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Research Article

Potential of *Dendrobium officinale* oligosaccharides to alleviate chronic colitis by modulating inflammation and gut microbiota

Dong-Cheng Shi[1, 2, †], Pei-Yi Wang[1, 2, †], Lei Xu[1, 2], Hua Zhu[1, 2], Wang-Yue Zhang[1, 2], Qi-Yong Wu[1, 2], Ting-Ting Bu[1, 2], Bao-Ming Tian[1, 2, 3], Pei-Long Sun[1, 2, 3], Ming Cai[1, 2, 3] (✉)

[1] *College of Food Science and Technology, Zhejiang University of Technology, Hangzhou 310014, China*
[2] *Key Laboratory of Food Macromolecular Resources Processing Technology Research (Zhejiang University of Technology), China National Light Industry, China*
[3] *Eco-Industrial Innovation Institute ZJUT, Longyou, 324400, China*
[†] *Dong-Cheng Shi and Pei-Yi Wang contributed equally to this work.*

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Abstract: *Dendrobium officinale* oligosaccharides (DOO), as a novel prebiotic, offer promising therapeutic potential for the treatment of inflammatory diseases. In this study, the alleviating effect of DOO on dextran sulfate sodium (DSS)-induced chronic colitis in mice was investigated. DOO treatment relieved the main symptoms of weight loss, colon shortening, and bloody stool caused by colitis. DOO ameliorated histopathological colon tissue damage and reduced levels of pro-inflammatory factors (TNF-α, IL-6, IL-1β). Western blot results suggested that DOO downregulated the phosphorylation levels of key proteins in the NF-κB signaling pathway, including IKKα/β, IκBα and p65. 16S rRNA sequencing suggested that DOO altered the diversity and composition of gut microbiota in DSS-induced mice. It promoted the intestinal colonization of beneficial bacteria such as *Dubosiella*, *Lactobacillus* and *Alistipes*, and inhibited the abnormal overgrowth of *Akkermansia*. Furthermore, DOO increased the secretion of short-chain fatty acids (SCFAs) such as acetic acid, propionic acid, and butyric acid to maintain intestinal homeostasis. These findings suggest the potential of DOO as a therapeutic agent for ulcerative colitis (UC).

Keywords: *Dendrobium officinale*; oligosaccharides; chronic colitis; NF-κB signaling pathway; gut microbiota

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1 Introduction

UC is an inflammatory bowel disease (IBD) characterized by chronicity and susceptibility to recurrence[1]. Environmental, genetic, and gut microbiota factors interact with the immune system, resulting in dysregulated immune responses responsible for colitis[2,3]. UC typically causes inflammation in the mucosal and submucosal layers of the intestine, presenting in the lamina propria and crypts as abscesses characterized by large aggregates of neutrophils[4]. In the organism, it is manifested by overactivation of the NF-κB signaling pathway and dysregulation of cytokines, such as TNF-α, IL-1β, and IL-6[5]. In addition to the inflammatory response, individuals with UC are always accompanied by disturbances in the gut microbiota; for instance, decrease in the diversity of the flora, decrease in the abundance of beneficial bacteria, and increase in the proportion of pathogenic bacteria[6]. In patients with colitis, the composition of the intestinal microbiota is difficult to change appropriately as in healthy individuals, resulting in acute inflammation that often evolves into a chronic state[7-9]. Therefore, it is necessary to understand the changes in the microbiome in chronic colitis.

Pharmacotherapy, including glucocorticoids, immunosuppressive agents, and 5-aminosalicylic acid preparations, is the primary treatment option for UC. However, these medications have significant side effects and often fail to achieve satisfactory therapeutic outcomes, which has become an urgent problem to be solved[10]. Recently, polysaccharides and oligosaccharides derived from natural plants have attracted the attention of colitis researchers due to their wide availability, low toxicity, immunomodulatory properties, and potential prebiotic effects[11]. Compared with polysaccharides, oligosaccharides have superior solubility, bioavailability, and their structure is more clearly defined, which helps us to study the mechanism of their structural features on the alleviation of disease. According to Chen *et al.*[12], human milk oligosaccharide 2′-fucosyllactose could regulate the secretion of inflammatory cytokines through the Extracellular matrix (ECM) signaling pathway and utilized by gut microbiota to produce SCFAs such as butyric acid and acetic acid, reversing dysbiosis in colitis mice. Furthermore, functional oligosaccharides such as chitosan and alginate oligosaccharides all showed promise

Address correspondence to Ming Cai, caiming@zjut.edu.cn

in adjuvant therapy for UC[13,14].

Dendrobium officinale belongs to the orchid family and is mainly found in Asia and Europe[15]. As a valuable herb, its biological activity has always served as a focus of attention for researchers. *Dendrobium officinale* polysaccharides (DOP) are the main active substances present in the stems of *Dendrobium*, which have been shown to alleviate the clinical symptoms of UC through multiple pathways[16]. However, DOO has been poorly studied, especially regarding their biological activity as potential prebiotics. In our previous study[17], DOO has been shown an excellent regulatory effect on the gut microbiota during *in vitro* fermentations. However, the regulatory effects of DOO on inflammation and intestinal homeostasis have not been explored *in vivo*.

In this study, we established a chronic colitis mouse model using DSS to explore the potential alleviating effects of DOO on colitis and compared these effects with those of DOP. Intestinal inflammatory response, gut microbiota composition, and SCFA levels were the main focus and measured. Additionally, the role of the NF-κB signaling pathway in the anti-inflammatory effects of DOO was investigated. This research provides evidence for the use of DOO as a natural treatment for intestinal inflammation and offers new insights for further exploration of the active components of *Dendrobium officinale*.

2 Materials and methods

2.1 Materials and reagents

The spray-dried powder extracted from *Dendrobium officinale* stems was provided by Longevity Valley Botanical Co., Ltd. (Wuyi, Zhejiang, China). DSS (36-50 kDa) and sulfasalazine (SASP) were obtained from MeilunBio Biotechnology Co., Ltd. (Dalian, China). Enzyme-linked immunosorbent assay (ELISA) kits were obtained from Shanghai Meimian Industrial Co. Ltd. (Shanghai, China). The primers for PCRs were purchased from Sangon Biotech Co., Ltd. (Shanghai, China). The primary antibodies were purchased from Affinity Biosciences (Changzhou, China). All other chemical reagents were of analytical grade.

2.2 Preparation of DOO and DOP

The DOO and DOP in this study were extracted by the method described in our previous study[17]. Briefly, *Dendrobium* rhizome powder was mixed at a solid-liquid ratio of 1:50 (*w*/*v*) and added with cellulase, which was hydrolyzed at pH 5.0 and 55 °C for 8 h. The solution after enzymatic hydrolysis was treated with ultrasound at 480 W for 40 min. The resulting solution was then filtered using a 3,000 Da ultrafiltration membrane (Zhongke Ruiyang Membrane Technology Ltd., Beijing, China). The permeate underwent dialysis with a 500 Da membrane, followed by concentration and freeze-drying to obtain DOO. DOP (total sugar content of 86.3 ± 3.1%) was obtained from the membrane-trapped liquid through precipitation with four volumes of ethanol[18].

2.3 Animal experiment

The mouse model of chronic colitis was established as described in our previous study[19]. All animal experiments were conducted in accordance with the *National Institutes of Health Guide for the Care and Use of Laboratory Animals* and approved by the Animal Ethics Committee of Zhejiang University of Technology (Review Approval No. MGS20231017147). 6-week-old SPF C57BL /6 male mice (weight 20 ± 2 g) were acclimatized for 7 days with free dietary (AIN-93) and water in a pathogen-free barrier environment with constant temperature (22 ± 2 °C), relative humidity (60 ± 5%) and 12-hour alternating light and dark conditions. The colitis subgroup modeling scheme was shown in Fig. 1A. 60 mice were randomly divided into 6 groups: normal control group (NC), model control group (MC), positive drug group (SASP; 50 mg/kg), polysaccharide group (DOP; 200 mg/kg), oligosaccharide low-dose group (DOOL; 100 mg/kg), and oligosaccharide high-dose group (DOOH; 200 mg/kg). The dosage was determined according to the Chinese Pharmacopoeia and relevant experimental reports[20,21]. The chronic colitis model was established through three cycles of induction. Each cycle consisted of 5 days of free drinking of 3% DSS (*w*/*v*) followed by 7 days of normal sterile water. Only NC group was given sterile water throughout the experiment. SASP, DOP, DOOL, and DOOH were gavaged with 0.2 mL of the corresponding drug daily.

At the end of the experiment, mice were euthanized, and blood was taken from the heart by puncture. The serum was obtained at 4 °C with 4,000 g centrifugation for 20 min. At the same time, mice were dissected, and colon contents and colon tissue samples were collected after recording the length of the colon and stored at −80 °C.

2.4 Measurements of the DAI

The progression of colitis was monitored based on changes in body weight, stool morphology, and fecal occult blood. These indexes were used to determine DAI scores for each group. The scoring criteria were based on Shao *et al.*[22] with some modifications (Table 1). The presence of fecal occult blood was detected according to the instructions of the occult blood test kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China).

2.5 Histopathological analysis of colon tissues

The distal colon tissue was rinsed with saline and fixed in 4% paraformaldehyde solution for 24 h and then embedded in paraffin. Thin sections (5 μm thick slides) of cut tissue samples were stained with hematoxylin and eosin (H&E)[23]. The pathology of the colonic tissues in the sections was observed under the microscope and histopathological scores were given for each group. The scoring criteria was shown in Table 2.

2.6 Pro-inflammatory cytokine analysis

The secretion levels of pro-inflammatory cytokine (TNF-α, IL-1β, and IL-6) in serum were determined using commercially available ELISA kits. Additionally, total RNA was extracted from colon tissues (50 mg) using the Tissue Total RNA Isolation Kit V2 (Vazyme Biochemical Co., Ltd. Nanjing, China) to detect the expression level of mRNA. The reverse transcription (cDNA) was synthesized from total RNA using HiScript® III All-in-one RT SuperMix Perfect Kit. Subsequently, SYBR Green fluorescent dye and primers were added to detect the expression levels of pro-inflammatory cytokine genes in colon tissues by using RT-qPCR. All primer sequences used are listed in Table 3. *β-actin* was used as a reference to calculate the relative expression levels of the target genes using the $2^{-\Delta\Delta Ct}$ method[24].

2.7 Western blot analysis

Protease and phosphatase inhibitors were added to RIPA buffer

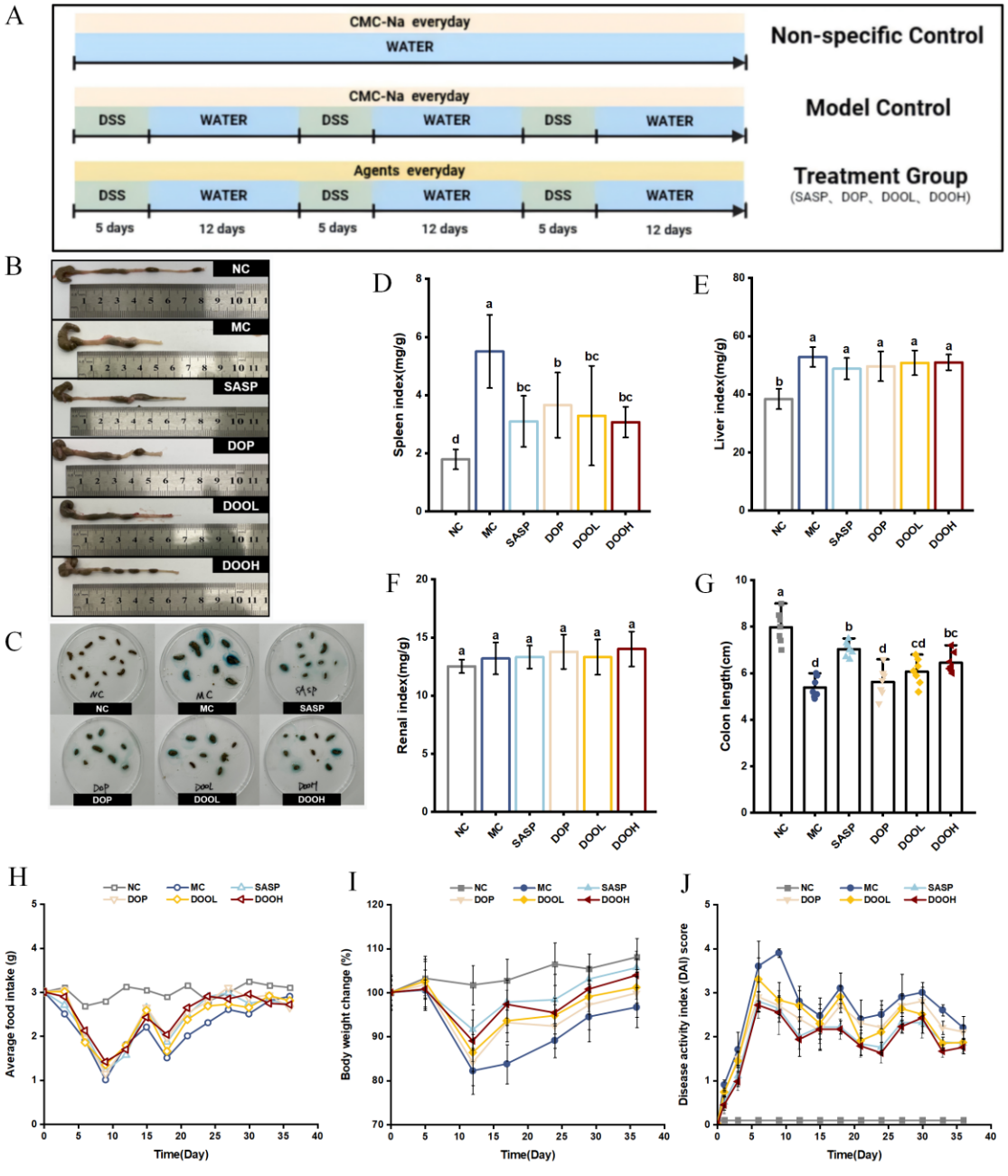

Figure 1 Effects of DOO on basic indicators in chronic UC mice. (A) Experimental design, (B) Representative pictures of the colon, (C) Fecal occult blood conditions, (D) Spleen index, (E) Liver index, (F) Renal index, (G) Colon length, (H) Average food intake, (I) Change in body weight, (J) Disease Activity Index (DAI) score. Results were displayed as means ± SD ($n = 7$). Different lowercase letters mean statistically significant differences ($P < 0.05$).

Table 1 The scoring criteria of DAI

Body weight loss	Feces status	Bloody stools	Score
No loss	Normal	None	0
1-5%	Loose stool (not attached to the anus)	None	1
5-10%	Loose stool (attached to the anus)	Mild bleeding	2
10-15%	Diarrhea	Mild bleeding	3
15-20%	Severe diarrhea	Gross bleeding	4

for protein extraction from colon tissue. Total protein was quantified using BCA and adjusted to the same concentration with loading buffer. All protein samples were boiled at 100 °C for 10 min and stored at −80 °C. Subsequently, proteins were separated by SDS-PAGE and transferred to a PVDF membrane. After blocking the membrane, it was incubated with specific primary antibodies, including p-IKKα/β, IKKα/β, p-NF-κB p65, NF-κB p65, p-IκBα, and IκBα[25]. After washing off the primary antibody from the membrane, the secondary antibody was used for incubation. Western blot analysis was analyzed using *β-actin* as

Table 2 The scoring criteria of Colonic histopathology.

Inflammation Severity	Inflammation Extent	Crypt Damage	Score
None	None	None	0
Mild	Mucosa	Basal 1/3 damaged	1
Moderate	Submucosa	Basal 1/3 damaged	2
Severe	Transmural	Complete crypt loss	4

Table 3 Primer sequence.

Gene	Forward Primer (5'-3')	Reverse Primer (5'-3')
IL-6	TAGTCCTTCCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC
IL-1β	GCAACTGTTCCTGAACTCAACT	ATCTTTTGGGGTCCGTCAACT
TNF-α	GACGTGGAACTGGCAGAAGAG	TTGGTGGTTTGTGAGTGTGAG
β-actin	GGCTGTATTCCCCTCCATCG	CCAGTTGGTAACAATGCCATGT

an internal reference and quantified by imageJ software.

2.8 Compositional analysis of gut microbiota

The composition of the gut microbiota was analyzed by 16S rRNA gene high-throughput sequencing[26]. The intestinal contents of mice from NC, MC, DOP and DOOH groups were selected and sent to Beijing Novozymes Technology Co., Ltd. (Beijing, China) for analysis.

2.9 Determination of SCFAs content

A quantity of 0.2 g of mouse intestinal contents was thoroughly mixed with 2 milliliters of PBS buffer solution. The mixture was transferred into a 2 mL centrifuge tube and added 100 μL of crotonic acid–para-phosphoric acid solution, and vortexed for 5 min. Samples were reacted overnight at −20 °C in a refrigerator and the supernatant was filtered through a 0.22 μm membrane. The resulting solution was analyzed on an Agilent 7820 A VL gas chromatography system with an Agilent DB-FFAP column (0.32 mm × 30 m × 0.5 μm) and FID detector. The detection conditions were conducted according to Xia *et al.*[27].

2.10 Statistical analysis

All statistical analyses were conducted using SPSS 26 (SPSS Inc.; Chicago, IL, USA) software. All experimental results were provided as mean ± SD. One-way ANOVA followed by the Tukey HSD test was used for statistical analysis. $P < 0.05$ and $P < 0.01$ showed significant and extremely significant differences between the different groups of mice, respectively.

3 Results and discussion

3.1 Effects of DOO on basic indicators in chronic UC mice

Based on the high recurrence and slow progression of UC, we designed a DSS-induced chronic colitis model in mice[8]. Compared to the common acute colitis model, this model was closer to the actual situation. The effects of DOO on basic physiological indicators in chronic UC mice were shown on Fig. 1. Changes in food intake and body weight in each group were consistent with the protocol of modeling. In the first two cycles, mice experienced a decrease in food intake and body weight during the initial 7 days of each cycle, particularly noticeable in the first cycle. This decline was followed by a gradual recovery over the subsequent 5 days (Figs. 1H, I). This indicates that during the administration of DSS, the symptoms in mice gradually worsened and improved during the 5-day recovery period. Furthermore, DOO could alleviate symptoms of weight loss and decreased food intake in mice. As shown in Figs. 1B, G, colon length was significantly shorter in the MC group (5.38 ± 0.44) compared to the NC group (7.97 ± 0.69) ($P < 0.05$), while DOO treatment significantly reversed this colon abnormality caused by intestinal inflammation. Colon length was significantly increased ($P < 0.05$) in the DOOL (6.07 ± 0.56) and DOOH (6.45 ± 0.44) groups. The MC group had the darkest fecal occult blood test, while DOO and DOP treatments reduced fecal occult blood (Fig. 1C).

Spleen, as an important immune organ of the body, its index is generally used to measure the immune function of the organism[28]. Therefore, the spleen index can reflect the severity of inflammation in the body. As shown in Fig. 1D, MC group mice showed a significant increase in splenic index after induction of colitis ($P < 0.05$). This might be due to chronic colitis causing abnormal immune function in the mice, resulting in inflammatory infiltration and enlargement of the spleen, which led to an increase in spleen weight. Spleen index was all significantly reduced after treatment with DOO ($P < 0.05$). DAI score is a recognized criterion for determining the severity of colitis. The scores in the MC group were significantly higher than those in the NC group ($P < 0.05$), and there was some recovery of DAI scores after treatment with different doses of DOO. All these results manifest that DOO had a positive effect on alleviating chronic colitis in mice.

3.2 Effects of DOO on the Intestinal Histological Morphology in chronic UC mice

To further determine the anti-inflammatory effects of DOO, the histopathological effects of DOO in chronic UC mice were assessed using HE stains. As shown in Fig. 2, in the NC group, numerous typical U-shaped crypts and goblet cells were clearly observed, and the basal thickness of the crypts was moderate, reflecting the normal colonic tissue structure of mice[29]. Compared with the NC group, the colonic tissues in the MC group showed distortion of the crypts, atrophy, and abnormal infiltration of inflammatory cells. In contrast, the colonic morphology of mice in the other experimental groups showed varying degrees of recovery. The SASP group exhibited the most favorable recovery, with a substantial number of crypt structures and goblet cells resembling normal conditions, although there was still some inflammatory cell infiltration. The degree of recovery of

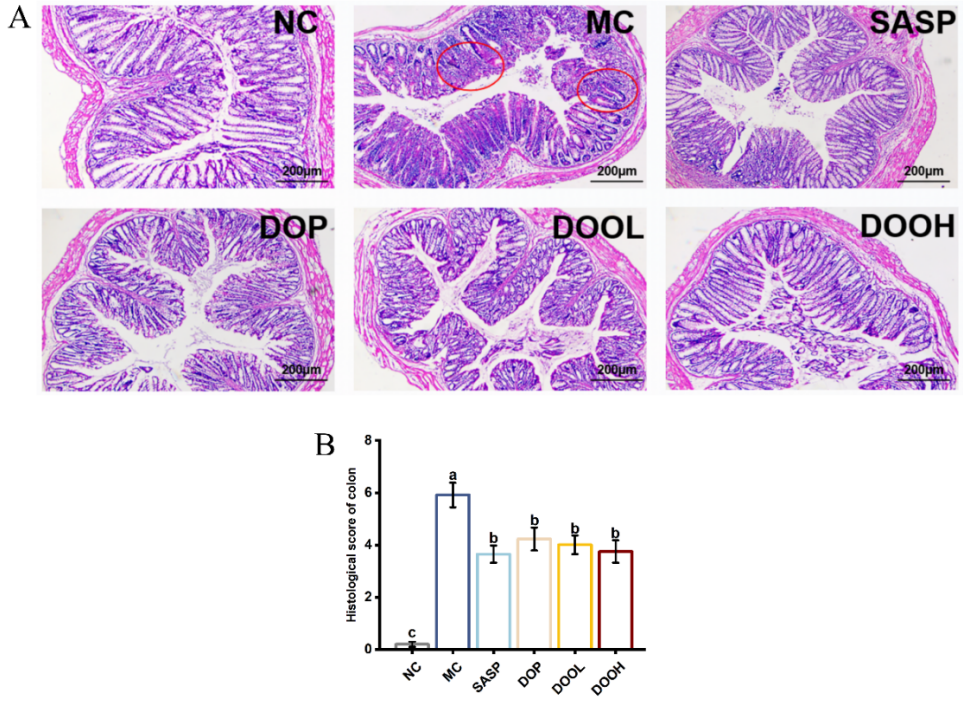

Figure 2 HE stains of mice colon tissues. (A) Stained images of each group. The magnification is 200 ×. (B) Histopathological score.

the DOO treatment was slightly inferior to that of SASP, but superior to that of DOP, which was in agreement with the results of histopathological score. These histological manifestations suggested that DOO could alleviate the colonic tissue damage in chronic UC mice.

3.3 Effects of DOO on the Pro-inflammatory cytokines in chronic UC mice

TNF-α, IL-1β and IL-6, as signaling molecules between immune cells, play an important role in the pathogenesis of colitis, and their high levels of expression could trigger an inflammatory environment that disrupts the integrity of the colonic mucosa, thereby impairing the normal function of intestinal epithelial tissue[30]. As shown in Figs. 3A-C, the levels of three pro-inflammatory cytokines were significantly increased in the serum of DSS-treated UC mice ($P < 0.05$), which was consistent with previous reports[31]. The changes suggest that intestinal inflammation was successfully induced by DSS. SASP, DOP, and DOO treatments inhibited the upregulation of serum pro-inflammatory cytokine levels to varying degrees compared to the MC group. Furthermore, RT-qPCR results showed that DOOH treatment could reduce the mRNA expression levels of three pro-inflammatory cytokines in colonic tissues ($P < 0.01$), bringing *IL-1β* and *IL-6* levels down to normal (Figs. 3D-F). These results suggested that DOO might alleviate colitis by reducing the production of pro-inflammatory cytokines.

3.4 Effects of DOO on the NF-κB signaling pathway in chronic UC mice

Abnormal activation of NF-κB is closely associated with the development of inflammatory diseases. Key members of the NF-κB family include IKKα/β, IκBα, and p65[32]. Typical NF-κB activation is associated with the degradation of IκB, which is rapidly phosphorylated by the active IκB kinase (IKK) complex. Phosphorylated IκB proteins are marked for degradation, allowing p65 to translocate into the nucleus and activate the transcription of inflammation-related genes[33]. To illustrate the mechanism of DOO in the treatment of colitis, we investigated its effect on the NF-κB signaling pathway. As shown in Fig. 4, the colonic protein expression levels of p-IKKα/β, p-IκBα, and p-NF-κB p65 were significantly higher in the MC group than in the NC group ($P < 0.01$). Intestinal inflammation activated the NF-κB signaling pathway, and DOO significantly inhibited the phosphorylation of IKKα/β, IκBα and p65 ($P < 0.01$) thereby reducing their expression levels. Notably, DOO exhibited a superior inhibitory effect compared to DOP, indicating that oligosaccharides with lower molecular weight might achieve better therapeutic outcomes than polysaccharides. Previous studies have reported that oligosaccharides from *Periplaneta americana* could alleviate DSS-induced colitis in mice by inhibiting the TLR4/MAPK/NF-κB pathway, hypothesizing that the structural characteristics and low molecular weight of oligosaccharides may enhance their bioactivity[34].

3.5 Effects of DOO on the Gut Microbiota in chronic UC mice

3.5.1 *Effects on gut microbiota in alpha- and beta-diversity*

Gut microbiota are key factors influencing host metabolism, and microbiota-focused interventions play a crucial role in the treatment of IBD[35]. Alpha diversity analysis is typically used to compare microbial diversity differences between different samples or treatment groups, primarily reflecting the richness and evenness of microbial communities within a sample. The Chao1 index and Observed species index show species richness while the Shannon and Simpson index reflect species evenness[36]. As shown in Figs. 5A-D, compared to the NC group, all indices in the MC group showed a significant decrease ($P < 0.01$), indicating that DSS-induced colitis adversely impacted the gut microbiota of

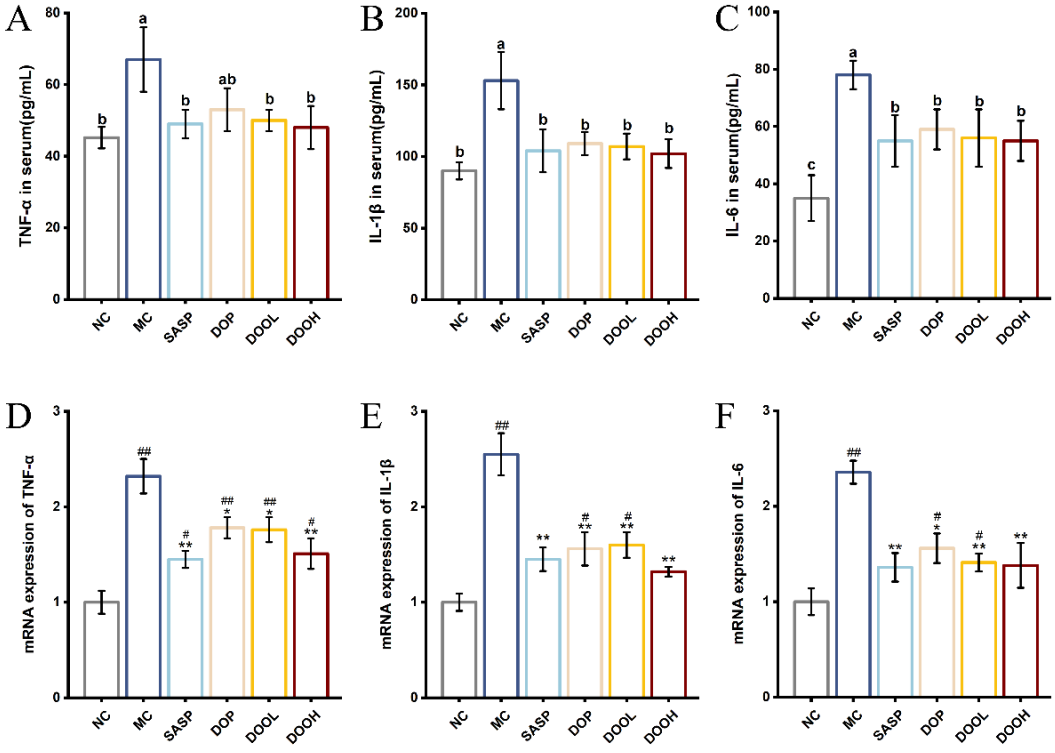

Figure 3 Effects of DOO on pro-inflammatory cytokines in chronic UC mice. Serum levels of TNF-α (A), IL-1β (B), IL-6 (C); mRNA expression levels of *TNF-α* (D), *IL-1β* (E), and *IL-6* (F) in colon tissue. Data are expressed as mean ± SEM (n = 3). The value bars with signs denote statistically significant differences, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ in comparison with the NC group; $^{*}P < 0.05$, $^{**}P < 0.01$ in comparison with the MC group.

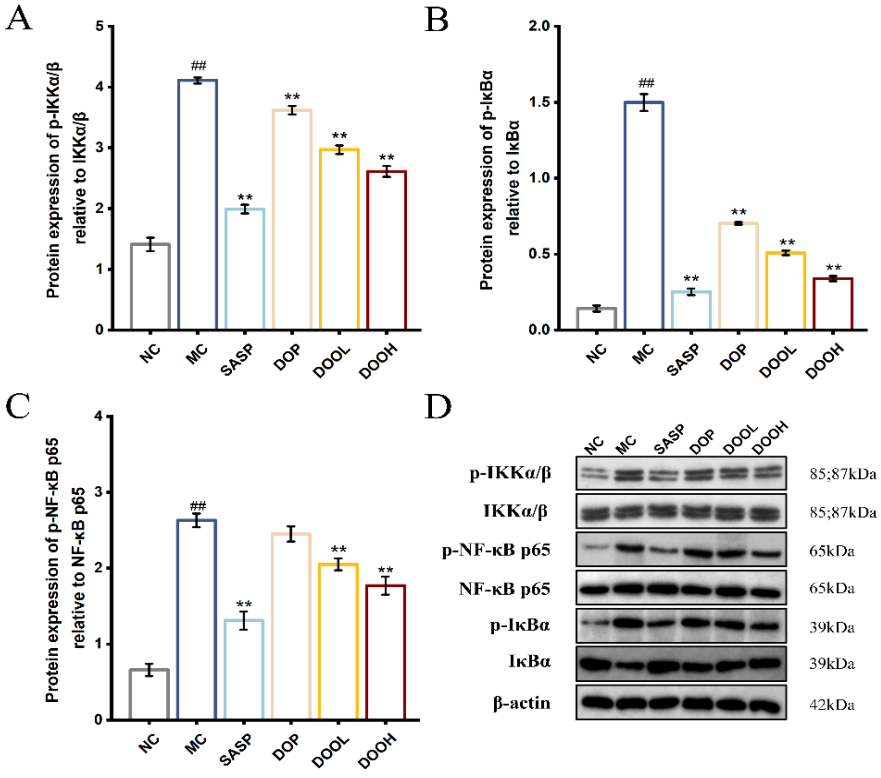

Figure 4 Effects of DOO on the expression levels of proteins related to the NF-κB signaling pathway. Protein expression levels of IKKα/β, IκBα, NF-κB in colon tissue (A-C). (D) Representative western blotting image. Data are expressed as mean ± SEM (n = 3). The value bars with signs denote statistically significant differences, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ in comparison with the NC group; $^{*}P < 0.05$, $^{**}P < 0.01$ in comparison with the MC group.

mice, leading to reduced microbial richness and evenness. After the intervention of DOP and DOO, all indices exhibited an increasing trend. Overall, the DOOH group showed a superior restorative effect on the Chao1 index, observed species index, and Shannon index compared to the DOP group. Beta diversity assessed the differences in species composition and abundance

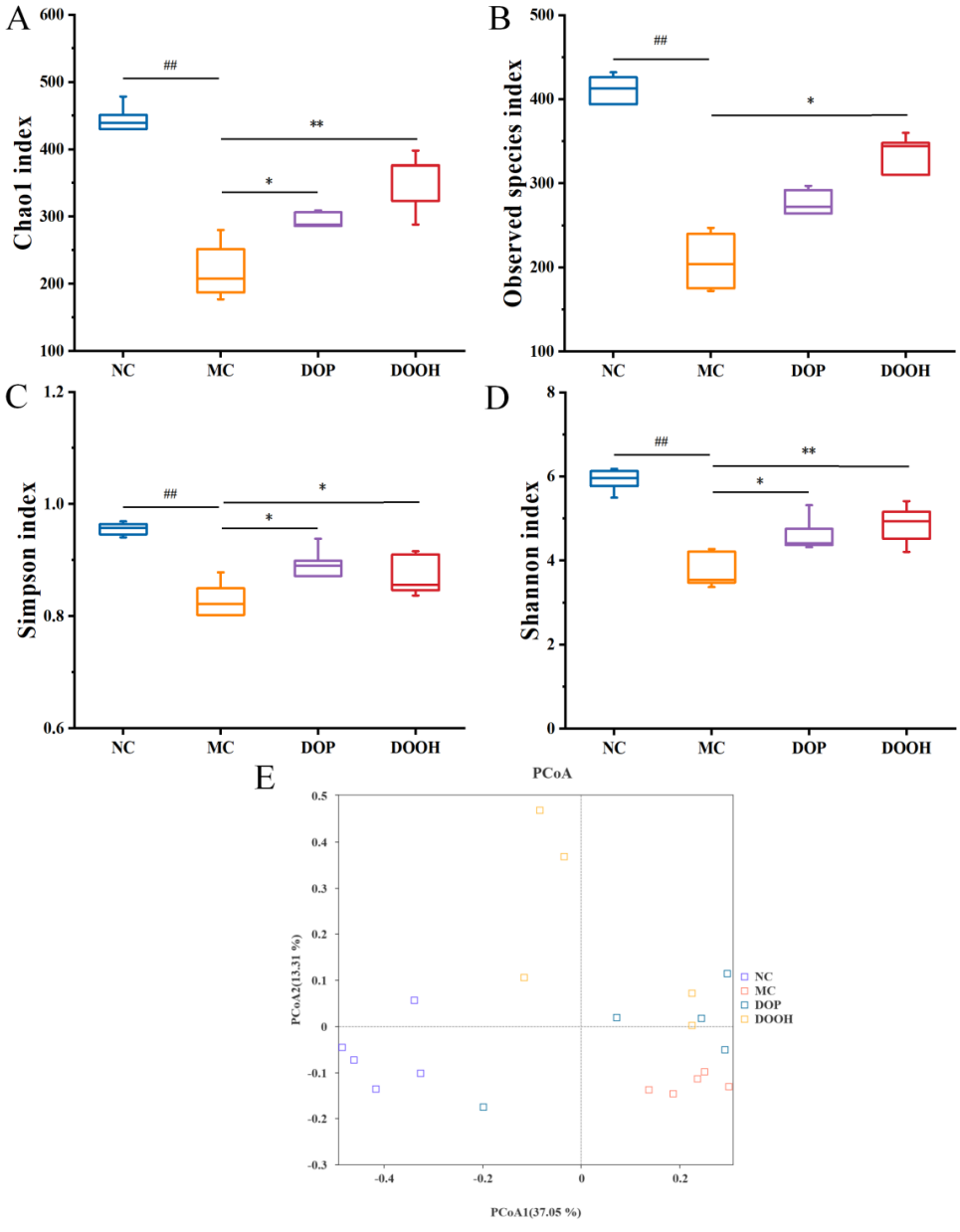

Figure 5 The alpha- and beta-diversity of the gut microbiota. (A) Chao1 index. (B) Observed species index. (C) Simpson index. (D) Shannon index. (E) Weighted Bray–Curtis algorithm-based PCoA.

across multiple samples. In the principal co-ordinates analysis (PCoA) (Fig. 5E), the NC group and MC group were positioned at opposite ends, indicating a significant difference in their gut microbiota composition. The DOOH and DOP groups had similar gut microbiota composition, which might be related to their homologous nature.

3.5.2 *Effects on different levels of microbial abundance*

Typically, dysbiosis of the gut microbiota in colitis includes an increase in pathogenic bacteria and a decrease in beneficial bacteria[37]. However, this microbial imbalance can be reversed following the intake of prebiotic oligosaccharides[34]. To explore these changes, we evaluated the differences in gut microbiota at both the phylum and genus levels after DOO intervention. At the phylum level (Fig. 6A), the gut microbiota of mice in each group was predominantly composed of three phyla: *Bacteroidota*, *Firmicutes*, and *Verrucomicrobiota*. Generally, *Bacteroidota* and *Firmicutes* were the predominant phylums in the gut microbial community. Compared to the NC group, the relative abundances of *Bacteroidota* and *Firmicutes* in the MC group significantly decreased, from 45.77% to 34.61% and 49.80% to 23.51%, respectively. Liu *et al.* reported that the relative abundances of *Bacteroidota* and *Firmicutes* were reduced in patients with colitis[38], which was consistent with our study. However, high doses of DOO effectively restored the abundance of *Bacteroidota,* reaching up to 48.46%. In addition, we found that DOO intervention significantly reduced the abnormal proliferation of *Verrucomicrobiota,* from 40.34% to 24.65%. *Verrucomicrobiota* predominantly colonized the intestinal mucus layer, where they broke down complex carbohydrates and produced SCFAs such as Propionic acid and Butyrate acid to maintain intestinal health and regulate the immune system[39]. However, the abnormal proliferation of *Verrucomicrobiota* can lead to reduced microbial diversity, resulting in gut microbiota imbalance. Certain bacteria

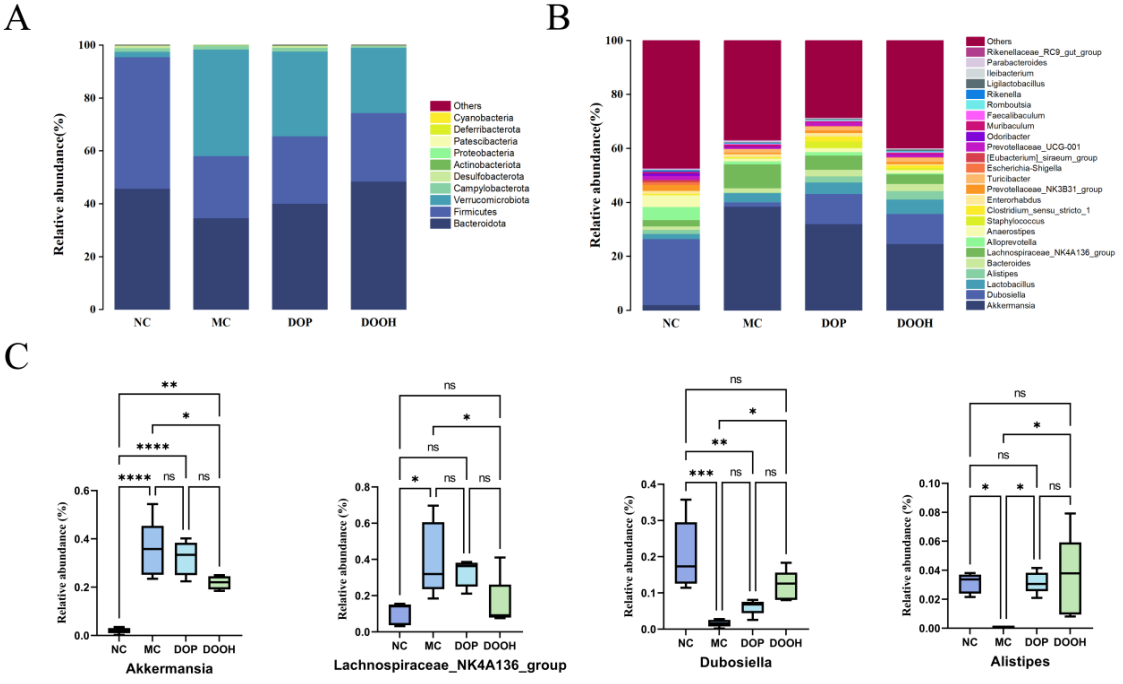

Figure 6 Analysis of gut microbiota composition. (A) Phylum level. (B) Genus level. (C) Analysis of representative species at genus level. The value bars with signs denote statistically significant differences, $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, $^{****}P < 0.0001$.

in *Verrucomicrobiota* can depolymerize DSS and proliferate[40], which may account for the expansion of the *Verrucomicrobiota* in the MC group.

In Fig. 6B, it showed the distribution of gut microbiota at the genus level. There were differences in the gut microbiota between the different groups, but overall consisted mainly of *Akkermansia*, *Dubosiella*, *Lactobacillus*, *Alistipes*, *Bacteroides*, *Lachnospiraceae_NK4A136*_group, *Alloprevotella*, and *Anaerostipes*. Compared to the NC group, the proportions of *Akkermansia* and *Lachnospiraceae_NK4A136*_group were significantly increased in the MC group ($P < 0.05$), and the proportions of *Dubosiella* and *Alistipes* were decreased in the MC group ($P < 0.05$) (Fig. 6C). After DOP and DOO intervention, the abundances of the above bacteria showed varying degrees of improvement, moving closer to the level of the NC group. Qu *et al.* reported that abnormal overgrowth of *Akkermansia* can decrease intestinal mucosal permeability, damage the gut barrier, and trigger intestinal inflammation[41]. *Lachnospiraceae_NK4A136*_group was considered a potential pathogenic bacterium, with increased levels of *Lachnospiraceae* observed in UC[42]. Compared to DOP, DOO intervention demonstrated a more effective inhibitory effect on the overgrowth of these two bacterial genera. Furthermore, DOO intervention increased the abundance of *Dubosiella*, *Lactobacillus*, and *Alistipes*, three genera that may play beneficial roles in gut microbiota. For example, *Dubosiella* can regulate immune tolerance in colitis by activating the AhR-IDO1-Kyn pathway through the production of propionic acid and lysine[43]. Taken together, these results suggest that DOO intervention can restore the diversity of gut microbiota by inhibiting the proliferation of harmful bacteria and promoting the growth of beneficial bacteria.

3.5.3 *Effects on the LEfSe analysis and correlation analysis*

As shown in Fig. 7A, there were 10, 7, 1 and 6 species with significant differences in the NC, MC, DOP and DOOH groups, respectively. *Firmicutes* in the NC group, *Verrucomicrobiota* in the MC group, and *Bacteroidota* in the DOOH group could be

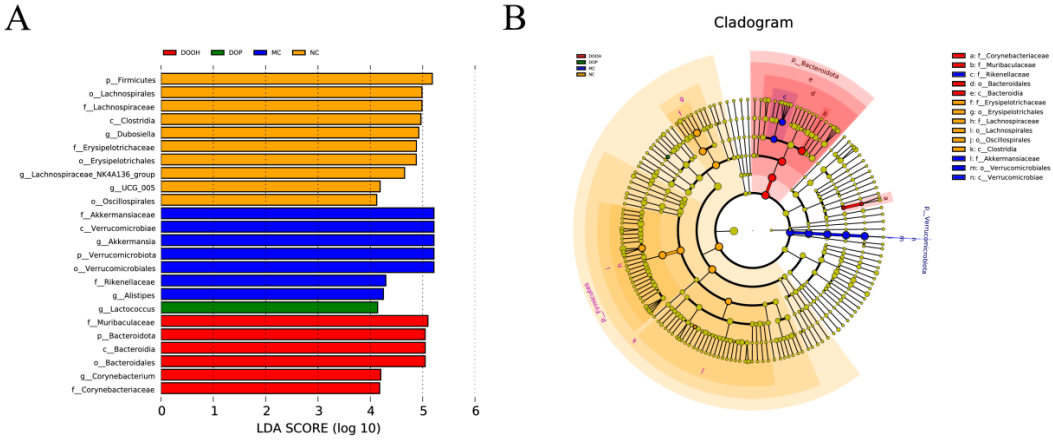

Figure 7 Effects on the LEfSe analysis of microbiota in the mice model. (A) Linear discriminant analysis (LDA) scores of the differentially abundant taxa, (B) Taxonomic cladogram obtained from LEfSe analysis of 16S sequences.

considered as biomarkers of their significant differences at the phylum level. *Dubosiella*, *Lachnospiraceae*_NK4A136_group and UCG_005 in the NC group, *Akkermansia* and *Alistipes* in the MC group, Lactococcus in the DOP group, and Corynebacterium in the DOOH group could be considered as biomarkers with significant differences at the genus level. Fig. 7B illustrates the taxonomic differences among the four groups of bacterial communities. Notably, the gut microbiota structure of mice in the MC group, induced by DSS with colitis, exhibited significant intergroup variability when compared to the DOOH group. Following DOOH treatment, the intestinal flora of the mice revealed distinct biomarkers across various taxonomic levels, including order, family, genus, and species. Importantly, the classes and abundance levels of these biomarkers were more closely aligned with those observed in the NC group. This observation further substantiates the beneficial impact of DOOH in mitigating DSS-induced colitis through the modulation of dysregulated intestinal flora.

In pursuit of a deeper understanding of the potential correlations between the gut microbiota and colitis, we employed Spearman correlation heatmap analysis to examine the relationships between the spleen index, DAI, inflammatory cytokines, and SCFAs (Fig. 8). The findings revealed a negative correlation between inflammatory cytokines and DAI with the phylum Firmicutes. Conversely, SCFAs (propionic acid and acetic acid) exhibited a negative correlation with the phyla Proteobacteria and Cyanobacteria, while a positive correlation was observed with Actinobacteriota. These results underscore the intricate interplay between gut microbiota composition and the inflammatory processes associated with colitis.

3.6 Effects of DOO on SCFAs Levels in chronic UC mice

SCFAs are the primary metabolic products of the gut microbiota and serve as a crucial bridge between the gut microbial community and the host[44]. Gut-derived SCFAs not only regulate intestinal homeostasis but also directly influence immune responses beyond the gut, being associated with a range of diseases[45]. As shown in Table 4, compared to the NC group, the levels of acetic acid, butyric acid, isobutyric acid, and valeric acid in the colonic contents of mice in the MC group were significantly reduced ($P < 0.05$), whereas the levels of propionic acid and isovaleric acid were not significantly different ($P > 0.05$). This may be due to the dysbiosis of gut microbiota caused by DSS-induced chronic colitis, which affected the production of SFCAs. Butyrate acid is an important SCFA that helps maintain intestinal homeostasis by promoting the generation of Treg cells and enhancing macrophage phagocytic activity[46, 47]. In cases of IBD, the number of butyrate-producing bacteria in the gut decreases, leading to reduced butyrate levels[6]. Under the intervention of DOO and DOP, the butyric acid content showed different degrees of recovery, suggesting that DOO can reverse the decrease in butyric acid content caused by DSS and contribute to the regeneration and repair of intestinal epithelial cells. Notably, following DOOH treatment, the levels of acetic acid, propionic acid, and total SCFAs were significantly elevated compared to both the NC and MC groups ($P < 0.05$). Acetic acid and propionic acid promote the secretion of anti-inflammatory cytokines and maintain the stability of the gut microbiota by stimulating

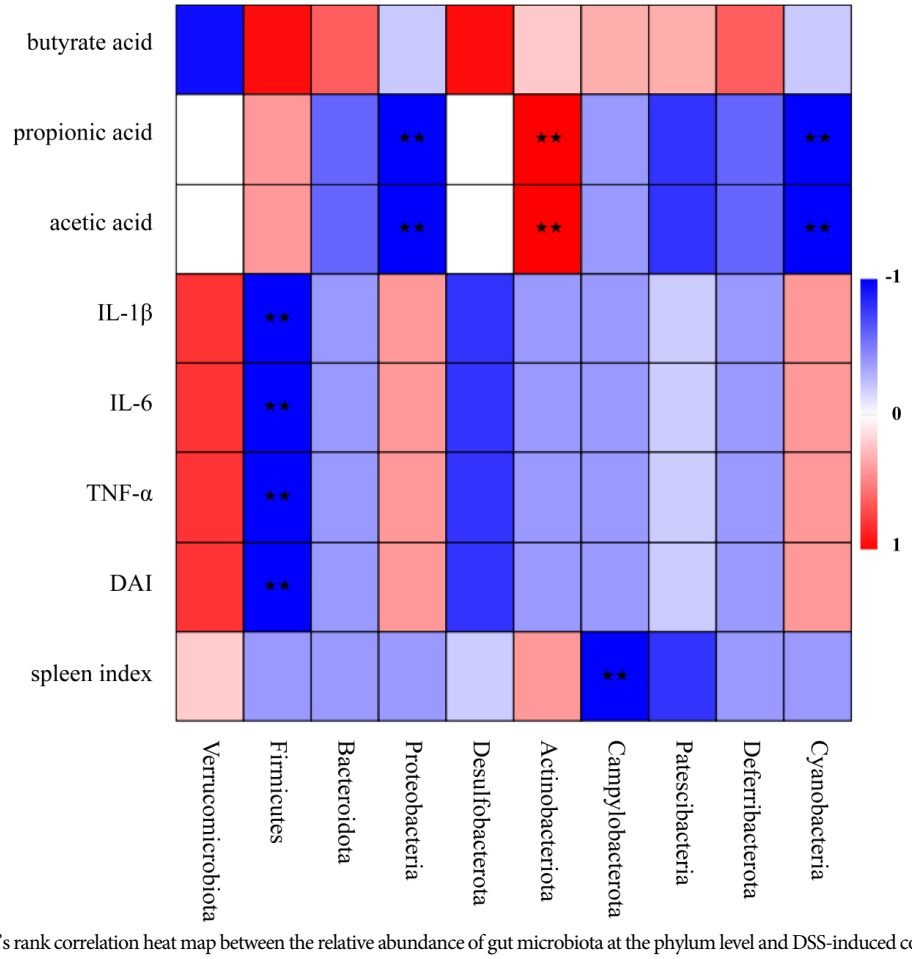

Figure 8 Spearman's rank correlation heat map between the relative abundance of gut microbiota at the phylum level and DSS-induced colitis-related indices.

Table 4 Effects of DOO on SCFAs content.

Group	SCFAs concentration (mmol/L)						
	Acetic acid	Propionic acid	Isobutyric acid	Butyrate acid	Isovaleric acid	Valeric acid	Total SCFAs
NC	2.838 ± 0.164 ^{c}	0.750 ± 0.073 ^{ab}	0.124 ± 0.040 ^{a}	0.750 ± 0.430 ^{a}	0.192 ± 0.102 ^{a}	0.260 ± 0.095 ^{a}	4.813 ± 1.003 ^{bc}
MC	2.443 ± 0.121 ^{d}	0.524 ± 0.097 ^{b}	0.055 ± 0.025 ^{b}	0.257 ± 0.123 ^{b}	0.110 ± 0.050 ^{ab}	0.073 ± 0.024 ^{b}	3.462 ± 0.441 ^{c}
SASP	3.127 ± 0.557 ^{bc}	1.054 ± 0.365 ^{ab}	0.070 ± 0.027 ^{b}	0.449 ± 0.120 ^{ab}	0.107 ± 0.034 ^{b}	0.069 ± 0.027 ^{b}	4.875 ± 1.131 ^{bc}
DOP	3.821 ± 0.385 ^{b}	1.156 ± 0.181 ^{ab}	0.071 ± 0.019 ^{b}	0.312 ± 0.146 ^{ab}	0.144 ± 0.020 ^{ab}	0.075 ± 0.018 ^{b}	5.578 ± 0.772 ^{ab}
DOOL	4.008 ± 0.245 ^{b}	0.871 ± 0.223 ^{ab}	0.066 ± 0.020 ^{b}	0.398 ± 0.154 ^{ab}	0.156 ± 0.027 ^{ab}	0.048 ± 0.021 ^{b}	5.547 ± 0.692 ^{ab}
DOOH	4.997 ± 0.578 ^{a}	1.277 ± 0.443 ^{a}	0.074 ± 0.028 ^{b}	0.563 ± 0.267 ^{ab}	0.111 ± 0.028 ^{ab}	0.062 ± 0.030 ^{b}	7.085 ± 1.374 ^{a}

Note: Results are presented as means ± SD (n = 3). Different lowercase letters represent significant differences at $P < 0.05$.

intestinal mucus production[48]. Previous studies have shown that gut microbiota can degrade complex carbohydrates and promote the release of acetic acid and propionic acid[49]. Due to its unique structural properties, DOO may be more readily degraded and utilized by gut microbiota in the colon, thereby enhancing the production of SCFAs. These results were consistent with the prebiotic effects of DOO in our previous *in vitro* fermentation studies. In conclusion, DOO may alleviate chronic colitis by restoring gut immune homeostasis through the regulation of SCFAs levels.

4 Conclusions

In this study, a DSS-induced chronic colitis mouse model was used to explore the effect of DOO on the improvement of UC symptoms and investigated the possible mechanisms. In terms of the underlying indicators, DOO had an ameliorating effect on the symptoms of weight loss, colon shortening, and bloody stool caused by colitis. DOO alleviated colonic inflammation injury by inhibiting the NF-κB signaling pathway. DOO positively modulated the gut microbiota structure and increased the secretion of SCFAs to maintain intestinal homeostasis in colitis mice. Therefore, DOO holds potential as a novel prebiotic for UC treatment.

Conflicts of interest

The authors declare that there are no conflicts of interest in this work.

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