Weilan gum oligosaccharide ameliorates dextran sulfate sodium-induced experimental ulcerative colitis

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Abstract. Ulcerative colitis (UC) is a global disease, characterized by periods of relapse that seriously affects the quality of life of patients. Oligosaccharides are considered to be a prospective strategy to alleviate the symptoms of UC. The present study aimed to evaluate the effect of weilan gum oligosaccharide (WLGO) on a mouse UC model induced by dextran sulfate sodium (DSS). WLGO structural physical properties were characterized by electrospray mass spectrometry and fourier tansform infrared spectroscopy. MTT assays were performed to evaluate the non-toxic concentration of WLGO. RT-qPCR and ELISAs were conducted to determine the levels of inflammatory factors. The clinical symptoms and mucosal integrity of the DSS-induced UC model were assessed by DAI and histological assessment. LPS-induced Caco-2 cells and DSS-induced UC mice were used to explore the effects of WLGO on UC. Treatment of the mice with 4.48 g/kg/day WLGO via gavage for 7 days significantly relieved the symptoms of DSS-induced UC model mice, whereas significant effects were not observed for all symptoms of DSS-induced UC in the WLGO-low group. The disease activity index score was decreased and the loss of body weight was reduced in DSS-induced UC model mice treated with WLGO. Moreover, colonic damage and abnormally short colon length shortenings were relieved following WLGO treatment. WLGO treatment also reduced the concentration and mRNA expression levels of proinflammatory cytokines, including interleukin-1β, interleukin-6 and tumor necrosis factor α, in DSS-induced UC model mice and lipopolysaccharide-treated Caco-2 cells. These results indicated that WLGO may be an effective strategy for UC treatment.

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Introduction

Ulcerative colitis (UC) is a chronic disease that is difficult to cure using conventional treatments. It is often accompanied by diarrhea, weight loss, abdominal pain and other clinical symptoms, such as blood in the stool, vomiting and nausea. The occurrence of UC is related to a variety of factors, including intestinal flora, intestinal mucosal immunity, external environments and genetic susceptibility. However, the pathogenesis of UC is not fully understood (1). Until now, anti-inflammatory drugs and immune modulators, including aminosalicylates, corticosteroids, monoclonal antibodies and immunosuppressants, have been used in the clinic to control severe symptoms (2,3). Although enteritis can be improved to some extent, these traditional medicines usually result in side effects, such as osteopenia and growth failure (4), thus are not suitable for long-term treatment. The cure rate of enteritis is very low (5). Therefore, safe therapeutic approaches with improved efficiency are needed.

Functional oligosaccharides are carbohydrates composed of 2-10 monosaccharide units linked together via O-glycosidic or N-glycosidic bonds (6). They are associated with numerous health benefits, including regulating the immune response and alleviating allergic inflammation, and can reduce intestinal inflammation and modulate the composition of the intestinal microbiota, particularly in UC, to improve the host response to pathogens. Studies have shown that the most common functional types of oligosaccharide primarily include galacto-oligosaccharide (GOS), chitosan-oligosaccharide (COS), pectin-oligosaccharide (POS), fructo-oligosaccharide and isomalto-oligosaccharide (IMO) (7-9). IMO is a commercial mixture of glucose monomers that can improve intestinal absorption by modulating epithelial functions and

stimulating enteric motor neurons (10). POS is composed of rhamnose and galacturonic acid (11), and it can promote the generation of short-chain fatty acids to inhibit pathogenic bacteria in the intestine and decrease the incidence of colon cancer (12).

Weilan gum (WLG) polysaccharide is a macromolecular exopolysaccharide secreted by *Alcaligenes* (13) that has broad commercial applications in the food industry as a thickener, suspending agent, binder and emulsifier (14). For example, WLG functions as a protective colloid and plays an important role in eliminating protein flocculation in dairy products (15,16). WLG can be used as a food additive in baked products to replace oils, fats, margarine and other fatty acids to improve the taste (17), and as a thickener in ice cream products (18). Therefore, WLG may be a safe approach to alleviate UC. According to a previous report, commercialized WLG is an acidic hetero-polysaccharide composed of glucose, rhamnose, glucuronic acid and mannose, with a molecular weight (Mw) of ~$1.0x10^6$ (19). Due to its complex structure and high Mw, it is difficult for WLG to permeate into epithelial cells and be utilized by intestinal microorganisms (13). Thus, trifluoroacetic acid (TFA) has been used to degrade WLG into oligosaccharides (20). Studies have shown that WLG has no side effects on clinical signs and body weight (21,22). Additionally, WLG is composed of glucose, rhamnose, glucuronic acid and mannose (23). Glucose is widely used in confectionery and medicine (24), rhamnose is used as a sweetener in the food industry (25), and glucuronic acid and mannose can be used directly to synthesize glycoproteins to participate in immune regulation and be used as glyconutrients in clinical medicine (26). All these data indicate that both WLG and WLG oligosaccharide (WLGO) are safe for use in humans. Thus, it was hypothesized in the present study that WLGO may display similar effects on intestinal health as POS and IMO.

The objectives of this study were to explore the therapeutic effects of WLGO on UC. WLGO structural and physical properties were characterized by electrospray mass spectrometry (ESI) and Fourier transform infrared spectroscopy (FTIR), and its effects on UC were assessed *in vitro* by using lipopolysaccharide (LPS)-induced Caco-2 cells and *in vivo* by using dextran sulfate sodium (DSS)-induced UC model mice. Body weight loss, disease activity index (DAI) scores, colon histopathological changes and inflammatory cytokine interleukin-1β (IL-1β), interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α) levels were measured to evaluate the therapeutic effect of WLGO on UC.

Materials and methods

Chemicals and reagents. WLG (purity >90%; Mw $1.0x10^6$) was obtained from Shandong Academy of Sciences. TFA (cat. no. 76-0501; purity >99%; Mw 114.02 g/mol) (76-05-1; https://www.sigmaaldrich.cn) was purchased from Shanghai Macklin Biochemical Co., Ltd. DSS (cat. no. 9011-18-1. MW40000) (https://www.aladdin-e.com) was purchased from Shanghai Aladdin Biochemical Technology Co., Ltd. LPS (purity >99%; *Escherichia coli* 055:B5; Sigma-Aldrich; Merck KGaA) was dissolved in ddH_2O to make a 1 mg/ml stock solution. Human IL-1β (cat. no. 1110122), human IL-6 (cat. no.1110602), human TNF-α (cat. no. 1117202), mouse IL-1β (cat. no. 1210122), mouse IL-6 (cat. no. 1210602) and mouse TNF-α (cat. no. 1317202) ELISA kits purchased from Beijing Dakowei Biotechnology Co., Ltd. were used. CYN (cat. no. Z36020518) was purchased from Zhejiang Jinhua Conba Biopharm Co., Ltd.

Preparation of WLGO. A total of 2 mg WLG was dissolved in 4 ml TFA (2 mol/l) in a hydrolysis tube and hydrolyzed at 110˚C for 3 h in an autoclave. WLGO solution was mixed with 5 ml methanol, and the solvent was evaporated in a rotary evaporator. The procedure was repeated four times, and the obtained WLGO was analyzed with ESI and FTIR. For the FTIR, WLGO was prepared as Kbr tablets and examined using a Bruker Tensor 27 model infrared spectrometer (scan range, 400-4,000 cm^{-1}; resolution, 4.0 cm^{-1}). The obtained spectra were the results of averaging 64 scans.

Cell culture and treatment. Caco-2 cells (presented by Professor Yanqing Li from Qilu Hospital of China) were used as the epithelial cell model for this study. Cells were cultured in high-glucose DMEM (DMEM-H; cat. no. 12800-017; Gibco; Thermo Fisher Scientific, Inc.) supplemented with 10% v/v FBS (cat. no. SV30087.02; HyClone; Cytiva) in a humidified incubator with 5% CO_2 at 37˚C. The cells were divided into the following five groups: i) Normal group (Nor); ii) LPS group; iii) LPS + 50 μg/ml WLGO group; iv) LPS + 100 μg/ml WLGO group; and v) LPS + 200 μg/ml WLGO group. For the induction of inflammatory conditions, cells in the LPS and LPS + WLGO groups were treated with 50 μg/ml LPS for 12 h in a humidified incubator with 5% CO_2 at 37˚C. After LPS treatment for 12 h, cells were cultured in the presence of WLGO for 12, 24 or 48 h. At the end of cell culture experiments, the supernatants were collected by centrifugation at 1,000 x g at 4˚C for 10 min. The samples stored at -80˚C for subsequent analyses.

Animals and treatment. A total of 30 male C57BL/6J mice (age, 6 weeks; weight, 22-25 g) were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. and used in the animal experiments. All mice were adapted to the environment for 7 days before the experiments. All mice were housed in Makrolon cages, maintained under an air-conditioned atmosphere at 22±2˚C with 50-60% humidity, 12 h light/dark cycles, and free access to tap water and standard rodent diet. These mice were randomly divided into the following five groups: i) Normal control group (Nor); ii) DSS group; iii) Chang Yan Ning group (CYN, 2.24 g/kg/day); iv) high WLGO group (4.48 g/kg/day); and v) low WLGO group (2.24 g/kg/day). C57BL/6J mice received an oral dose of 200 μl 2.5% DSS solution for 7 days to establish the UC mouse model. Mice in the Nor group were received sterile water via oral administration. For induction of UC, mice in the DSS, CYN and WLGO groups were treated with drinking water containing 2.5% (w/v) DSS once per day for 7 days. Subsequently, mice in the CYN and WLGO groups were treated with drinking water containing CYN or WLGO, respectively, dissolved in sterile water for 7 days. Body weight was recorded every day during the experiment (27). At the end of the experiment, all mice were anesthetized and euthanized by cervical dislocation. The

organs were removed and examined. Colon and blood samples were collected. Colons were measured to determine their length, then divided into three segments and stored at -80˚C for hematoxylin and eosin (H&E) staining, ELISA and reverse transcription-quantitative PCR (RT-qPCR) (28). All animal experiments were performed in compliance with the Animal Research: Reporting of *In Vivo* Experiments guidelines, the UK Animals (Scientific Procedures) Act (1986) and the associated guidelines, the EU Directive 2010/63/EU for Animal experiments, the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications no. 8023; revised 1978) and the Animal Management Rules of the Chinese Ministry of Health (http://www.gov.cn/gongbao/content/2017/content_5219148.htm) (29). This study was approved by the Animal Experiment Ethics Committee of Qilu University of Technology (Jinan, China) (30).

Cell viability assay. The 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltertrazolium bromide (MTT) assay was used to assess cell viability in response to WLGO. Caco-2 cells were seeded ($5x10^4$ cells/100 μl) in 96-well plates and treated with different concentrations (50-200 μg/ml) of WLGO. After 24 and 48 h, 20 μl MTT reagent (0.5 mg/ml) was added into each well for another 4 h at 37˚C. The medium was removed and 100 μl DMSO (0.04 M) was added. The optical density was measured at a wavelength of 570 nm using a SpectraMax ABS microplate spectrophotometer (Molecular Devices, LLC) (31).

DAI and histological assessment. The DAI was calculated and recorded in accordance with stool consistency, body weight loss and blood in feces. Colon samples were fixed in optimal cutting temperature embedding medium and stained with H&E. Each colon was assessed using four consecutive 8-μm sections taken every 40 μm to cover the entire colon. Histopathological changes were estimated according to a previously established scoring system. In brief, the scoring system comprised two parameters, tissue damage and infiltration of lamina propria by inflammatory cells, and scored from 0 (no changes) to 6 (widespread cellular infiltration and extensive tissue damage). The intestinal inflammation was graded blindly by two observers under the guidance of previously established scoring system using a light microscope (32,33).

ELISA. The colon tissues were weighed, and homogenized with NP40 lysis buffer (Beyotime Institute of Biotechnology) on ice. The mixture was centrifuged at 5,000 x g and 4˚C for 15 min, and the supernatants were collected and quantified using a BCA assay (Beyotime Institute of Biotechnology) as reported previously (34). The serum was prepared by centrifugation at 3,000 x g for 20 min at 4˚C and stored at -80˚C for biochemical analysis. ELISA kits were used to measure IL-1β, IL-6 and TNF-α levels in mouse colon tissue and blood serum samples.

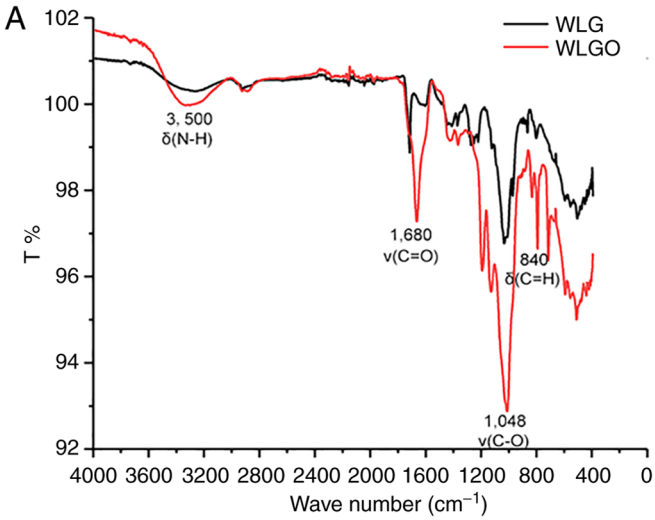
RT-qPCR. Total RNA was extracted from colon tissues using TRIzol® (Invitrogen; Thermo Fisher Scientific, Inc.). The purity and concentration of the isolated RNA were determined using NanoDrop 1000 (NanoDrop Technologies; Thermo Fisher Scientific, Inc.), RNA samples were reverse transcribed into cDNAs using an ABScript II RT Master Mix (ABclonal Biotech Co., Ltd.) according to the manufacturer's protocol. The cDNAs were subjected to qPCR using the following primers (purchased from Sangon Biotech Co., Ltd.): IL-1β forward, 5'-GCCACCTTTTGACAGTGATGAG-3' and reverse, 5'-ATGTGCTGCTGCGAGATTTG-3'; IL-6 forward, 5'-ACCCCAATTTCCAATGCTCTCC-3' and reverse, 5'-GCATAACGCACTAGGTTTGCC-3'; TNF-α forward, 5'-GGACTAGCCAGGAGGGAGAACAG-3' and reverse, 5'-GCCAGTGAGTGAAAGGGACAGAAC-3'; and GAPDH forward, 5'-TGTGTCCGTCGTGGATCTGA-3' and reverse, 5'-TTGCTGTTGAAGTCGCAGGAG-3'. The mRNA expression levels were examined on a Rotor-Gene Q instrument (Qiagen China Co., Ltd.) using a SYBR Green Fast qPCR Mix (ABclonal Biotech Co., Ltd.) to evaluate the amount of double stranded DNA. The primer amplification efficiencies were measured in cDNA dilutions from 1x10 to $1x10^5$ copies, and the amplification was linear over the range of 1x10 to $1x10^5$ copies. The efficiencies of the primers in the standard curves ranged from 97 to 102%. The following thermocycling conditions were used for qPCR: 95˚C for 30 sec; 40 cycles at 58˚C for 30 sec and at 72˚C for 30 sec; and a final cycle at 72˚C for 5 min. IL-1β, IL-6 and TNF-α gene expression levels were normalized against GAPDH using the $2^{-\Delta\Delta Cq}$ method (35).

Statistical analysis. All experiments were repeated at least three times independently. Data were recorded and analyzed using Excel (version 2010; Microsoft Corporation) and GraphPad Prism software (version 8.0.2; GraphPad Software, Inc.) Data are presented as the mean ± SEM. Differences among Nor, DSS, CYN and WLGO groups were analyzed by Kruskal-Wallis followed by Dunn's post hoc test or one-way ANOVA followed by Tukey's post hoc test using SPSS software (version 11.5; SPSS, Inc.). $P<0.05$ was considered to indicate a statistically significant difference.

Results

Chemical profile of WLGO. The molecular mass and structure of WLGO were characterized using ESI and FTIR analysis. FTIR analysis showed the bands of 840 cm^{-1} (C-H deformation vibration), 1,048 cm^{-1} (C-O deformation vibration), 1,680 cm^{-1} (C=O stretching vibration) and 3,500 cm^{-1} (N-H deformation vibration) in WLGO (Fig. 1A), which was consistent with a previous study reporting that WLG is composed of repeating units with glucose, rhamnose, glucuronic acid and mannose (19). ESI analysis showed that the polymerization degrees of WLGO were mainly between 2 and 6 (Fig. 1B). WLGO with a polymerization degree of 2 consists of glucuronic acid and rhamnose units. WLGO with a polymerization degree of 3 consists of three rhamnose units. WLGO with a polymerization degree of 4 consists of four rhamnose units. WLGO with a polymerization degree of 5 contains four mannoses units and one rhamnose unit. WLGO with a polymerization degree of 6 contains six rhamnose units. The m/z of WLGO was mainly concentrated in 381.10113 and 1007.40085. Overall, the ESI and FTIR results confirmed that the WLG was successfully degraded into WLGO.

A



B

No.	Observed mass, m/z	Charge	Calculated mass, m/z	Theoretical mass, m/z	Formula	Error	DP
1	381.1619	1	381.1619	381.10113	$[C_{12}H_{22}N_3O_{17}+Na]^+$	1.60	2
2	515.0397	1	515.0397	515.195425	$[C_{18}H_{36}O_{15}+Na]^+$	3.02	3
3	605.3855	1	605.3855	605.117965	$[C_{18}H_{30}O_{21}+Na]^+$	4.42	3
4	679.0429	1	679.0429	679.2639	$[C_{24}H_{48}O_{20}+Na]^+$	3.25	4
5	923.014	1	923.014	923.420035	$[C_{30}H_{60}O_{29}+K]^+$	4.40	5
6	1007.0593	1	1007.0593	1007.40085	$[C_{36}H_{72}N_3O_{30}+Na]^+$	3.40	6

Figure 1. Characterization of the degraded WLGO. (A) Infrared spectrum of WLGO. (B) Analysis of electrospray mass spectrometry results of WLGO. WLGO, weilan gum oligosaccharide; WLG, weilan gum; T, (%); DP, degree of polymerization.

Effect of WLGO on Caco-2 cell viability. To evaluate the non-toxic concentration of WLGO, Caco-2 cells were incubated with 50, 100 and 200 μg/ml WLGO for 24 and 48 h. The results showed that WLGO had no cytotoxic effects on the Caco-2 cells after treatment for 24 h (Fig. 2A).

WLGO decreases LPS-induced upregulation of TNF-α, IL-6 and IL-1β. To explore the anti-inflammatory effects of WLGO *in vitro*, LPS-induced Caco-2 cells were used (19). Compared with those in the Nor group, LPS significantly increased the concentrations of IL-1β, IL-6 and TNF-α (Fig. 2B-D). WLGO treatment significantly decreased IL-1β levels in a concentration-dependent manner in LPS-induced Caco-2 cells (Fig. 2B). In addition, 50, 100 and 200 μg/ml WLGO treatment significantly decreased IL-6 levels in LPS-induced Caco-2 cells (Fig. 2C). Moreover, 200 μg/ml WLGO treatment significantly decreased TNF-α levels both at 12 and 24 h (Fig. 2D), and 50 and 100 μg/ml WLGO treatment significantly decreased TNF-α levels at 24 h. These results suggested that WLGO had an anti-inflammatory effect on LPS-induced cells.

WLGO alleviates the clinical symptoms of DSS-induced colitis. To investigate the anti-inflammatory role of WLGO, C57BL/6J mice received an oral dose of 200 μl 2.5% DSS solution for 7 days to establish the UC mouse model. During DSS treatment, body weight was measured. Compared with that in the Nor group, the body weight of mice in the DSS group reduced significantly, reaching the lowest value at day 10 (Fig. S1). The percentage of body weight loss was reduced by 10.9% in the DSS group on day 14 compared with that in the Nor group. However, the percentage of body weight loss was reduced by 7.9% in the WLGO-High group at the 14 day time point, indicating that WLGO could relieve the reduction in body weight in mice with DSS-induced colitis ($P<0.05$; Figs. 3A and S1).

Mice in the DSS group exhibited apparent rectal bleeding and diarrhea after DSS treatment. As the treatment continued, the severity of inflammation was evident in the mice, as measured by the DAI score. Compared with that in the Nor group, the DAI score in the DSS group increased (Figs. 3B and S2). Moreover, compared with the DSS group, the DAI scores of DSS-treated mice were significantly reduced in the WLGO treatment groups (Fig. S2). These results indicated that WLGO could relieve the symptoms of DSS-induced UC. The organs (heart, spleen, kidney, lung and liver) of mice in each treatment group were examined. There were no notable changes in any of the organs among the different groups (Fig. S3).

WLGO protects mucosal integrity against injury. The colon length of mice in the DSS group was significantly shorter compared with that in the Nor group ($P<0.05$; Fig. 3C and D). The colon length of mice in all three treatment groups was notably longer compared with that in the DSS group. Moreover, the colon length of mice in the CYN group almost returned to the level observed in the Nor group, which significantly relieved the colon length loss in DSS-induced UC.

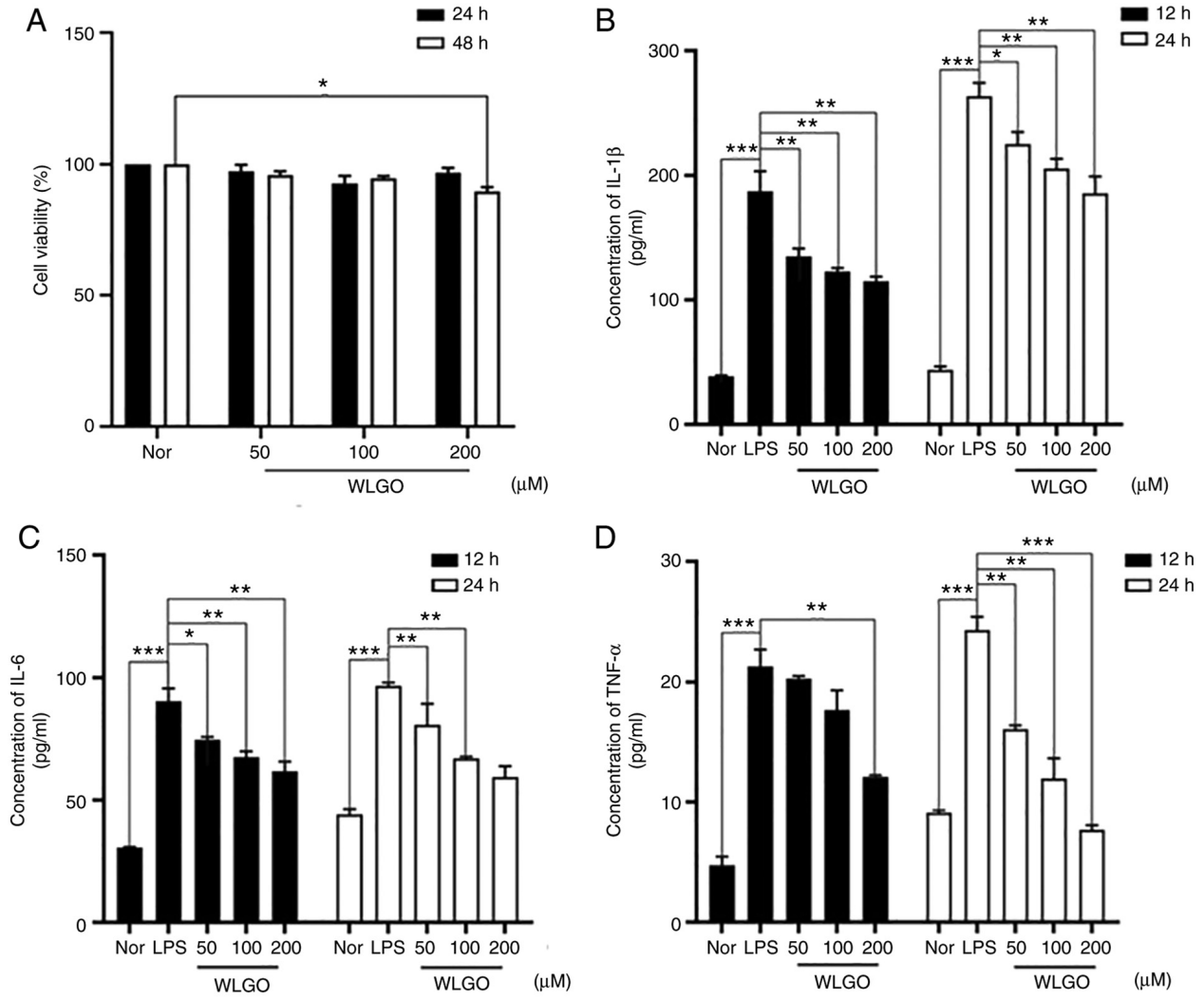


Figure 2. Caco-2 cell viability and IL-1β, IL-6 and TNF-α levels in Caco-2 cells after treatment with WLGO at different doses. (A) Caco-2 cells were treated with different concentrations (50-200 μg/ml) of WLGO for 24 and 48 h. (B) IL-1β, (C) IL-6 and (D) TNF-α levels were examined in the supernatant of Caco-2 cells that were pretreated with 50 μg/ml LPS for 12 h and then treated with different concentrations of WLGO for 12 or 24 h. The following groups were assessed: i) Nor, normal group; ii) LPS, Caco-2 cells stimulated with LPS; and iii) WLGO, Caco-2 cells stimulated with LPS and 50, 100 or 200 μg/ml WLGO, as indicated. Data are expressed as the mean ± SEM. All experiments were performed in triplicate. *P<0.05, **P<0.01, ***P<0.001; IL, interleukin; TNF-α, tumor necrosis factor α; WLGO, weilan gum oligosaccharide; LPS, lipopolysaccharide; Nor, normal.

DSS-induced intestinal inflammation was accompanied by mucosal infiltration of inflammatory cells. Histological examination of frozen colon tissue sections showed the degree of inflammation and epithelial damage. Multifocal erosion areas and severe inflammatory cell infiltration were observed on the colonic surface epithelium of mice in the DSS group (Fig. 4A). By contrast, WLGO-High treatment notably ameliorated histological alterations and decreased histological scores compared with those in the DSS group (Fig. 4B). These results indicated that WLGO at a high dose exhibited a protective effect against mucosal injury.

WLGO decreases proinflammatory cytokines in DSS-induced mice. To investigate the treatment effect of WLGO on DSS-induced UC, the levels of proinflammatory cytokines (IL-1β, IL-6 and TNF-α) in serum and colon tissues were measured using ELISAs. The results showed that the levels of IL-1β, IL-6 and TNF-α were significantly increased in mice in the DSS group compared with those in the Nor group (Fig. 5A-C). In addition, compared with those in the DSS group, levels of IL-1β, IL-6 and TNF-α in both serum and colon tissues were significantly decreased in the WLGO-High group (Figs. 5A-C and 6A-C). These results suggested that all three proinflammatory cytokines were decreased after WLGO treatment, and the most significant changes were observed with a high dose of WLGO.

To explore the anti-inflammatory effect of WLGO on mRNA expression levels, the mRNA expression levels of IL-1β, IL-6 and TNF-α in the colons of mice with DSS-induced UC were measured via RT-qPCR. The results showed that, compared with those in the Nor group, the mRNA expression levels of IL-1β, IL-6 and TNF-α increased significantly in the DSS group, whereas WLGO-High treatment significantly reversed these increases (Fig. 7A-C). The results demonstrated that WLGO inhibited the expression of proinflammatory cytokines (IL-1β, IL-6 and TNF-α) in mice with DSS-induced UC, and WLGO at a high dose exhibited stronger effects than WLGO at a low dose.

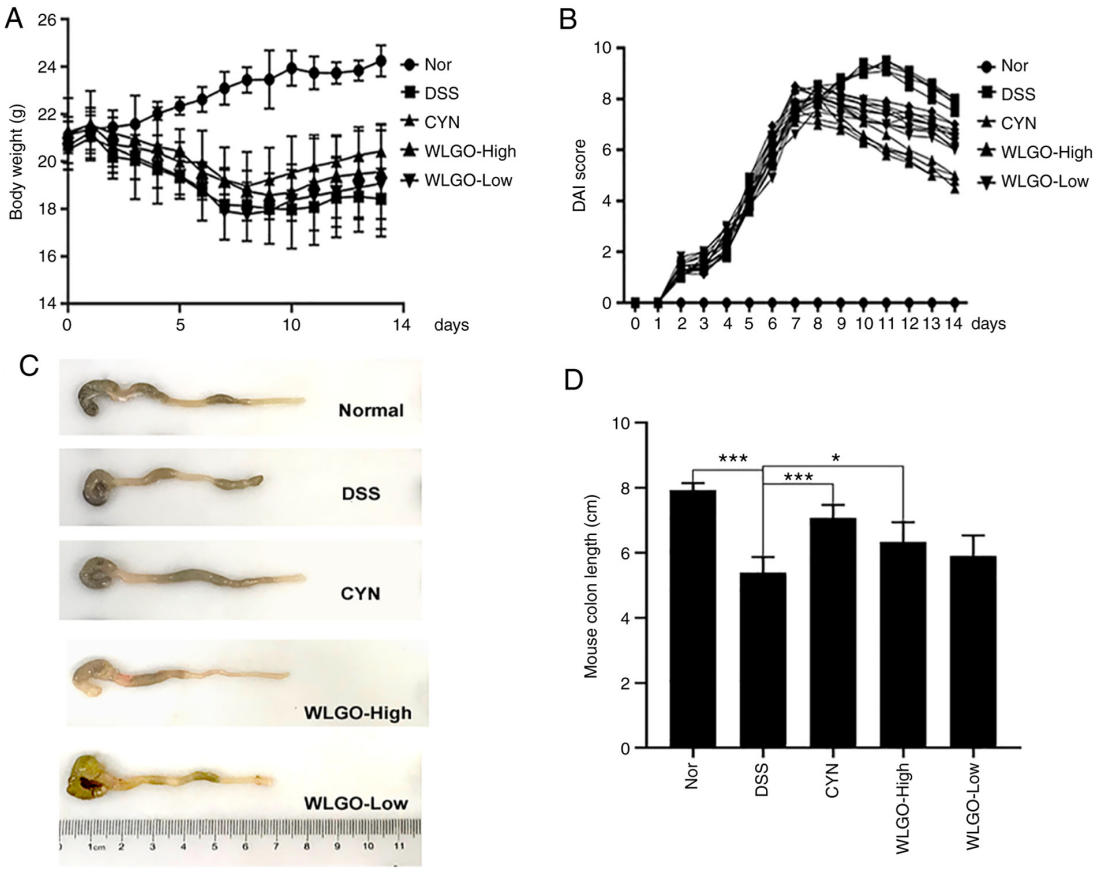
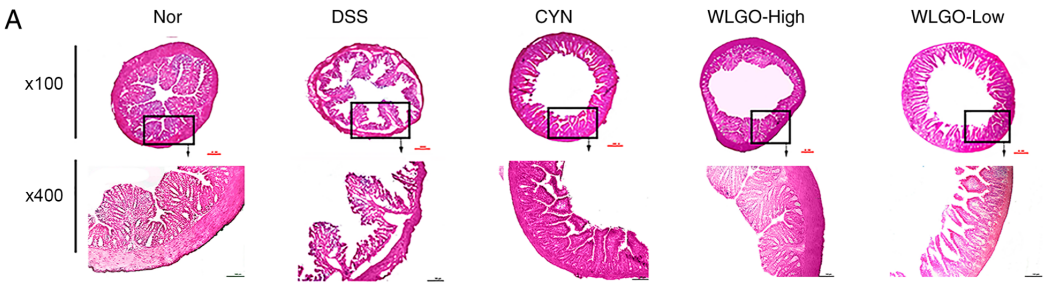


Figure 3. Effects of WLGO treatment on DSS-induced ulcerative colitis model mice. (A) Body weight of mice was measured every day. (B) DAI score of mice was examined every day. (C) Images of colons. (D) Calculated colon lengths based on images from C. The following groups were assessed: i) Nor, mice treated with saline; ii) DSS, mice treated with drinking water containing 2.5% DSS; iii) CYN, mice treated with 2.24 g/kg/day CYN; iv) WLGO-High, mice treated with 4.48 g/kg/day WLGO; and v) WLGO-Low, mice treated with 2.24 g/kg/day WLGO. Data are expressed as the mean ± SEM. All experiments were performed in triplicate. *P<0.05, ***P<0.001. WLGO, weilan gum oligosaccharide; DSS, dextran sulfate sodium; DAI, disease activity index; CYN, Chang Yan Ning; Nor, normal.



B

Parameter	Nor	DSS	CYN	WLGO-High	WLGO-Low
Inflammation					
Severity	100±0	3.50±0.58$^{\#}$	1.25±0.50	2.75±0.50	3.58±0.58
Thickness	0.50±0.58	4.00±0$^{\#}$	0.75±0.50*	1.50±0.58*	2.00±0.82
Epithelial damage					
Character	0.50±0.58	3.75±0.5$^{\#}$	1.25±0.50*	1.75±0.50	2.75±0.50
Extent	1.00±0	3.50±0.58$^{\#}$	1.00±0.82	2.25±0.50	3.00±0
Total lesion score	3.00±1.16	14.75±1.66$^{\#}$	4.25±2.32*	8.25±2.08	10.83±1.90

Figure 4. Histological sections of colonic tissues stained with H&E. (A) Histological sections of colonic tissues stained with H&E under a microscope. (B) Effects of WLGO on colon pathology of DSS-induced ulcerative colitis model mice. The following groups were assessed: i) Nor, mice treated with saline; ii) DSS, mice treated with drinking water containing 2.5% DSS; iii) CYN, mice treated with 2.24 g/kg/day CYN; iv) WLGO-High, mice treated with 4.48 g/kg/day WLGO; and v) WLGO-Low, mice treated with 2.24 g/kg/day WLGO. Data are expressed as the mean ± SEM. All experiments were performed in triplicate. $^{\#}$P<0.001 vs. Nor; *P<0.05 vs. DSS. H&E, hematoxylin and eosin; WLGO, weilan gum oligosaccharide; DSS, dextran sulfate sodium; CYN, Chang Yan Ning; Nor, normal.

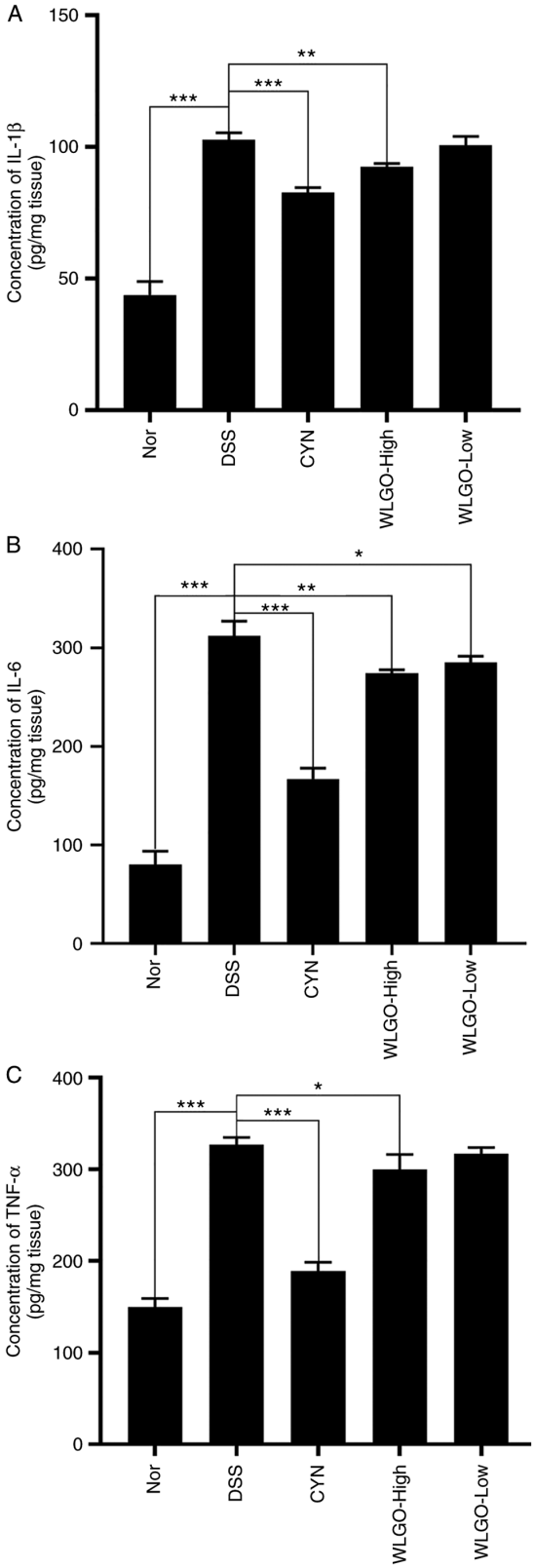


Figure 5. Effects of WLGO on the levels of inflammatory cytokines in colonic tissues of mice. Levels of (A) IL-1β, (B) IL-6 and (C) TNF-α in the colonic homogenates of DSS-induced ulcerative colitis model mice were detected using ELISA kits. The following groups were assessed: i) Nor, mice treated with saline; ii) DSS, mice treated with drinking water containing 2.5% DSS; iii) CYN, mice treated with 2.24 g/kg/day CYN; iv) WLGO-High, mice treated with 4.48 g/kg/day WLGO; and v) WLGO-Low, mice treated with 2.24 g/kg/day WLGO. Data are expressed as the mean ± SEM. All experiments were performed in triplicate. *P<0.05, **P<0.01, ***P<0.001. WLGO, weilan gum oligosaccharide; DSS, dextran sulfate sodium; CYN, Chang Yan Ning; IL, interleukin; TNF-α, tumor necrosis factor α; Nor, normal.

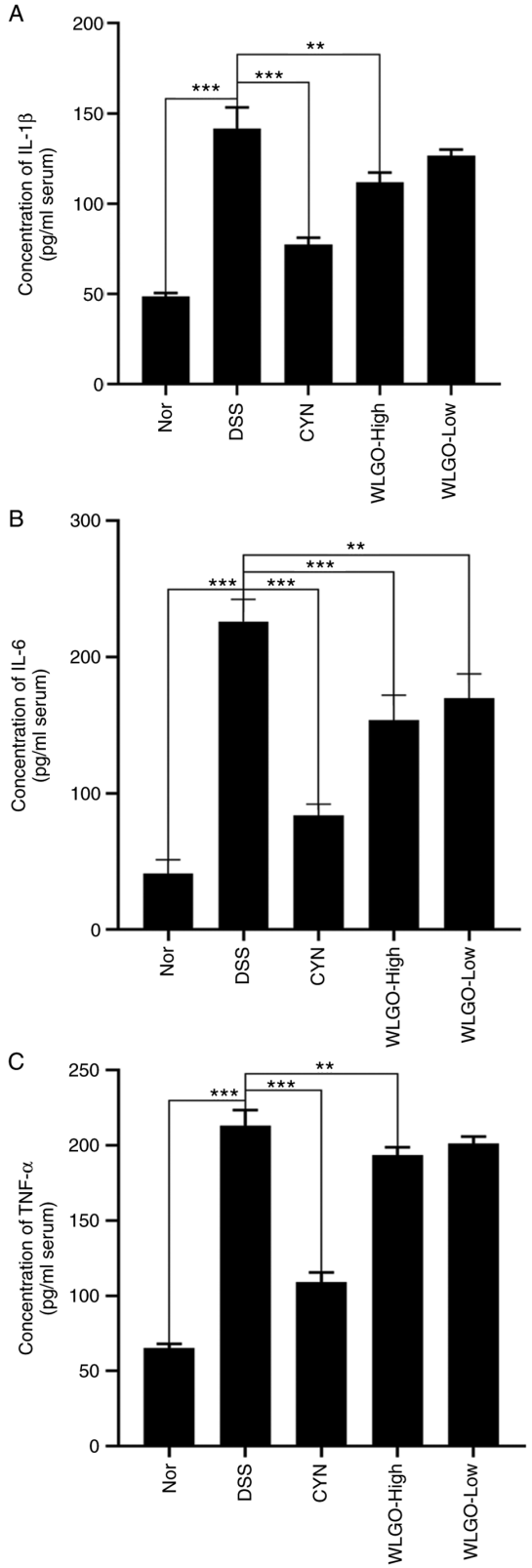


Figure 6. Effects of WLGO on the serum levels of inflammatory cytokines. Levels of (A) IL-1β, (B) IL-6 and (C) TNF-α in the blood serum of DSS-induced ulcerative colitis model mice were detected using ELISA kits. The following groups were assessed: i) Nor, mice treated with saline; ii) DSS, mice treated with drinking water containing 2.5% DSS; iii) CYN, mice treated with 2.24 g/kg/day CYN; iv) WLGO-High, mice treated with 4.48 g/kg/day WLGO; v) WLGO-Low, mice treated with 2.24 g/kg/day WLGO. Data are expressed as the mean ± SEM. All experiments were performed in triplicate. **P<0.01, ***P<0.001. WLGO, weilan gum oligosaccharide; DSS, dextran sulfate sodium; CYN, Chang Yan Ning; IL, interleukin; TNF-α, tumor necrosis factor α; Nor, normal.

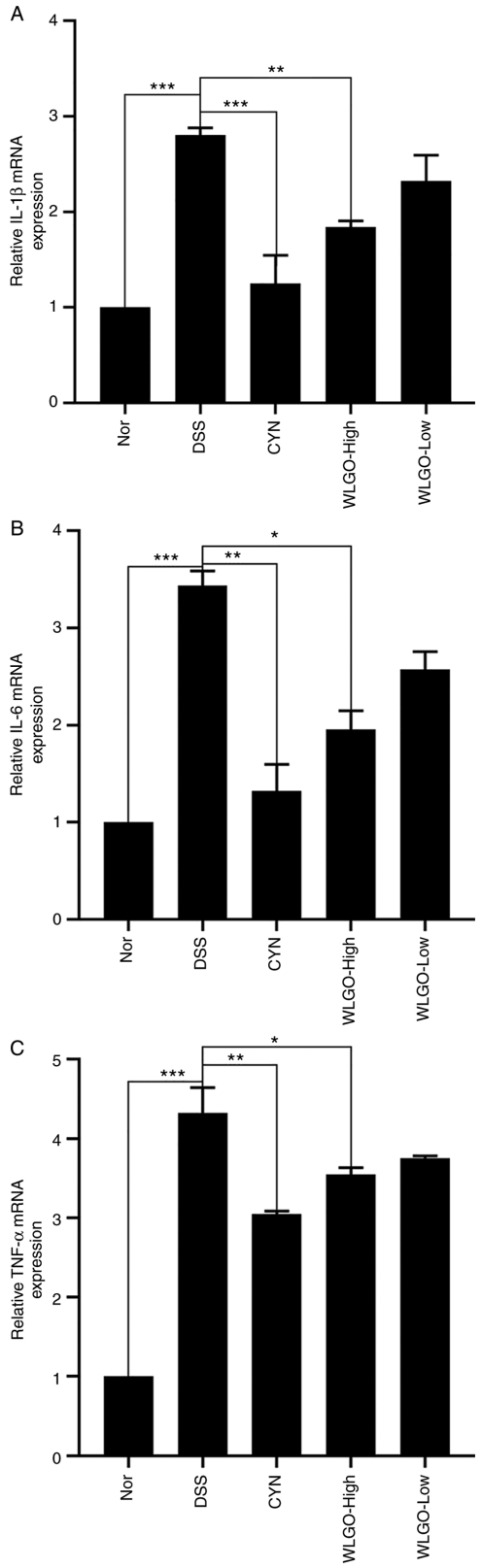


Figure 7. Effects of WLGO on the mRNA expression levels of inflammatory cytokines in the colons of mice. mRNA expression levels of (A) IL-1β, (B) IL-6 and (C) TNF-α in the colons of DSS-induced ulcerative colitis model mice were detected. The following groups were assessed: i) Nor, mice treated with saline; ii) DSS, mice treated with drinking water containing 2.5% DSS; iii) CYN, mice treated with 2.24 g/kg/day CYN; iv) WLGO-High, mice treated with 4.48 g/kg/day WLGO; and v) WLGO-Low, mice treated with 2.24 g/kg/day WLGO. Data are expressed as the mean ± SEM. All experiments were performed in triplicate. *P<0.05, **P<0.01, ***P<0.001. WLGO, weilan gum oligosaccharide; DSS, dextran sulfate sodium; CYN, Chang Yan Ning; IL, interleukin; TNF-α, tumor necrosis factor α; Nor, normal.

Discussion

WLGO is composed of glucose, rhamnose, glucuronic acid and mannose, and its structure is similar to that of POS (36). Compared with other oligosaccharides, WLGO is more diverse in its monosaccharide composition (37). Among its components, glucuronic acid has a wide variety of pharmacological activities, including anti-inflammatory, cell protective and immune regulatory effects (38-40). Mannose can inhibit tumor growth and prolong the survival of patients with cancer (41). Therefore, it was proposed that WLGO may display more biological activities than other oligosaccharides.

Although UC can be triggered by bacteria, viruses and other environmental factors, the inflammatory process of the intestinal mucosa is ultimately induced by soluble inflammatory mediators (42). The inflammatory mediators IL-1β, IL-6 and TNF-α play leading roles in the development of UC (43). Therefore, the effective reduction of IL-1β, IL-6 and TNF-α in the serum and colon tissues is a reasonable modality for UC treatment (44,45). The present data suggested that IL-1β, IL-6 and TNF-α levels were decreased by WLGO in the colon tissues and serum samples of DSS-induced UC model mice. These results demonstrated that WLGO alleviated DSS-induced UC in mice by reducing the levels of inflammatory cytokines.

The present study showed that WLGO treatment reduced LPS-induced secretion of cytokines (IL-6, IL-β and TNF-α) in Caco-2 cells. Moreover, the MTT assay demonstrated that treatment with 200 μM WLGO for 48 h reduced the percentage of viable cells. A previous study reported that oligosaccharides at various concentrations have different effects, exhibiting adverse effects at high dosages (46). For example, COS at ≥600 μM markedly decreases cell viability, whereas COS at low doses shows a protective effect on intestinal porcine epithelial cells (IPEC-J2 cells) (47). Acute and chronic toxicology tests are required to verify the safe concentration of WLGO. Therefore, the therapeutic WLGO dose in UC should be carefully evaluated in clinical settings.

CYN is the most common drug to relieve chronic enteritis in China (48). It is a mixture of extracts from Euphorbia humifusa Willd, Herba hedyotids chrysotrichae and *Cinnamomum camphora* (48). It has been shown that Euphorbia humifusa Willd can significantly reduce the production of inflammatory mediators nitric oxide and TNF-α in LPS-treated RAW 264.7 cells (49). Herba hedyotids chrysotrichae has broad pharmacological effects as an antioxidant, antibacterial and antitumor agent (50). It has been shown that Euphorbia humifusa Willd can protect against DSS-induced experimental UC in mice (51). Additionally, it has been reported that a variety of natural ingredients in CYN, such as flavones and organic acid, can protect epithelial cells (52). Thus, CYN was chosen as the positive control in the present study.

The present data showed that both CYN and WLGO could attenuate the symptoms of DSS-induced UC, and the therapeutic effect of WLGO on UC was less potent than CYN. It has been highlighted that patients with UC usually show complications, such as nausea, vomiting and stomach pain, after receiving CYN treatment (53). Thus, CYN cannot be used for an extended period. Moreover, WLGO is an oligosaccharide

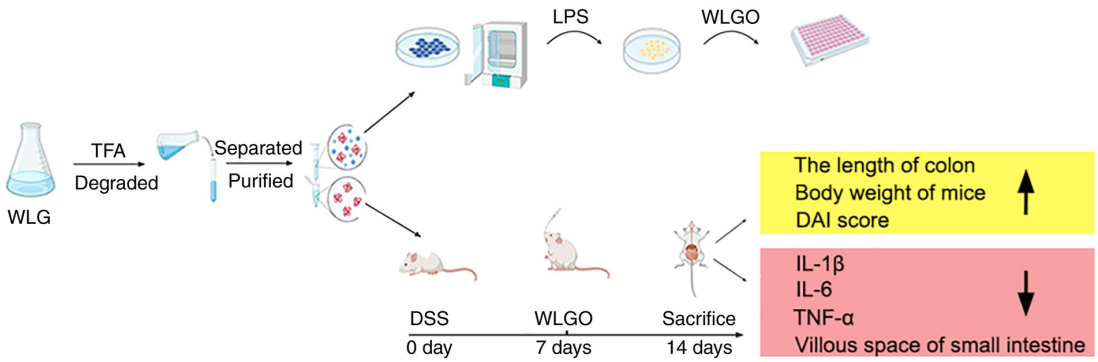


Figure 8. Diagram of experimental procedures and results. WLG was degraded by TFA into WLGO. The effects of WLGO on UC were investigated in LPS-induced Caco-2 cells and DSS-induced mouse UC model. WLG, weilan gum; WLGO, weilan gum oligosaccharide; TFA, trifluoroacetic acid; UC, ulcerative colitis; LPS, lipopolysaccharide; DSS, dextran sulfate sodium; DAI, disease activity index; IL, interleukin; TNF-α, tumor necrosis factor α; Nor, normal.

composed of four monosaccharide units (25), glucose, rhamnose, glucuronic acid and mannose. These units are widely used in the food industry and clinical settings (25), indicating that WLGO is stable and does not result in adverse reactions. Additionally, the present study aimed to identify potential treatment strategies for UC through lowering inflammatory cytokines and alleviating UC symptoms. The current results showed that WLGO had similar beneficial effects to CYN on colon length recovery, DAI score and anti-inflammatory activities (IL-1β, IL-6 and TNF-α) in mice with DSS-induced UC.

It has been pointed that the reduction of inflammatory cytokines in the LPS-induced Caco-2 cells represents a logical target for UC therapy (44). The inflammatory factors need to be expressed before they can be secreted from the cells. It has been shown that the accumulation of inflammatory factors induces cell apoptosis and alters cell survival (54). Thus, changes in the levels of inflammatory cytokines precede the changes in cell survival. Cell viability was measured during the cell cycle at 24 and 48 h. The results showed that WLGO had no cytotoxic effects on the Caco-2 cells after treatment for 24 h. For subsequent inflammatory factor assays, time points within 24 h were chosen. WLGO treatment could decrease the levels of cytokines in LPS-induced Caco-2 cells.

It has been suggested that lowering the levels of inflammatory factors is a reasonable target for UC therapy, and the intestinal inflammatory response is mediated by a complex network of cytokines (proinflammatory cytokines, chemokines, growth factors and adhesion molecules) released from epithelial cells within the lamina propria (55). TNF-α is an essential factor in the inflammatory cytokine network and is the target of numerous novel biological therapies to attenuate the symptoms of UC in patients (56). It has become apparent that the progressive release of cytokines, such as IL-6 and IL-1β, from T cells and macrophages also plays critical roles in the development of UC (57). Therefore, proinflammatory cytokines (TNF-α, IL-6 and IL-1β) could be inflammatory biomarkers for UC. Reducing the production of these biomarkers is critical to explore the efficacy of medicine to treat UC. In the present study, WLGO inhibited the production of proinflammatory cytokines in LPS-treated Caco-2 cells and the intestine of DSS-induced UC model mice, suggesting that this effect was the primary response of the anti-UC activity of WLGO. According to previous reports, rhamnose and glucose are the monomer units of WLGO (23). It has been demonstrated that rhamnose exhibits anti-inflammatory effects via inhibiting the toll-like receptor 4 (TLR4)/NF-κB signaling pathway (58). We speculated that the TLR4/NF-κB signaling pathway may be involved in the suppressive effect of WLGO on the production of proinflammatory cytokines in UC. Further studies are required to confirm this hypothesis.

In summary, WLG was degraded into WLGO using TFA, and the effects of WLGO on LPS-induced Caco-2 cells and DSS-induced UC mouse model were explored. The results showed that WLGO markedly attenuated the inflammatory responses to DSS-induced UC, increased mouse body weight and colon length, improved DAI score and microscopic damages, and decreased mRNA expression levels of IL-1β, IL-6 and TNF-α in mice. Moreover, WLGO treatment also decreased the levels of cytokines in LPS-induced Caco-2 cells (Fig. 8). This study revealed that WLGO may be an effective drug for the treatment of UC and laid a solid foundation for future studies on the underlying mechanisms of WLGO in the treatment of UC.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

PZ designed the work, performed the experiments, analyzed the data and wrote the manuscript. LS performed the experiments and analyzed the data. FM performed the experiments. XJ performed the experiments. YS analyzed the data. QY analyzed the data. CZ acquired the data. SZ revised the manuscript and analyzed the data. XS revised the manuscript and designed the study. LZ supplied the funds, analyzed the data, drafted the manuscript, revised the manuscript and provided final approval of the version to be published and supplied the funds. LZ and LS confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

All animal experiments were performed in compliance with the ARRIVE guidelines and in accordance with the UK Animals (Scientific Procedures) Act (1986) and the associated guidelines, the EU Directive 2010/63/EU for Animal experiments, the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Publications no. 8023, revised 1978) and the Animal Management Rules of the Chinese Ministry of Health (no. 55, 2001). This study was approved by the Animal Experiment Ethics Committee of Qilu University of Technology (Jinan, China).

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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