

## *Gracilaria lemaneiformis* polysaccharides alleviate colitis by modulating the gut microbiota and intestinal barrier in mice

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### ABSTRACT

*Gracilaria lemaneiformis* polysaccharide (GLP) has varieties of antioxidation, however, the therapeutic effects of GLP on ulcerative colitis (UC) and the potential mechanisms involved are still incomplete. In the study, the analysis of the  $\zeta$ -potential, thermal, and morphology properties demonstrated that GLP was a negatively charged polymer, and had great thermostability and irregular network. Moreover, the GLP treatment has the effects of reducing the severity of colitis caused by dextran sulfate sodium by alleviating the colon damage of mice, and increasing the amount of short-chain fatty acids in the intestines, alleviating histopathological inflammation. The sequencing results and  $\alpha$ -diversity analysis showed that GLP could improve biodiversity, restore the abundance of Bacteroidetes, and decrease the proportion of Firmicutes. The level of CCL-25 and CCR-9 were inhibited, CD40 and TGF- $\beta$ 1 were increased. In summary, GLP has potentiality to be utilized as a hopeful functional food to the UC patients.

### Introduction

Nowadays, due to the booming food industry and the increasing temptation of junk food, unhealthy food consumption is common. Unhealthy diets include high-sugar beverages, candies, high-fat foods, salty snacks, and animal fat, coupled with reduced consumption of fruits and vegetables. Intake of such unhealthy foods is associated with a higher risk of chronic diseases, especially inflammatory bowel disease (IBD) (Lo et al., 2020). IBD, which includes Crohn's disease (CD) and ulcerative colitis (UC), is a kind of chronic, idiopathic, relapsing inflammatory, and long-term disorders of the gastrointestinal tract. Its prevalence and incidence have been raising in the past decades and have been a public health issue around the world (Ananthakrishnan, 2015). Although the IBD pathogenesis is not fully understood, recent evidence suggests that IBD is strongly associated with an unhealthy diet, imbalance in gut microbiota, and destruction of the intestinal barrier (Kudelka, Stowell, Cummings, & Neish, 2020). There are some medical strategies for IBDs, including therapeutic drugs and surgical biologics. Indeed, the currently approved drugs, for example, immunomodulators and antibodies, have

severe side-effects such as the risk of vomiting, infections, abdominal pain, and malignancies (Salice, Rizzello, Calabrese, Calandriani, & Gionchetti, 2019). But, functional foods derived from natural resources provide great beneficial effects to human health such as influencing the communication between the gut and peripheral tissues and enhancing intestinal barrier function. Recently, these have also showed promising properties in decreasing the risk of IBD and colon cancer (Green, Miller, Suskind, & Lee, 2019). For all the above mentioned reasons, it is necessary to find out an alternative functional-food-based approach in a safer, efficacious, and cheaper manner for preventing or treating IBD.

Marine seaweeds have been consumed since ancient times in Asian countries, however, currently, their use has also increased worldwide due to their high nutritional value and several health benefits. *Gracilaria lemaneiformis*, belonging to the family *Gracilariaeae* (Rhodophyta), has a long edible history as traditional Chinese medicine (Huang et al., 2019). The nutritional value of *G. lemaneiformis* is high in humans owing to the presence of essential components such as significant amounts of polysaccharides, amino acids, vitamins, and minerals. It is one of the edible seaweeds widely cultivated in China. Numerous studies have

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proven that *G. lemaneiformis* polysaccharide (GLP) has anti-inflammatory, antioxidation, blood lipid reduction, antidiabetic, wound healing, and metabolic syndrome regulating activities (Fan et al., 2012; Ren et al., 2017; Veeraperumal et al., 2020). Therefore, GLP has the potential to serve as an ingredient of function food for the prevention and treatment of various diseases.

Gut microbiota is a complex microbial ecosystem that occupies a considerable position in human health. In the last few years, numerous studies concluded that the gut microbiota had been closely related to different physiological functions, such as regulation of the host's immune system, nutrients production or absorption, and metabolic phenotype (Wu et al., 2019). Researchers have proposed various therapeutic strategies for probiotics for modulating the gut microbiome and regulating gut dysbiosis in IBD patients (Ni, Wu, Albenberg, & Tomov, 2017). Accumulating evidence shows that polysaccharides play a critical role in the treatment of acute colitis and have the potential to improve current IBD treatment regimens. It could be a supplementary source of the treatment (Xu, Xu, Ma, Tang, & Zhang, 2013). Currently, marine algal polysaccharides have attracted extensive attention for regulating gut microbiota by increasing the beneficial microorganisms and inhibiting the levels of pathogens, thus creating new possibilities in functional foods and nutraceutical industries (Shang et al., 2018; Zheng, Chen, & Cheong, 2020). Marine algae are renewable resources, which have an abundant supply. Moreover, the structures of marine algal polysaccharides are unique and are different from terrestrial plants (Xu, Huang, & Cheong, 2017). However, many marine algal polysaccharides have not been explored, and there is a lack of knowledge with respect to their effects on intestinal barrier functions. The present study thus explored the beneficial effects of GLP on the intestinal barrier.

In one of our previous studies, the GLP was purified and had showed to be a homogenous and repeating structure of alternating 4-linked 3,6-anhydro- $\alpha$ -L-galactopyranosyl and 3-linked  $\beta$ -D-galactopyranosyl units with sulfate residues. GLP helps cell proliferation and migration by activating the signaling of PI3K/aPKC during human keratinocytes wound healing (Veeraperumal et al., 2020). In addition, we studied the fermentation of GLP by human fecal inocula in vitro. It can be found the benefits for the gastrointestinal tract functions via producing short chain fatty acids (SCFAs) and modulating intestinal microbes, indicating that it has potentiality to be a source of prebiotics for functional food (Zhang et al., 2020). A recent study has showed that sulfated polysaccharide derived from *G. lemaneiformis* had beneficial effects on intestinal health through regulating the production of SCFAs (Han et al., 2020). However, the protecting effects on the intestinal function and the structure-function relationship between GLP and in vivo data are still unclear. Therefore, we aimed at investigating the potential therapeutic effects of GLP on UC by use of DSS-induced colitis as an in vivo model. The results of the beneficial effects of GLP on the intestinal microenvironment could offer new insights for *G. lemaneiformis* in dietary therapy and its corresponding applications in the functional food industry.

## Materials and methods

### Materials

*Gracilaria lemaneiformis* was gained from the Nan'ao Island (Guangdong, China). Dextran sulfate sodium (DSS, 50 kDa) and the standards of six major SCFAs included acetate, butyrate, isobutyrate, propionate, isovalerate and valerate were provided by Aladdin Chemical Co. (Shanghai, China). Fructo-oligosaccharide was provided by Yuanye Biotechnology Co. (Shanghai, China). The primary antibodies, rabbit monoclonal CC-chemokine 9 receptor (CCR-9) and CC-chemokine ligand 25 (CCL-25), which are related to transport lymphocytes to intestine, were purchased from ABclonal, China. Moreover, we bought all of the secondary antibodies and primary antibodies for mouse monoclonal glyceraldehyde-3-phosphate dehydrogenase (GAPDH) in Beyotime, China. The reagents in the study were of analytical grade.

### Isolation and purification of GLP

*Gracilaria lemaneiformis* was oven-dried until a constant weight was achieved, then crushed in a mill to a homogeneous powder and weighed the powder quality. By using distilled water to extracted at 90 °C and 40:1 (v/w, mL/g) ratio for two hours to obtain dry algae powder. And centrifuged at 4000 × rpm for two minutes. The supernatant was concentrated with a rotary evaporator and precipitated with a 2.5-folds volume of 95% ethanol overnight at 4 °C (Khan, Qiu, Xu, Liu, & Cheong, 2020). Then, the precipitate was collected by centrifugation at 4000 × g. Finally, we dissolved it in water and then dried it to obtain GLP.

### Microelectrophoresis measurement

The study used microelectrophoresis to detect the  $\zeta$ -potential for GLP to gain insight into system stability by a Zetasizer Nano ZS90 (Malvern Instruments, UK). The solution of GLP was prepared by adding distilled water to 1 mg/mL, and adding diluted hydrochloric acid or sodium hydroxide to adjust the pH value of the solution in the range of 2–12. Finally, the mixture was vortexed for 1 min and filtered by the hydrophilic membrane (0.45  $\mu$ m). All determinations were carried out at room temperature (25 °C) and repeated in triplicate.

### Thermal properties analysis

According to a presented report with slight modifications (Khatkar, Barak, & Mudgil, 2013), the thermal properties of GLP were studied using thermogravimetric analysis (TGA) utilizing a thermal analyzer (TA-50H, Shimadzu, Japan) instrument. During the experiment, nearly 6 mg of GLP was placed in the aluminum crucible, protected by high-purity nitrogen, and the temperature was ranged from 30 to 500 °C at a heating growth rate of 10 °C /min.

### Microstructure characterization

The cryogenic scanning electron microscope (JSM-6360LA), which was purchased from JEOL in Japan, was used to observe the microstructure characterization and the surface of GLP. The sample was placed in brass rivets and immersed in liquid nitrogen. Then these were frozen in the cryo-chamber and immediately broken by using a razor blade. The surface of lyophilized GLP was coated with a slice of gold, analyzed in a high vacuum at 10 kV for accelerating voltage.

### Animals and treatment

Kunming Male mice (weighing 50 ± 2 g, 7–8 weeks old, specific pathogen-free) were obtained from the Laboratory Animal Center of Guangdong. They were housed in clean plastic cages under standard circumstance (constant temperature of 23 ± 2 °C; 12 h light/dark cycle; humidity 50 ± 10%) with tap water and free access to food for a week before setting up a DSS-induced model. This study protocol was performed following the Director of Lab Animal Center of Shantou University and approved by Shantou University Medical Animal Care & Welfare Committee (SUMC 2020-382).

In animal experiments, we randomly divided mice into two groups. The first group was the control group (NC, n = 6). In the NC group, they were provided potable water. The second group was suffered from the colitis group (IBD, n = 18). In the IBD group, they were administered 3% DSS (w/v) for drinking water which initiated acute colitis. The states of mice were carefully recorded and monitored daily for signs of disease (the mice weight, stool consistency and rectal bleeding). Through these indicators, colonic inflammation was evaluated by determining the disease activity index (DAI) with minor modification (Friedman et al., 2009). After 7 days for induction of colitis, we randomly divided the IBD group into 3 experiment groups (n = 6), including the GLP group, the fructo-oligosaccharides (FOS) group, and the DSS group. The positive

control, FOS, is a kind of oligosaccharide that has been studied (Matsumoto et al., 2017), and scholars have been performed clinical trials to determine the wholesome functional of fecal microbiota (Li, Lu, & Yang, 2017). During this treatment period, the DSS group and the NC group were provided with equal amounts of normal rodent diet, the GLP group and the FOS group were respectively gavaged with GLP (0.5 mL, 300 mg/kg) (Han et al., 2021) and FOS (0.5 mL, 300 mg/kg). After two weeks, mice were sacrificed by cervical dislocation. The individual colon samples were dissected, and the colon length of samples were measured precisely, then frozen in -80 °C for future analysis. At the time of collection, the feces were collected from all the mice and frozen at -80 °C for subsequent analysis. Supernatants were stored at -20 °C immediately until finish the study. The liver, kidney and spleen were removed and washed with normal saline. Then weighed the quality for fresh liver, spleen, and kidney aiming to gain the organs coefficients.

#### Determination of SCFAs in feces

Fecal SCFAs levels in these groups were prepared as previously with slight modification (Xu et al., 2019), which were assayed by gas chromatography. In brief, dissolving 100 mg fecal samples at a ratio of 1:9 (w/v) in the de-ionized water. Next, the solution was mixed sufficiently by ultrasound vortexed for 3 min, centrifuged at 4 °C and 12000 × g for 10 min. Subsequently, filtering it through 0.45 μm-members to obtain the supernatant comprising the polysaccharides. Next, the supernatant (0.45 mL) was mixed with 0.03 mL 2-ethylbutyric acid (0.04 mol/L) and 0.02 mL HCl (6 mol/L). The resulting supernatant was analyzed for the levels and ingredients of SCFAs by gas chromatography using a GC-2010 Plus system (Shimadzu, Japan) equipped with a DB-WAXetr column (30 cm × 0.25 mm × 0.25 μm; Agilent Technologies, Stockport, UK) and a flame ionization detector. The split ratio was set at 1:9, and the injection volume was 1 μL. Under the following temperature program: first, the initial column temperature was held at 90 °C for 60 sec; second, raising the temperature to 200 °C at a rate of 5 °C /min. The peak areas were used to calculate the content of SCFAs compared to standard calibration equations.

#### 16S ribosomal RNA isolation and quantitative real-time PCR

Centrifugation (4 °C, 12000 × g, 10 min) was used to collect the pellets. Then add 1 mL phosphate buffer (0.1 mol/L) (Zhang et al., 2020). Genomic DNA of mouse feces were obtained using the QIA AMP DNA Stool Mini Kit (Qiagen, Shanghai China). Subsequently, the region V4 of the 16S rRNA was amplified with primers: 338F and 518R. PCR products were carried out in a PCR system (Biometra, Göttingen, Germany) and were extracted by the agarose gel, then purified by the poly-Gel DNA Extraction Kit (Omega Engineering, Inc., Norwalk, CT, USA). After that, we sequenced the purified amplicons which were measured by Illumina Hiseq 2500 platform (San Diego, CA, USA).

#### Bioinformatic analysis

The sequencing data were processed using the quantitative insights into microbial ecology (QIIME) v1.9.1 software package. The operational taxonomic units (OTUs) data (97% sequence similarity) were used to calculate the richness and diversity indices of the intestinal bacterial community and the relative abundance in stool samples. Finally, analysis of groups differences was based on weighted or unweighted UniFrac distance metrics. Analysis of alpha-diversity included three indexes. Community richness was calculated by the Chao 1 index. Community diversity was calculated by the Shannon index and Simpson diversity index. The visualization was by weighted UniFrac-based principal coordinate analysis (PCoA).

#### Histopathological examination

Freshly collected colon tissues were washed with PBS and fixed in 10% neutral formalin at room temperature overnight (Khan et al., 2020). The colon tissues were then followed by dehydration and embedded in paraffin wax, sectioned at five μm, stained with hematoxylin and eosin (H & E). Colon tissue samples were visualized microscopically.

#### Western blotting analysis

We Extracted the total protein from the colonic tissue by use of RIPA lysis buffer. The total protein contents were determined by the use of the Bicinchoninic acid protein assay kit. After boiling (90 °C, 10 min) in protein sample buffer, total proteins were separated by 10% SDS-PAGE and electrotransferred to polyvinylidene fluoride (PVDF) membranes. PVDF membranes were immediately blocked with 5 % w/v non-fat dry milk in TBST buffer for one hour at room temperature. The primary antibody [TGF-β1, CD40, CCL-25, CCR-9, and GAPDH (1:1000 dilution)] was incubated overnight at 4 °C and HRP-conjugated secondary antibody (1:1000 dilution) for one hour at room temperature. Blots were quantified by the Amersham Image 600 gel imaging system (GE Healthcare Life Science, UK) after added of the enhanced chemiluminescence (ECL) detection reagent (Beyond, China). Expression of GAPDH was served as an internal control.

#### Statistical analysis

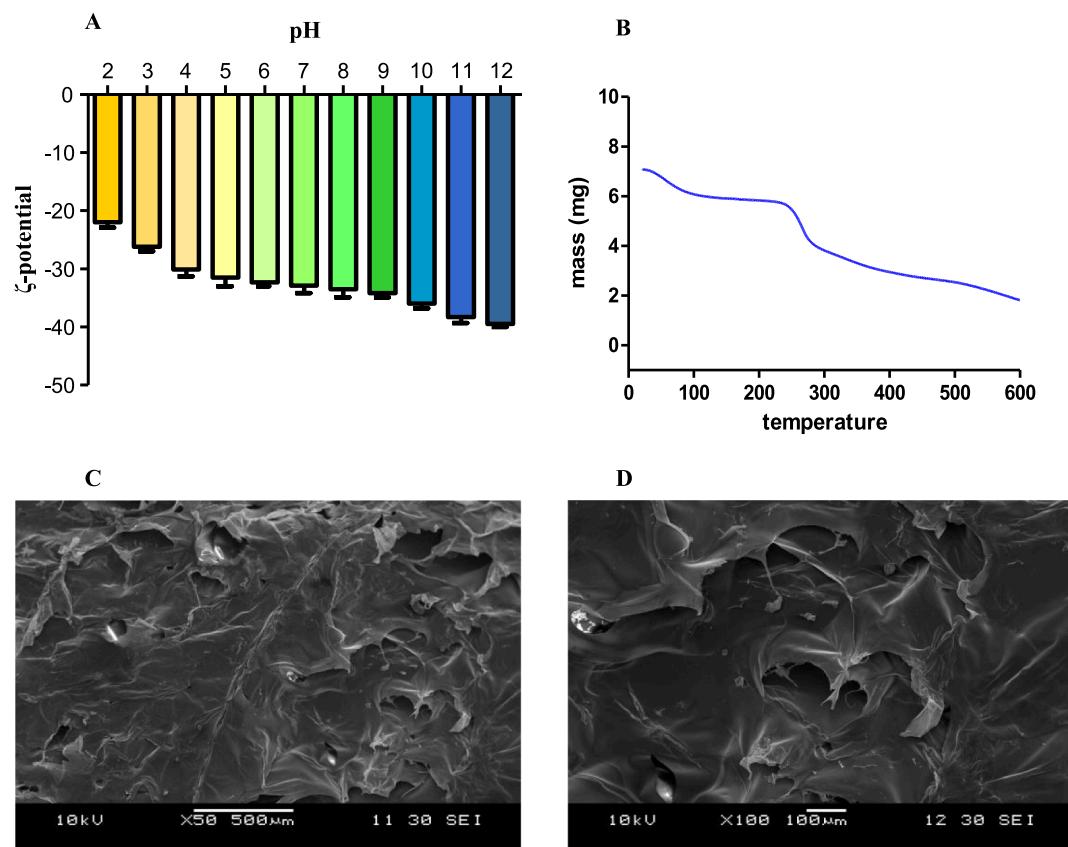
The value of the above experiments was expressed as mean ± SD. Two sets of data were subjected to statistical analysis using one-way analysis of variance followed by Duncan's multiple range tests. The significance of individual variations between groups was carried out by the statistical analysis software SPSS 23.0, and p < 0.05 was considered significant. The biomarkers between the groups were found by using the linear discriminant via richness and diversity of bioinformatics and genetic sequencing data. The sequence of each OTU was assigned to different taxonomic levels using Ribosomal Database Project Naïve Bayesian. Furthermore, alpha values were set to 0.05 whereas the threshold on the logarithmic score of linear discriminant analysis was ≥ 2.0.

#### Results

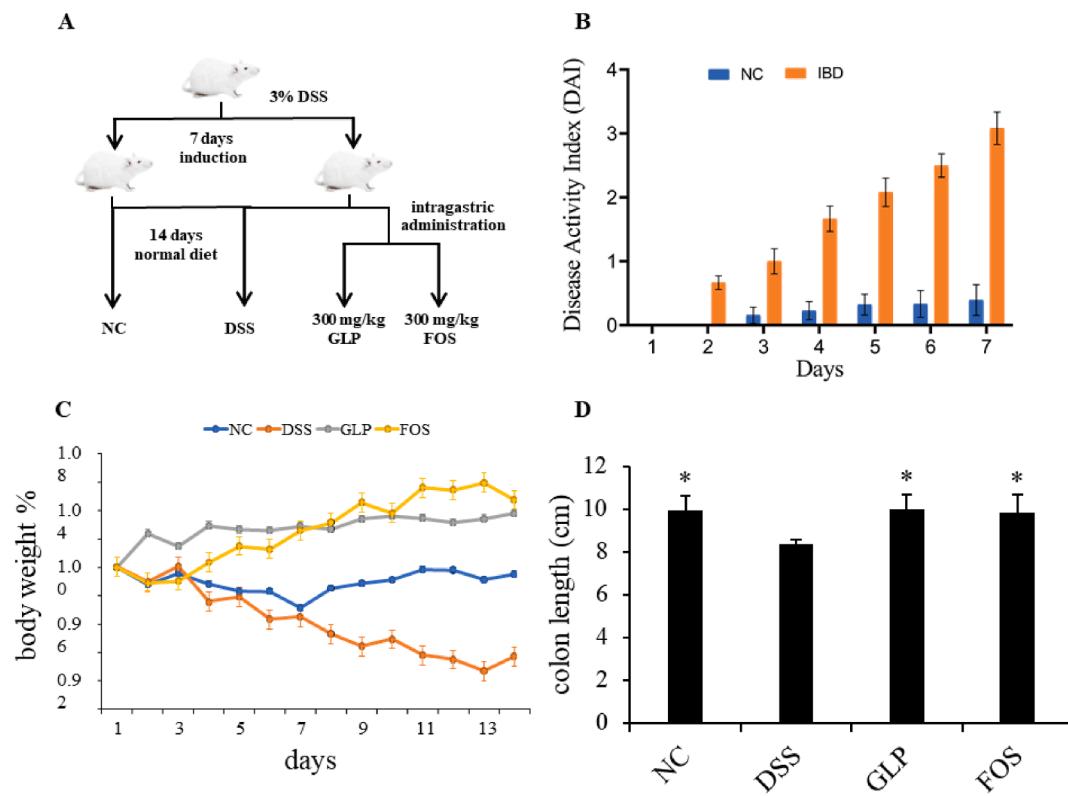
##### $\zeta$ -potential, thermal properties, and surface morphological characteristics of GLP

Since the pharmaceutical activities of polysaccharides are mainly based on their physicochemical properties (Yan et al., 2021), in one of our previous studies, the chemical structure and physical properties of GLP were determined. GLP has a flexible chain in an aqueous solution with a weight average molecular weight of  $1.570 \times 10^5$  Da and an intrinsic viscosity of 133.94 mL/g. The structure of GLP is repeating, homogenous, and alternating 4-linked 3,6-anhydro- $\alpha$ -L-galactopyranosyl and 3-linked  $\beta$ -D-galactopyranosyl units with sulfate residues. The parameters included  $\zeta$ -potential, thermal properties, and morphological features of GLP were determined in this study, to apply more usefully for the industry.

The  $\zeta$ -potential value, as an essential surface property, is one of the critical parameters in many fields such as functional food, pharmacy, and cosmetics. The  $\zeta$ -potential plays an important role in detecting the stability of the colloidal system by reviewing the electrostatic attraction and steric repulsion. Zeta potential analysis (Fig. 1A) revealed that GLP in the aqueous phase was negatively charged and was below -30 mV in the pH range of 4–12. We range from pH 2 to pH 14, the absolute value of  $\zeta$ -potential increased with the increase of pH. The higher absolute  $\zeta$ -potential value of polysaccharides conferred a greater electrostatic



**Fig. 1.** The  $\zeta$ -potentials of GLP aqueous solutions with pH ranged from 2 to 12 (A); TGA profiles of GLP (B); The microstructure of GLP under scanning electron microscopy: zoomed 50 times (C), zoomed 100 times (D).



**Fig. 2.** GLP protects mice against DSS-induced colitis. (A) Schematic representation of the experimental plan; (B) DAI score; (C) Body weight change; and (D) colon length. Data are expressed as mean  $\pm$  SD ( $n = 6$ ) and significantly different at the level of  $p < 0.05$ .

repulsion and prevented the aggregation of polysaccharides. The absolute value of  $\zeta$ -potential values  $> 30$  mV is considered that the dispersion system is stable (Wu et al., 2018). Therefore, GLP has good stability both in the aqueous solutions at a pH range of 4–12.

The thermal properties of polysaccharides affect various industrial purposes. Generally, polysaccharides possess some special features that high resistance to high temperatures (i.e., those above 200 °C), and that temperature should stay in the production processes strictly. The decomposition pattern and thermal behavior of GLP were investigated by TGA (Fig. 1B). The TGA plot of GLP was lost upon heating at a temperature of 100 °C due to the dehydration of the absorbed water by evaporation. At 175 °C, all free and bound water was desorbed, and consequently, at 500 °C, thermal degradation occurred because of the arbitrary breakage of glycosidic bonds. Our results demonstrated that GLP possessed good thermal stability, further, GLP could be applied in the functional foods, nutraceuticals, and pharmacy industry.

As showed in Fig. 1C–D, a 50 and 100 times magnified image, respectively, the surface and morphology of GLP revealed a smooth and highly porous irregular network, which demonstrated that GLP was composed of a loose microstructure with high porosity. The morphology presented here was consistent with the results of the study of other red seaweed polysaccharides (Khan et al., 2020). The highly porous and loose structure of polysaccharides enables its use in various applications such as the production of functional foods with improved physicochemical and mechanical properties (Selvasekaran & Chidambaram, 2021).

#### *GLP alleviates the symptoms of DSS-induced colitis in mice*

This study evaluated the protective effects of GLP on the animal model with DSS induced. The experimental timelines are displayed in Fig. 2A. Severe diarrhea, hematochezia, and body weight loss showed that the UC model was successfully established. In general, the clinical symptoms of DSS-induced colitis in mice are reflected by the DAI score (Fig. 2B). The DAI score was higher in the IBD group when compared with the results of the NC group. DSS-induced injury in experimental animals resulted in a significant decrease in body weights. Fig. 2C shows that the weight of the DSS group was substantially lower than that of the NC group.

DSS typically caused colonic shortening and it is one of the typical symptoms, clearly marked the severity of intestinal inflammation. The effects of the DSS group upon colon length was visibly decreased than that of the NC group, while the colon length was significantly restored in the GLP and FOS treatment (Fig. 2D). Our results indicated that GLP treatment can relieve the clinical symptoms of colon damage induced by DSS.

#### *Effects of GLP on organ index in DSS-treated mice*

Since the model of colitis with DSS-induced in mice is used widely and its histopathology is similar to human UC. Thus, in the present study, this model was used to assess the therapeutic effects of GLP on colitis.

The immune organs included the kidney, liver, and spleen, the corresponding indices can indicate the levels of inflammation (Wu et al., 2022; Zong et al., 2018). Therefore, the organ indices were determined. The results of kidney, liver, and spleen indices are showed in Table 1. The kidney and liver indices of the DSS group were decreased than those of the NC group, whereas the kidney and liver indices of the GLP and FOS groups were recovered. The inflammation induced by DSS could increase spleen weights as the spleen index of the DSS group was higher than that of the NC group, indicating an inflammatory response; further, after being treated by GLP and FOS, the spleen index was found similar to that of the NC group. This is because that the spleen is a lymphoid organ and plays a crucial role in initiating adaptive immune responses to pathogens in the bloodstream (Bronte & Pittet, 2013). In addition, the

**Table 1**

Effects of the GLP on the kidney, liver, and spleen indices in different organs in mice.

| Group | kidney index (mg/g)     | liver index (mg/g)      | spleen index (mg/g)    |
|-------|-------------------------|-------------------------|------------------------|
| NC    | 15.2 ± 1.9 <sup>a</sup> | 54.9 ± 5.9 <sup>a</sup> | 2.9 ± 1.0 <sup>b</sup> |
| DSS   | 14.0 ± 1.5 <sup>a</sup> | 53.4 ± 4.4 <sup>a</sup> | 7.0 ± 3.7 <sup>a</sup> |
| GLP   | 16.1 ± 2.3 <sup>a</sup> | 54.0 ± 3.4 <sup>a</sup> | 3.7 ± 1.6 <sup>b</sup> |
| FOS   | 14.6 ± 1.1 <sup>a</sup> | 51.3 ± 2.8 <sup>a</sup> | 2.8 ± 0.5 <sup>b</sup> |

Data within a column without the same superscripts differ significantly ( $p < 0.05$ ). Data are represented as the mean ± SD;  $n = 6$  in each group.

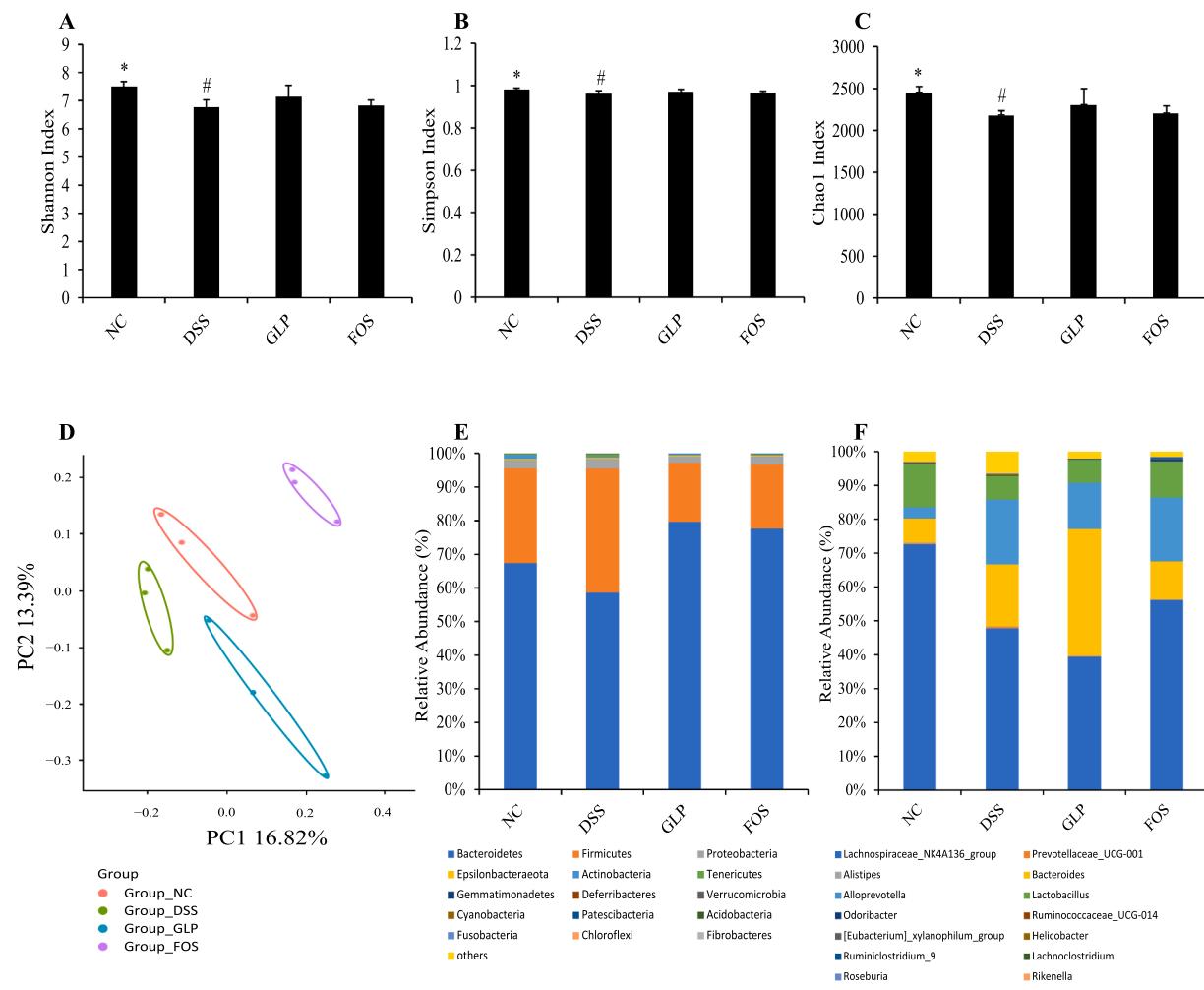
spleen index is one of the important indicators to evaluate the protective effects of substances on the immune system. Our results showed that GLP inhibited intestinal inflammation of DSS colitis, thus protecting the immune organs.

#### *GLP improved DSS-induced imbalances in intestinal microbiota*

The effectiveness of GLP on DSS-induced intestinal microbiota dysbiosis was obtained by 16S rRNA sequencing. The sequence data could analyze the alpha diversity. Alpha diversity is reflected by Shannon (Fig. 3A), Simpson (Fig. 3B), and Chao 1 (Fig. 3C) indices. Among them, the Shannon and Simpson indices are used to indicate the gut microbiota diversity, wherein the higher values of the Shannon index signify higher diversity but the Simpson index is inversely associated with community diversity. Moreover, Chao 1 index reflects the intestinal microbiota richness, and the higher values mean the higher community richness. The results showed that the Chao1 and Shannon indices of the DSS group were lower than those of the other three groups, indicating that the diversity of intestinal flora was the lowest and the number of species was the least in the group. Nonetheless, the Shannon and Chao1 indices of both GLP and FOS groups were increased, the results indicated that GLP and FOS played a role in enhancing the intestinal microbial community diversity (or species number) in the mice. Moreover, the GLP group has displayed a stronger increase than the FOS group, but no significant difference. The community diversity of the two groups was not significantly different in the Simpson index, however, the DSS group demonstrated the lowest value of the Simpson index. Loss of intestinal diversity can trigger inflammatory reactions and promote the progression of inflammatory diseases. As it is proved that GLP treatment can reduce the loss of intestinal flora diversity, it has a certain clinical significance in the treatment of intestinal inflammatory diseases and metabolic diseases.

To compare the relationships among these gut microbiota communities, the weighted PCoA was used to evaluate. The PCoA was adopted for visualizing the multivariate structure of the data and dissimilarity within the intestinal microbiota. As showed in Fig. 3D, there was a clear separation among these samples. PC1 showed a 16.82% difference, which was the most important loading value to segregate the DSS group from other groups. PC2 showed a 13.39% difference, which was used to separate the GLP and FOS groups from the NC group. According to the results of PCoA, different samples from the model and treatment groups have obvious differences in the overall gut microbiota composition.

In the DSS-induced mice, the gut barrier was destroyed and the gut microecosystem was altered, which resulted in changes in gut microflora components. Fig. 3E–F shows the classification of bacteria in the intestines of each group of mice at the phylum and genus levels. A total of 15 phyla were detected. The predominant phyla were Bacteroidetes, Firmicutes, and Proteobacteria, followed by Epsilonbacteraeota, Actinobacteria, Tenericutes, Chloroflexi, and Fibrobacteres, which were less abundant. Bacteroidetes and Firmicutes are the most predominant phyla in the intestinal tract because they can secrete hydrolytic enzymes, designated carbohydrate-active enzymes, to degrade the polysaccharides into smaller carbohydrate molecules. Moreover, their high polysaccharide utilization ability can provide suitable glycans to other



**Fig. 3.** Alpha diversity analysis of fecal microflora for (A) Shannon index analysis, (B) Simpson index analysis, (C) Chao1 index analysis. (D) Principal co-ordinates analysis of the gut microbiota of rats of different groups, while the different colors in the figure represent samples in different groups. Effect of GLP administration on the gut microbiome composition in DSS-induced colitis mice at the (E) phylum level and (F) genus level. # $p < 0.05$  compared with the control group and \* $p < 0.05$  compared with the DSS group.

bacteria and contribute to the symbiosis of complex intestinal communities (Ye et al., 2020). As showed in Fig. 3E, compared with the NC group, the relative abundance of Bacteroidetes in the DSS group decreased, while the proportion of Firmicutes had a significant increase. Contrary to the result of the DSS group, the relative abundance of the Bacteroidetes increased significantly in both the GLP and FOS groups, and the proportion of Bacteroidetes in the GLP group was the highest, however, the ratio of Firmicutes in both the groups decreased significantly. The Firmicutes/Bacteroidetes (F/B) ratio can be a possible biomarker-related gut dysfunction to identify pathological conditions in intestinal microorganisms. A low F/B ratio was found to be associated with a balanced immune system and is normally considered beneficial for health (Wang et al., 2019). As showed in our result, the DSS group had a high F/B ratio, whereas GLP and FOS treatment groups had decreased values of the F/B ratio. Furthermore, the group of GLP has lower F/B ratio than the group of FOS, suggesting that GLP has beneficial effect to balance intestinal function.

In addition, Proteobacteria is a major phylum of pathogenic bacteria and suggested as a potential diagnostic criterion for dysbiosis, they can accelerate producing excessive pro-inflammatory cytokines. The proportion of the Proteobacteria phylum in the DSS group was higher than the other three groups, whereas it was the lowest in the GLP group. As has been mentioned, the UC typical characteristic is a large amount of Proteobacteria, which increasing colonic susceptibility that leads to the

defect in innate and adaptive immune systems of DSS-induced murine (Shin, Whon, & Bae, 2015).

The *Lachnospiraceae NK4A136* group, at the level of genus (Fig. 3F), was regarded as a probiotic in the intestine and the more amounts mean the less inflammation (Jiang et al., 2020). The relative abundance of the *Lachnospiraceae NK4A136* group of the GLP group and the DSS group demonstrated a distinct diminution. Whereas, compared with the DSS group, the FOS group advances the amount of *Lachnospiraceae NK4A136* group more significantly. In addition, after GLP and FOS treatment, the proportion of *Lactobacillus* had been improved distinctly. *Lactobacillus* is a beneficial probiotic genus, which could balance gut microbiota and help maintain the mucus layer (Valitutti, Cucchiara, & Fasano, 2019). Furthermore, the composition of intestinal flora was enhanced by increasing the relative abundance of beneficial bacteria. Beneficial bacteria, including *Alloprevotella*, *Lactobacillus*, *Prevotella*, and *Ruminococcus* can produce SCFAs, demonstrating the ability for producing energy to intestinal cells and protecting the intestinal barrier (Kong, Gao, Yan, Huang, & Qin, 2019). Taken together, the information suggests that DSS-induced UC make intestinal flora disorder, but when administrated with GLP, the gut microbiota dysbiosis could be prevented.

#### GLP improved DSS-induced decrease in functional microbial metabolites

The content of SCFAs was detected to value the effects of GLP on

microbial metabolites. As showed in Table 2, the composition of SCFAs includes acetate, propionate, isobutyrate, butyrate, isovalerate and valerate. The total SCFAs, acetic acid, isobutyric acid, and isovaleric acid in the DSS group were lower than the NC group ( $p < 0.05$ ). Whereas, the levels of SCFAs, especially propionate, isovalerate, and the total SCFAs, were recovered in GLP-treated mice ( $p < 0.05$ ). The evidence was in line with our preceding study, that the in vitro fermentation of GLP by human fecal inoculum can increase the SCFAs content (Zhang et al., 2020). In addition, our finding is similar to the outcomes of Sun et al.'s report, which alleviated the high-fat diet-induced obesity by reducing dysbiosis in the gut microbiota and increasing the levels of SCFAs (Sun et al., 2018). Higher contents of propionate and valerate were observed than the NC group, indicating that GLP can be better utilized by gut microbiota.

Many studies have suggested that human intestinal microbiota can digest dietary polysaccharides to produce functional metabolites, such as SCFAs, in the large intestine. The SCFAs were identified and quantified in fecal samples, they exert numerous beneficial effects on host energy metabolism and are closely involved in immune, antitumor, and anti-inflammatory activities (Yin et al., 2020). SCFAs not only contribute to preventing pathology by their action in the lumen and on the colonic musculature and by their metabolism by colonocytes, but also play an important role in maintaining the energy metabolism balance. A change in SCFAs levels can reflect the homeostasis of the gut microbiota that maintains the human intestine health (Han et al., 2021). SCFAs can enter the systemic circulation and directly influence metabolism and peripheral tissue functions. As previous studies indicated that SCFAs had therapeutic effects on IBD patients (Parada Venegas et al., 2019). According to our results, we demonstrated that GLP can elevate the content of SCFAs in a DSS-induced colitis mouse model, and had beneficial effects to alleviate UC by reconstructing the microbiota compositions.

#### GLP reversed the histological injury of colonic epithelium induced by DSS

DSS-induced colitis in mice is mimicking typical histological characteristics of colonic injury in IBD. Histological analyses of the colon are showed in Fig. 4A. In the NC group, the colon was showed normal pathological morphologies with no obvious signs of injury and inflammation (Fig. 4A1). On the contrary, the mice with DSS-induced colitis exhibited severe acute colitis, which is a typical structural injury accompanied by malformation of the gland and infiltration of inflammatory cells in mucosa and submucosa layers (Fig. 4A2). However, the colonic pathological damage in GLP and FOS treatment groups was significantly alleviated (Fig. 4A3-4). The pathological morphologies showed well-shaped compact columnar epithelium and a separated and well-defined layer between mucosa and submucosa with deep narrow-spaced intact intestinal crypt. The result indicated that GLP could significantly ameliorate colonic inflammation and injury.

#### GLP promoted protein expression in the colon tissue of DSS-exposed mice

To explore the mechanism behind the reduction in colitis by utilization of GLP, we evaluated the effects of specific proteins: CCL25,

CCR9, transforming growth factor- $\beta$ 1 (TGF- $\beta$ 1) and CD40.

CCL25 and CCR9 are involved in colonic inflammation. Chemokines are a family of low-molecular-weight proteins, and are primarily recognized by guiding leukocyte migration under both homeostatic and inflammatory environments. The corresponding functional receptor of CCL25 is CCR9 (Rivera-Nieves et al., 2006). It is previously well known that CCL25 is largely absent in healthy human colon, however, its expression is highly upregulated in colitis and is associated with inflammatory responses (Rivera-Nieves et al., 2006). When there was affected with UC, CCL25/CCR9 interactions began to drive lymphocytes to the intestine (Svensson & Agace, 2006). It is a tissue-specific multistep process involving adhesion molecules and chemoattractant receptor signaling by chemokines (Feagan et al., 2015). As evidenced in Fig. 4B, CCL25 and CCR9 were expressed at high levels in the DSS group, which suggested that the function of gut barrier was weakened, leading to UC. However, GLP and FOS significantly inhibited the production of CCL25 and CCR9; the CCL25 inhibition rate of GLP was higher than that of FOS, whereas the CCR9 inhibition rate of FOS was higher than that of GLP.

TGF- $\beta$  is an inhibitory cytokine with potent immunological homeostasis and inflammatory responses (Becker, Fantini, & Neurath, 2006). The targeted disruption of TGF- $\beta$ 1 gene that from mouse will result in an acute multifocal inflammatory response and leads to premature death. The prevention from colitis development is strictly associated with the upregulation of TGF- $\beta$ 1. As showed in Fig. 4C, TGF- $\beta$ 1 was significantly reduced in the DSS group, in the GLP group, the expression of the TGF- $\beta$ 1 was upregulated, and in the FOS group, the TGF- $\beta$ 1 expression increased significantly. The possible mechanism of TGF- $\beta$ 1 in colitis is by mediating the Wnt pathway (Lin et al., 2015). Intestinal immune homeostasis depends on the production of regulatory T (Treg) cells. The mechanism of CD40 is able to inhibit iTreg (induced Treg) cell induction, and the CD40L/CD40-signaling axis might intervene to produce new iTreg cells to improve immunity (Barthels et al., 2017). Protein analysis revealed that DSS induction could lead to remarkable expression of CD40. Positive control FOS could upregulate CD40 with extreme significance, GLP has a weaker effect of upregulating CD40. In summary, GLP can guide the targeted cytokine to alleviate the UC by affecting the expression of CCL25, CCR9, TGF- $\beta$ 1, and CD40, thus, it has significant potential to be a supplemental UC dietary remedy in the field of functional foods.

#### Conclusion

Maintaining the intestinal barrier function and balancing the gut microbiota are the main approaches for an effective remedy for UC. Hence, it is crucial to seek an efficient and safe mode, such as food-based therapeutic strategies, to repair and reestablish the intestinal functions and restore the diversity of the intestinal microbiota. Considering that *G. lemaneiformis* is a centuried food and provides abundant sources of polysaccharides, it could become a potential UC treatment.

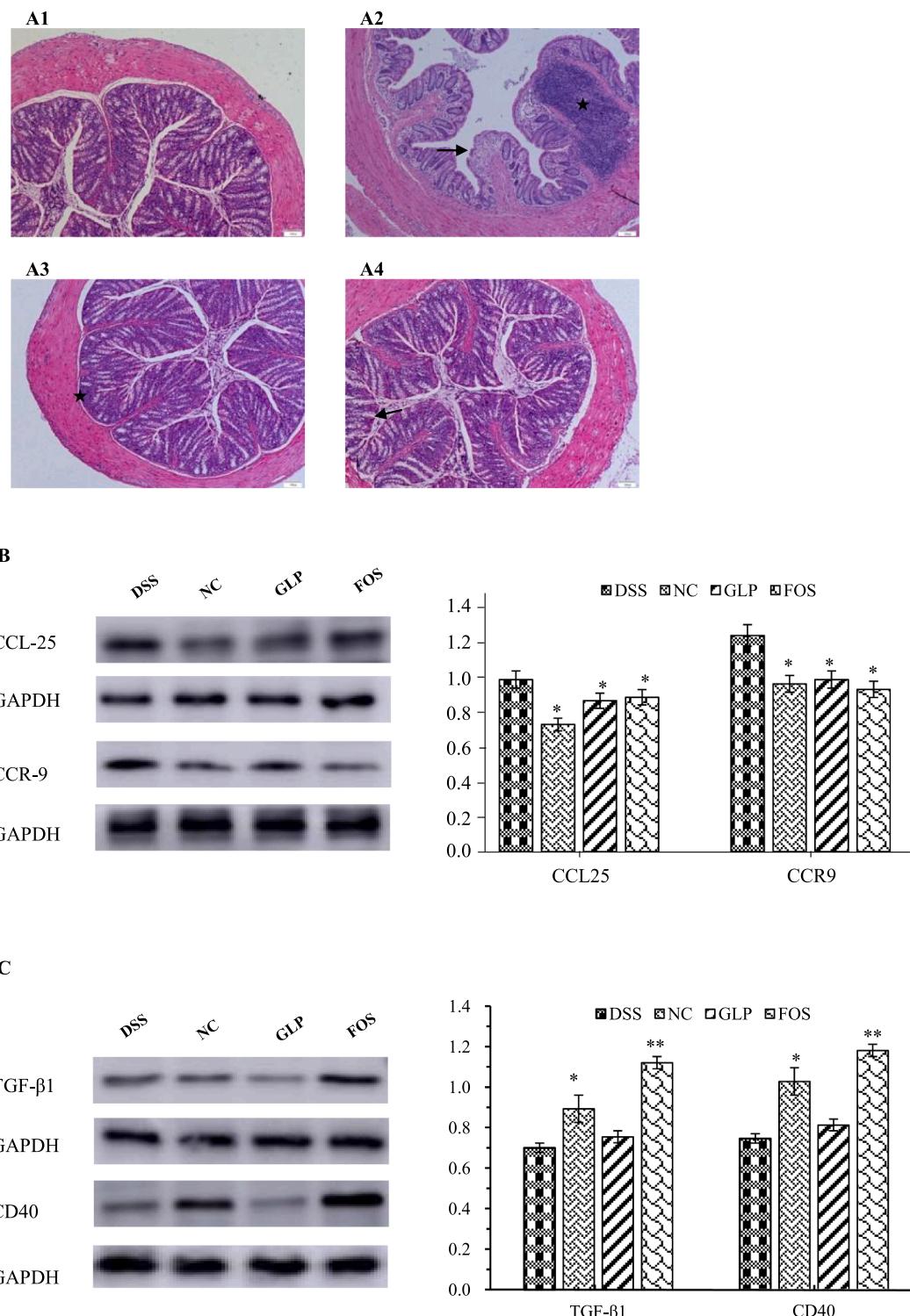
GLP has a good performance on DSS-induced UC in mice by alleviating pathological features of mucosal barrier function. Additionally, GLP can regulate the content of the intestinal flora, recover the relative abundances of crucial bacteria especially Bacteroidetes, prevent the dysbiosis of the gut microbiota, and improve the content of SCFAs.

**Table 2**

Effect of *G. lemaneiformis* polysaccharides on the content of short-chain fatty acids in mice feces.

| Group           | SCFAs (mmol/L)            |                           |                          |                          |                          |                          |                           |
|-----------------|---------------------------|---------------------------|--------------------------|--------------------------|--------------------------|--------------------------|---------------------------|
|                 | Acetic acid               | Propionic acid            | Isobutyric acid          | Butyric acid             | Isovaleric acid          | Valeric acid             | Total                     |
| NC <sup>1</sup> | 2.311±0.215 <sup>a</sup>  | 0.576±0.043 <sup>ab</sup> | 0.444±0.293 <sup>a</sup> | 0.634±0.226 <sup>a</sup> | 0.152±0.010 <sup>a</sup> | 0.138±0.001 <sup>a</sup> | 4.255±0.787 <sup>a</sup>  |
| DSS             | 1.855±0.342 <sup>b</sup>  | 0.331±0.076 <sup>b</sup>  | 0.244±0.093 <sup>b</sup> | 0.566±0.101 <sup>a</sup> | - <sup>2</sup>           | 0.135±0.001 <sup>a</sup> | 3.181±0.662 <sup>b</sup>  |
| GLP             | 2.020±0.138 <sup>ab</sup> | 0.638±0.086 <sup>a</sup>  | 0.223±0.010 <sup>b</sup> | 0.559±0.098 <sup>a</sup> | 0.162±0.008 <sup>a</sup> | 0.161±0.009 <sup>a</sup> | 3.747±0.372 <sup>ab</sup> |
| FOS             | 2.016±0.479 <sup>ab</sup> | 0.471±0.128 <sup>ab</sup> | -                        | 0.765±0.311 <sup>a</sup> | 0.148±0.004 <sup>a</sup> | 0.147±0.011 <sup>a</sup> | 3.548±0.932 <sup>ab</sup> |

<sup>1</sup> NC: negative control group; DSS: DSS-induced colitis group; GLP: *G. lemaneiformis* polysaccharides group; FOS: fructo-oligosaccharides group. 2 -: not detected. Values are presented as mean ± SD, n = 6, different lowercase letters mean significant difference in the different treatment groups ( $p < 0.05$ ).



**Fig. 4.** (A) Representative sections of proximal colon stained with haematoxylin and eosin (magnification  $\times 4$ ) demonstrating the effect of GLP on the recovery of colon tissue damage. (A1) Control group, NC; (A2) DSS model group; (A3) GLP group; (A4) FOS group. Arrows indicate ulceration, asterisks indicate inflammatory infiltrate. DSS group showed complete loss of crypt structure and goblet cells, while GLP groups partly restored crypt structure and goblet cells. Effects of GLP on intestine protein expression in (B) CCL25 and CCR9, (C) TGF- $\beta$ 1 and CD40 signaling of DSS induced mice. Data are expressed as mean  $\pm$  SD ( $n = 3$ ). \* $p < 0.05$ , \*\* $p < 0.01$  compared with the DSS-induced mice.

Furthermore, GLP treatment relieved the inflammatory symptoms of the DSS-induced colitis mice by suppressing the levels of CCL25/CCR9 and increasing TGF- $\beta$ 1 and CD40. The findings of the present study assisted in gaining a further comprehension of the related mechanisms and helped in the development of novel treatment by utilization of GLP in

dietary supplements or functional foods for treating UC patients. However, further *in vivo* and clinical researches are required to support this hypothesis.

## Author contributions

S.Y. L participated in analyses and interpreted the results, along with drafting the manuscript; Y. L., Q. Y., and C. S. critically revised the final draft; S. T., W. Z., and K. L. C conceived and designed the study and approved the final draft for submission; all authors have read and agreed to the published version of the manuscript.

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## Declaration of Competing Interest

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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