Ginseng Polysaccharides and its Effective Subfraction Protects Against Dextran Sodium Sulfate-Induced Colitis via Regulating NF-κB Signaling Pathway, Recovering Intestinal Barrier and Adjusting Gut Microbiota

Shanshan Li
Chinese Academy of Agricultural Sciences Institute of Special Animal and Plant Sciences

Yuli Qi
Chinese Academy of Agricultural Sciences Institute of Special Animal and Plant Sciences

Duoduo Ren
Chinese Academy of Agricultural Sciences Institute of Special Animal and Plant Sciences

Yue Zhang
Chinese Academy of Agricultural Sciences Institute of Special Animal and Plant Sciences

Yinshi Sun (sunyinshi2015@163.com)
Chinese Academy of Agricultural Sciences Institute of Special Animal and Plant Sciences

https://orcid.org/0000-0002-1889-4984

Research Article

Keywords: gut microbiota, colitis, Panax ginseng polysaccharides, pectin, short-chain fatty acids

Posted Date: August 18th, 2021

DOI: https://doi.org/10.21203/rs.3.rs-801098/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License
Abstract

**Background:** Polysaccharides from *Panax ginseng* is a natural carbohydrates with multiple activities. Our previous work found that ginseng polysaccharide could recover the gut microbiota dysbiosis caused by antibiotic. However, it was little known about its functions on colitis. In this study, we aim to investigate the protective effects of ginseng polysaccharide (WGP), its neutral fraction (WGPN) and acidic fraction (WGPA) on dextran sodium sulfate (DSS) induced colitis and the potential mechanisms with pharmacological experiments.

**Methods:** Animal model of DSS-induced colitis was constructed by male Wistar rats. Disease activity index (DAI) scores, weight increment, colon weights, and colon length were recorded. Physiological and histological parameters, tight junctions proteins, inflammatory factors, gut microbiota, short-chain fatty acids (SCFAs) and NF-kB signalling pathway related proteins were compared among the five experimental groups, which were treated with a normal diet (Con group), DSS (DSS group), WGP (WGP group), WGPN (WGPN group), and WGPA (WGPA group), respectively.

**Results:** Both WGP and WGPA alleviated the colitis symptoms and colon structure changes of colitis rats. They can decreased the DAIs and improved colon health; reduced colon damage and recovered intestinal barrier via regulating the tight-junction related proteins (ZO-1 and Occludin); downregulated inflammatory cytokines (IL-1β, IL-2, IL-6, and IL-17) and inhibited the TLR4/MyD88/NF-kB signalling pathway in the colon; regulated the diversity and composition of gut microbiota, especially the relative abundance of Ruminococcus; enhanced the production of SCFAs.

**Conclusions:** WGP exerted had a protective effect against colitis through regulating NF-kB signaling pathway, recovering intestinal barrier and adjusting gut microbiota, with its acidic fraction (WGPA) primarily contributing to this activity. The results support to the utilization and investigation of ginseng polysaccharides as potential intervention strategy for the prevention of colitis.

1. **Background**

Colitis is a chronic gastrointestinal disease with a high incidence and an unclear pathogenesis (1). Patients with colitis exhibit a series of inflammatory symptoms, such as diarrhea, vomiting, abdominal pain, rapid weight loss, and blood and/or pus in the stool. These symptoms easily affect the daily life of patients. During the process of development of colitis, a significant shift in the nature of the gut microbiota is observed, i.e., an increase in pathogenic bacteria and a decrease in beneficial bacteria are always detected (2, 3). Moreover, the gut microbiota regulates the gut microecological environment by acting on the production of short-chain fatty acids (SCFAs) or other metabolites, thus affecting the signaling pathways and immune cytokines of the host (4). The balance between pro-inflammatory and anti-inflammatory factors is also important for the colitis status. Once this balance is destroyed in the gastrointestinal system, the intestinal mucosa are damaged and exhibit a disease status, which might
also have adverse effects on the isolation of intestinal contents and nutrient absorption and may even endanger lives (5, 6).

Treatment of colitis via the application of human monoclonal antibodies and recombinant cytokines has achieved positive results. However, sometimes, these approaches are accompanied by varying levels of side effects and a high cost (7). In addition, the drugs approved currently for the treatment of UC, such as antibiotics, amino salicylate, and glucocorticoids, might cause serious side effects (8). Because of their low cost and high efficiency without side effects, the natural active extracts of plants might become safer and more effective potential substances to treat colitis. Polysaccharides, as bioactive macromolecules, are also reportedly beneficial to intestinal health. For example, *Astragalus* polysaccharides alleviate dextran sodium sulfate (DSS)-induced colitis by inhibiting the NF-κB activation pathway (9). Therefore, plant polysaccharides might be a potential active ingredient in the treatment of colitis.

*Panax ginseng* has been listed as a health-care food in China since 2012. Polysaccharides are the most important active components of ginseng. Our previous studies showed that ginseng polysaccharides can regulate the composition and diversity of the gut microbiota and promote the recovery of the mucosa in mice with antibiotic-associated diarrhea (10). It has been indicated that ginseng polysaccharides might have some positive therapeutic effects on colitis. Therefore, this study aimed to analyze the relieving effects of ginseng polysaccharide fractions on DSS-induced colitis in a rat model and provide new approaches to the treatment and prevention of colitis.

### 2. Experimental Section

#### 2.1. Preparation of WGP, WGPN, and WGPA

*Panax ginseng* roots were extracted in hot distilled water three times, precipitated by ethanol with a final concentration of 80%, and deproteinized in Sevag reagent three times. Subsequently, the aqueous layer was freeze dried and the total soluble polysaccharide extract (WGP) was obtained. Next, WGP was loaded onto a DEAE-Sepharose Fast flow column (4 cm × 37 cm), eluted with distilled water and 0.5 mol L⁻¹ NaCl, then dialyzed (0.5 mol L⁻¹ NaCl fraction; Mw, 3500 Da) and freeze dried; the neutral fraction (WGPN, eluted with distilled water) and the acidic fraction (WGPA, eluted with 0.5 mol L⁻¹ NaCl) were obtained (11, 12). The total carbohydrate and uronic acid content was determined according to methods reported previously (13, 14). Protein content was determined using the Dumas nitrogen analyzer (NDA 701). The monosaccharide composition of the WGP, WGPN, and WGPA samples was analyzed by HPLC (Shimadzu, Japan) after acid hydrolysis and PMP derivatization (15).

#### 2.2. Animal Groups and Experimental Design

Five-week-old male Wistar rats (weight, 160–180 g) were purchased from Changsheng Biotechnology Co., Ltd. (Liaoning, China), caged separately, and raised in a temperature- and humidity-controlled lab environment (22°C ± 1°C; relative humidity, 50% ± 5%) with a strict 12 h light/dark cycle. The rats were domesticated for 3 days before the experiment. The study protocol (No. TCS-2017019, January 2017)
was reviewed and approved by the Laboratory Animal Management and Ethics Committee of the Institute of Special Animal and Plant Sciences, Chinese Academy of Agricultural Sciences. All animal experiments were performed in strict accordance with the Guidelines for the Care and Use of Laboratory Animals recommended by the Institute of Special Animal and Plant Sciences, Chinese Academy of Agricultural Sciences and the Chinese Legislation on Laboratory Animals. Every effort was made to maximize the well-being of rats and to minimize their suffering.

Forty rats were equally distributed into five groups (Con, DSS, WGP, WGPN, and WGPA). The rats in the DSS, WGP, WGPN, and WGPA groups were administered 5% DSS (molecular weight, 36–50 kDa; MP Biomedical Solon, OH, USA) in the drinking water on a modeling period of 7 days, whereas the rats in the Con group were administered normal drinking water without DSS. After the modeling period, the rats in the WGP, WGPN, and WGPA groups received WGP (100 mg kg$^{-1}$), WGPN (60 mg kg$^{-1}$), and WGPA (35 mg kg$^{-1}$), respectively, twice daily by gastric gavage during a recovery period of 7 days. Dose of WGP was selected by as per our previous study, whereas doses of WGPN and WGPA were determined by their yields from WGP, i.e., 61.8% and 26.5%, respectively. Concomitantly, the rats in the Con and DSS groups received physiological saline.

During the experimental period, body weight was measured daily. The disease activity index (DAI) was used to assess the severity of colitis (16). The DAI score is the average of the following three scores: (I) weight loss (0: none; 1: 1–5%; 2: 5–10%; 3: 10–15%; and 4: >15%), (II) stool consistency (0: normal; 1 and 2: loose; and 3 and 4: diarrhea), and (III) stool blood (0: normal; 1: +; 2: ±; 3: ++; 4: +++).

### 2.3. Sample Collection

Fresh fecal samples were collected aseptically from rats on the eighth day of the recovery period. Subsequently, the rats were anesthetized with isoflurane. Measured the length of the colon. The bowel contents were rinsed with pre-cooled normal saline and the colon tissue was collected, weighed and segmented. The end segment of the colon tissue (∼5–8 cm) was collected and divided into three parts, in which the parts of near the rectum were as fixed in 10% neutral formalin solution for histological observation, whereas other parts was stored at −80°C for use in mRNA, and protein extraction.

### 2.4. Histological Assessment

The histological analysis was performed as previously reported (15).

### 2.5. Western Blotting

Proteins of colon samples (0.1 mg) were extracted using a protein extraction kit (No. C510003-0050, Sangon Biotech. Inc.) and followed the procedures by instructions. The protein content was determined using the BCA assay (No. C503021-0500, Sangon Biotech. Inc.). The buffer solution (0.1 M Tris–HCl, 20% glycerol, 4% SDS, and 0.01% bromophenol blue) was added to the protein lysates in a ratio of 1:4 and boiled for 10 min. Aliquots of protein were subjected to 10% SDS-PAGE. The separated proteins were transferred to a PVDF membrane for 1 h at 200 mA. The membrane was pre-blocked with 5% nonfat milk in TBST, gentle shaking at 4°C overnight. Each membrane was washed 5 times for 5 min and incubated...
with the secondary horseradish peroxidase-linked antibody. Quantitation of detected bands was performed with the Image Pro-Plus software. Each density was normalized using the intensity of the corresponding β-actin protein band as loading controls. The density of the control group for relative comparison was standardized as 1.0 to compare across groups or the ratio of aim protein/β-actin. Anti-IL-1β (A1112), anti-IL-2 (A16317), anti-IL-6 (A0286), anti-IL-17 (A10052), anti-Caspase-3 (A16793), and anti-NF-κB (A19653) antibodies were purchased from ABclonal (Wuhan) Biotechnology Co., LTD. Anti Cyclin E1 (A14225) was purchased from Abclonal Biotechnology, Inc. and p21 (sc-6246) was purchased from Santa Cruz Biotechnology, Inc.

2.6. Real-time Quantitative Reverse Transcription PCR (qRT–PCR)

The total RNA of colonic samples (100 mg) was extracted using a TaKaRa ultra-pure RNA extraction kit (Code, D9108B; TaKaRa Bio. Inc., Japan) and quantified using a NanoDrop oneC Microvolume UV-Vis Spectrophotometer (Thermo Fisher Scientific Inc., USA). First-strand cDNA synthesis reactions (total amount of RNA was about approximately 500–800 ng) were conducted using a TakaRa kit (Code, DRR047A;TaKaRa Bio. Inc., Japan). Quantitative real-time PCR (RT–qPCR) was performed on an ABI7500 real-time fluorescence quantitative PCR instrument (Thermo Fisher Scientific Inc., USA), according to the protocol of the manufacturer. PCR was performed using a 20 µL reaction mix containing: 50 ng of cDNA with PCR grade water, 12.5 µL 2× SYBR Premix Ex Taq (Tli RNaseH Plus), 0.5 µL forward primer and 0.5 µL reverse primer. A relative quantitative analysis of targets was performed using the $2^{-\Delta\Delta C_{\text{t}}}$ method and data were normalized to the levels of the β-actin mRNA.

2.7. Gut Microbiota Analysis

Before further analysis, total bacterial genomic DNA was extracted from the rat fecal samples using a Fast DNA SPIN Extraction Kit (MP Biomedicals, Santa Ana, CA, USA), followed by quantification using a NanoDrop ND-1000 Spectrophotometer (Thermo Fisher Scientific, Inc., USA) and agarose gel electrophoresis. The V3–V4 region of the 16S rRNA gene was amplified by PCR using a forward primer (5′–ACTCCTACGGGAGGCAGCA–3′) and a reverse primer (5′–GGACTACHVGGGTWTCTAAT–3′). Moreover, a DNA library was constructed, optimized, and sequenced using the Illumina MiSeq System. The original data from high-throughput sequencing were screened and controlled according to the sequence quality using the QIIME software (version 1.8.0). Length distribution of the sequences contained in all samples was analyzed statistically using the R software package (v3.2.0). Sequences with a similarity greater than 97% were defined as an operational taxonomic unit (OUT) using the online QIIME software, and the most abundant sequence within each OUT was selected as the representative sequence (17). All representative reads were annotated and compared with the template sequence in the Greengenes database (18).

2.8. Contents of SCFAs

The SCFAs of each rat were analyzed as previously reported (12).
2.9. Statistical Analyses

Statistical analyses were performed using the GraphPad Prism software (version 5.01), and the significance of differences was analyzed using ANOVA (Tukey’s test). Significance was set at $P < 0.05$.

3. Results

3.1. Physiochemical Features of the Ginseng Polysaccharide Fractions

The water-soluble polysaccharide (WGP) from ginseng roots was obtained after water extraction, ethanol precipitation, and deproteination as a yield of 10.1%. Total carbohydrate contents, uronic acid contents, protein contents and monosaccharide composition were analyzed and are shown in Table 1. WGP composed of rhamnose (Rha, 2.7%), galacturonic acid (GalA, 25.7%), galactose (Gal, 15.1%), arabinose (Ara, 13.9%), glucose (Glc, 42.6%). WGPN and WGPA were purified from WGP using DEAE-Sepharose Fastflow chromatography via elution with distilled water and 0.5 mol L$^{-1}$ NaCl, respectively. WGPN was neutral polysaccharide fraction and exhibited the main structure features of a starch-like glucan composed by Gal, Ara, and Glc with a ratio of 3.3%, 1.4%, 95.3%, respectively. WGPA was mainly composed of Rha (3.8%), GalA (44.2%), Gal (18.0%), Ara (15.4%), and Glc (13.6%). WGPA contains type I rhamnogalacturonan (RG-I) and homogalacturonan (HG) regions, which serve as an acidic pectin that is rich in uronic acid (11, 12).

| Fraction | Yield (%) | Total carbohydrate contents (%) | Uronic acid contents (%) | Protein contents (%) | Monosaccharide composition (%) |
|----------|-----------|---------------------------------|-------------------------|----------------------|--------------------------------|
| WGP      | 10.1$^a$  | 89.2                            | 22.9                    | 0.6                  | 42.6  15.1  13.9  25.7  2.7   |
| WGPN     | 61.8$^b$  | 92.8                            | 0                       | 0.3                  | 95.3  3.3  1.4  --  --       |
| WGPA     | 26.5$^b$  | 92.1                            | 38.5                    | 0.2                  | 13.6  18.0  15.4  44.2  3.8   |

$^a$ Yield in relation to the dried ginseng roots; $^b$ Yield in relation to the weight of WGP applied into column.

3.2. WGP and Its Subfractions Alleviated DSS-Induced Colitis

Both WGP and WGPA decreased the DAIs significantly compared with the DSS group (Fig. 1A), with WGPA exhibiting the lowest DAI value. Weight increment results showed that WGPA increased the body weight after the recovery period compared with DSS treatment (Fig. 1B). The colon length and colon
weight were significantly reduced in the DSS group compared with the Con group (Fig. 1C, 1D). Moreover, the colon weight and length of the WGPA group was significantly different from that of the DSS group. These results suggested that WGP obviously alleviated the gut injury resulting from colitis, while WGPA was the effective subfraction.

3.3. Ginseng Polysaccharide Fractions Reduced Colon Damage and Recovered Intestinal Barrier

The colon of the DSS model group exhibited significant tissue damage, such as the decrease in, or disappearance of, glands and goblet cells; a shallower crypt; disordered arrangement of intestinal villi; and severe epithelial injury and inflammatory cell infiltration in the mucosa and submucosa (Fig. 2A). However, these damage signs were noticeably improved by ginseng polysaccharide fractions. The intestinal villi of the WGP and WGPA groups were longer and the crypts were deeper, structure of colonic gland were fuller and more complete, and the recovery effect was better, than that of the WGPN group. Compared with the Con group, the expression of the tight-junction protein ZO-1 and Occludin were decreased in the DSS group (Fig. 2B), suggesting the integrity and function of the colon–intestinal barrier were destroyed by DSS. Ginseng polysaccharide and its subfractions enhanced the expression of ZO-1 significantly; however, only WGP increased the expression of Occludin significantly (Fig. 2B). These results indicated that WGP and WGPA protected intestinal integrity and attenuated DSS-induced structure damage.

3.4. Ginseng Polysaccharide Fractions Decreased Intestinal Inflammation Level and inhibited NF-κB Signaling pathway

The levels of inflammatory cytokines IL-1β, IL-2, IL-6, and IL-17 in the colonic mucosa were significantly increased in the DSS group compared with the Con group (Fig. 3A), which suggests an inflammation-causing effect of DSS in rats. WGP and its subfractions significantly improved the level of inflammation of the colon caused by DSS and down-regulated the four inflammatory cytokines, among which the inflammatory cytokine levels in WGPN and WGPA were closer to those of the Con group. Both WGPN and WGPA decreased the proinflammatory factors more effectively compared with WGP.

NF-κB pathway related protein level was detected using Western Blotting (Fig. 3B). DSS treatment increased the TLR4, MyD88 and NF-κB expression levels, which suggested the activation of NF-κB pathway. After treatment with WGP, WGPN and WGPA, the expression levels of the three proteins was downregulated, especially that of the WGPA group. It can be concluded that ginseng polysaccharide could improve the colitis inflammation via inhibit the TLR4/MyD88/NF-κB pathway.

3.5. Ginseng Polysaccharide Fractions Adjusted the Diversity and Composition Changes of Gut Microbiota
In the DSS group, the diversity of the gut microbiota was significantly decreased, as assessed using the Simpson index (Fig. 4A) and the Shannon diversity index (Fig. 4B). After the recovery treatment, the diversity indexes were obviously improved in the WGP and WGPA groups compared with the DSS group. However, the two α diversity indexes in the WGPN group did not show beneficial effects compared with the DSS group, although there were significant differences between the WGPN and the Con groups.

Figure 4C showed the changes in the relative abundance of bacteria at the phylum level. The fecal microbiota of the five groups was mainly composed of Firmicutes, Verrucomicrobia, and Bacteroidetes. Compared with the Con group, the relative abundance of Firmicutes and Bacteroidetes was increased, while that of Verrucomicrobia was decreased significantly in the DSS group, which suggested changes in the fecal microbiota composition under the DSS treatment. After treatment with the ginseng polysaccharide and its subfractions, the composition of the fecal microbiota was also recovered, especially in the WGP and WGPA groups. Compared with the DSS group, the relative abundance of Verrucomicrobia was increased significantly, while that of Firmicutes and Bacteroidetes was decreased, in both WGP and WGPA groups, which was similar to the composition of the Con group. The microbiota composition of the WGPN group was not similar to that of the DSS or Con group and showed irregular variations after the recovery period, which is also consistent with the results obtained for the DAI, colon length, structure observation, and cytokine levels. Figure 4D shows the key changes in the fecal microbiota observed at the genus level. The relative abundance of Ruminococcus was significantly decreased in the DSS group compared with the Con group. However, WGP and WGPA groups exhibited a significant improvement in dysbiosis and an increase in the relative abundance of Ruminococcus.

### 3.6 Content of SCFAs in Feces

All types of SCFAs detected were significantly decreased in the DSS group compared with the Con group (Fig. 5). After treatment with WGP, WGAN, or WGPA, the content of SCFAs was significantly increased in feces compared with that observed in the DSS group. The contents of acetate, propionate, butyrate, and total SCFAs was enhanced most obviously in the three treatment groups ($P < 0.001$). However, WGPA increased the production of valerate and decreased that of isobutyrate, while WGPN yielded the opposite results compared with WGP. In general, the effects of WGP, WGPN, and WGPA on SCFA production were similar, as all three polysaccharide fractions improved the levels of SCFAs in feces.

### 4. Discussion

#### 4.1 WGPA is the Effective Subfraction For the Anti-Colitis Activity of WGP

In this study, the colitis model was induced by adding 5% DSS orally to the drinking water of rats for 7 days and was used to investigate the effects of ginseng polysaccharides and their sub-fractions on colitis rats. The results showed that the Wistar rats exhibited physiological and pathological manifestations of colitis. Compared with the DSS group, WGP and WGPA significantly improved the
diarrhea status of rats, including a decrease in the DAI index, and an increase in the colon length, colon weight, reduced the intestinal injury. The recovery effects of WGP and WGPA were better than that of WGPN.

The function of the intestinal mucosal barrier is largely dependent on intercellular tight junctions (TJs) (19). TJs are the most apical intercellular complexes in epithelial cells and are composed of transmembrane barrier proteins (e.g., Claudins, Occludin, and junctional adhesion molecules) and cytoplasmic scaffolding proteins (e.g., the ZO family, AF-6, and Cingulin) (20, 21). The downregulation of TJ proteins leads to an increase in the permeability of the intestinal epithelium, which might induce bacterial translocation, the risk of intestinal infection, and inflammation (22). In this study, the expression of the Occludin and ZO-1 proteins was significantly downregulated in the DSS group, which suggests that DSS destroys the mucosal layer and increases intestinal permeability. In contrast, WGP promoted the expression of ZO-1 and Occludin in the colon significantly, suggesting that ginseng polysaccharides repair the intestinal epithelium after DSS damage, while the WGPN and WGPA treatments only promoted the levels of ZO-1. Therefore, regarding colon structure repair, WGP was more effective than WGPN and WGPA.

4.2 Potential Mechanism of WGP and WGPA on Anti-Colitis Activity

The lamina propria T cells increase the production of Th17-associated cytokines (IL17A and a little IFN-γ), which in turn upregulate the expression of IL-1, IL-6, and IL-8 during the process of colitis (23, 24). The levels of inflammatory cytokines reflect the degree of inflammation of the intestinal mucosa. Here, we chose IL-1β, IL-2, IL-6, and IL-17 as representative cytokine factors, with the aim of detecting changes in the levels of inflammatory molecules in the colon. It was shown that DSS aggravated the inflammation levels, while the three ginseng polysaccharide fractions significantly reduced the expression of these inflammatory cytokines in the colon. Concomitantly, the WGPN and WGPA sub-fractions exerted stronger effects on inflammation than did WGP.

NF-κB pathway is a very important signal pathway in inflammation response. The occurrence of colitis was closely related with NF-κB pathway (6). The results of our study also identified the activation of NF-κB pathway in colitis rats that was induced by DSS. Ginseng polysaccharide could significantly inhibit the activation of the NF-κB pathway via regulation of the TLR4 and MyD88 protein expression, especially WGPA treatment, further inhibiting the production of inflammation factors. Notably, we found that WGNP can also suppress this signaling pathway to a certain level, which was in accordance with the results of cytokine levels. These results suggested that although WGPN did not play a role in colitis treatment, it might have functions in other inflammatory diseases.

Dysbiosis of gut microbiota is an important feature of colitis. The diversity of gut microbiota is reduced in patients with colitis, together with the decrease in SCFA-producing bacteria and the increase in mucus-dissolving bacteria, sulfate-reducing bacteria, and pathogenic bacteria (3). Here, we found that the Simpson and Shannon indexes of fecal microbiota were decreased significantly in the DSS group.
compared with the Con group, suggested the dysbiosis and the destruction of the structure of the gut microbiota. After treatment with WGP and WGPA, the diversity of fecal microbiota was increased and became similar to that of the Con group, which was indicative of recovery from dysbiosis. WGPN did not exert obvious effects on the diversity of fecal microbiota. These results indicate that the beneficial effects of WGP and WGPA on colitis might be associated with their ability of adjusting the diversity of the gut microbiota. We also found that WGP and WGPA recovered the composition of fecal microbiota at the phylum level. The key microbiota changes afforded by WGP and WGPA that played a role in the DSS treatment were also observed at the genus level. The relative abundance of *Ruminococcus* was significantly decreased in the DSS group compared with the Con group; however, this abundance was increased to normal levels after treatment with WGP or WGPA, while there was no significant change after treatment with WGPN. *Ruminococcus* is a common bacterium that produces SCFAs and has beneficial effects on hosts (25). SCFAs are important metabolites in the intestinal microbial environment and are closely related to immune, anti-tumor, and anti-inflammatory activities (26). Some plant polysaccharides that are not digested by the host are fermented by a series of anaerobic probiotics. Because of the different structure and sources of polysaccharides, different kinds of SCFAs are produced (27). WGPA was rich in acidic pectin, which is often referred to as a prebiotic that ferments in the colon and produces beneficial metabolites, especially SCFAs. Since the gut microbiota adjusting ability, especially the increasing effects on *Ruminococcus* and the SCFAs production, ginseng polysaccharide WGP and WGPA might used as potential prebiotics, which help for against diarrhea, gut microbiota dysbiosis, or other colonic related diseases.

WGPN and WGPA were purified from the ginseng polysaccharide extract, WGP. WGPN is a starch-like glucan that belongs to the group of neutral polysaccharides. In contrast, WGPA is an acidic pectin that is rich in type I rhamnogalacturonan and homogalacturonan (12). The different structural features of these sub-fractions determine their different activities. Of the two purified fractions, WGPA showed more obvious beneficial effects on colitis compared with WGPN, not only regarding the normal status, but also regarding gut microbiota diversity and composition. WGP could protect against colitis through inhibit the NF-κB pathway, adjust the diversity and composition of gut microbiota and recover the intestinal barrier.

### 4.3 The Comparison of WGP and Other Herbal Polysaccharides on Anti-Colitis Activity

The Chinese herbal medicine has a long history of application in Asia, especially in China, and had the characteristics of natural and non-toxic; certain medicinal food was the primary choice for daily healthcare, but the mechanisms underlying some of these activities were not clear. Polysaccharides from many traditional Chinese medicines can be used to treat and relieve colitis, such as certain non-starch polysaccharides, including glucan from oat bran, mushroom, seaweed, pectin, gum, prebiotics, etc (28). Other types of polysaccharide, such as *Dictyophora indusiata* polysaccharide could alleviate the severity of colitis by improving the gut epithelial integrity and inflammatory reactions, which was similar to the WGP and its effective fraction WGPA (29). Polysaccharides extracted from *Blidingia minima* showed an
anti-inflammatory effect on DSS-treated colitis by repaired colonic dysfunction, and improved colonic morphology, infiltration, and the expression of tight junction, pro-inflammatory cytokines, as well as the protein levels of NF-κB in colonic tissue (30). A novel alkali-soluble polysaccharide from purple sweet potato could restore the immune organ indices, increased colon length, improved colonic histopathology in colitis mice as well as inhibited the levels of pro-inflammatory cytokines (IL-1β, IL-6, and TNF-α) in colonic tissue, ameliorated the compositions and functions of gut microbiota in DSS-induced colitis mice (31). Different from the WGP and WGPA, it could adjust the relative abundance of Parasutterella, Desulfovibrio, Lachnospiraceae, Lactobacillus, Erysipelotrichaceae, and Bacteroidetes. Altogether, it seems that most of the polysaccharides that could work against colitis can alleviate colonic inflammation via blocking pro-inflammatory cytokines, however, not all of them could affect the same type of gut microbiota. Further investigation of the crosstalk between epithelial cells and gut microbiota for evaluating the effects of different types functional polysaccharide will lead to well-designed clinical intervention trials and improved treatment and prevention of colitis.

Although some positive results have been obtained on the effects and mechanism of ginseng polysaccharide on colitis in this research, it should be noted that there are still some deficiencies and limitations. For example, the relationship between initial body weight, final body weight, weight increment, food intake, and energy conversion rate has not been compared and discussed, which may also have an impact on the physical condition of rats. In terms of mechanism research, the detail of the signaling pathway was not fully discussed in this research. In the following work, we will focus more on these aspects and further study the therapeutic effect of ginseng polysaccharides on colitis and its specific mechanism, so as to provide data basis for conquer with colitis.

5. Conclusion

In conclusion, the ginseng polysaccharide extract WGP improved the symptoms of DSS-induced colitis in rats, with the acidic pectin fraction WGPA playing the main role in this activity. WGP and WGPA improved the symptoms of colitis by reducing the expression level of inflammatory cytokines in the colon; maintaining the integrity of the intestinal barrier via the upregulation of tight junction related proteins; regulating the diversity and composition of gut microbiota; increasing the relative abundance of Ruminococcus; increasing the production of SCFAs and inhibiting the TLR4/MyD88/NF-κB signaling pathway. The results of this study may provide basic data for improving the effects of natural polysaccharides on colitis and promote the applications of Panax ginseng and its active components.

Abbreviations

WGP, polysaccharides from Panax Ginseng; WGPN, neutral ginseng polysaccharide fraction; WGPA, acidic ginseng polysaccharide fraction; DSS, dextran sodium sulfate; SCFAs, short-chain fatty acids; HE, hematoxylin and eosin; OUT, operational taxonomic unit; QIIME, quantitative insights into microbial ecology; Rha, rhamnose; GalA, galacturonic acid; Gal, galactose; Ara, arabinose; Glc, glucose; RG-I, type I
rhamnogalacturonan; HG, homogalacturonan; TJs, tight junctions; TLR4, toll-like receptor 4; DAI, disease activity index.

Declarations

Acknowledgments

The authors thank Shanghai Personal Biotechnology Co., Ltd for the 16s rRNA sequences analysis.

Author’s contributions

conceptualization, writing - original draft and writing - review & editing, S.S. Li; methodology, Y.L. Qi; data curation, D.D. Ren and Y. Zhang; supervision and project administration, Y.S. Sun.

Funding

This work was supported by the Scientific and Technologic Foundation of Jilin Province (grant numbers 20200201116JC).

Availability of data and materials

The datasets used in the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The animal care and experimental procedures used in this study were approved by the Laboratory Animal Management and Ethics Committee of the Institute of Special Animal and Plant Sciences, Chinese Academy of Agricultural Sciences.

Consent for publication

Not applicable.

Competing interests

The authors declare no conflict of interest.

References

1. Steed H, Macfarlane GT, Macfarlane S. Prebiotics, synbiotics and inflammatory bowel disease. Mol Nutr Food Res. 2008;52:898–905.
2. Meleine M, Matricon J. Gender-related differences in irritable bowel syndrome: potential mechanisms of sex hormones. World J Gastroentero. 2014;20:6725–43.

3. Nishida A, Inoue R, Inatomi O, Bamba S, Naito Y, Andoh A. Gut microbiota in the pathogenesis of inflammatory bowel disease. Clinical Journal of Gastroenterology Clin J Gastroentero. 2018;11:1–10.

4. Gonçalves P, Araújo JR, Santo JPD. A cross-talk between microbiota-derived short-chain fatty acids and the host mucosal immune system regulates intestinal homeostasis and inflammatory bowel disease. Inflamm Bowel Dis. 2018;24:558–72.

5. Yin MM, Yan XB, Weng WH, Yang YZ, Gao RY, Liu MF, et al. Micro integral membrane protein (MIMP), a newly discovered anti-inflammatory protein of Lactobacillus Plantarum, enhances the gut barrier and modulates microbiota and inflammatory cytokines. Cell Physiol Biochem. 2018;45:474–90.

6. Xavier RJ, Podolsky DK. Unravelling the pathogenesis of inflammatory bowel disease. Nature. 2007;448:427–34.

7. Wilhelm SM, Mckenney KA, Rivait KN, Kale-Pradhan PB. A review of infliximab use in ulcerative colitis. Clin Ther. 2008;30:223–30.

8. Creed TJ, Probert CSJ, Norman MN, Moorghen M, Shepherd NA, Hearing SD, et al. Basiliximab for the treatment of steroid-resistant ulcerative colitis: further experience in moderate and severe disease. Aliment Pharm Therap. 2010;23:1435–42.

9. Lv J, Zhang YH, Tian ZQ, Liu F, Shi Y, Liu Y, et al. Astragalus polysaccharides protect against dextran sulfate sodium-induced colitis by inhibiting NF-κB activation. Int J Biol Macromol. 2017;98:723–9.

10. Li SS, Qi YL, Chen LX, Qu D, Li ZM, Gao K, et al. Effects of Panax ginseng polysaccharides on the gut microbiota in mice with antibiotic-associated diarrhea. Int J Biol Macromol. 2019;124:931–7.

11. Zhang X, Yu L, Bi HT, Li XH, Ni WH, Han H, et al. Total fractionation and characterization of the water-soluble polysaccharides isolated from Panax ginseng C. A. Meyer Carbohyd Polym. 2009;77:544–52.

12. Wang J, Li SS, Fan YY, Chen Y, Liu D, Cheng HR, et al. Anti-fatigue activity of the water-soluble polysaccharides isolated from Panax ginseng C. A. Meyer J Ethnopharmacol. 2010;130:421–3.

13. Li SS, Qi YL, Ren DD, Qu D, Sun YS. The structure features and improving effects of polysaccharide from Astragalus membranaceus on antibiotic-associated diarrhea. Antibiotics-Basel. 2020;9:8.

14. Huang XJ, Ma JB, Wei LX, Song JY, Li C, Yang HX, et al. An antioxidant α-glucan from Cladina rangiferina (L.) Nyl. and its protective effect on alveolar epithelial cells from Pb2+-induced oxidative damage. Int J Biol Macromol. 2018;112:101–9.

15. Qi YL, Chen LX, Gao K, Shao ZJ, Huo XH, Hua M, et al. Effects of Schisandra chinensis polysaccharides on rats with antibiotic-associated diarrhea. Int J Biol Macromol. 2019;124:627–34.

16. Wang LF, Xue PP, Tong MQ, Chen R, Yang WG, ZhuGe DL, et al. Injected laquinimod D-α-tocopheryl polyethylene glycol-1000 succinate polymeric micelles for the treatment of inflammatory bowel disease. Colloid Surface B. 2020;185:110575.
17. Blaxter M, Mann J, Chapman T, Thomas F, Whitton C, Floyd R, et al. Defining operational taxonomic units using DNA barcode data. Philos Trans R Biol Sci. 2005;360:1935–43.

18. DeSantis TZ, Hugenholtz P, Larsen N, Rojas M, Brodie EL, Keller K, et al. Greengenes, a chimera-checked 16S rRNA gene database and workbench compatible with ARB. Appl Environ Microbiol. 2006;72:5069–72.

19. Suzuki T, Yoshinaga N, Tanabe S. Interleukin-6 (IL-6) regulates claudin-2 expression and tight junction permeability in intestinal epithelium. J Biol Chem. 2011;286:31263–71.

20. Dokladny K, Zuhl MN, Moseley PL. Intestinal epithelial barrier function and tight junction proteins with heat and exercise. J Appl Physiol. 2016;120:692–701.

21. Medina de FS, Romero-Calvo I, Mascaraque C, Martínez-Augustin O. Intestinal inflammation and mucosal barrier function. Inflamm Bowel Dis. 2014;20:2394–404.

22. Xie SZ, Liu B, Ye HY, Li QM, Pan LH, Zha XQ, et al. Dendrobium huoshanense polysaccharide regionally regulates intestinal mucosal barriers function and intestinal microbiota in mice. Carbohydr Polym. 2019;206:149–62.

23. Sarra M, Monteleone I, Stolfi C, Fantini MC, Sileri P, Sica G, et al. Interferon-gamma-expressing cells are a major source of interleukin-21 in inflammatory bowel diseases. Inflamm Bowel Dis. 2010;16:1332–9.

24. Rovedatti L, Kudo T, Biancheri P, Sarra M, Knowles CH, Rampton DS, et al. Differential regulation of interleukin 17 and interferon gamma production in inflammatory bowel disease. Gut. 2009;58:1629–36.

25. Baxter NT, Schmidt AW, Venkataraman A, Kim KS, Waldron C, Schmidt TM. Dynamics of human gut microbiota and short-chain fatty acids in response to dietary interventions with three fermentable fibers. mBio. 2019;10:e02566–18.

26. Caspary WF. Physiology and pathophysiology of intestinal absorption. Am J Clin Nutr. 1992;55:299–308.

27. Fernández J, Redondo-Blanco S, Gutiérrez-del-Río I, Miguélez EM, Villar CJ, Lombó F. Colon microbiota fermentation of dietary prebiotics towards short-chain fatty acids and their roles as anti-inflammatory and antitumour agents: A review. J Funct Foods. 2016;25:511–22.

28. Nie Y, Lin QL, Luo FJ. Effects of Non-Starch Polysaccharides on Inflammatory Bowel Disease. Int J Mol Sci. 2017;18:1372.

29. Kanwal S, Joseph TP, Aliya S, Song S, Saleem MZ, Nisar MA, et al. Attenuation of DSS induced colitis by Dictyophora indusiata polysaccharide (DIP) via modulation of gut microbiota and inflammatory related signaling pathways. J Funct Foods. 2020;64:103641.

30. Song W, Li Y, Zhang XL, Wang ZL. Potent anti-inflammatory activity of polysaccharides extracted from Blidingia minima and their effect in a mouse model of inflammatory bowel disease. J Funct Foods. 2019;61:103494.

31. Sun J, Chen H, Kan J, Gou YR, Liu J, Zhang X, et al. Anti-inflammatory properties and gut microbiota modulation of an alkali-soluble polysaccharide from purple sweet potato in DSS-induced colitis.
Figures

Figure 1

The influence of ginseng polysaccharide fractions on colon health. (A) DAI scores; (B) weight increment; (C) colon weight; (D) colon length. The bars represent the mean ± SD, n=8; #P< 0.05, ##P< 0.01, ###P< 0.001 compared with the Con group; *P< 0.05, **P< 0.01, ***P< 0.001 compared with the DSS group. DAI, disease activity index; Con, control group; DSS, colitis group; WGP, WGP treatment group; WGPN, WGPN treatment group; WGPA, WGPA treatment group.
Figure 2

Changes of the colon structure and intestinal barrier. (A) histopathological observation; (B) intestinal barrier related protein expression by Western Blotting. Con, control group; DSS, colitis group; WGP, WGP treatment group; WGPN, WGPN treatment group; WGPA, WGPA treatment group.
Figure 3

Changes of inflammatory related protein expression in colon. (A) expression of cytokine using Western Blotting and their relative densitometry; (B) expression of NF-κB pathway related proteins using Western Blotting and their relative densitometry. β-actin as loading control. The bars represent the mean ± SD, n=3; ###P< 0.001 compared with the Con group; ***P< 0.001, **P< 0.01 compared with the DSS group. Con, control group; DSS, colitis group; WGP, WGP treatment group; WGPN, WGPN treatment group; WGPA, WGPA treatment group.
Figure 4

Diversity and composition analysis of the gut microbiota. (A) Simpson index. (B) Shannon index. (C) Gut microbiota composition at the phylum level. (D) Relative abundance of Ruminococcus. The bars represent the mean ± SD (A and B) or the mean ± SEM (C), n=8.*P< 0.05, **P< 0.01, and ***P< 0.001. Con, control group; DSS, colitis group; WGP, WGP treatment group; WGPN, WGPN treatment group; WGPA, WGPA treatment group.
Figure 5

SCFA content in the feces of rats. The bars represent the mean ± SD, n=8; #P< 0.05 and ##P< 0.01 compared with the Con group; *P< 0.05 and ***P< 0.001 compared with the DSS group. Con, control group; DSS, colitis group; WGP, WGP treatment group; WGPN, WGPN treatment group; WGPA, WGPA treatment group.