram

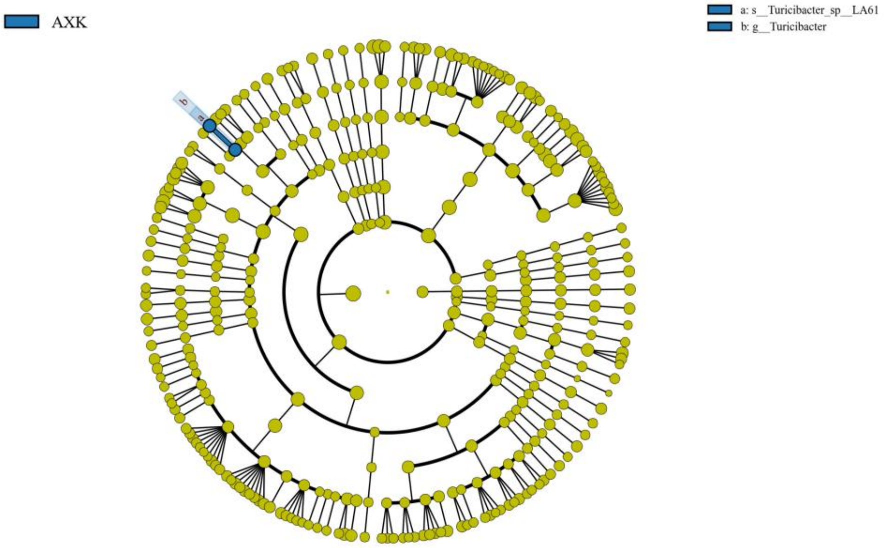


Fig. 9: LEfSe analysis of intestinal microbiota. Linear discriminant analysis (LDA) effect size (LEfSe) analysis and bacterial LDA score representation.

Additional analysis via multiple t-tests revealed genus-level alterations. Phylum of Deferribacterota was significantly higher in AXK than AC ($P<0.05$), and Nitrospirota in AM was higher than AXK ($P<0.05$) (Fig. 5a). Relative to controls, the AM mice showed significantly elevated abundances of *Colidextribacter* and *NK4A214* ($P<0.05$), but substantial reductions in *Erysipelatoclostridium*, *Coriobacteriales_Incertae_Sedis* and *Streptomyces* ($P<0.05$). Following *P. vulgaris* L. treatment, *Lachnospiraceae_UCG_006*, *Parasutterella*, *Bacillus*, and *Mammaliicoccus* abundances increased dramatically ($P<0.05$), while *Eubacterium* dropped ($P<0.05$) (Fig. 5b). In PICRUSt2-based predictive functional analysis (KEGG level 2), CCl_4 exposure was associated with altered relative abundances of pathways related to 'Global and overview maps', amino acid metabolism, nucleotide metabolism, and carbohydrate metabolism (Fig. 6).

DISCUSSION

Our study demonstrated that *Prunella vulgaris* L. polysaccharides exert significant hepatoprotective effects against CCl_4-induced chronic liver damage in mice. CCl_4 is extensively utilized as an agent to induce liver injury in animal models, primarily mediated by cytochrome P450 2E1 (CYP2E1)-dependent biotransformation, producing trichloromethyl and trichloromethyl peroxy radicals that initiate lipid peroxidation, disrupt membrane integrity, and impair hepatocyte function. Oxidative insult depletes endogenous antioxidant defenses, leading to hepatocellular necrosis and fibrosis (Abdelmegeed *et al.,* 2021; Hafeez *et al.,* 2024). Accordingly, CCl4 exposure is associated with acute hepatic injury, as evidenced by increased serum ALT and AST activities, considered critical biomarkers of liver injury. Increases in these enzymes reflect hepatocyte deformation, increased membrane permeability, and subsequent leakage of enzymes into the bloodstream (Chen *et al.,* 2018; Liu *et al.,* 2020). Overall, our findings are in good agreement with recent investigations into hepatoprotective plant polysaccharides, which have been reported to exert complementary antioxidant, anti-inflammatory, and gut microbiota-modulating activities in experimental liver injury (Wei *et al.,* 2024). In such a context, *P. vulgaris* L. could be considered a representative example with dual actions on hepatic biochemical indices and gut microbial composition.

In this study, the administration of *P. vulgaris* L. polysaccharide significantly reduced ALT and AST activities and normalized liver indices. These findings are in accordance with Ahmad et al. (2020), who found reduced ALT and AST activities after *P. vulgaris* L. extract treatment in hepatotoxic models. However, extending prior studies using crude extracts, our results now show that the polysaccharide fraction alone significantly alters the overall composition of gut microbiota, supporting an effective prebiotic function besides hepatoprotection. Under normal conditions, a balance between oxidative and antioxidative systems is maintained by the body. Oxidative stress upsets this balance, which accentuates the damage to organs (Wang *et al.,* 2019). Large amounts of free radicals CCl3· and CCl3OO· are produced during CCl4 metabolism, which interrupts the phospholipid bilayer of the hepatocyte membrane and initiates lipid peroxidation, thereby contributing to liver injury (Mondal *et al.,* 2023). One of the primary results from lipid peroxidation is malondialdehyde (MDA), which results in the disruption of membranes, causing cell swelling and death (Ling *et al.,* 2025). SOD and GSH, on the other hand, are vital protective antioxidants that, respectively, reduce and defend against oxidative damage (Lőrincz and Szarka, 2017; Ramachandran and Jaeschke, 2017). The administration of *P. vulgaris* L. polysaccharide markedly increased levels of hepatic SOD, GSH-Px, and T-AOC while decreasing levels of MDA, which indicates increased scavenging of ROS and decreased oxidative damage. Thus, *P. vulgaris* L. provides hepatoprotection by reducing oxidative stress and increasing the activity of antioxidant enzymes.

Oxidative stress and systemic inflammation are closely interlinked in the pathogenesis of chronic liver injury (Kouroumichakis *et al.,* 2011; Qian *et al.,* 2023). In this study, *P. vulgaris* L. treatment profoundly reduced TNF-α, IL-1β, and IL-6 and increased the level of anti-inflammatory cytokine IL-10. These observations are consistent with modulation of redox-sensitive and inflammatory signaling cascades, such as the Nrf2–ARE axis and TLR4/MyD88/NF-κB, that have been implicated in chemically induced liver injury (Arango-De la Pava *et al.,* 2021; Qian *et al.,* 2023; Yao *et al.,* 2025). However, we did not directly quantify Nrf2 activation, antioxidant response element transcription, or TLR4/MyD88/NF-κB pathway components in liver tissue, and thus our mechanistic interpretation remains speculative. Furthermore, histopathological evaluation provided direct morphological evidence of *P. vulgaris* L. protective effects. CCl4 induces structural and inflammatory damage to the liver (Mohammed and Kadhim, 2025). Excess collagen deposition is driven by hepatic stellate cell activation under persistent oxidative and inflammatory stress. Histopathological examination confirmed that *P. vulgaris* L. exerts hepatoprotective activity against CCl4-induced liver fibrosis, inflammatory infiltration, and structural changes. Because these are all potential upstream triggers, this action might suppress stellate cell activation, with such suppression preventing extracellular matrix deposition and progression to fibrosis (Kong *et al.,* 2024). The absence of the measurement of the collagen area or molecular markers like α-SMA, type I collagen, TGF-β, or liver hydroxyproline content, along with qualitative histology, limits our quantitative assertions about fibrosis to histological fibrosis. While we interpret the decrease in Sirius Red staining as indicating decreased fibrosis, the lack of quantitative and molecular assessments obviates saying this with greater certainty.

The gut microbiome is now widely regarded as a key component of the health of the host organism and is especially important when looking at liver disease because of the gut-liver axis (Yao *et al.,* 2025). Gut microbiota dysbiosis contributes to liver disease development, as there is mounting evidence that demonstrates their essential role in this process. Most of the time, liver injuries will disrupt the balance of the intestines, making the intestines more permeable and increasing the movement of microbes, which accelerates liver diseases (Albillos *et al.,* 2020). CCl_4-related liver injury impairs barriers in the intestines and allows for the passage of the bacterial component lipopolysaccharide (LPS), which enters the portal circulation and induces liver inflammation through activating TLR4. In this paper, we did gut microbiome analysis, and we found colonization of different microbes in CCl4-exposed liver-injured rodents. In comparison to the other rodents, the CCl_4 rodents had a significantly higher abundance of the microbes `*Colidextribacter*` and `*Mammaliicoccus*` and had a significantly lower abundance of `*Mammaliicoccus*` and the microbes `*Lachnospiraceae UCG006*`, `*Coriobacteriales Incertae Sedis*`, and `*Streptomyces*`. Prior research demonstrated that `*Colidextribacter*` was positively associated with high-fat diet-induced mice with non-alcoholic fatty liver disease (Hu *et al.,* 2021), and `*Mammaliicoccus*` was a pathogenic bacterium that caused host tissue damage through `glutamyl` specific endopeptidase and `exfoliative` toxin C (Gismene *et al.,* 2024). In contrast, Lachnospiraceae is known for its carbohydrate fermentation activities, producing short-chain fatty acids, primarily butyric acid and acetic acid, which have anti-inflammatory properties (Abdugheni *et al.,* 2022). For example, Lachnospiraceae has been associated with lower inflammation in the liver due to pyruvate production, the inhibition of ferroptosis, and the suppression of inflammatory cell infiltration (Deng *et al.,* 2025). *Coriobacteriales Incertae Sedis* is associated with propionic acid level and bile acid metabolism (Zhao *et al.,* 2024), while Streptomyces exhibits strong antioxidant properties and antibacterial activity against Gramella (Konwar *et al.,* 2024; Shah *et al.,* 2024). These findings revealed the remit effect of *P. vulgaris* L. on CCl_4-challenged mice by reducing the abundance of harmful bacteria and enriching beneficial microbes. Furthermore, *P. vulgaris* L. modulated gut microbial communities in a manner consistent with enhanced production of potentially beneficial metabolites (e.g., SCFAs). However, we did not directly quantify intestinal barrier integrity markers (such as ZO-1, claudin-1, occludin) or circulating lipopolysaccharide, and thus any conclusion regarding ‘intestinal barrier repair’ remains speculative at this stage. The multi-dimensional protective profile observed suggests that *P. vulgaris* L. polysaccharides act through integrated mechanisms targeting the gut–liver axis, rather than through a single biochemical pathway. Although *P. vulgaris* L. significantly improved several histological and biochemical parameters. We acknowledge that the present work is preliminary and recommend future studies with expanded cohorts, extended intervention periods, and additional mechanistic assays to further delineate the molecular targets of *P. vulgaris* L. polysaccharides and establish dose–response relationships.

Although our findings support a link between *P. vulgaris* L. induced microbiota remodeling and improved hepatic outcomes, the present study cannot establish causality. We did not perform microbiota manipulation experiments such as antibiotic-mediated depletion, gnotobiotic colonization, or fecal microbiota transplantation (FMT). Such interventions would be required to determine whether the hepatoprotective effects of *P. vulgaris* L. are primarily microbiota-dependent or reflect a combination of direct hepatic and microbiota-mediated actions. Future studies employing antibiotic-treated or germ-free mice, coupled with FMT from *P. vulgaris* L.-treated donors, will be critical to dissect the causal contribution of the microbiota to *P. vulgaris* L.-induced hepatoprotection.

Conclusions: *Prunella vulgaris* L. alleviates CCl4-induced chemical liver injury by inhibiting abnormal aminotransferase and oxidative stress, increasing antioxidant enzyme activity, and reducing inflammatory factor levels. Moreover, *P. vulgaris* L. positively affected the gut microbiome by increasing the abundance of the beneficial bacteria *Lachnospiraceae UCG*006, *Coriobacteriales Incertae Sedis*, and Streptomyces, while decreasing the abundance of the harmful bacteria Colidextribacter and Mammaliicoccus. This is the first study that demonstrates the dual role of *P. vulgaris* L. polysaccharides, which provides the basis for future translational and mechanistic research.

Data availability: All raw sequence data from mice were deposited in the NCBI Sequence Read Archive database under accession number: PRJNA1244574.

Authors contribution: JG, XZ, LY and LL conceptualization and methodology; JG, XZ, XL, QL and QH reagents, materials and analysis tools; LL original draft writing and preparation. JG, LY, MAA, AMA and LL review and edit, visualization and supervision; All authors reviewed and approved the final manuscript.

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