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RESEARCH ARTICLE

Prunella vulgaris Polysaccharides Counteract Chromium-Induced Liver Injury via Antioxidant and Gut Microbiota Pathways

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ABSTRACT

Hexavalent chromium ($K_2Cr_2O_7$) induces liver damage in livestock, causing health and productivity losses. *Prunella vulgaris* polysaccharides (PVP) possess anti-inflammatory and antioxidant activities. This study applied network pharmacology, network toxicology, animal experiments and *in-vitro* assays to explore the protective effects of PVP on $K_2Cr_2O_7$-induced hepatotoxicity. Network toxicology and pharmacology identified TP53, HIF1A, MYC as potential targets and revealed a connection to oxidative stress and apoptosis pathways. *In-vitro*, PVP showed strong antioxidant activity. In $K_2Cr_2O_7$-challenged mice given PVP by gavage (75mg/kg, 32 days), body weight loss was rescued, liver fibrosis and serum ALT/AST were reduced ($P<0.01$), serum inflammation cytokines IL-6, TNF-α, IL-1β and IL-10 were balanced ($P<0.05$), oxidative stress enzymes T-AOC, GSH-Px and SOD were restored ($P<0.05$). 16S rRNA sequencing data suggested that PVP could alleviate intestinal flora disorders induced by $K_2Cr_2O_7$ and increased Lachnospiraceae_NK4A136_group levels. In AML-12 cells exposed to $K_2Cr_2O_7$ *in-vitro*, NQO1/HO-1/SOD2 levels were increased, MDA levels decreased, GSH concentrations elevated and ROS scavenged when PVP-containing serum was added to culture medium. Our findings reveal that PVP can protect mice from $K_2Cr_2O_7$-induced hepatotoxicity by inhibiting oxidative stress damage, blocking inflammatory responses and regulating the gut microbiota balance.

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INTRODUCTION

Chromium is a heavy metal widely used in steel manufacturing and tannery industries that increasingly penetrates environmental compartments through industrial discharge (Koppel *et al*., 2021; Su *et al*., 2021). Livestock are at higher risk of exposure due to $K_2Cr_2O_7$ use as a flotation agent for fecal egg detection in livestock production plants and as a milk preservative agent in some regions; this enables Cr(VI) accumulation in animal body tissues through forage feed consumption (Imran, 2022; Yue *et al*., 2023). Chromium-contaminated food products pose a dual risk to human health—direct toxicity from Cr(VI) via ingestion of contaminated animal products (Dheeba *et al*., 2015) and indirect Cr (VI) toxicity via association with fecal contaminants or soil particles. Chromium compounds also have extensive applications in leather tanning industries where they initiate shift from hexavalent to trivalent chromium forms during leather processing (Su *et al*., 2021). This can be further aggravated through co-exposure of sewage sludge-treated pastures with animal waste products during manure application which is

exacerbated by quadruple food chain transfer from Cr-contaminated farmlands.

Tissue damage results from excessive ROS production upon Cr(VI) exposure in hepatic cells; it generates ROS intermediates with lipid peroxidation leading to exacerbation of hepatic injury and inflammation thereby inducing genotoxicity (Zhao *et al*., 2019; Nde *et al*., 2021; Deng *et al*., 2024). Su *et al*. (2024) also observed elevated ALT/AST levels in rats treated with Cr(VI) at 9mg/kg/day for 15 days; toxic histopathological changes were triggered by genotoxic as well as immuno-inflammatory triggers. Currently, there is no specific treatment regimen for patients exposed to high levels of chromium and management strategy is largely supportive in nature, driven by their genotoxicity; supportive care provides tissue protection through supportive adjustments in antioxidant status yet no antidote is available. Plants enrich medicinal metabolites such as rosmarinic acid contained in herb *Rosmarinus officinalis* and trace element selenium offer protection against oxidative lesions caused by Cr(VI) molecules, which upregulate cellular antioxidant pathways (Khalaf *et al*., 2020; Wang *et al*., 2022; Gao *et al*., 2024). Similarly, the protection provided by plant polysaccharides remains largely unknown.

Prunella vulgaris L., Chinese name xiakucao or Xia-Ku-Cao is an important traditional herb-medicinal formulas widely used for several hundred years widely or consumed as condiments (Sun *et al*., 2025); a preparation made from *Prunella vulgaris* L- XIa Ku Cao. Flowers & leaves containing polysaccharides exhibited immunomodulating properties contrary to free radical scavenging activities (Mir *et al*., 2022; Zhang *et al*., 2024a); children consume alcoholic syrup containing *Prunella vulgaris* L. extract daily in spring season to prevent pediatric diseases. Polysaccharides from seaweed (Masudani *et al*., 2015) have been shown to alleviate Cr(VI)-induced apoptotic tissue lesions in rabbit colon cells although *Prunella vulgaris* polysaccharides could protect against Cr(VI)-induced liver injury remains unclear (Gerbst *et al*., 2021; Jing *et al*., 2024).

To fill this gap, we extracted PVP and evaluated its hepatoprotective efficacy in a $K_2Cr_2O_7$-challenged murine model, with the broader goal of informing the development of natural feed additives or veterinary protective agents that safeguard livestock health and curb production losses associated with chromium toxicity.

MATERIALS AND METHODS

Experimental Animals: Forty-five male KM mice (4-week-old, 24.56±0.75g) were randomly assigned to three groups (n=15). They were housed under standard conditions (12 h light/dark cycle, *ad-libitum* access to food and water). All procedures were approved by the Animal Use Committee of Yunnan Agricultural University (Permission code: 202407012, Date: 10 July 2024; License: SCXK(Dian)K2015-0002).

Network Toxicology and Network Pharmacology Analysis: Toxicological targets of potassium dichromate were retrieved from the CTD database (http://ctdbase.org/), returning 2,403 genes, while liver injury-associated genes—12,069 in total—were sourced from GeneCards (https://www.genecards.org/). For *Prunella vulgaris*, bioactive components were screened from TCMSP (https://tcmsp-e.com/) and BATMAN-TCM (http://bionet.ncpsb.org.cn/batman-tcm/) using DL>0.18 and OB>30% as cutoffs; polysaccharide constituents were manually supplemented from prior reports (Zhang *et al*., 2020). Target prediction for these components, performed via CTD, yielded 3,493 non-redundant genes after deduplication. Overlapping targets among the three gene sets were subjected to PPI network construction through the STRING database (https://string-db.org/, version 11.5; confidence >0.7). Visualization and topological filtering—based on degree, betweenness and closeness centrality—were carried out using Cytoscape (version 3.9.1) to extract core targets. For functional annotation, GO enrichment and KEGG pathway analyses were run on Metascape (https://metascape.org/), with an adjusted $P<0.01$ set as the significance bar.

Extraction and Purification of PVP: PVP was extracted following a patented protocol with minor modifications. Dried *P. vulgaris* (purchased from herbal market in Kunming, China) powder was defatted with 95% ethanol under reflux (three times, 2 h each). The residue was boiled in water (solid: liquid = 1:5 g/mL) for three 2-h cycles. Pooled extracts were centrifuged and the supernatant was precipitated with 3 volumes of 95% ethanol (4°C, 24h). The resulting crude polysaccharides were deproteinized by Sewage treatment (chloroform:n-butanol = 4:1, v/v; 5–8 times), dialyzed and lyophilized. Polysaccharide content was 88.06% as determined by phenol-sulfuric acid assay.

Animal Experimental Design and Grouping: After 3 days of acclimatization, mice were divided randomly into 3 groups (n=15): Control (NC), Model (MD) and PVP Intervention group (PVP). Mice in the PVP group were orally administered PVP (75mg/kg) for 32 consecutive days, while the NC and MD received equal volume of saline. The MD and PVP groups simultaneously received daily intraperitoneal injections of $K_2Cr_2O_7$ (25mg/kg, 2mL/kg, prepared 1:1 in olive oil) to induce hepatitis. Deep anesthesia was induced by intraperitoneal injection of sodium pentobarbital (80mg/kg), followed by euthanasia via cardiac puncture and blood drainage. Death was confirmed by cessation of respiration, dilated pupils and absence of response to a noxious stimulus. Serum, liver, and rectal tissue samples were collected. Liver tissues were stored at −80°C and fixed in 4% paraformaldehyde, respectively.

Measurement of Body Weight and Liver Index: Body weight was recorded every 8 days during the 32-day modeling and administration period. After euthanasia, complete liver tissue was dissected, rinsed with pre-cooled saline, blotted dry and weighed. Liver Index (%)=(liver weight ÷body weight)×100%.

Detection of Liver Function and Biochemical Indicators by ELISA: Serum ALT, AST, T-AOC, SOD, GSH-Px, MDA, IL-6, TNF-α and IL-1βlevels were detected using ELISA kits (Nanjing Jiancheng Bioengineering Institute;

Nanjing, China) according to the manufacturer's instructions.

Sirius Red Staining: Liver tissues were fixed in 10% neutral buffered formalin for 48 h and embedded in paraffin. 4μM sections were deparaffinized, rehydrated and stained with 0.1% Sirius Red (Solarbio; Beijing, China) in saturated picric acid for 60min, washed in 0.5% acetic acid, counterstained with Weigert's iron hematoxylin for 5min, blued in ammonia solution, dehydrated and mounted in neutral resin. Collagen-positive area was determined with Image J

Gut Microbiota 16S rRNA Sequencing Analysis: Genomic DNA was extracted from cecal contents using a DNA extraction kit (Majorbio; Shanghai, China). The variable regions V3-V4 of the bacterial 16S rRNA gene were amplified using common primers F338 (ACTCCTACGGGAGGCAGCAG) and R806 (GGACTACHVGGGTWTCTAAT). Amplicons were sequenced on an Illumina NovaSeq platform. Raw data were quality-filtered and denoised for chimeras using QIIME2 (version 2019.4) software to obtain high-quality sequences (ASVs). Sequence comparison with the Silva database enabled the taxonomic assignment of different ASVs. At the same time, we analyzed alpha diversity based on ACE Chao1 Shannon Simpson and PD_whole_tree metrics as well as beta diversity through PCoA and NMDS metrics, LEfSe analysis was used to identify taxa differences between two groups.

***In-vitro* Cellular Experiments:** Preparation of PVP-containing serum, Male SD rats received oral PVP—80mg/kg on day 1, then 53mg/kg for 6 consecutive days, while controls got distilled water. 2h after the last gavage on day 7, serum was collected and then heat-inactivated (56°C, 30min), passed through 0.22μM filters and aliquoted at −20°C until use.

AML-12 hepatocytes (alpha mouse liver 12) were grown in AML-12 complete medium (MedChemExpress (MCE); NJ, USA). For viability testing, cells plated in 96-well plates (1×10^4/well) received PVP-serum at 5, 10, 20, or 30% for 24h; 10% yielded the highest survival and was chosen for follow-up assays. Separate dose-response experiments with $K_2Cr_2O_7$ (0–400μM, 12h) allowed calculation of the IC_{50} (cck-8 method).

ROS production was monitored by flow cytometry using DCFH-DA, with untreated cells set as 100%. MDA, GSH, T-SOD in cell lysates and IL-6, IL-10, IL-1β, TNF-α in culture supernatants were measured with ELISA kits. For mRNA expression analysis, RNA was isolated with TRIzol, reverse-transcribed (Nanjing Vazyme Biotech Co., Ltd; Nanjing, China), and amplified with gene-specific primers for NRF2, HO-1, NQO1 on a real-time PCR platform (reagents obtained from Nanjing Vazyme Biotech Co., Ltd; Nanjing, China). β-actin served as the endogenous reference and relative quantities were derived by the $2^{-\Delta\Delta Ct}$ method. The primer sequences used for qRT-PCR are listed in Table 1.

Statistical Analysis: Data are presented as mean±SD. One-way ANOVA with Tukey's post hoc test was performed using SPSS 22.0 for group comparisons. A $P<0.05$ was considered statistically significant.

Table 1: Primer sequences

Gene	Forward (5'→3')	Reverse (5'→3')
NRF2	TTCACCTCGATCTCCTGTTG	AATGTGCTGGCTGTGCTTTA
HO-1	ACAGATGGCGTCACTTCG	TGAGGACCCACTGGAGGAG
NQO1	AGGATGGGAGGTACTCGAATC	AGGCGTCCTTCCTTATATGCTA
β-actin	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT

RESULTS

Combination of network toxicology and pharmacology with *in-vitro* antioxidant activity: Network toxicology and pharmacological approaches were employed to identify the overlapping targets between $K_2Cr_2O_7$-induced toxicity and the therapeutic effects of *Prunella vulgaris* (PVP). Toxicological screening yielded 367 core genes from the CTD and GeneCards databases, which were significantly enriched in stress and inflammation-related biological processes, such as the positive regulation of cell migration, cellular responses to cytokine stimulation, leukocyte activation and adaptation to hypoxia (Fig. 1A–B). The corresponding PPI network featured TP53, STAT3 and TNF as high-degree hub nodes governing these stress and inflammatory responses (Fig. 1C). Concurrently, pharmacological screening retrieved 375 genes associated with PVP and DILI, showing marked enrichment in inflammatory signaling cascades (e.g., IL-11-mediated signaling, TNF signaling and cytokine–cytokine receptor interactions) alongside cancer-related pathways (e.g., proteoglycans in cancer, EGFR resistance and apoptosis) (Fig. 1D–E). The topological analysis of this network delineated an apoptosis module (CASP3, BCL2, TP53) and a signaling/inflammation module (EGFR, SRC, MAPK3, TNF), with TP53 persisting as a shared hub node across both networks (Fig. 1F). Collectively, these bioinformatic results imply that PVP may attenuate $K_2Cr_2O_7$-induced inflammation and oxidative stress by modulating inflammatory pathways and apoptotic signaling. Corroborating these *in-silico* predictions, *in-vitro* assays verified the potent antioxidant capacity of PVP: T-AOC levels plateaued at 4.02±0.15μmol/L beyond 5mg/mL, whereas ABTS and DPPH radical scavenging rates both surpassed 80% at concentrations above 0.02mg/Ml, underscoring its direct radical-scavenging efficacy (Fig. 1G).

PVP Ameliorate $K_2Cr_2O_7$-Induced Liver Injury in Mice: Recording of mouse body weight revealed that PVP improved the body weight loss induced by $K_2Cr_2O_7$induced hepatitis in mice (Fig. 2B); compared with the model group, they significantly reduced liver weight ($P<0.01$) (Fig. 2C). Sirius Red staining of mouse livers showed that PVP significantly reduced the area of fibroblasts and type I collagen fibers in the liver ($P<0.01$) (Fig. 2 D-E). These results indicate that PVP can alleviate the clinical symptoms of $K_2Cr_2O_7$ -induced liver injury in mice.

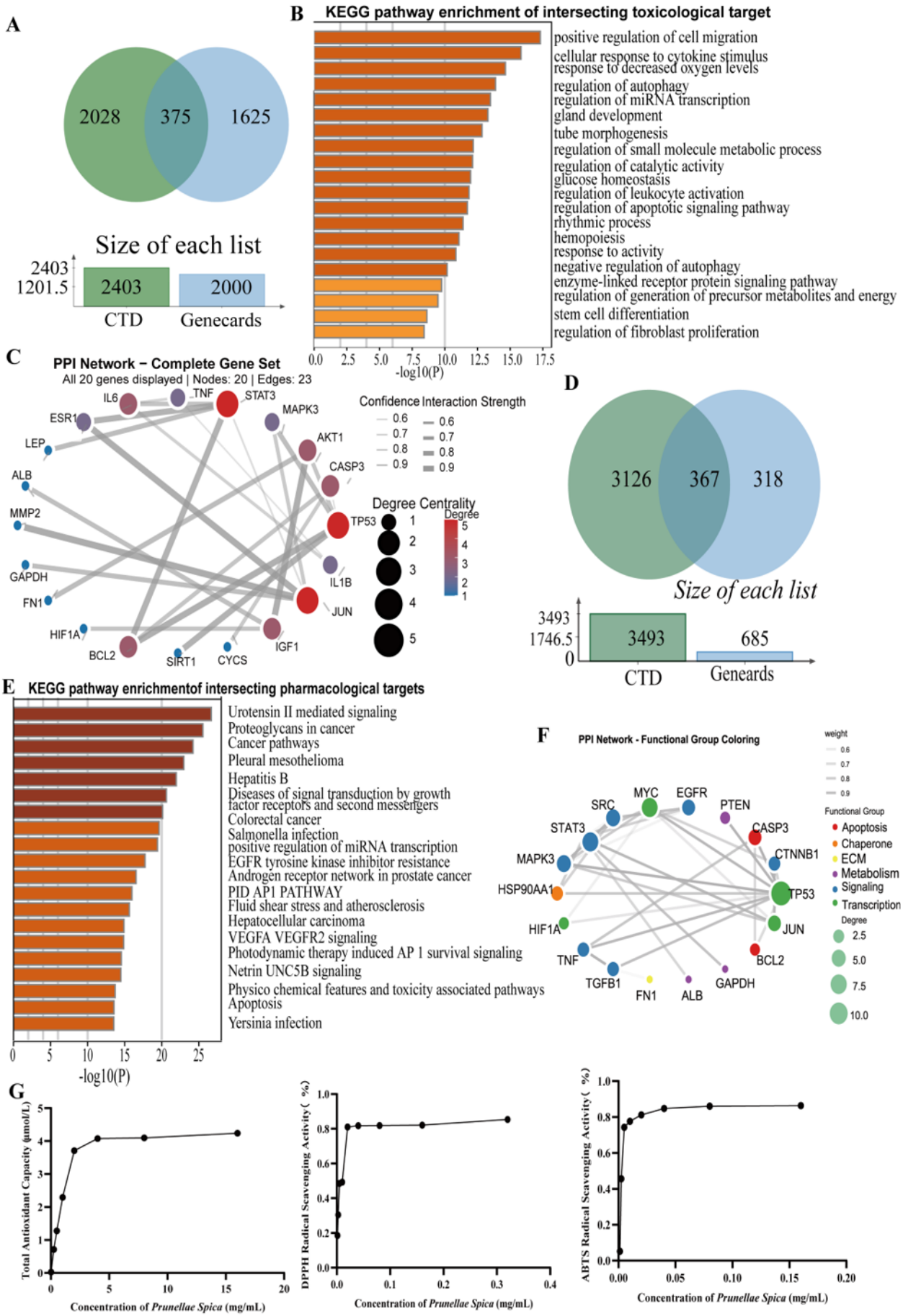


Fig. 1: Integrated network analysis and *in-vitro* antioxidant activity of PVP. (A) Venn diagram of toxicological targets. (B) KEGG pathway enrichment of intersecting toxicological targets. (C) PPI network of core toxicological targets. (D) Venn diagram of pharmacological targets. (E) KEGG pathway enrichment of intersecting pharmacological targets. (F) PPI network of core pharmacological targets. (G) *In-vitro* antioxidant capacity assessed by T-AOC, ABTS and DPPH assays.

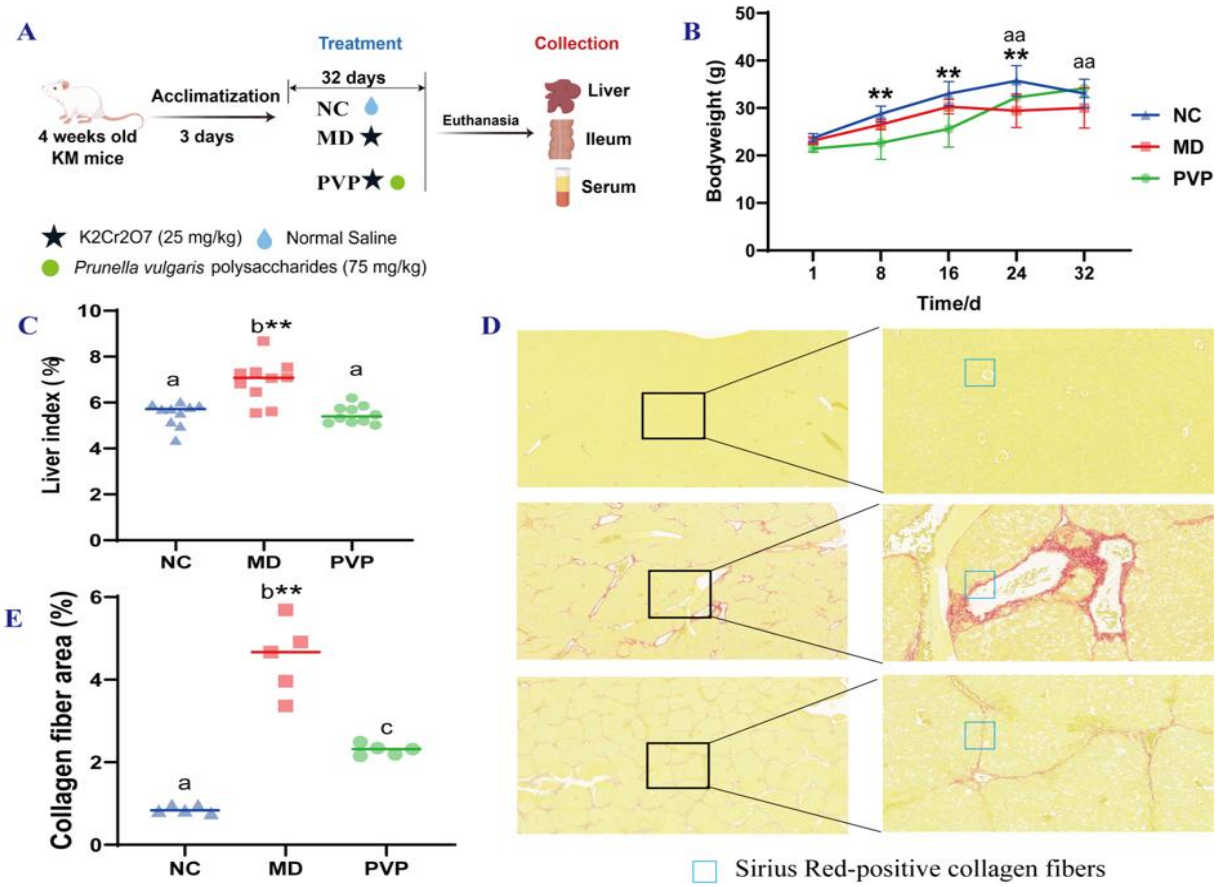


Fig. 2: PVP ameliorates $K_2Cr_2O_7$-induced liver injury. (A) Study design. (B) Body weight. (C) Liver index. (D) Sirius Red staining (scale bar, 100μM). (E) Fibrotic area quantification.

PVP Alleviate Hepatic Oxidative Stress and Inflammatory Response: Measurements were taken on mouse serum for liver injury markers ALT and AST; oxidative stress markers T-AOC, GSH-Px and SOD; and inflammatory markers IL-6, TNF-α, IL-1β and IL-10. The results showed that after $K_2Cr_2O_7$ induction, serum levels of ALT, AST, IL-6, TNF-α and IL-1β in mice increased, with highly significant differences compared to the control group ($P<0.001$); serum T-AOC, GSH-Px, SOD and IL-10 decreased ($P<0.001$ or $P<0.05$). After treatment with PVP, serum levels of ALT, AST, IL-6, TNF-α and IL-1β in mice decreased, with highly significant differences compared to the model group ($P<0.001$); serum T-AOC, GSH-Px, SOD and IL-10 increased, with highly significant differences compared to the model group ($P<0.001$) (Fig. 3A-C). These results indicate that PVP can improve the condition of $K_2Cr_2O_7$-induced liver injury in mice, enhancing their *in-vivo* antioxidant and anti-inflammatory capacities.

PVP Regulate Gut Microbiota Dysbiosis: PVP's impact on gut ecology was evaluated through 16S rRNA sequencing of cecal contents. In the Model group, ACE and Chao1 indices dropped below control levels ($P<0.05$), signaling a loss of microbial richness following $K_2Cr_2O_7$ challenge. PVP treatment raised these indices ($P<0.05$ vs. Model), indicative of restored richness (Fig. 4A–B). Rarefaction curves plateaued across all groups as sequencing depth increased (about > 30K), ensuring adequate coverage of microbial diversity (Fig. 4C). Beta diversity analysis (PcoA, NMDS) showed clear separation of the microbiota structure among the three groups, with the greatest difference between the control and model groups, while the microbiota structure of the PVP group was closer to that of the control (Fig. 5B, C, D).

PVP Reshapes Gut Microbial Composition: In terms of microbiota composition, PVP intervention significantly reversed the dysbiosis caused by $K_2Cr_2O_7$. At the phylum level, the abundance of Bacteroidota in the PVP group was significantly higher than that in the Model group ($P<0.05$) (Fig. 6A). At the genus level, the PVP group significantly increased the abundance of beneficial bacteria or short-chain fatty acid-producing bacteria suppressed by $K_2Cr_2O_7$, such as *Lachnospiraceae*_NK4A136_group, unclassified_Lachnospiraceae, etc. and decreased the abundance of some potential pathobionts (Streptococcus) (Fig. 6J-L). These results indicate that PVP can effectively ameliorate $K_2Cr_2O_7$-induced gut microbiota dysbiosis and restore microbiota homeostasis.

PVP Protects AML-12 Cells Against $K_2Cr_2O_7$-Induced Oxidative Stress: 10% PVP-containing serum showed the highest cell viability and was used for subsequent experiments (Fig. 7A). The IC_{50} of $K_2Cr_2O_7$ was 29.4μM (Fig. 7B). PVP pretreatment significantly reversed $K_2Cr_2O_7$-induced ROS elevation (5.2-fold, $P<0.001$, Fig. 7C). QPCR analysis showed that $K_2Cr_2O_7$ upregulated Nrf2 (1.6-fold), HO-1 (5.5-fold), NQO1 (2.1-fold) and SOD2 (1.8-fold). PVP further increased NQO1 and SOD2 (2.7-fold and 1.9-fold vs. MD) while Nrf2 returned to baseline (Fig. 8A). Consistently, $K_2Cr_2O_7$ decreased T-SOD (37%) and GSH (28%) and increased MDA (27%); PVP restored all markers to near-normal levels ($P<0.05$ or $P<0.01$, Fig. 8B).

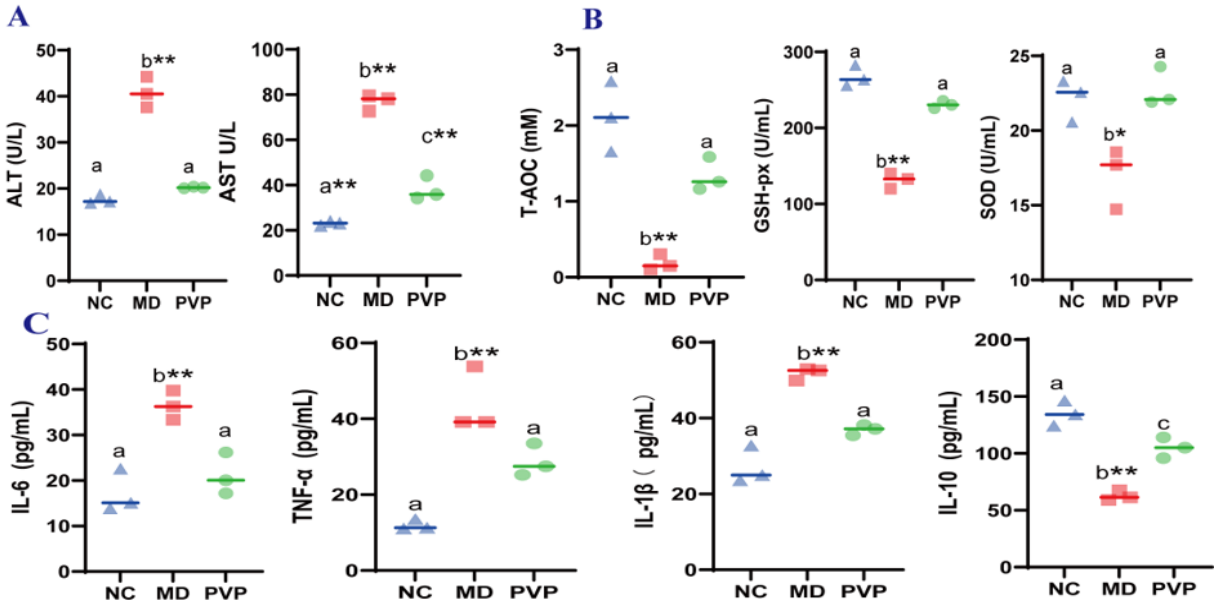


Fig. 3: Effects of PVP on serum biochemical parameters in $K_2Cr_2O_7$-induced mice. (A) Liver transaminases (ALT, AST). (B) Antioxidant indicators (T-AOC, GSH-Px, SOD). (C) Inflammatory cytokines (IL-6, TNF-α, IL-1β, IL-10).

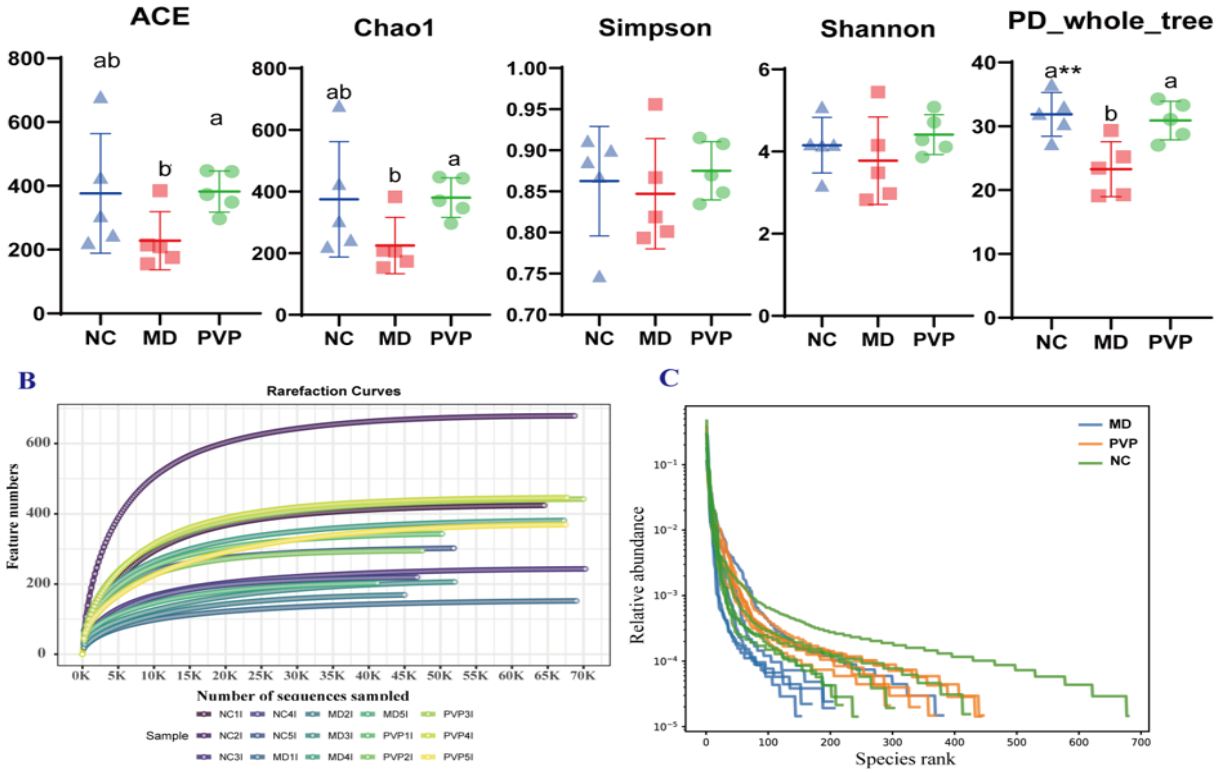


Fig. 4: Effects of PVP on the alpha diversity of gut microbiota in $K_2Cr_2O_7$-induced mice. (A) Alpha diversity indices, including ACE, Chao1, Simpson, Shannon and PD_whole_tree. (B) Rarefaction curves (C) Rank-abundance curves.

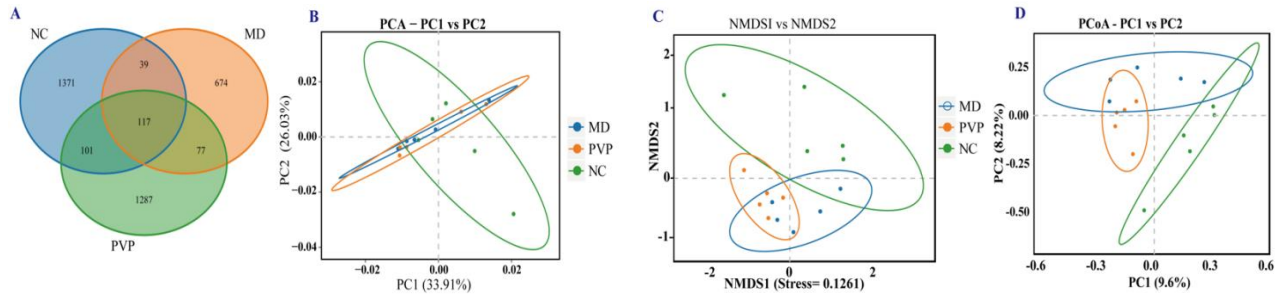


Fig. 5: PVP reshapes gut microbial community structure in $K_2Cr_2O_7$-exposed mice. (A) Venn diagram of ASVs across groups (B) PCA (C) NMDS (D) PcoA.

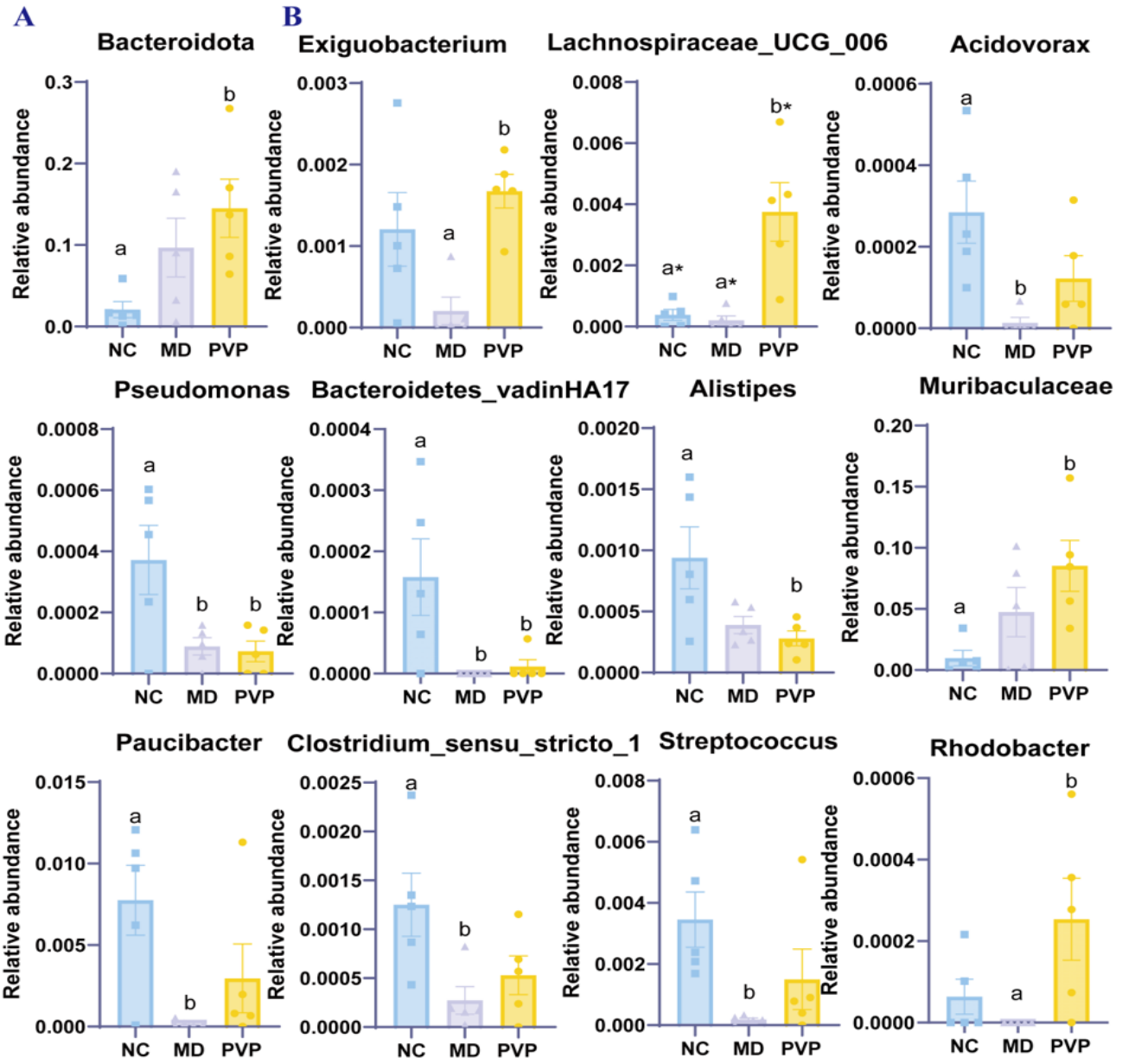


Fig. 6: Effects of PVP on the relative abundance of gut microbiota at different taxonomic levels in $K_2Cr_2O_7$-induced mice (A) Composition and changes at the phylum level (B) Composition and changes at the genus level.

DISCUSSION

Chromium contamination in livestock production systems has become an increasing concern due to its dual role as both an environmental pollutant, intentional additive in fecal parasite detection and milk sample preservation. This study comprehensively evaluated the protective effects of PVP against $K_2Cr_2O_7$-induced liver injury through an integrated approach combining experimental validation and computational prediction. PVP acts as a potent antioxidant *in-vitro* while counteracting $K_2Cr_2O_7$-driven hepatic damage and gut dysbiosis *in-vivo*;a profile that positions it as a natural hepatoprotective candidate for veterinary use.

The network toxicology and pharmacology analysis uncovered the multi-target nature of PVP action. A total of 375 overlapping genes between $K_2Cr_2O_7$ toxicity targets and PVP-associated active component targets, coupled with PPI network prioritization of HIF1A and MYC as core nodes, offers crucial mechanistic insights. HIF1A, a master regulator of cellular oxygen response, is consistent with PVP's observed antioxidant and anti-inflammatory activities and may contribute to the attenuation of $K_2Cr_2O_7$-induced oxidative damage in hepatocytes. Similarly, MYC enrichment implies that PVP may confer hepatoprotective benefits through modulating cell survival pathways, potentially opposing $K_2Cr_2O_7$-driven apoptotic signaling. GO and KEGG enrichment analyses corroborated these observations, with notable enrichment in terms related to oxidative stress, inflammatory regulation and apoptosis (Kanehisa *et al*., 2023).

Experimentally, PVP displayed marked free radical scavenging activity at low doses (>0.02mg/mL), with total antioxidant capacity leveling off at 5mg/mL. Upon liver injury, diverse cells release abundant inflammatory cytokines, which trigger quiescent hepatic stellate cells to undergo trans-differentiation into myofibroblasts and drive excessive extracellular matrix (ECM) secretion, chiefly

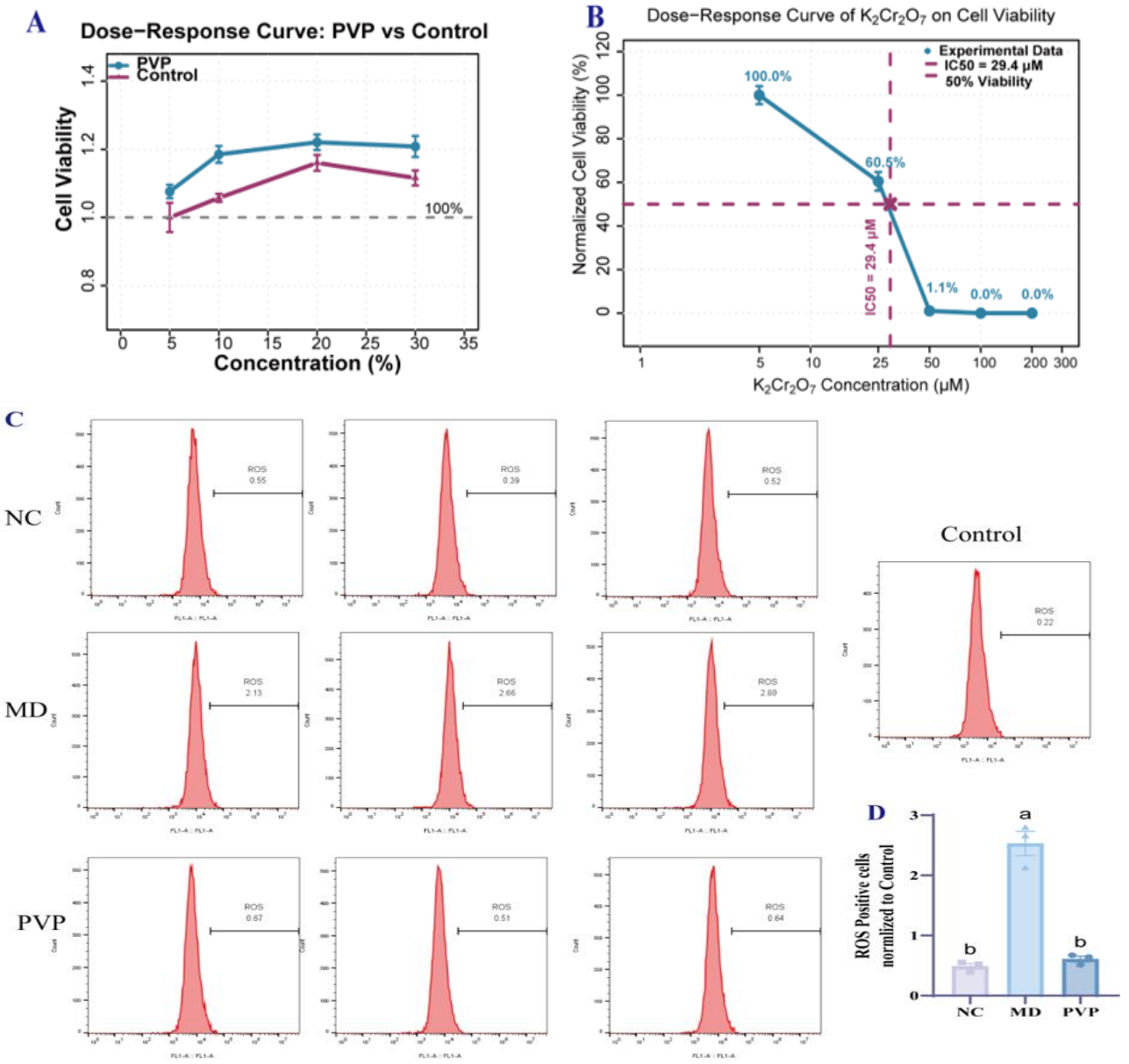


Fig. 7: PVP-containing serum reduces $K_2Cr_2O_7$-induced cytotoxicity and ROS production in AML-12 cells (A) PVP serum CCK-8 (B) $K_2Cr_2O_7$ IC_{50} = 29.4 μM (C) ROS (flow).

collagen (Rogers *et al*., 1995; Zhang *et al*., 2022). This disturbs the balance between ECM synthesis and degradation, resulting in excessive deposition within liver tissue, eventually structural damage and promotion of liver fibrosis (Rho *et al*., 2023). $K_2Cr_2O_7$ is known to induce hepatocyte necrosis, collagen accumulation and fibrosis via oxidative stress and inflammatory cascades (Park *et al*., 2023). In this study, model group mice exhibited weight loss, increased liver index and collagen fiber deposition, indicating successful replication of the liver injury model (Jaeschke and Ramachandran, 2024; Zhang *et al*., 2024b). PVP treatment significantly improved weight loss, reduced the liver index and decreased the fibrotic area in Sirius Red staining (Wang *et al*., 2025). This suggests that PVP can alleviate $K_2Cr_2O_7$-induced liver injury and fibrosis, potentially related to its inhibition of fibroblast activation and collagen deposition (Kisseleva and Brenner, 2021).

Furthermore, this study found that PVP regulated serum biochemical and inflammatory factors (Chen *et al*., 2024). ALT and AST are common indicators reflecting liver function damage; PVP significantly reduced their levels, suggesting a protective effect against hepatocyte damage (Alvarez-Garcia *et al*., 2024). Simultaneously, PVP upregulated the antioxidant enzymes T-AOC, GSH-Px and SOD, downregulated the pro-inflammatory factors IL-6, TNF-α and IL-1β, and upregulated the anti-inflammatory factor IL-10 (Wang *et al*., 2022). This indicates that PVP can exert both antioxidant and anti-inflammatory effects, thereby mitigating liver damage caused by oxidative stress and the inflammatory cascade (Du *et al*., 2025).

The liver and gut are linked via the "gut-liver axis," and gut microbiota dysbiosis can promote the release of inflammatory factors, exacerbating liver injury (Tripathi *et al*., 2018; Hov and Karlsen, 2023). This study found that the alpha diversity of the gut microbiota was significantly decreased in the model group, while PVP partially restored this diversity, showing no significant difference from the control group, indicating its ability to improve $K_2Cr_2O_7$-induced microecological disorder (Wang *et al*., 2024). In

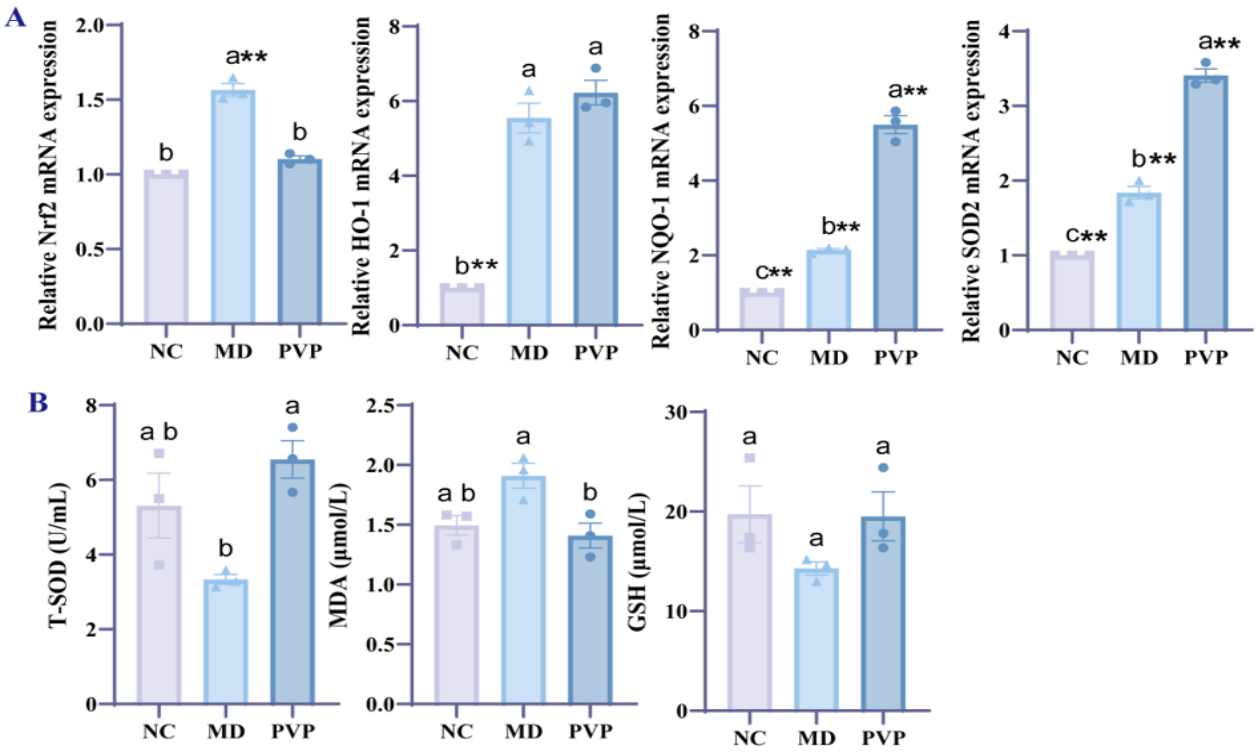


Fig. 8: PVP-containing serum activates Nrf2 pathway and restores redox homeostasis in AML-12 cells (A) Qpcr of Nrf2/HO-1/NQO1/SOD2 (B) T-SOD/MDA/GSH Different letters (a, b, c) above the bars indicate statistically significant differences among groups (one-way ANOVA followed by Tukey's post hoc test, P<0.05, **P<0.01).

terms of microbiota structure, PVP significantly increased the abundance of Bacteroidota and reversed the reduction of several beneficial genera (Zhang *et al*., 2025). As dietary glycans are known to act as prebiotics—shaping gut microbiota composition and stimulating short-chain fatty acid (SCFA) production-they contribute critically to digestive health, intestinal barrier integrity and immune regulation (Yang *et al*., 2025). Consistent with this notion, our data show that PVP, a plant-derived polysaccharide, effectively modulates the gut microbial community. This places PVP as a candidate prebiotic agent, capable of both regulating intestinal flora and mitigating chromium-induced liver injury. Further expanding its potential, PVP holds promise not only as a prebiotic and detoxifying agent for chromium exposure in human health, but also as a feed supplement to enhance overall health and productivity in animal farming. Given the widespread presence of chromium contamination in livestock production and its detrimental effects on animal health, PVP also shows great potential as a feed supplement for farm animals to enhance liver health, improve growth performance and reduce the risk of chromium-induced hepatotoxicity in veterinary practice (Campbell *et al*., 2023).

Based on the *in-vivo* hepatoprotective effects of PVP, we prepared PVP-containing serum and found that it restored all oxidative stress markers (ROS, MDA, GSH, T-SOD) to near-normal levels, upregulated NQO1 and SOD2 and maintained HO-1 expression without increasing Nrf2. Notably, SOD2 was upregulated at both mRNA and activity levels, indicating enhanced mitochondrial antioxidant capacity.

It is worth noting that PVP-containing serum, rather than PVP itself, was used in these cellular experiments. Since PVP is a polysaccharide, it may not be directly absorbed. One possibility is that PVP modulates the gut microbiota and that circulating microbial metabolites contribute to hepatocyte protection. This interpretation remains tentative. One caveat is that PVP was not directly tested on cells. Subsequent work should compare PVP directly with PVP-containing serum and probe the gut-liver axis more deeply—especially in agricultural animal settings—to solidify the translational case for PVP as a veterinary hepato-protectant.

Conclusions: In summary, this work establishes that PVP confer substantial protection against $K_2Cr_2O_7$-induced liver injury via multiple complementary routes. The polysaccharides both scavenge free radicals directly and bolster endogenous antioxidant defenses, while curbing inflammatory responses and reinstating gut microbial equilibrium. Network analysis additionally points to modulation of candidate molecular hubs, including HIF1A and MYC. Taken together, these observations lay a scientific groundwork for regarding PVP as a natural countermeasure against heavy metal-induced hepatopathy—meriting further exploration for both clinical translation and veterinary application in livestock settings to reduce chromium-associated health risks and advance animal welfare.

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

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