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Integrated microbiome and metabolomic analysis uncover the amelioration mechanisms of *Prunella vulgaris* L. polysaccharides on gut health of ulcerative colitis

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ABSTRACT

Prunella vulgaris L. has been reported to exhibit certain remission potential for ulcerative colitis (UC). However, research that specifically focused on the *P. vulgaris* L. polysaccharides (PVP) is limited. The present study evaluated the anti-UC effects of PVP in mice with UC and investigated the underlying mechanism. The results indicated that PVP ameliorated the symptoms of UC by inhibiting colon shortening and weight loss, suppressing serum inflammatory cytokine (tumor necrosis factor (TNF)- α , interleukin (IL)- β , and IL-6) levels, preventing the activation of the colonic nod-like receptor family pyrin domain containing 3 (NLRP3) inflammasome, and enhancing the expression of tight junction proteins to maintain gut barrier integrity. Additionally, PVP treatment significantly altered gut microbiota perturbation by decreasing the abundance of norank_f_norank_o_Clostridia_UCG-014, Rikenellaceae_RC9_gut_group, and unclassified_f_Prevotellaceae. In contrast, it increased the abundance of *Lactobacillus*, *Desulfovibrio*, *Enterorhabdus*, and norank_f_Oscillospiraceae. Metabolomic analysis revealed that 23 altered metabolites were selected as potential biomarkers of mice with UC following PVP treatment. These metabolites significantly enriched the pathways of glycerophospholipid metabolism and sphingolipid metabolism. Furthermore, there was a strong correlation between the alterations in gut microbiota at the genus level and the changes in metabolites. These results provide insights into the ameliorative effects of PVP on UC, suggesting its potential of PVP as a functional food or natural agent for UC management.

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1. Introduction

Ulcerative colitis (UC), a type of inflammatory bowel disease (IBD), is characterized by weight loss, diarrhea, and bloody stools. These symptoms are closely linked to the risk of colorectal carcinoma. Epidemiological studies have demonstrated a significant rise in the

incidence and prevalence of UC in China over recent decades, posing a serious risk to human health^[1]. Although the precise pathogenesis of UC remains inconclusive, its development is influenced by complex factors, including environmental factors and genetics. Moreover, UC severely disrupts intestinal health, particularly impacting the gut microbiota disorders, the gut barrier, and metabolic abnormalities^[2]. Intestinal flora is vital in modulating gut metabolites and inflammation, thereby improving the gut barrier^[3]. Animals and patients with UC exhibit increased harmful bacteria and decreased beneficial bacteria in the gut microbiome. This imbalance worsens metabolic chaos and inflammation, ultimately damaging the intestinal barrier^[4]. Therefore, targeting the gut microbiota is a promising therapy for UC.

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Polysaccharides provide various biological benefits for bowel health and are recognized as prebiotics that can improve bowel disease by regulating gut microbiota^[5]. Numerous studies have reported that polysaccharides alleviate UC symptoms by regulating gut microbiota and restoring the intestinal barrier, demonstrating their polysaccharides may have therapeutic potential for UC^[6–8]. Recent studies have demonstrated that *Cyclocarya paliurus* (Batal.) Iljinskaja polysaccharide^[9], Chinese yam polysaccharide^[9] and *Mesona chinensis* Benth polysaccharides^[10] alleviate dextran sulfate sodium (DSS)-induced UC *via* inhibiting inflammation and modulating gut microbiota. However, polysaccharides derived from different sources and with varying structures exhibit diverse effects against UC. They can display various beneficial effects through microbiota-dependent and -independent mechanisms, including anti-inflammation, intestinal barrier protection, and immune regulation. Therefore, long-term use of bioactive polysaccharides for treating UC is associated with fewer side effects than the standard medication.

Prunella vulgaris L. (PV), also known as the “self-healing” or “all-healing” plant, is traditionally used as a folk medicine in China, Korea, Japan, and Europe^[11]. It is typically utilized to treat sore throats, diarrhea, fevers, pulmonary tuberculosis, infectious hepatitis, and arterial hypertension. Notably, PV serves as a primary ingredient in Guangdong Herbal Tea (known as Wanglaoji-Liang-Cha), which is consumed as a healthy beverage in Southern China. Modern research has revealed that PV contains various bioactive components, including polysaccharides, flavonoids, triterpenoids, sterols, phenolic acid, coumarins, and phenylpropanoids, which exhibit the properties of anti-inflammation, antioxidant, antitumor, antimicrobial activities, and antiviral^[12]. In addition, emerging evidence indicates that the anti-UC activity of PV primarily stems from 4 bioactive constituents: triterpenoids, flavonoids, organic acids, and sterols^[13]. However, whether PV polysaccharides (PVP) have anti-UC activity remains unknown. PVP has demonstrated significant antioxidant^[14], anti-inflammatory^[15], anti-hyperlipidaemic^[16], anti-cancer and immunoregulatory effects^[17]. Our previous studies indicated that PVP mitigates inflammation in renal tubular epithelial cells by enhancing antioxidant capacities and inhibiting nuclear factor kappa-B (NF-κB) inflammatory pathways^[18]. Accordingly, we hypothesized that PVP may be a potential therapy for UC. The present study evaluated the effects of PVP on UC using a mouse model. It investigated the underlying mechanisms, providing foundational data to support future applications of PV in UC prevention and treatment.

2. Materials and methods

2.1 Chemicals

PV was obtained from the Chinese Medicine Raw Material Processing Plant of the Guangdong Traditional Chinese Medicine Corp (Guangzhou, China). DSS (molecular weight 36–50 kDa) was acquired from MP Biomedical (Illkirch, France). Additionally, ELISA kits for tumor necrosis factor (TNF)-α, interleukin (IL)-β, and IL-6 were sourced from MultiSciences Biotech Co., Ltd. (Hangzhou, China). The BCA kit was procured from Beyotime (Shanghai, China). The anti-zonula occludens-1 (ZO-1), anti-Occludin, and anti-Claudin-1 antibodies were procured from Servicebio (Wuhan, China). All other chemicals were of reagent grade.

2.2 Preparation of PVP

PVP was prepared according to our previous study^[18]. Briefly, PV powders were extracted twice with distilled water at a solid-liquid ratio of 1:30 and for a duration of 2 h for each extraction. After centrifugation, the supernatant was equally mixed with organic solvents (chloroform/*n*-butyl alcohol, 4:1, *V/V*), followed by shaking for another 2 h to remove proteins. After centrifugation, the supernatant was dialyzed for 48 h. Subsequently, ethanol thrice the volume of the solution was added into the solution and kept in a 4 °C refrigerator for 24 h. Lastly, the supernatant was vacuum concentrated and dried using a vacuum freeze dryer to obtain PVP for subsequent assay. The polysaccharides from PV plant were obtained, with final yield of 2.14%. Among them, the sugar content of PVP is 65.00%, the proportion of uronic acid is 3.59%, and the total polyphenols content is 2.21%.

2.3 Animal experiment design

A total of 24 male C57BL/6J mice (6–8 weeks, 20 ± 2 g) were acquired from the Guangdong Experimental Animal Center. All the experiment procedures were approved by the Dongguan People's Hospital Laboratory Animal Welfare and Ethics Committee (IACUC-AWEC-202401030). The authors adhered to all applicable international, national, and institutional guidelines for the care and use of animals were followed by the authors. The mice were acclimated for 7 days in a controlled 12-h light-dark cycle environment. All mice were randomly divided into 4 groups ($n = 6$): Control group, DSS group, DSS + low PVP group (LPVP), and DSS + high PVP group (HPVP). From days 1–14, LPVP and HPVP groups were orally administrated with PVP at dosages of 50 and 200 mg/kg bw, respectively, as a preventive measure. Conversely, control and DSS groups received distilled water. From days 15 to 21, drinking water with 2% (*m/V*) DSS was administrated to the DSS, LPVP, and HPVP groups, while distilled water was administered to the control group. Our previous studies found aqueous extract of PV (25–100 mg/kg bw) inhibited the growth of papillary thyroid carcinoma in BALB/c *nu/nu* mice and ameliorated cadmium-induced bone loss by promoting osteogenic differentiation in female rats with concentration of 50–250 mg/kg bw^[19–20]. Our doses aimed to span this range. In addition, in our preliminary experiments, 50 mg/kg showed significant biological activity, and 200 mg/kg was chosen to evaluate potential saturation effects without exceeding safety thresholds (no adverse effects observed). Throughout the experiments, the body weight of the mice was recorded daily. After the study, all mice were sacrificed for measurements of colon length and spleen weight. Additionally, the serum, colon tissues, and cecum samples were collected for analysis.

2.3.1 Evaluation of colitis symptoms

The disease activity index (DAI) was analyzed to evaluate the severity of UC based on weight loss, stool consistency, and stool bleeding^[21]. DAI score and body weight change rate were calculated as follows:

DAI score = weight loss score + stool consistency score + stool bleeding score (1)

$$\text{Body weight change rate (\%)} = \frac{\text{Current weight (g)}}{\text{Initial mice weight (g)}} \times 100 \quad (2)$$

2.3.2 Inflammatory mediator measurement

According to the manufacturer's instructions, serum cytokines (TNF- α , IL-1 β , and IL-6) were detected using ELISA reagent kits (Neobioscience Technology Co., Ltd., Shenzhen, China).

2.3.3 Histopathological evaluation and immunohistochemistry (IHC) analysis

Distal colon tissues were fixed with paraformaldehyde, dehydrated, and then embedded in paraffin. Colonic slices, each sectioned at a thickness of 5 μm , were stained with hematoxylin and eosin (H&E) or Alcian blue-periodic acid Schiff (AB-PAS). The stained tissues were examined under a microscope (ZEISS, Germany).

IHC was performed on paraffin-embedded colon slices (5 μm) following a previously established protocol with some modifications^[22]. Briefly, the slices were incubated with the anti-ZO-1, Occludin, and Claudin-1 primary antibodies for 12 h at 4 °C. They were subsequently incubated with secondary antibodies conjugated to avidin-labeled horseradish peroxidase (HRP). Subsequently, diaminobenzidine (DAB) chromogenic solution was used for staining, and the stained tissues were imaged using a light microscope (ZEISS, Germany). The protein levels were evaluated using the ImageJ software.

2.3.4 Western blot analysis

Tissue proteins of the colon were prepared by radio immunoprecipitation assay (RIPA) buffer with protease inhibitors for 30 min at 4 °C. The protein concentrations were tested using a BCA protein assay kit. The quantified proteins (20 mg) were transferred onto polyvinylidene fluoride (PVDF) membranes following separation on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE). The membranes were blocked with 5% non-fat milk and incubated with the primary antibodies overnight at 4 °C. On the second day, the membranes were washed with tris-buffered saline with tween-20 (TBST) and incubated with appropriate secondary antibodies at room temperature for 1 h. The membranes were washed 3 times in TBST and visualized using a chemiluminescence western blotting detection system. Band intensities were quantified with ImageJ software.

2.3.5 Cecal microbiota analysis

Total bacterial RNA was extracted from cecal samples using the PF Mag-Bind Stool DNA Kit (Omega Bio-Tek, Georgia, USA). The V3–V4 region of the 16S rRNA gene was amplified with the following prime pairs: 338F, 5'-ACTCCTACGGGAGGCAGCAG-3'; 806R, 5'-GGACTACHVGGGTWTCTAAT-3'. The polymerase chain reaction (PCR) product was extracted with 2% agarose gel. Subsequently, the purified product was quantified using

the AxyPrep DNA Gel Extraction Kit (Axygen, USA) and the Quantus™ Fluorometer (Promega, USA). Sequencing was performed on the Illumina PE300/PE250 platform (Illumina, San Diego, USA) according to the standard protocols by Majorbio Bio-Pharm Technology Co., Ltd. (Shanghai, China). Raw data were de-multiplexed and quality-filtered using fastp (version 0.19.6). The sequences were then merged with FLASH (version 1.2.11). The filtered sequences were clustered into operational taxonomic units (OTUs) using UPARSE 11. We assessed alpha diversity (Shannon and Simpson indices) and beta diversity (principal coordinate analysis (PCoA) and hierarchical clustering) of the bacterial communities based on the OTUs. Linear discriminant analysis effect size (LEfSe) (threshold value with linear discriminant analysis (LDA) > 4) was also performed to identify the significantly abundant taxa among groups at various taxonomic levels.

2.3.6 Untargeted metabolomics analysis

Cecal sample (50 mg) was mixed with organic solvent (methanol: water = 4:1, *V/V*) containing 0.02 mg/mL of internal standard (*L*-2-chlorophenylalanine). The mixture was ground using the Wonbio-96c frozen tissue grinder (Shanghai Wanbo Biotechnology Co., Ltd.) frozen tissue grinder for 6 min (−10 °C, 50 Hz). The samples were then extracted at 5 °C with ultrasound for 30 min, followed by maintained at −20 °C for 30 min. After centrifugation (13 000 $\times$ g, 15 min), the supernatant was collected for subsequent analysis. The metabolic samples were analyzed using the Acquity ultra performance liquid chromatography (UPLC) system coupled to a quadrupole-time-of-flight mass spectrometer (Triple TOF™5600+, Sciex, USA) equipped with an electrospray ionization (ESI) source. Solvents A and B, both containing 0.1% formic acid, consisted of water:acetonitrile (95:5, *V/V*) and acetonitrile:water (47.5:47.5, *V/V*), respectively. The linear gradient for the analysis was as follows: 0–0.1 min, 5% mobile phase B; 0.1–2 min, 5%–25% mobile phase B; 2–9 min, 25%–100% mobile phase B; 9–13 min, 100% mobile phase B; 13–13.1 min, 100%–0% mobile phase B; 13.1–16 min, 100% mobile phase B. The flow rate, column temperature, and injection volume were 0.40 mL/min, 40 °C, and 2 μL , respectively. The optimal MS parameters were as follows: Scan type (*m/z*): 70–1 050; source temperature at 550 °C; ion-spray voltage floating (ISVF) at −2 800 V in negative mode; and 3 500 V in positive mode; collision energy (CE), 20–60 eV. The metabolites were identified by searching database, and the main databases were the HMDB (<http://www.hmdb.ca/>), Metlin (<https://metlin.scripps.edu/>) and the self-compiled Majorbio Database (MJDB) of Majorbio Biotechnology Co., Ltd. (Shanghai, China).

2.4 Statistical analysis

Data are presented as mean $\pm$ standard deviation (SD). Graphs were drawn using GraphPad Prism software (version 8.0). Significance analysis was performed using the One-way analysis of variance (ANOVA) of SPSS (version 18.0) based on Duncan's multiple range test. Furthermore, $P < 0.05$ was considered as a significant level.

3. Results and discussion

3.1 PVP ameliorated the symptoms of colitis mice

A DSS-induced colitis model was established to evaluate the therapeutic effects of PVP on DSS-induced colitis (Fig. 1A). As illustrated in Figs. 1B–C, mice in the model group experienced a gradual decrease in weight following DSS administration compared to the control group, while DAI scores continually increased. There was a significant difference in weight loss and DAI scores between the control and DSS groups at day 21 ($P < 0.01$), confirming the successful establishment of the DSS-induced colitis model. Notably, high DAI scores reflect more severe weight loss, stool consistency, and bloody stools^[23], supporting the colitis phenotype. PVP significantly ameliorated DSS-induced colitis, as evidenced by the weight gain of mice in HPVP ($P < 0.01$) and the reduction in DAI scores in LPVP ($P < 0.01$) and HPVP ($P < 0.01$). Furthermore, mice in the DSS group displayed a shorter colon and a greater spleen weight than the control group (Figs. 1D–F, $P < 0.01$). Notably, PVP restored the symptoms ($P < 0.01$), demonstrating that PVP has a beneficial effect in alleviating colitis symptoms in mice. Supplementation of mice with PVP relieved the symptoms of DSS-induced colitis. It has been demonstrated that natural polysaccharides display an obvious therapeutic effect on UC because of their great anti-inflammatory activity^[24]. According to the previous report^[13], PV capsules can notably decrease ulcer numbers and congestion index, enhance colon and intestinal weight indices, implying that PV capsule could be involved in UC protection. Our findings introduce a new perspective on the potential role of PVP in protecting against UC, although the precise mechanism is not yet understood.

3.2 PVP relieved DSS-induced serum and colon inflammation of colitis mice

UC is characterized by an overabundance of inflammatory cytokines, which accelerate the pathological process of colitis. Serum TNF- α , IL- β , and IL-6 levels in the DSS group were significantly higher than those of the control group ($P < 0.01$; Figs. 2A–C), suggesting that DSS causes severe inflammatory cytokines secretion in mice with UC. Our results corroborated with previous findings that secretion of inflammatory cytokines was increased in mice with UC^[25–26]. Besides, PVP at 50 and 200 mg/kg reduced TNF- α and IL- β levels in a dose-dependent manner ($P < 0.05$) whereas PVP at 200 mg/kg effectively decreased IL-6 level ($P < 0.01$). These results indicated that PVP treatment alleviated the DSS-induced inflammatory response. Gut microbiota disorders in mice with UC may contribute to intestinal inflammation and barrier damage, facilitating the leakage of gut-derived LPS into the blood circulation, and thereby triggering an increase in serum pro-inflammatory factors^[27].

The NLRP3 inflammasome, composed of NLRP3, Caspase-1, and the apoptosis-associated speck-like protein (ASC), represents the most extensively characterized and prototypical member among inflammasome complexes^[28]. Upon activation, NLRP3 initiates Caspase-1 proteolytic processing, which subsequently cleaves pro-IL-1 β and pro-IL-18 into their biologically active forms, IL-1 β and IL-18, respectively. Meanwhile, the activation of NLRP3 inflammasome accelerates the maturation and secretion of IL-1 β , thereby perturbing the equilibrium of cytokines and aggravating UC^[24]. In the present study, PVP significantly inhibited serum IL-1 β in mice with UC, indicating the suppression of NLRP3 inflammasome activation. As displayed in Fig. 2D, the notable activation of NLRP3

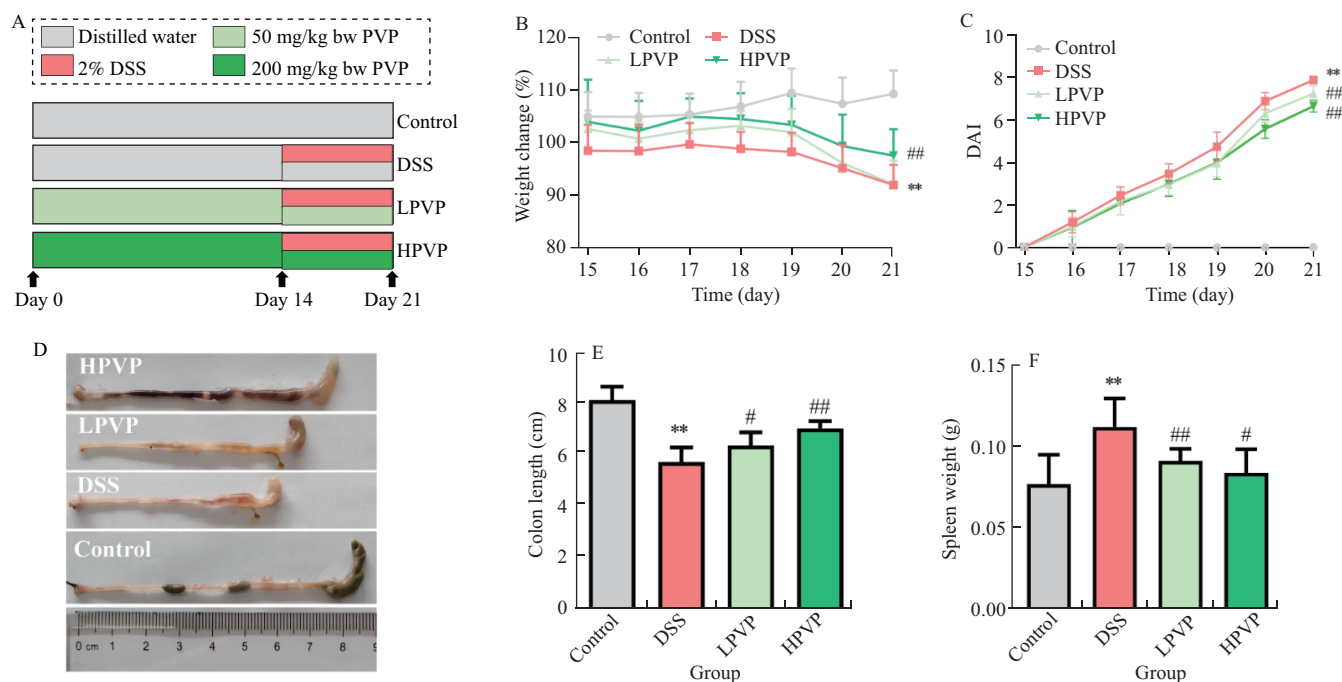


Fig. 1 PVP relieved the symptoms of colitis mice. (A) Experimental protocol; (B) Weight change of the mice; (C) DAI score in each group; (D) Representative graphs of the colon; (E) Colon length; (F) Spleen weight. Control vs. DSS, $**P < 0.01$; DSS vs. PVP, $*P < 0.05$, $^{##}P < 0.01$.

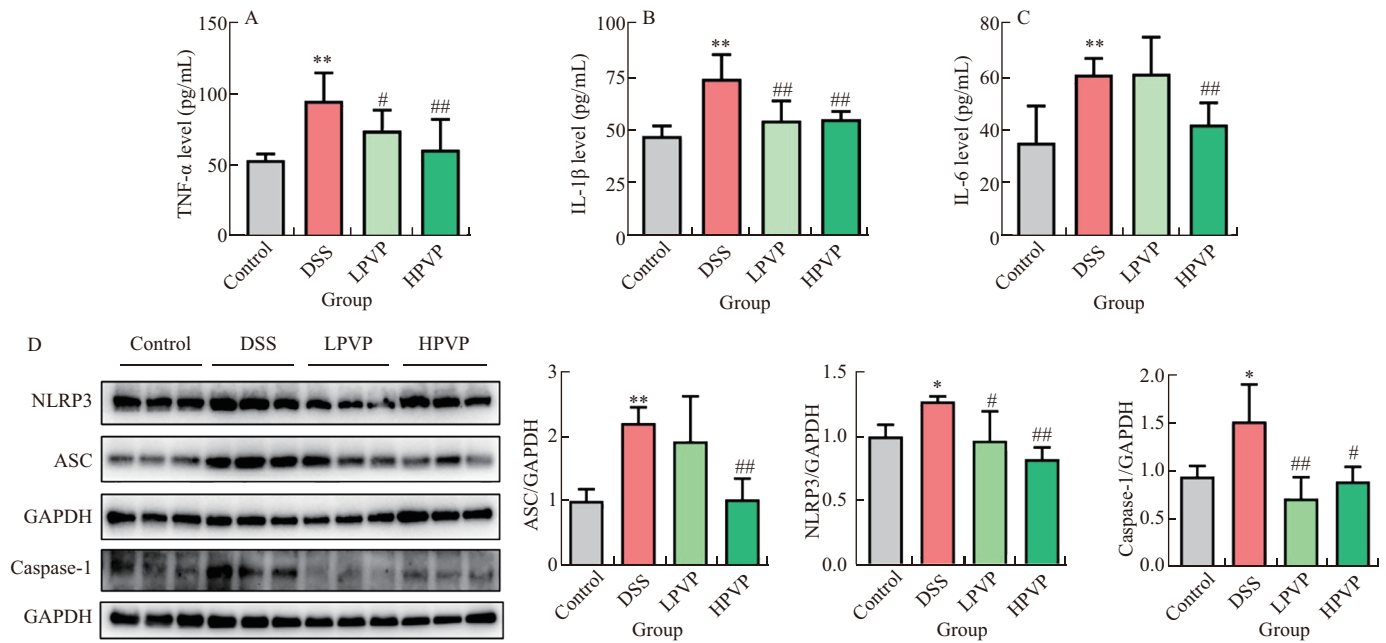


Fig. 2 PVP relieved DSS-induced intestinal inflammation in colitis mice. (A-C) Serum TNF- α , IL- β , and IL-6 levels; (D) Western blot analysis of NLRP3, ASC and Caspase-1 proteins. Control vs. DSS, ** $P < 0.05$, ** $P < 0.01$; DSS vs. PVP, # $P < 0.05$, ## $P < 0.01$.

inflammasome was observed in the colon tissues of mice with UC. This finding is consistent with prior research^[29]. However, PVP suppressed the activation of NLRP3 inflammasome, as evidenced by the downregulation of NLRP3, ACS and Caspase-1 expression in colon tissues. These data indicated that PVP might exert anti-UC effects by suppressing the activation of NLRP3 inflammasome and inflammatory cytokine secretion. In addition, polysaccharides derived from *Herichium erinaceus*^[30], *Scutellaria baicalensis* Georgi^[26] and *Smilax china* L.^[31] help reduce UC in mice by suppressing NLRP3 inflammasomes and restoring intestinal balance. NLRP3 serves as the main link in inflammatory response. Chinese plant-derived polysaccharides can regulate the gut microbial imbalances, repair intestinal barrier issues, and mitigate intestinal inflammation through the action of NLRP3^[32].

3.3 PVP mitigated DSS-induced gut barrier defects of colitis mice

We performed H&E and AB-PAS staining of the colon to evaluate pathological conditions of colon tissue. As illustrated in Fig. 3A, compared with the control group, the colon of mice in the DSS group caused severe inflammatory cell infiltration, reduction in crypts and goblet cells, and disruption of epithelial cells, suggesting the disturbed gut barrier integrity after DSS management. Similarly, UC patients are accompanied by impaired intestinal barrier function, which causes the increased permeability of intestinal mucosa to endotoxins and antigens and ultimately damages gut health^[29]. Conversely, PVP attenuated the severe histopathological injury, as evidenced by reduced inflammatory infiltration, relatively intact crypt structure, and epithelial cells, particularly in cases involving high doses of PVP. These results corroborate previous studies which indicate that natural

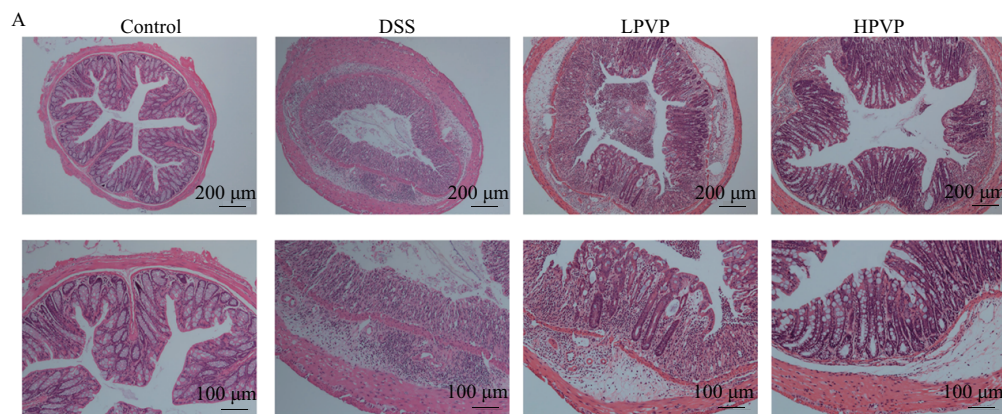


Fig. 3 Effects of PVP on histopathological damage and goblet cells in mice colons. (A) Representative H&E staining images of the colon; (B) Representative AB-PAS staining images of the colon; (C) Number of goblet cells. Mucin-containing goblet cells, marked by black arrows, were stained bright purple. Control vs. DSS, ** $P < 0.01$; DSS vs. PVP, ## $P < 0.01$.

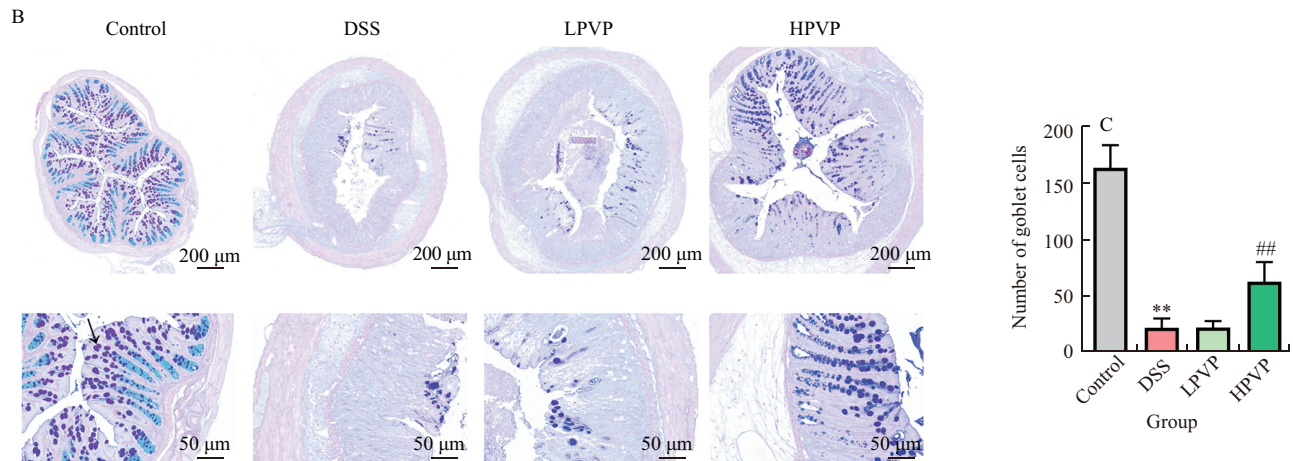


Fig. 3 (Continued)

polysaccharides significantly benefit the intestinal barrier integrity in UC^[7]. Moreover, mice with UC or patients are distinguished by the absence of identifiable goblet cells in the colonic epithelium, leading to less mucin secretion and consequently complicating the maintenance of the intestinal mucosa and mucus layer^[33]. AB-PAS staining results indicated that the intestinal layer and goblet cells in the DSS group were significantly destroyed compared with the control group, while 200 mg/kg bw PVP significantly increased the number of goblet cells that decreased in the DSS group (Figs. 3B-C). The repaired goblet cells secreted enough mucus in the lamina propria of the colon and released into the gut cavity, forming the first line of defense of the host to protect the gut from invasion^[34]. These findings suggest that PVP could improve DSS-induced intestinal damage of mice with UC by restoring the integrity of the intestinal barrier and increasing the number of goblet cells.

Immunohistochemical analysis of tight junction proteins (TJs) (Occludin, Claudin-1, and ZO-1) in colon tissues was conducted

to further determine the mitigation effects of PVP on the colonic mucosal barrier of mice with colitis. As illustrated in Fig. 4A, DSS significantly notably decreased the expression of Occludin, Claudin-1, and ZO-1, thereby triggering the impaired gut barrier. Additionally, TJs consisting of Occludin, Claudin-1, ZO-1, and junctional adhesion molecules—are crucial components in linking epithelial cells to form the intestinal barrier^[35]. The suppression of DSS-induced TJs expression severely destroyed the intestinal epithelial barrier and increased gut permeability, facilitating further damage to gut health caused by external factors. However, PVP partially restored the reduction of these TJs in different degrees, suggesting that PVP effectively promotes the TJ of epithelial cells to repair the intestinal barrier (Figs. 4A-B). TJs are considered an indispensable role in cell adhesion, intestinal permeability, and pathogen prevention. Consequently, PVP could restore DSS-induced intestinal barrier integrity by upregulating the expression of Occludin, Claudin-1, and ZO-1.

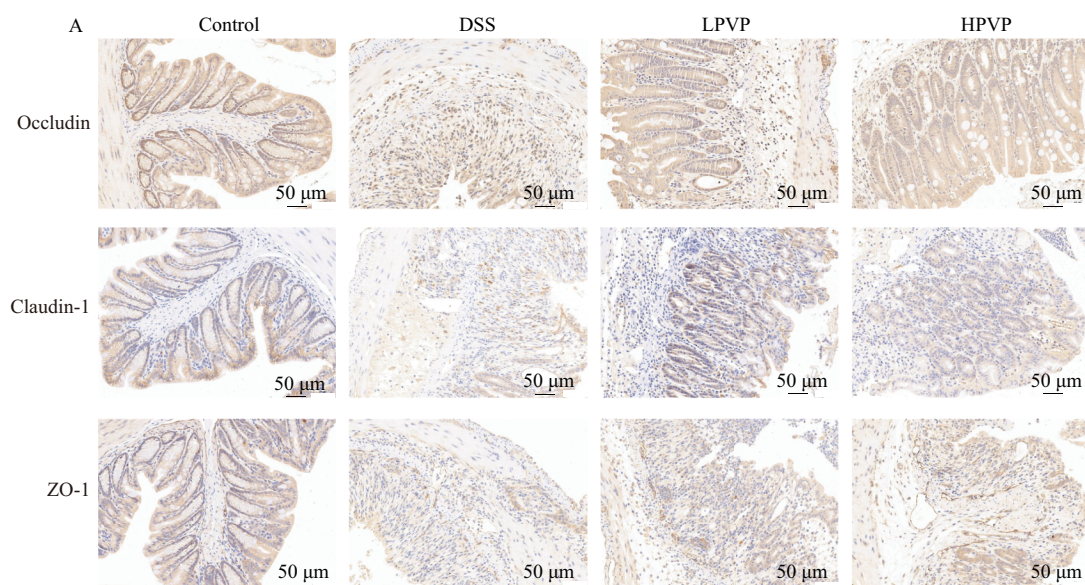


Fig. 4 PVP mitigated DSS-induced gut barrier defects of colitis mice. (A) Representative IHC images. (B) Relative Occludin, Claudin-1, and ZO-1 expression level. Control vs. DSS, ** $P < 0.01$; DSS vs. PVP, * $P < 0.05$, ## $P < 0.01$.

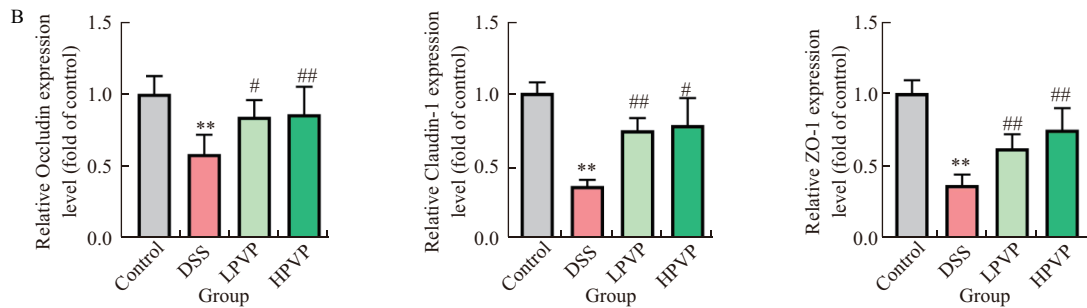


Fig. 4 (Continued)

3.4 PVP restored gut microbiota disorders of colitis mice

Gut microbiota dysbiosis is typically attributed to accelerated UC development^[36]. To explore whether the efficacy of PVP on UC is correlated with intestinal flora, we performed 16S rRNA sequencing on cecal content in mice. The alpha diversity results displayed that PVP significantly increased the Shannon index and decreased the Simpson index of the gut microbes compared with the DSS group (Figs. 5A-B; $P < 0.01$), indicating that PVP effectively reverses the decline in microbial community diversity. Similar studies have reported that gut microbiome diversity is

recovered by administering several polysaccharides^[37-38], which support our results. The Venn diagram (Fig. 5C) reflects the common or unique taxa among the treatment groups. The beta diversity analysis containing a hierarchical clustering tree and PCoA displayed a significant separation in diversity between the control and DSS groups, suggesting that the microbial profile composition in mice varies following DSS treatment (Figs. 5D-E). Notably, PVP reversed the alterations of DSS-induced microbiota community structure. The beneficial effects of PVP treatment on mice with UC are associated with an overall improvement in the diversity and structure of intestinal flora.

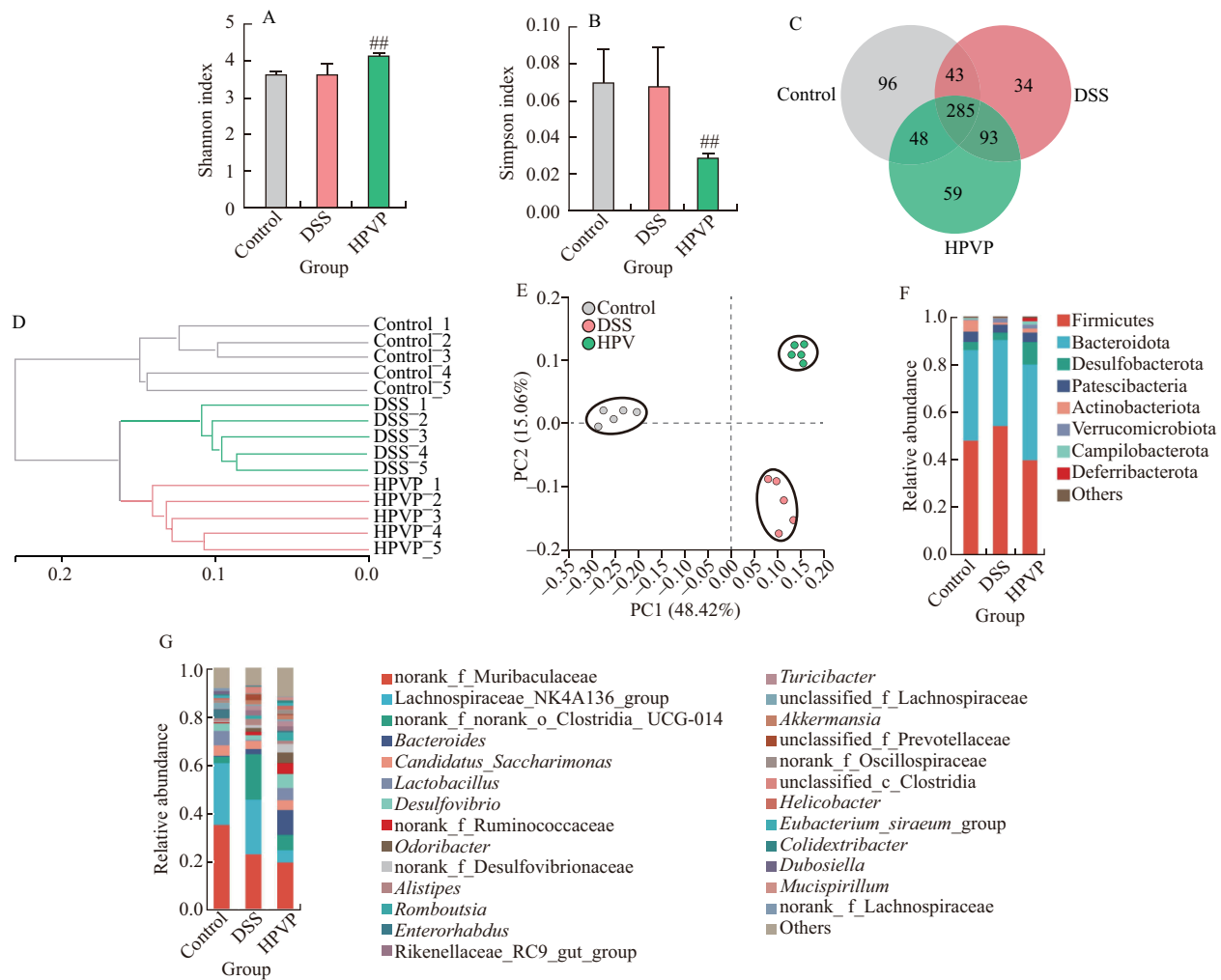


Fig. 5 Effects of PVP supplementation on gut microbiota in DSS-induced colitis mice. (A) Shannon index; (B) Simpson index; (C) Venn diagram; (D) hierarchical clustering tree based on Bray-Curtis distance matrix; (E) PCoA; (F) Composition of gut microbiota among the treatment groups at phylum levels; (G) Composition of gut microbiota among the treatment groups at genus levels; (H) LefSe analysis based on the LDA; (I) Score of the LDA analysis (LDA > 4). DSS vs. PVP, * $P < 0.05$, ** $P < 0.01$, ### $P < 0.001$.

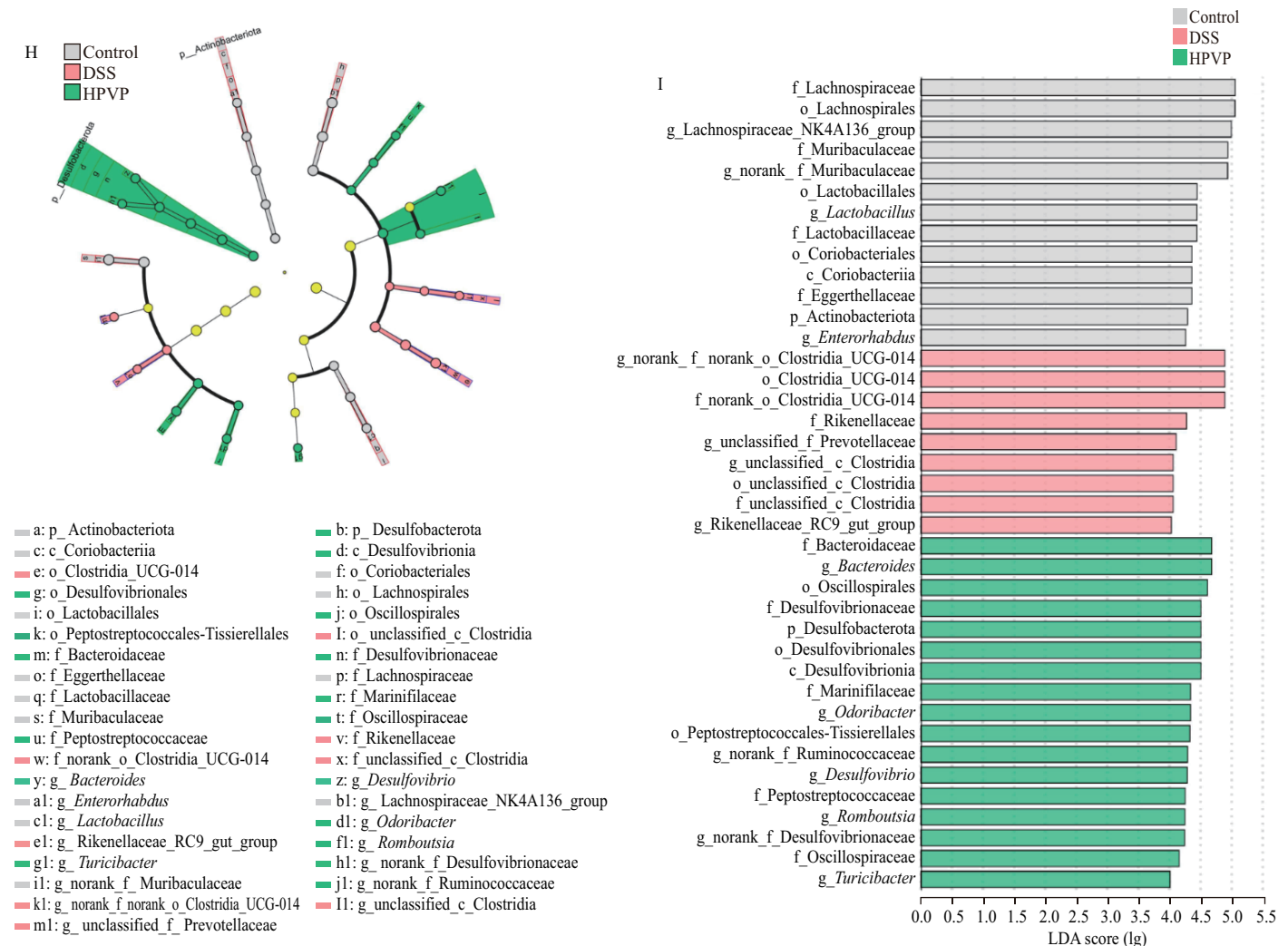


Fig. 5 (Continued)

The gut microbiota composition of cecum in mice at phylum and genus levels was further analyzed to evaluate the effect of PVP on the intestinal flora of mice with UC (Figs. 5F-G). At the phylum level, the bacterial composition was mainly comprised of Firmicutes, Bacteroidetes, Desulfovibrionia, Patescibacteria, and Actinobacteriota (relative abundance > 1%). DSS increased the relative abundance of Firmicutes and decreased the relative abundance of Bacteroidetes compared with the control group ($P > 0.05$), resulting in a high Firmicutes/Bacteroidetes (F/B) ratio (Figs. 6A-C). A high F/B ratio is a typical characteristic of gut microbiota disorders, which always happen in UC. Fu et al.^[39] found similar results reporting that a relatively high abundance of Firmicutes and a low abundance of Bacteroidetes were observed in the DSS group. Another clinical study reported that patients with UC exhibited a relatively low abundance of Bacteroidetes spp.^[40] However, patients with UC displayed inconsistent bacterial dysregulation, which significantly in gut microbiota composition among the patients^[41]. In the present study, PVP remarkably restored the Firmicutes abundance and F/B ratio ($P < 0.05$), indicating the potential beneficial effects of PVP on the bacterial composition of mice with UC. Additionally, the relative abundance of Desulfovibrionia in the DSS group was significantly lower than that of the control group ($P < 0.05$); however, restored

by PVP treatment (Fig. 6D, $P < 0.01$). Moreover, the function of Desulfovibrionia in UC treatment has not been clearly discovered. Notably, a similar study reported that *Cordyceps militaris* polysaccharide ameliorated diabetic symptoms by regulating gut microbiota, such as the upregulated Desulfovibrionia abundance^[42]. At the genus level, the abundance of norank_f_norank_o_Clostridia_UCG-014, Rikenellaceae_RC9_gut_group, unclassified_f_Prevotellaceae in the DSS group significantly increased than that of the control group. Conversely, the abundance of *Lactobacillus*, *Desulfovibrio*, *Enterorhabdus*, and norank_f_Oscillospiraceae notably decreased (Fig. 6E, $P < 0.05$). PVP effectively reversed these variations. Similar studies have reported that a high abundance of norank_f_norank_o_Clostridia_UCG-014, Rikenellaceae_RC9_gut_group, and unclassified_f_Prevotellaceae after DSS treatment^[43-44], which could aggravate intestinal damage. By contrast, *Lactobacillus* is generally considered a beneficial bacteria, functioning to reduce tissue oxidative stress and inflammation^[45]. *Lactobacillus* produces various short-chain fatty acids, including acetic acid, propionic acid, and butyric acid, which can benefit gut health. Notably, the downregulated *Lactobacillus* abundance with DSS treatment was observably restored by PVP, indicating the positive effects of PVP on beneficial bacteria. Moreover, the upregulated abundance

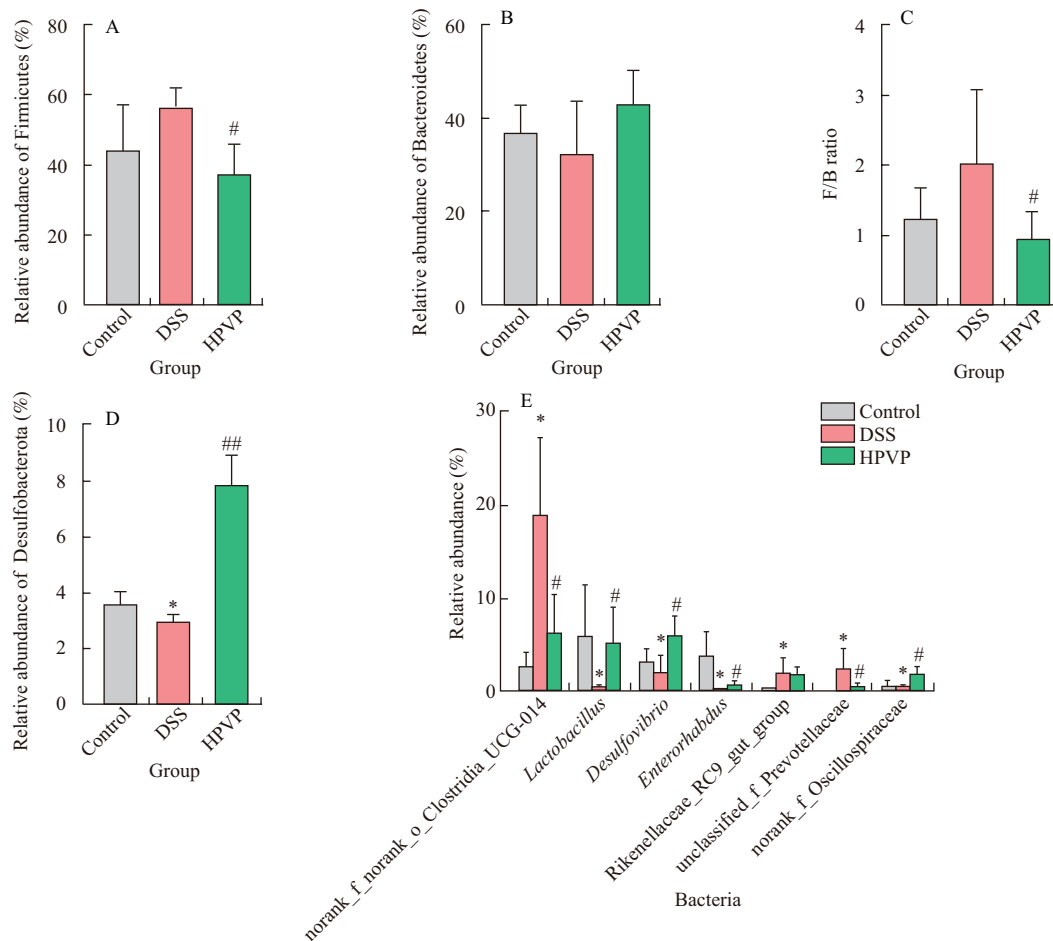


Fig. 6 Analysis of differences in gut microbiomes abundance at the (A-D) phylum level (E) genus level among 3 groups. Control vs. DSS, * $P < 0.05$, ** $P < 0.01$; DSS vs. PVP, # $P < 0.05$, ## $P < 0.01$.

of *Desulfovibrio*, *Enterorhabdus*, and norank_f_Oscillospiraceae in the PVP group supported the amelioration effects of PVP on gut microbiota dysbiosis. *Desulfovibrio*, associated with mucosal function and intestinal environment, is a kind of opportunistic pathogen. A study reported that the *Lactobacillus gasseri* G098 strain alleviated IBD, partially attributed to enhancing *Desulfovibrio* abundance in mice with UC^[46]. *Desulfovibrio* abundance was positively correlated with anti-inflammatory factors, such as IL-10^[47]. Similarly, Oscillospiraceae is a conditioned pathogen associated with inflammation. Low norank_f_Oscillospiraceae abundance was observed with DSS or cyclophosphamide administration and restored by active substances, such as Fucoidan and *Lactobacillus plantarum* H6^[48-49]. These findings lioration effect of PVP on colitis is associated with alterations in intestinal flora.

LEfSe was implemented among the treatment groups to evaluate the differences in gut microbiome populations. The control group was enriched in the o_Lachnospirales, f_Lachnospiraceae, o_Lactobacillales, f_Lactobacillaceae, g_Lactobacillus, c_Coriobacteriia, o_Coriobacteriales, p_Actinobacteriota phylum, and g_Enterorhabdus. Conversely, the DSS group was enriched in the Clostridia_UCG-014, f_Rikenellaceae, Rikenellaceae_RC9_gut_group, unclassified_c_Clostridia and unclassified_f_Prevotellaceae (Figs. 5H-I). With PVP intervention, the relative abundances of p_Desulfobacterota, c_Desulfovibrionia o_Desulfovibrionales, f_Desulfovibrionaceae,

g_Desulfovibrio, o_Oscillospirales, f_Oscillospiraceae, g_Odoribacter were multiplied. Notably, norank_f_Ruminococcaceae and *Romboutsia*, which were positively associated with synthesizing short-chain fatty acids and reducing inflammation, were multiplied following PVP treatment. These findings suggest that PVP could restore DSS-induced bacterial dysbiosis and alterations at different taxonomy levels.

3.5 PVP regulated host metabolomics of colitis mice

Metabolites originating from the intestinal flora play a vital role in the interactions between the host and the gut microbiomes, which exhibit various biological functions in host physiology. Metabolites of cecal content were analyzed using UPLC-quadrupole time-of-flight-tandem mass spectrometry (Q-TOF/MS) to explore the effect of PVP on gut microbiota metabolism. The samples were observed with a clear separation between the control and DSS groups, indicating that DSS significantly altered gut microbiota metabolism (Figs. S1A-B). Samples from the PVP group were closer to the control group than the DSS group, revealing that the metabolite profiles damaged by DSS were restored following PVP administration. The partial least squares-discriminant analysis (PLS-DA) score plots in positive and negative ion modes displayed similar results in which the control and DSS groups were separated from each other. Furthermore, PVP treatment partially reverses DSS-induced metabolic changes (Figs. S1C-D).

Similarly, the orthogonal partial least squares-discriminant analysis (OPLS-DA) score plot displayed a separation between the control and DSS groups (Figs. S1E-F) and between the DSS and PVP groups (Figs. S1G-H), thereby validating DSS-induced metabolic variation and PVP-induced amelioration effects. The significantly altered metabolites (SAMs; $P < 0.05$, VIP > 1 , and FC > 1.2 or < 0.83)

between control and DSS groups and between DSS and PVP groups were identified and analyzed for subsequent analysis. As displayed in Figs. 7A-B, there were 173 SAMs (98 upregulated SAMs and 75 downregulated SAMs) in the DSS group compared with the control group. Conversely, there were 74 SAMs (17 upregulated SAMs and 57 downregulated SAMs) between DSS and PVP groups. The Venn

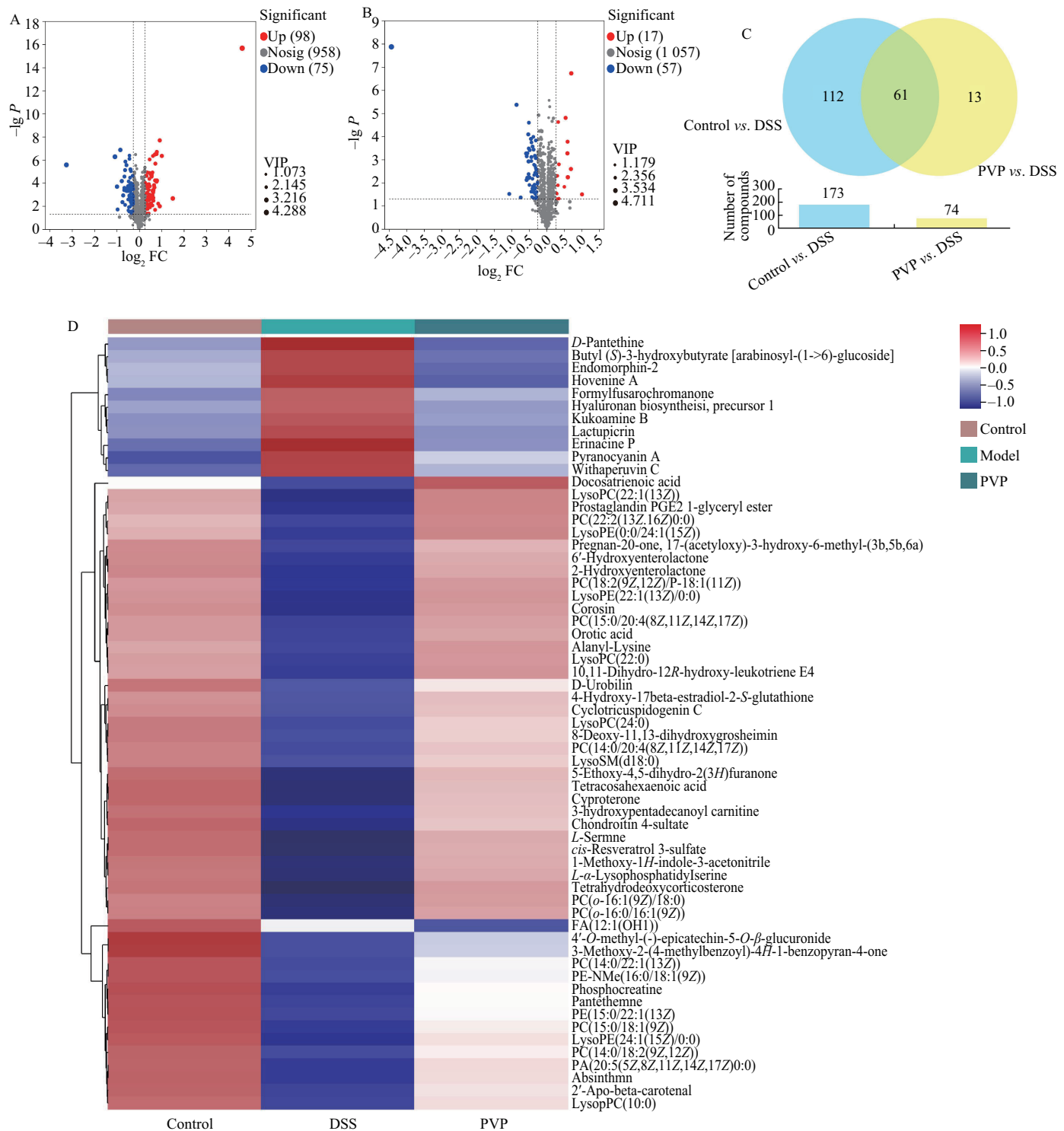


Fig. 7 Effects of PVP supplementation on metabolites and metabolic pathways in DSS-induced colitis mice. Volcano plot of differentially altered metabolites (A) between the control and DSS groups and (B) between DSS and PVP groups; (C) Venn diagram of the distribution of differentially altered metabolites; (D) heatmap of the shared significantly differential metabolites among the treatment groups; KEGG enrichment pathways for metabolites altered (E) between the control and DSS groups and (F) between the DSS and PVP groups.

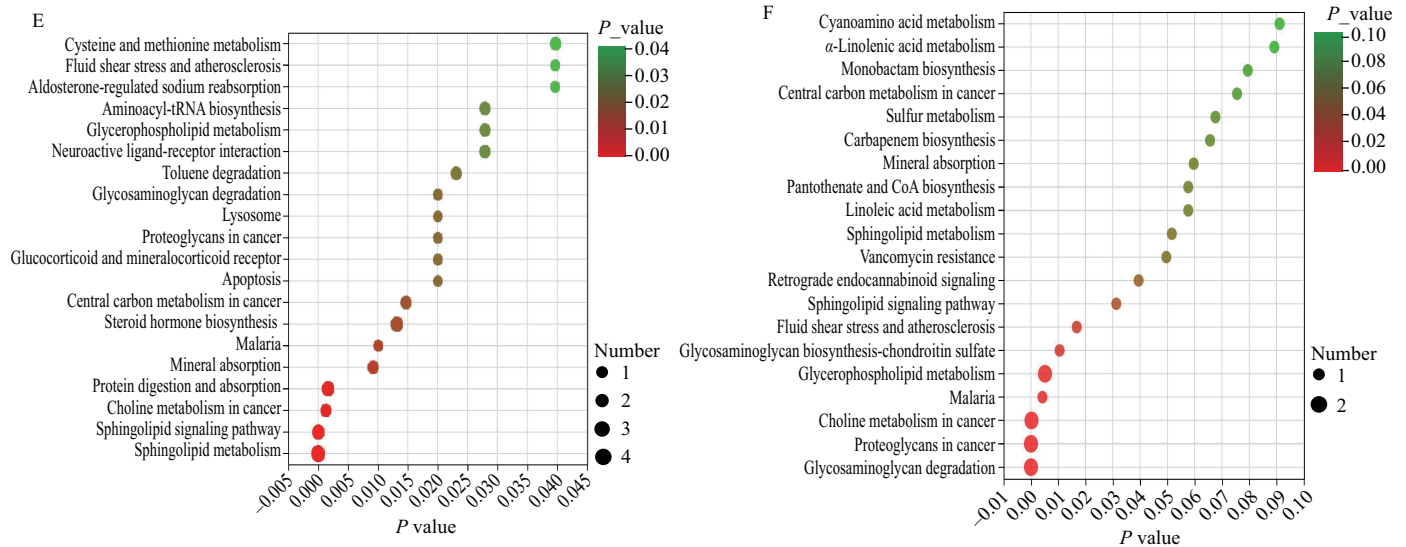


Fig. 7 (Continued)

diagram illustrates that 61 shared metabolites were observed by “Control group and DSS group” and “DSS group and PVP group” (Fig. 7C). Among them, 60 metabolites (49 upregulated SAMs and 11 downregulated SAMs) were restored to levels close to the control group following PVP treatment and are summarized in the heatmap of Fig. 7D and Table S1. Furthermore, metabolic pathway enrichment analysis revealed that sphingolipid metabolism, sphingolipid signaling pathway, choline metabolism in cancer, aminoacyl-tRNA biosynthesis, glycerophospholipid metabolism, aldosterone-regulated sodium reabsorption, and cysteine and methionine metabolism were notably enriched in the DSS group compared with those in the control group (Fig. 7E). However, proteoglycans in cancer, glycosaminoglycan degradation, choline metabolism in cancer, glycerophospholipid metabolism, glycosaminoglycan biosynthesis-chondroitin sulfate/dermatan sulfate, sphingolipid signaling pathway, sphingolipid metabolism, among others, were the most changed metabolic pathways in the PVP group (Fig. 7F).

Phosphatidylcholine (PC) and phosphatidylethanolamine (PE) belong to glycerophospholipids and constitute the main lipid components of biological membranes. Furthermore, they maintain membrane structure, reduce serum cholesterol, and promote lipid metabolism. In this study, the metabolism of glycerophospholipids in the cecum of mice with UC was significantly disrupted by DSS compared to the control group. This disruption was evidenced by a decrease in 7 PCs, 4 lysophosphatidylcholines (LysoPCs), 2 PEs, and 3 LysoPEs ($P < 0.05$). However, PVP played a protective role by reversion. As reported, glycerophospholipid (mainly PE and PC) metabolic disorders are often associated with the onset and progression of UC^[50]. The mucus secreted by intestinal epithelial cells contains a continuous lamellar layer of PC (mucus stabilizer) and glycoprotein, which protect the mucosal epithelium from external damage^[51]. PC comprised nearly 70% of the total phospholipids in the intestinal mucus layer and played a crucial role in glycerophospholipid, choline, and arachidonic acid metabolism. However, the quantity and composition of mucus were reduced, leading to decreased PC content. This reduction diminished the hydrophobic and protective properties of the mucus^[52]. Our results indicated that DSS significantly decreased PC levels (PC(14:0/20:4(8Z,11Z,14Z,17Z)), PC(14:0/18:2(9Z,12Z)), PC(14:0/22:1(13Z)), PC(15:0/20:4(8Z,11Z,14Z,17Z)),

PC(15:0/18:1(11Z)), PC(18:2(9Z,12Z)/P-18:1(11Z)), PC(22:2(13Z,16Z)/0:0)); however, the PC levels were restored by PVP treatment. This suggests that the disordered glycerophospholipid metabolism is partially restored. Low PC levels were observed in patients with UC. Subsequently, the anti-inflammatory effects of PC treatment on UC were confirmed^[53]. Similarly, Yu et al.^[54] reported that *Sanguisorba saponin* extract alleviated the metabolic pathway disorder of PCs in mice with UC and enhanced the PC(34:1) level to normal by upregulating phosphocholine cytidyltransferase. A clinical study also demonstrated that the restored PC levels were negatively associated with UC progression and produced curative results^[55]. Moreover, PC can be hydrolyzed into lysoPC by phospholipase A2, involving in the occurrence and regression of inflammation. An imbalance between PC and lysoPC levels is strongly linked to UC, potentially leading to disorders in phospholipid metabolism that can harm the cell membrane^[56-57]. Previous studies have confirmed that the downregulation of glycerophospholipid synthesis happens in the serum and gut of mice with UC^[58]. This reduction leads to decreased ATP biosynthesis, thereby impairing the gut barrier repair function. In our study, a significant decrease of LysoPCs (LysoPC(10:0), LysoPC(22:0), LysoPC(22:1(13Z)), LysoPC(24:0)) in DSS group were reversed by PVP. A similar study demonstrated that PC and LysoPC levels in mice with UC were disturbed and restored by Shaoyao decoction^[59]. Additionally, PE is an essential component of the cell lipid bilayer and plays a crucial role in host metabolism and signal transduction, which could be translated into PC by PE methyltransferase. LysoPE plays a vital role in modulating inflammatory mediators with TRP channels, which indirectly regulate the inflammatory environment in tissue^[60]. Numerous studies have manifested that DSS decreases PE or LysoPE levels in mice, accompanied by significant lipid metabolism dysbiosis^[58]. This reduction in PE content is negatively correlated with mitochondrial energy metabolism and can impair cell survival^[52]. Our data indicated that DSS suppressed the levels of PEs (PE(15:0/22:1(13Z)), PE-NMe(16:0/18:1(9Z)) and LysoPEs (LysoPE(0:0/24:1(15Z)), LysoPE(22:1(13Z)/0:0), and LysoPE(24:1(15Z)/0:0)) in cecum of mice with UC. Consequently, PVP positively modulates glycerophospholipid metabolism in these mice by downregulating PEs and LysoPEs. Furthermore, PC and PE are hydrolyzed into free

fatty acid (FFA), followed by β -oxidation of FFA, providing ATP as an energy source for cell growth, survival, and metastasis^[61]. However, ATP depletion is positively linked with reduced unsaturated fatty acid (UFA) biosynthesis with disordered glycerophospholipid hydrolyzation in UC. This may cause difficulties for intestinal epithelial cell regeneration and subsequently accelerate the barrier damage of UC. Our results found that PVP increased the low level of UFA (tetracosahexaenoic acid and docosatrienoic acid) in the DSS group ($P < 0.05$), indicating that PVP administration may relieve colonic barrier damage. Therefore, PVP could ameliorate DSS-induced glycerophospholipid metabolism by modulating the aberrant decreased content of PCs, LysoPCs, PEs, LysoPEs, and UFA.

Sphingolipids, which are derived from the catabolism of endogenous cellular sphingosine, play a significant role in transmembrane signal transduction processes, including inflammation, apoptosis, and cellular immune responses^[59]. Sphingolipids are crucial components of intestinal membranes, protecting the integrity of the intestinal mucosa and regulating the intestinal barrier^[62]. However, the disruption of sphingolipid metabolism in UC animals or patients exacerbates the severity of UC. In our study, sphinganine, phytosphingosine, and sphingosine in the DSS group exhibited a significant increase compared with the control group ($P < 0.05$), while the *L*-serine level decreased ($P < 0.05$), suggesting that sphingolipid metabolism in mice with UC is aberrant. Wen et al.^[63] found similar

results in that DSS treatment increased the levels of sphinganine and sphingosine in mice. Notably, sphinganine, phytosphingosine, and sphingosine can be converted into dihydroceramide, phytoceramide, and ceramide by adding an acyl chain to the amine group^[64]. Excessive accumulation of sphingolipids in mice with UC may contribute to the deleterious effects of ceramide and sphingosine, mainly inducing apoptosis and suppressing cell growth while promoting inflammation^[65]. Moreover, *L*-serine effectively ameliorates DSS-induced UC by modulating gut microbiota and inhibiting cell apoptosis^[66]. In the present study, the administration of PVP partially reduced the levels of sphinganine, phytosphingosine, and sphingosine ($P > 0.05$) and increased *L*-serine level ($P < 0.05$), partially restoring the disturbance of sphingolipid metabolism.

3.6 Correlation analysis among the gut microbiota, metabolites, and disease symptoms

Spearman's rank correlation analysis was conducted to explore the relationship among the gut microbiota, metabolites, and disease symptoms. As illustrated in Fig. 8, norank_f_norank_o_Clostridia_UCG-014, Rikenellaceae_RC9_gut_group, and unclassified_f_Prevotellaceae, whose relative abundance obviously increased in the DSS group, exhibited negative correlations with the content of

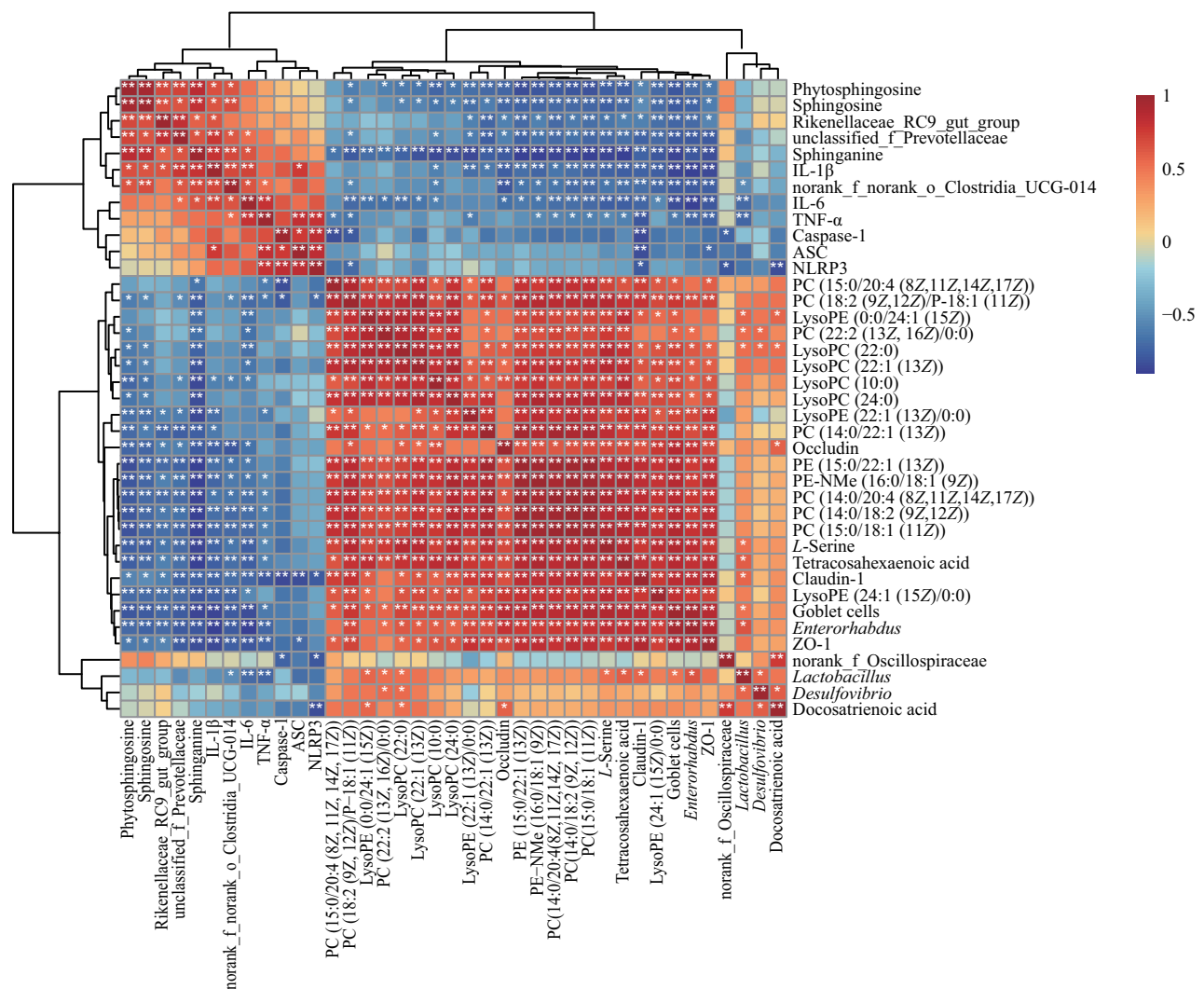


Fig. 8 Correlation analysis among the gut microbiota, metabolites, and disease symptoms using Spearman's analysis. * $P < 0.05$, ** $P < 0.01$ represent significant difference.

3 PCs, 2 PEs, 2 LysoPEs, *L*-serine, tetracosahexaenoic acid, goblet cells, TJs (Occludin, Claudin-1, ZO-1) ($P < 0.05$), and positive correlations with sphinganine, phytosphingosine, sphingosine levels, inflammatory cytokines (TNF- α , IL-1 β , IL-6), NLRP3 inflammasome (Casepase-1, ACS, NLRP3) ($P < 0.05$). These results hinted that DSS treatment significantly triggered inflammatory damage, gut microbiota dysbiosis, lipid metabolism disorder in mice. Furthermore, the relative abundance of *Lactobacillus*, *Desulfovibrio*, *Enterorhabdus*, and norank_f_Oscillospiraceae remarkably increased by PVP intervention were closely related with glycerophospholipid metabolites and phenotypic indexes. Specifically, *Enterorhabdus* was significantly positively correlated with the content of 5 PCs, 4 LysoPCs, 2 PEs, 2 LysoPEs, *L*-serine, tetracosahexaenoic acid, goblet cells, and TJs (Occludin, Claudin-1, ZO-1) ($P < 0.05$), whereas negatively related to sphinganine, phytosphingosine, sphingosine, inflammatory cytokines (TNF- α , IL-1 β , IL-6), and NLRP3 inflammasome (Casepase-1, ACS, NLRP3) ($P < 0.01$). *Desulfovibrio* and norank_f_Oscillospiraceae were markedly positively correlated with docosatrienoic acid ($P < 0.05$), whereas *Lactobacillus* displayed positive correlations with LysoPC (22:0), LysoPE (0:0/24:1(15Z)), PC (22:2(13Z,16Z)/0:0), *L*-serine, tetracosahexaenoic acid, Claudin-1, and goblet cells ($P < 0.05$). Therefore, we hypothesized that PVP alleviated DSS-induced intestinal homeostasis imbalance by modulating gut metabolites and barrier. Noteworthy, the expression of TJs (Occludin, Claudin-1, ZO-1) were positively correlated with most glycerophospholipid metabolites, goblet cells, *L*-serine, and tetracosahexaenoic acid, but were negatively correlated with sphinganine, phytosphingosine,

sphingosine, inflammatory cytokines (TNF- α , IL-1 β , IL-6), and NLRP3 inflammasome (Casepase-1, ACS, NLRP3). One study supported our results that the upregulated tight junction proteins and downregulated inflammatory cytokines intervened by bergamot polysaccharides benefited to gut health in DSS-induced mice^[34]. Overall, we initially hypothesized that DSS disrupted the equilibrium of gut microbiota, which in turn affected certain metabolite levels and worsened UC severity. These findings suggest that the aforementioned altered floras and metabolites may contribute to the occurrence and progress of UC, whereas their levels could be modulated through PVP treatment.

4. Conclusion

In conclusion, PVP intervention has the potential to alleviate DSS-induced colitis in mice by increasing colon length and reducing weight loss. Moreover, PVP suppressed excessive pro-inflammatory cytokines, colonic NLRP3 inflammasome activation and improved intestinal barrier integrity. Notably, PVP also restored the structure and composition of the disrupted gut microbiota, reversed changes in key metabolites by regulating glycerophospholipid metabolism and sphingolipid metabolism. Fig. 9 illustrates the potential mechanisms through which PVP ameliorates DSS-induced colitis. These findings suggest that PVP could be a valuable alternative or complementary therapy for preventing and treating UC. Furthermore, it is important to explore the bioactive polysaccharide components of PVP in future research.

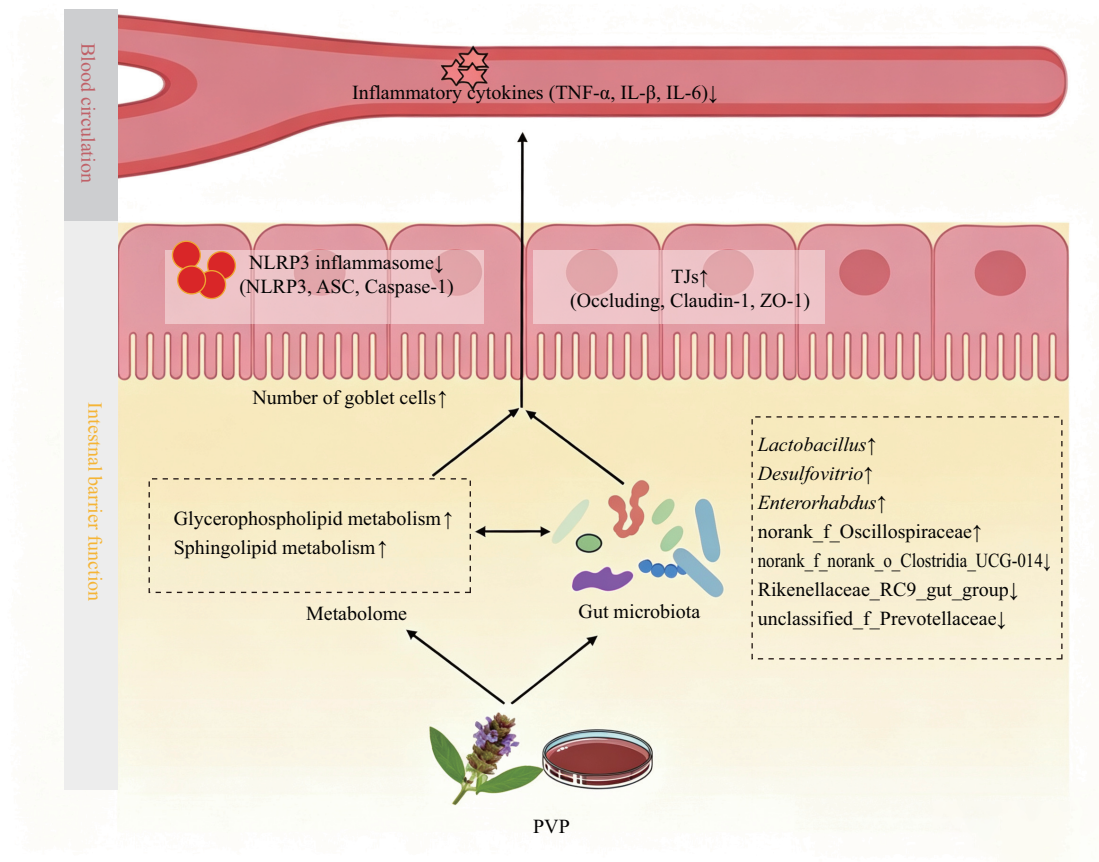


Fig. 9 Potentially amelioration mechanism of PVP on DSS-induced colitis.

Conflict of interest

Xinhui Xing is an editorial board member for *Food Science and Human Wellness* and was not involved in the editorial review or the decision to publish this article. The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://doi.org/10.26599/FSHW.2025.9250652>.

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