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Oat β-glucan ameliorates dextran sulfate sodium (DSS)-induced ulcerative colitis in mice

Bo Liu*, Qinlu Lin*, Tao Yang*, Linna Zeng, Limin Shi, Yaya Chen, Feijun Luo*

Department of Molecular Nutrition, College of Food Science and Engineering, Central South University of Forestry and Technology, Changsha, 41004, PR China

* Contributed equally to this work.
* Co-corresponding author, Dr. Feijun Luo and Qinlu Lin, Central South University of Forestry and Technology, No. 498, Raoshan Road, Changsha, 410004, PR China.

Tel: 0086-731-85623240, Feijun Luo Email: luofeijun@hotmail.com.
Prof. Qinlu Lin Email: linql0403@126.com
Abstract

Ulcerative colitis is a major inflammatory bowel diseases (IBD), characterized by inflammation within the gastrointestinal tract through chronic or relapsing immune system activation. The aim of this study is to investigate the potential protective effect of oat β-glucan (βG) against colitis induced by DSS in mice. Eighty mice were randomly divided into control group (no DSS, no βG), DSS group (DSS only), DSS+L-βG group (DSS plus 500 mg/kg βG), and DSS+H-βG group (DSS plus 1000 mg/kg βG). Compared with the DSS group, administration of βG significantly reduced clinical symptoms with less weight loss, diarrhea and shortening of the colon, the severity of colitis was significantly inhibited as evidenced by reduced disease activity index (DAI) and degree of histological damage in colon. Moreover, treatment with βG not only decreased myeloperoxidase activity (MPO), nitric oxide (NO) and malondialdehyde (MDA) level, but also inhibited mRNA and protein expression of pro-inflammatory factors such as TNF-α, IL-1β, IL-6 and iNOS. It suggests that oat βG in diet might exhibits an anti-inflammatory function against colitis through inhibition of expression pro-inflammatory factors.

Key Words: Oat β-glucan; colitis; TNF-α; IL-1β; IL-6; iNOS
1. Introduction

Inflammatory bowel disease (IBD) is inflammation within the gastrointestinal (GI) tract characterized by chronic or relapsing immune system activation. There are two types of IBD: ulcerative colitis (UC) and Crohn’s disease. While the clinical features of Crohn’s disease include pain, diarrhea, narrowing of the intestines lumen leading to strictural and bowel obstruction, abscess formation, and fistulization of the skin and internal organs; the clinical features of UC include severe diarrhea, blood loss, and progressive loss of peristaltic function. Although the precise etiology of colitis is not very clear, it is now well recognized that inflammation of the intestinal mucosa is characterized by chronic inflammatory cell infiltration composed mainly of neutrophils and macrophages, an effect that is accompanied by production of pro-inflammatory cytokines, like interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α), inducible nitric oxide synthase (iNOS) and so on.

UC can be treated with a number of medications including 5-ASA drugs such as sulfasalazine and mesalazine. Corticosteroids such as prednisone can also be used due to their immunosuppressing and short term healing properties. However, due to the high risks of adverse effects such as sleep, mood disturbance, dyspepsia, or glucose intolerance, corticosteroids are not suitable as long term therapies, and new strategies for adjunct therapies are needed. To imitate the IBD, a classic model of colitis model induced by dextran sulfate sodium (DSS) was set up. DSS is a heparin-like polysaccharide that has been successfully used to induce colonic mucosal injury in mice. Colitis induced by this model exhibits characteristics resembling human UC,
including weight loss, severe diarrhea, rectal bleeding, loss of epithelium followed by ulceration and leukocyte infiltration. Several studies reported that plant extracts such as arvelexin and flavonoids show anti-inflammatory activity in this model. Kimchi, betaine and isorhamnetin can inhibit expression of TNF-α. Some other plant extracts like Serpylli herba extract showed beneficial effect against inflammation via inhibiting expression of IL-1β. Plants contain many beneficial nutrients (phytochemicals) which may protect against inflammation such as colitis without side effect.

β-glucans are major structural components of cell walls of fungi and plants such as mushrooms, yeast, bacteria, oat, barley, seaweeds and algae. β-glucans are polysaccharides of D-glucose monomers linked by β-glycosidic bonds with different molecular mass, solubility, viscosity, and three-dimensional configurations. β-glucans can promote the functional activities of macrophages and enhance the anti-microbial activities of mononuclear cells and neutrophils. β-hydroxy-β-methyl-butyrate (HMB), a β-1,3/1,6-D-glucan, showed strong therapeutic effect on canine colitis by decreasing IL-6 and increasing IL-10 concentrations. Yeast glucan, also a β-1, 3/1, 6-D-glucan, possess a beneficial effect on mice intestinal inflammation caused by DSS. Schizophyllan (SPG), a member of β-(1-3) glucan family, can effectively alleviate the colitis in vivo. Lentinan, a β-glucan isolated from Lentinula edodes, ameliorated DSS induced colitis and reduced IL-8 in Caco-2 cells.

Oat β-glucan (βG) is a β-1,3/1,4-D-glucan and its construct is different from β-1,3/1,6-D-glucan of mushrooms and yeast. It has been reported that βG possess
many physiological functions such as anti-insulin resistance, anti-obesity and anti-oxidant. It is demonstrated that the βG can fight against high risk of cardiovascular diseases by increasing insulin sensitivity index and increasing activity of intestinal Na\(^+/K^+/ATPase\) and Ca\(^{2+}/Mg^{2+}-ATPase\). Oat β-glucan improved metabolic indexes of obesity mice and controlled human appetite. Interestingly, βG may also inhibit nonalcoholic steatohepatitis and ameliorate inflammation, but the molecular mechanism is not clear. Until today, there is no report about the anti-colitis effect of βG. In this study, we assessed the effect of βG on the DSS-induced colitis in mice and found that it not only suppressed the shortening and swelling of the intestine, but also inhibited the expression of pro-inflammatory factors in colonic tissues as well.

2 Materials and Methods

2.1 Experimental Animals

Male, 8-week-old ICR mice were purchased from Hunan SJA Laboratory Animal Co., Ltd (SLAC, Changsha, China). Mice were housed in an air-conditioned animal room at 23±2°C with a 12h light/dark cycle. Before treatment, mice were fed with a laboratory diet (SLAC, Changsha, China) and water ad libitum. The formula of mice feed (SLAC) was corn 20%, soybean meal 18%, wheat 38%, fish meal 10%, wheat bran 5%, soybean oil 3%, maltodextrin 2%, minerals and vitamins 2%. The care and use of the animals and experimental protocols were approved by the Guidelines for the Care and Use of Experimental Animals, Central South University of Forestry and
Technology, and the study was approved by the Office of Animal Experiment Ethics, Central South University of Forestry and Technology.

2.2 DSS and β-glucan Treatment

9-week-old mice were divided into four groups (20 mice per group). Control group was not treated with DSS (Purity:100% MP Biomedicals, Illkirch France, molecular weight 36–50 KD) and βG (Purity:97% Barido, Wuxi, China). DSS group only received DSS treatment. For βG treatment, mice were received either 500 mg/kg or 1,000 mg/kg of βG by intragastric administration 7 days before DSS treatment and lasted to the end of the experiment. Colitis was induced by administration of 3% (w/v) DSS in drinking water for 7 days as described previously 22.

2.3 Physiological Index and Histology

Body weight of each mouse was scored during the DSS treatment. The number of mice with diarrhea and/or hematochezia was recorded before sacrifice. The mice were sacrificed under anesthesia 4 h after receiving the last gavage. Spleen weight and colon length were recorded after sacrifice. The disease activity index (DAI) was calculated for each animal by body weight, stool consistency, and stool blood 23. Each score was determined as follow: change in weight (0:<1%, 1: 1–5%, 2: 5–10%, 3: 10%-15% 4:>15%), stool blood (0: negative, 2: positive) or gross bleeding (0: negative, 4: positive), and stool consistency (0: normal, 2: loose stools, 4: diarrhea) as previously described 24. For histological analysis, colon biopsies were fixed in 10%
(v/v) buffered formalin, embedded in paraffin, sectioned at 4 µm (Thermo histostar, USA), and then stained with hematoxyline and eosin (H&E). Stained tissue sections were examined for infiltration of inflammatory cells using fluorescence microscope (Leica, Solms, Germany).

2.4 Myeloperoxidase (MPO) activity Assay

Myeloperoxidase (MPO) activity determination was assessed as modified by Song JL\textsuperscript{25}, Colon tissues (50mg) were washed, homogenized in cooled phosphate buffered saline (PBS, 80mM, pH5.4) containing 0.5% hexadecyl trimethyl ammonium bromide (TCI chemicals, Japan) and centrifuged at 12,000rpm, for 20min at 4˚C. The supernatant was added to a mixture of 150µl 2mM 3,3’,5,5’-tetramethylbenzidine (Sigma-Aldrich, Munich, Germany), 50 µl H\textsubscript{2}O\textsubscript{2} (300mM), 250 µl PBS and incubated for 30min at 25\textdegree. The reaction was quenched by adding 2.5ml H\textsubscript{2}SO\textsubscript{4} (200mM) and the absorbance of the resulting mixture was measured at 450nm with a UV-2401PC spectrophotometer (Shimadzu Corporation, Kyoto, Japan).

2.5 Malondialdehyde (MDA) Assay

Malondialdehyde (MDA) was determined by the method of Xu BL\textsuperscript{26}. Colon tissue (100 mg) was washed, homogenized in cooled PBS. Total protein was determined with a bicinchoninic acid (BCA) assay kit (Pierce, Rockford, USA). The suspension was mixed with 1 ml 0.67% thiobarbituric acid and 1 ml 25% trichloroacetic acid, heated for 45 min at 95˚C and centrifuged at 12,000 rpm for 20
min at 4°C. The volume of MDA was measured at 535nm using spectrophotometer.

2.6 Nitric Oxide (NO) Assay

Nitric oxide (NO) content was calculated by measuring its stable metabolites, nitrite (NO\textsuperscript{2-}) and nitrate (NO\textsuperscript{3-}) as described by Miranda et al\textsuperscript{27}. In brief, colonic homogenate (0.1ml, 20%) was added to 0.1ml of methanol and centrifuged at 3000 rpm for 10 min. An aliquot of the supernatant (0.1 ml) was aspirated and mixed with 0.1 ml of vanadium (III) chloride. Then, 50µl of sulphanilamide solution and 50µl of N-(1-naphthyl) ethylenediamine dihydrochloride (Santa Cruz, Dallas, USA) were added, and the mixture was incubated at 37°C for 30 min. The optical density was measured at 540 nm using the spectrophotometer.

2.7 RNA Isolation and Quantitative RT-PCR

Total RNA was isolated from colon tissues by using Transzol Up (Transgen, Beijing, China). Then the RNA was aliquot, stored in -80°C. 2 µg RNA was reverse-transcribed by High-Capacity cDNA Reverse Transcription Kits (Applied Biosystems, Foster City, USA). Quantitative PCR was performed by the CFX96 Real Time PCR system (Applied Biosystems) using SYBR® Select Master Mix (Applied Biosystems). Gene Expression Assays for mouse proinflammatory cytokines (TNF-α, IL-1β, iNOS, IL-6) and β-actin (control for qPCR) were performed according to the manufacturer’s protocol (Applied Biosystems). For all panels, the bars represent the ratio of target gene to endogenous gene expression, as determined by the software of
2.8 Western Blot Analysis

For Western blot analysis, total proteins were extracted with RIPA buffer (0.1% deoxycholate, 1% Triton X-100, 0.5% SDS, 2 mM PMSF, 2 mM EDTA and 2 mM orthovanadate) supplemented with protease inhibitors (Roche, Basel, Switzerland). The concentration of protein was measured using the BCA protein assay kit. The samples were equally (10-20µg) mixed with sample buffer [125mM Tris-HCl, pH6.8, 4% SDS, 10% 2-mercaptoethanol, 0.3% bromophenol blue, and 20% glycerol], then boiled for 10 min, and subjected to electrophoresis on 10% SDS-PAGE gel. The electrophoresed proteins were transferred from the gel onto a nitrocellulose membrane (Pall, New York, USA). The membrane was then blocked with TBST (Tris-buffered saline containing 0.1%, Tween 20) containing 5% skimmed milk for 1h at room temperature. After being blocked, the membrane was incubated with primary antibody for 1 h. Then, the membrane was incubated with secondary antibody for 1 h. After final wash, the membrane was developed with ECL Plus<sup>TM</sup> Western blotting detection system (Pierce, Rockford, USA) according to the manufacturer’s protocol. The film was imaged in the imaging system (ChemiDoc<sup>TM</sup> XRS+, BIO-RAD). The monoclonal antibody for β-actin (control for WB) and rabbit antibodies for IL-1β, IL-6, TNF-α were obtained from Cell Signaling (Cell Signaling, Boston, USA) and were used at 1:2,000 dilution except for β-actin which was 1:5,000. Peroxidase conjugated goat anti-mouse IgG was from Cell Signaling and used at 1:2,000. Peroxidase conjugated
goat anti-rabbit IgG was from Thermo (Thermo Scientific, USA) and was used at 1:10,000.

2.9 Immunohistochemical (IHC) Analysis

For IHC analysis, tissues were fixed and sectioned as in 2.3. Sections were deparaffinized in xylene, and rehydrated through graded alcohols to distilled water. Slides were then treated with 0.01M citrate buffer (pH 6.0) at 100°C for 3.5 min, followed by cooling on ice for 20 min. Endogenous peroxidase activity was blocked with 1% H$_2$O$_2$ solution for 20 min. Non-specific staining was blocked by incubation of slides in 10% normal goat serum (Vector Laboratories Ltd, Peterborough, UK). The anti-IL-1β primary antibody, anti-TNF-α primary antibody, anti-iNOS primary antibody, anti-IL-6 primary antibodies (1/100 dilution) were incubated with slides either for 1 h at room temperature, or for 16h at 4°C. Following washing with PBS, the slides were incubated with HRP-conjugated secondary antibody for 1 h and then detected with Vectastain Elite ABC kit (Vector Laboratories Ltd, UK) using 3',3'-diaminobenzidine (DAB) as substrate (DAB Peroxidase Substrate Kit, Vector Laboratories Ltd). Slides were counterstained with Mayer’s hematoxylin (sigma-Aldrich, Munich, Germany) and images were captured using microscope.

2.10 Statistical Analysis

Data were expressed as the mean±SD and analyzed using SPSS 17.0 statistical software (SPSS, Chicago, USA). Comparisons among groups were performed using
Bonferroni’s test. For body weight curve, log rank test was used to compare weight change. A value of \( p < 0.05 \) was considered statistically significant.

### 3 Results

#### 3.1 Treatment of oat βG significantly reduced the symptoms of mice

To investigate the effect of βG on colitis, a DSS-induced inflammatory model was established. The DSS induced mice developed a typical IBD-like colitis such as body weight loss, weak movements, reduced food intake, diarrhea and even hematochezia. DSS treatments resulted in significant loss of body weight from 29.94±1.73g to 24.83±1.44g at day 7. The body weights of 500mg/kg.bw and 1000mg/kg.bw of oat β-glucan were 26.74 ± 1.08 and 27.75 ± 1.43, respectively (Figure 1A). For body weight curve, log rank test was used to compare weight change and it has significant difference between DSS group and H-βG group (\( p < 0.05 \)). DSS significantly increased the scores of DAI from about 0.2 to 9.0 (\( p < 0.01 \)), and β-glucan treatment suppressed DSS-induced DAI score increase (\( p < 0.01 \)) (Figure 1B). 80% mice of DSS groups had hematochezia and only 55% and 35% mice in β-glucan groups had the symptom (Figure 1C). All mice in DSS group developed a diarrhea condition at day 7 (100%), and only 60% and 40% of mice in L-βG and H-βG groups developed diarrhea (Figure 1D). The results indicated clearly that β-glucan partially
ameliorated DSS-induced colitis of mice.

3.2 The effect of oat β-glucan on alterations of colon and spleen

DSS administration resulted in colon inflammation associated with hyperemia, ulceration and bowel wall thickening, leading to an increase in macroscopic colon damage and decrease in colon length. In the current study, the average lengths of colon in control group was 10.38 ± 0.76 cm and the average lengths of colon in DSS group was 7.32 ± 0.74 cm, a significant reduction ($p<0.01$). β-glucan at 500 and 1000mg/kg partially prevented shortening of colon length to 7.91±0.82cm and 8.36±0.67cm respectively (comparing with DSS group, both $p<0.01$). Spleen is very important immunity organ and spleen size was related to the severity of inflammatory bowel disease. In this study, the average weight of spleen in DSS group was 0.27±0.05g, significantly higher than that in control group (0.17±0.02g); nevertheless, the spleen weights in two βG groups were 0.24±0.05g and 0.20±0.03g respectively (comparing with DSS group, both $p<0.05$), reduced from DSS group but still higher than that in control group (Figure 2). These data suggested that the administration of βG can ameliorate the DSS-induced colitis.

3.3 The effect of oat β-glucan on histological inflammation characteristics of colon

DSS-induced mice developed immunological abnormalities, such as the prominent inflammatory cell infiltration in the lamina propria of the colon, thickening
of the muscular layer, and crypt damage in the inflamed areas. A histological
inflammation inspection was performed after the sacrifice at day 7 and DSS-treated
mice developed a severely colitis when compared with control group. Oral
administration of βG protected mucosal structural damages of colonic tissues. Mice in
the DSS group developed colonic inflammation such as mucosal hyperemia (red
arrow area in Figure 3 B, F), thickening of the muscular layer (double-headed arrow
area in Figure 3 B), and crypt damage in the inflamed areas, in contrast, mice in the
β-glucan treatment groups exhibited a thinner muscular layer (double-headed arrow
area in Figure 3 C, D). These results indicated that βG can protect colonic tissue
structural damage.

3.4 The effect of oat β-glucan on the content of MPO, MDA and nitrite in colon
tissues
MPO activity, contents of MDA and nitrite are well established biomarkers of
ulcerative colitis. 7 days after DSS treatment, mucosal neutrophils infiltration into the
colon was indirectly assessed by measuring MPO activity. Compared with the control
group, the MPO level in DSS group increased from 9.1±2.1 to 23.1±5.2mU/mg
colonic tissues protein, a dramatic increase (p<0.01). MPO in L-βG and H-βG groups
were 19.1±3.2 and 13.2±5.1, respectively (Fig. 4A). The activity of MPO in β-glucan
administrated groups was obviously lower than that in DSS group (p<0.05) (Figure
4A). Compared with the control group, administration of DSS increased the amount
of MDA from 1.44±0.45 to 5.01±1.37nmol/mg protein in colon (p<0.01). The MDA
in low and high dose βG groups were 3.84±0.41 and 2.36±0.67 nM/mg, respectively, greatly lower than that in DSS group (p< 0.05 and 0.01, respectively) (Figure 4B). The colon tissue nitrile level in DSS group was 4.80±1.25µM, higher than that in control group which 1.81±0.51nmol/mg (p<0.01). Administration of oat β-glucan with low dose produced mild decrease (3.93±1.14, p>0.05) and high dose β-glucan resulted in significant amelioration (2.39±0.81, p<0.01) in nitrate level (Figure 4C).

3.5 The effect of oat β-glucan on the expression of inflammatory factors in colon tissues

To explore the anti-inflammation mechanism of oat β-glucan, the mRNA expression levels of several cytokines and chemokine of colon tissues were analyzed by using real-time PCR. Compared with the control group, the mRNA expression of pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6 and iNOS were increased dramatically in DSS-treated mice. The aberrant mRNA expression of TNF-α and IL-1β induced by DSS was significantly inhibited by oat β-glucan, (500mg/kg and 1000mg/kg βG) (Figure 5). In agreement with qPCR results, western blot show similar results for protein expression of these inflammatory factors (Figure 6). Furthermore, IHC in tissue crosssections confirmed that βG inhibited expression of inflammatory factors in colonic tissues (Figure 7). These results indicated that the oat βG may exert an anti-colitis effect through inhibition of the expression of inflammatory cytokines and chemokine in the gut of colitis mouse by unknown mechanisms.
4 Discussions

In recent years, the use of medicinal foods or phytochemicals extracted from food has become a recognized strategy to combat human diseases such as IBD\textsuperscript{28,29}. Oat is generally considered as healthy food for it contains beneficial components such as $\beta$-glucan. $\beta$-glucan is a linear polymer of D-glucose bonded by $\beta$-(1-4) and $\beta$-(1-3) glucosidic linkages and it has a wide distribution of molecular weight. Different extract methods may turn out different molecular weight products and results in different biological effects\textsuperscript{30}. In this study, the $\beta$-glucan was extracted in hot water, and then starch was removed by amylase and protein was removed by the method of isoelectric precipitation. The molecular weight of oat $\beta$-glucan (about 80\%) is around $2.4 \times 10^6$ Da in this study. Our data showed that the range of oat $\beta$-glucan can effectively ameliorates DSS-induced colitis in mice.

The $\beta$-glucan was proven to be effective in treating metabolism-associated diseases, such as anti-hypercholesterolaemia\textsuperscript{31}, anti-insulin resistance\textsuperscript{32} and anti-obesity\textsuperscript{33}. In this study, the treatments of $\beta$-glucan not only remarkably reduced the clinical symptoms of mice such as body weight loss, diarrhea, hematochezia, shortening of colon length and increasing of spleen weight but also reduced the histological score. The present study is the first study to demonstrate the effect of $\beta$-glucan on ameliorating IBD-like colitis.

Histopathological evaluation further confirmed that $\beta$-glucan prevented DSS-mediated destruction of epithelium crypt structure. Since DSS-induced mice
developed immunological abnormalities, such as the prominent inflammatory cell infiltration in the lamina propria of the colon, thickening of the muscular layer and crypt damage in the inflamed areas and extensive infiltration of leukocytes in the mucosa. β-glucan treatment obviously reduced the infiltration of leukocytes and mucosal damage, which may be related with the down-regulation of MPO activity and MDA and NO level. These results were supported by some other natural extracts such as mangiferin\textsuperscript{30} and myricetin\textsuperscript{34}, which were all proven to be effective in preventing colitis as well as decreasing the MPO activity and MDA and NO contents.

Moreover, oral administration of β-glucan significantly reduced TNF-α, IL-1β, IL-6 and iNOS mRNA and protein expression in the colonic tissues of DSS-induced colitis and were confirmed by IHC. TNF-α is regarded as a pro-inflammatory cytokine that plays a pivotal role in the DSS-induced colitis. Indeed, clinical studies have discovered that TNF-α level in serum is elevated in patients with colitis\textsuperscript{35}. A lot of a natural extracts such as mangiferin\textsuperscript{30} and lentinan\textsuperscript{17} were proven to possess an anti-colitis function correlated with decreasing TNF-α expression level. IL-1β is up-regulated in colitis patients\textsuperscript{36} and in animal models\textsuperscript{37} since it is one of the primary drivers of inflammation and is mainly produced by infiltrating lamina properia monocytes including macrophages in the colitis mucosa\textsuperscript{38}. Macrophages are recruited and activated from peripheral blood into the inflamed colon\textsuperscript{39}. The mature IL-1β together with other cytokines causes cascade of inflammatory responses and tissue damage\textsuperscript{40}. The binding between IL-1 and IL-1 receptor activates the NF-κB signal-transduction pathway, resulting in the upregulation of other pro-inflammatory
mediators such as TNF-α and IL-6, which would cause more inflammation. The later one was now determined as an important cytokine in the pathogenesis of IBD. Some strategies such as ustekinumab, a monoclonal antibody, have in later years been developed to target the IL-6 family of receptors in IBD patients. DSS-induced colitis increased the expression level of iNOS protein in the surface epithelial cells. Similar to the IL-1β, the iNOS expression increased during inflammation is not surprising since iNOS has been localized in macrophages and infiltrating neutrophils in the colonic mucosa and submucosa in animal models of colitis as well as in colitis patients. During the established DSS-induced colitis, the role of iNOS was even more extensive, since iNOS was responsible for the increased colonic mucus thickness seen during the colitis model.

These pro-inflammatory cytokines amplify the inflammatory cascade of inflammatory mediators, destructive enzymes, and free radicals that cause tissue damage. Therefore, drugs or natural extracts which show the capacity of suppressing the eruption of these cytokines turned out to be considerable ways of treating IBD. Actually, several biologic agents or medicines such as infliximab and ustekinumab have been shown to be effective in human trials by blockade of these cytokines.

Different concentrations of β-glucan have been proven to decrease the expression of inflammatory cytokines in this study. These results indicated that β-glucan attenuates colon inflammation through the blockage of the expression of these cytokines in DSS-induced colitis model. β-glucan varies in different origins in molecular weight and fine structure, ratio,
lengths, number and distribution of cellulosic oligosaccharides. Different sizes, branching patterns and conformation may have significantly variable anti-inflammatory potency. Besides the anti-inflammatory effects of yeast glucan\textsuperscript{15}, schizophyllan\textsuperscript{16} and lentinan\textsuperscript{17}, there was even a report showed that an insoluble/particle glucan strongly induced inflammatory cytokine production instead of decreased them\textsuperscript{47}. Therefore, the relationship between structure and anti-inflammatory activity of \(\beta\)-glucan was not clear. Future effort should be focused on this field.

In conclusion, our results demonstrated that the \(\beta\)-glucan, when given orally, exerted an anti-inflammatory effect in DSS-induced colitis in mice. The anti-inflammatory properties of \(\beta\)-glucan were associated with the inhibition of the DSS-induced overexpression of cytokines such as TNF-\(\alpha\), IL-1\(\beta\) and iNOS. Our data suggest that \(\beta\)-glucan has the potential to serve as an effective anti-IBD therapy.

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**Figure Legends**

**Figure 1.** Effect of oat β-glucan on the phenotype (body weight, disease activity) in DSS-induced colitis.

Oat β-glucan and/or DSS were administrated for 7 days, and all mice were scarified and tissue samples were taken for analysis. **A:** Body weight. **B:** DAI score. **C:** Percentage of hematochezia. **D:** Percentage of diarrhea of mice. The data represented mean±SD of 20 mice per group; data were compared between DSS and β-glucan treated groups; #: $p$ value is less than 0.05; *: $p$ value is less than 0.01, ##: $p$ value is more than 0.05. **L-βG:** low dosage β-glucan (500 mg/kg) and **H-βG:** high dosage β-glucan (1000 mg/kg).

**Figure 2.** Effect of oat β-glucan on the changes of colon and spleen in DSS-induced colitis.

After mice were scarified, colons and spleens were rapidly removed and processed for analysis. **A:** The representative picture of colon after DSS or/and β-glucan treatments. **B:** The length of colon after DSS or/and β-glucan treatments. **C:** The representative picture of spleen after DSS or/and β-glucan treatments. **D:** The weights of spleen after DSS or/and β-glucan treatments. The data represented mean±SD of 20 mice per group; data were compared between DSS and β-glucan treated groups; #: $p$ value is less than 0.05; *: $p$ value is less than 0.01. **L-βG:** low dosage β-glucan (500 mg/kg) and **H-βG:** high dosage β-glucan (1000 mg/kg).
Figure 3. Effect of oat β-glucan on the morphology of colon tissues by HE staining in DSS-induced colitis.

A: HE staining of colon tissues (100× magnification). B: HE staining of colon tissues (200× magnification). Colon tissues from control mice did not show any histological modifications, DSS-induced colon tissue injury was associated with partial destruction of epithelial architecture such as: loss of crypts and epithelial integrity, submucosal edema, and intense inflammatory cellular infiltration. Treatments with various dosages attenuated the injury of colon tissues. Black arrow: muscular layer. Red arrow: mucosal hyperemia. L-βG: low dosage β-glucan (500 mg/kg) and H-βG: high dosage β-glucan (1000 mg/kg). The scale bar represents 50µm.

Figure 4. Changes of MPO, MDA, and nitrate level of colon tissues after treatment with DSS and/or oat β-glucan.

A: Effect of oat β-glucan on MPO activity of colon tissues. B: Effect of oat β-glucan on MDA level of colon tissues. C: Effect of oat β-glucan on nitrite level as expressed as sum of nitrite and nitrate. #: p value is less than 0.05; *: p value is less than 0.01. L-βG: low dosage β-glucan (500 mg/kg) and H-βG: high dosage β-glucan (1000 mg/kg).

Figure 5. The mRNA expression levels of inflammatory factors in colonic tissues were inhibited by β-glucan treatment. Gene Expression Assays for mouse proinflammatory cytokines were performed according to the manufacturer’s
protocol

A: The relative mRNA expression levels of TNF-α by RT-qPCR analysis. B: The relative expression levels of IL-1β. C: The relative expression levels of IL-6. D: The relative expression levels of iNOS. #: p value is less than 0.05; *: p value is less than 0.01, #: p value is more than 0.05. L-βG: low dosage β-glucan (500mg/kg) and H-βG: high dosage β-glucan (1000mg/kg).

Figure 6. The protein expression levels of inflammatory factors in colonic tissues were inhibited by oat βG treatment.

L-βG: low dosage β-glucan (500mg/kg) and H-βG: high dosage β-glucan (1000mg/kg).

Figure 7. IHC staining for protein expression of inflammatory factors in colonic tissues after treatments of DSS and/or oat β-glucan.

The colon was washed and cut into 4 μm sections and IHC staining was performed as described in the Materials and methods. Positive staining was seen as brown for TNF-α, IL-1, IL-6 and iNOS in each row respectively. Representative colon tissue sections are shown at 200× magnification and the scale bar represents 50μm in length. L-βG: low dosage β-glucan (500mg/kg) and H-βG: high dosage β-glucan (1000mg/kg).
Oral administration of oat β-glucan ameliorates DSS induced colitis in mice through decreasing the expression of inflammatory cytokines TNF-α, IL-1β, IL-6 and iNOS.
3% (w/v) DSS 7 days

TNF-α ↑↓
IL-1β ↑↓
IL-6 ↑↓
iNOS ↑↓

Oat β-Glucan

Severe IBD: DAI ↑↓
body weight ↓↑
colon length ↓↑
colon weight ↑↓