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1 Title:

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3 **Oat β-glucan ameliorates dextran dolfate sodium (DSS)-induced**

4 **ulcerative colitis in mice**

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22 ## Abstract

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

38 **Key Words:** Oat β-glucan; colitis; TNF-α; IL-1β; IL-6; iNOS

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41 ## 1. Introduction

42 Inflammatory bowel disease (IBD) is inflammation within the gastrointestinal
43 (GI) tract characterized by chronic or relapsing immune system activation. There are
44 two types of IBD: ulcerative colitis (UC) and Crohn's disease[1,2]. While the clinical
45 features of Crohn's disease include pain, diarrhea, narrowing of the intestines lumen
46 leading to strictural and bowel obstruction, abscess formation, and fistulization of the
47 skin and internal organs; the clinical features of UC include severe diarrhea, blood
48 loss, and progressive loss of peristaltic function[2,3]. Although the precise etiology of
49 colitis is not very clear, it is now well recognized that inflammation of the intestinal
50 mucosa is characterized by chronic inflammatory cell infiltration composed mainly of
51 neutrophils and macrophages, an effect that is accompanied by production of
52 pro-inflammatory cytokines, like interleukin-1β (IL-1β), interleukin-6 (IL-6), tumor
53 necrosis factor-α (TNF-α), inducible nitric oxide synthase (iNOS) and so on[4].

54 UC can be treated with a number of medications including 5-ASA drugs such as
55 sulfasalazine and mesalazine. Corticosteroids such as prednisone can also be used due
56 to their immunosuppressing and short term healing properties. However, due to the
57 high risks of adverse effects such as sleep, mood disturbance, dyspepsia, or glucose
58 intolerance, corticosteroids are not suitable as long term therapies[5], and new strategies
59 for adjunct therapies are needed. To imitate the IBD, a classic model of colitis model
60 induced by dextran sulfate sodium (DSS) was set up. DSS is a heparin-like
61 polysaccharide that has been successfully used to induce colonic mucosal injury in
62 mice. Colitis induced by this model exhibits characteristics resembling human UC,

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63 including weight loss, severe diarrhea, rectal bleeding, loss of epithelium followed by
64 ulceration and leukocyte infiltration[6]. Several studies reported that plant extracts such
65 as arvelexin and flavonoids show anti-inflammatory activity in this model[7,8]. Kimchi,
66 betaine and isorhamnetin can inhibit expression of TNF-α[9-11]. Some other plant
67 extracts like Serpylli herba extract showed beneficial effect against inflammation via
68 inhibiting expression of IL-1β[9]. Plants contain many beneficial nutrients
69 (phytochemicals) which may protect against inflammation such as colitis without side
70 effect.

71 β-glucans are major structural components of cell walls of fungi and plants such
72 as mushrooms, yeast, bacteria, oat, barley, seaweeds and algae. β-glucans are
73 polysaccharides of D-glucose monomers linked by β-glycosidic bonds with different
74 molecular mass, solubility, viscosity, and three-dimensional configurations. β-glucans
75 can promote the functional activities of macrophages and enhance the anti-microbial
76 activities of mononuclear cells and neutrophils[12,13]. β-hydroxy-β-methyl-butyrate
77 (HMB), a β-1,3/1,6-D-glucan, showed strong therapeutic effect on canine colitis by
78 decreasing IL-6 and increasing IL-10 concentrations[14]. Yeast glucan, also a β-1, 3/1,
79 6-D-glucan, possess a beneficial effect on mice intestinal inflammation caused by
80 DSS[15]. Schizophyllan (SPG), a member of β-(1-3) glucan family, can effectively
81 alleviate the colitis *in vivo*[16]. Lentinan, a β-glucan isolated from Lentinula edodes,
82 ameliorated DSS induced colitis and reduced IL-8 in Caco-2 cells[17].

83 Oat β-glucan (βG) is a β-1,3/1,4-D-glucan and its construct is different from
84 β-1,3/1,6-D-glucan of mushrooms and yeast. It has been reported that βG possess

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85 many physiological functions such as anti-insulin resistance, anti-obesity and
86 anti-oxidant[18,19]. It is demonstrated that the βG can fight against high risk of
87 cardiovascular diseases by increasing insulin sensitivity index and increasing activity
88 of intestinal Na^+/K^+-ATPase and Ca^{2+}/Mg^{2+}-ATPase[18]. Oat β-glucan improved
89 metabolic indexes of obesity mice [19] and controlled human appetite[20]. Interestingly,
90 βG may also inhibit nonalcoholic steatohepatitis and ameliorate inflammation, but the
91 molecular mechanism is not clear[21]. Until today, there is no report about the anti-
92 colitis effect of βG. In this study, we assessed the effect of βG on the DSS-induced
93 colitis in mice and found that it not only suppressed the shortening and swelling of the
94 intestine, but also inhibited the expression of pro-inflammatory factors in colonic
95 tissues as well.

96

97 2 Materials and Methods

98 2.1 Experimental Animals

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

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107 Technology, and the study was approved by the Office of Animal Experiment Ethnics,
108 Central South University of Forestry and Technology.
109

110 **2.2 DSS and β-glucan Treatment**

111 9-week-old mice were divided into four groups (20 mice per group). Control
112 group was not treated with DSS (Purity:100% MP Biomedicals , Illkirch France.
113 molecular weight 36–50 KD) and βG (Purity:97% Barido, Wuxi, China). DSS group
114 only received DSS treatment. For βG treatment, mice were received either 500 mg/kg
115 or 1,000mg/kg of βG by intragastric administration 7 days before DSS treatment and
116 lasted to the end of the experiment. Colitis was induced by administration of 3% (w/v)
117 DSS in drinking water for 7 days as described previously[22].
118

119 **2.3 Physiological Index and Histology**

120 Body weight of each mouse was scored during the DSS treatment. The number
121 of mice with diarrhea and/or hematochezia was recorded before sacrifice. The mice
122 were sacrificed under anesthesia 4 h after receiving the last gavage. Spleen weight and
123 colon length were recorded after sacrifice. The disease activity index (DAI) was
124 calculated for each animal by body weight, stool consistency, and stool blood[23]. Each
125 score was determined as follow: change in weight (0:<1%, 1: 1–5%, 2: 5–10%,3:
126 10%-15% 4:>15%), stool blood (0: negative, 2: positive) or gross bleeding (0:
127 negative, 4: positive), and stool consistency (0: normal, 2: loose stools, 4: diarrhea) as
128 previously described[24]. For histological analysis, colon biopsies were fixed in 10%

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129 (v/v) buffered formalin, embedded in paraffin, sectioned at 4 μm (Thermo histostar,
130 USA), and then stained with hematoxyline and eosin（H&E）. Stained tissue sections
131 were examined for infiltration of inflammatory cells using fluorescence microscope
132 (Leica, Solms, Germany).
133
134 **2.4 Myeloperoxidase (MPO) activity Assay**
135 Myeloperoxidase (MPO) activity determination was assessed as modified by
136 Song JL[25], Colon tissues (50mg) were washed, homogenized in cooled phosphate
137 buffered saline (PBS, 80mM, pH5.4) containing 0.5% hexadecyl trimethyl ammonium
138 bromide (TCI chemicals, Japan) and centrifuged at 12,000rpm, for 20min at 4˚C. The
139 supernatant was added to a mixture of 150μl 2mM 3,3’,5,5’-tetramethylbenzidine
140 (Sigma-Aldrich, Munich, Germany), 50 μl H_2O_2 (300mM), 250 μl PBS and incubated
141 for 30min at 25 . The reaction was quenched by adding 2.5ml H_2SO_4 (200mM) and
142 the absorbance of the resulting mixture was measured at 450nm with a UV-2401PC
143 spectrophotometer (Shimadzu Corporation, Kyoto, Japan).
144
145 **2.5 Malondialdehyde (MDA) Assay**
146 Malondialdehyde (MDA) was determined by the method of Xu BL[26]. Colon
147 tissue (100 mg) was washed, homogenized in cooled PBS. Total protein was
148 determined with a bicinchoninic acid (BCA) assay kit (Pierce, Rockford, USA). The
149 suspension was mixed with 1 ml 0.67% thiobarbituric acid and 1 ml 25%
150 trichloroacetic acid, heated for 45 min at 95˚C and centrifuged at 12,000 rpm for 20

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151 min at 4˚C. The volume of MDA was measured at 535nm using spectrophotometer.

152

153 **2.6 Nitric Oxide (NO) Assay**

154 Nitric oxide (NO) content was calculated by measuring its stable metabolites,
155 nitrite (NO^{2-}) and nitrate (NO^{3-}) as described by Miranda et al[27]. In brief, colonic
156 homogenate (0.1ml, 20%) was added to 0.1ml of methanol and centrifuged at 3000
157 rpm for 10 min. An aliquot of the supernatant (0.1 ml) was aspirated and mixed with
158 0.1 ml of vanadium (III) chloride. Then, 50μl of sulphanilamide solution and 50μl of
159 N-(1-naphthyl) ethylenediamine dihydrochloride (Santa Cruz, Dallas, USA) were
160 added, and the mixture was incubated at 37˚C for 30 min. The optical density was
161 measured at 540 nm using the spectrophotometer.

162

163 **2.7 RNA Isolation and Quantitative RT-PCR**

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

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173 PCR system (Applied Biosystems)

174

175 2.8 Western Blot Analysis

176 For Western blot analysis, total proteins were extracted with RIPA buffer (0.1%
177 deoxycholate, 1% Triton X-100, 0.5% SDS, 2 mM PMSF, 2 mM EDTA and 2 mM
178 orthovanadate) supplemented with protease inhibitors (Roche, Basel, Switzerland).
179 The concentration of protein was measured using the BCA protein assay kit. The
180 samples were equally (10-20μg) mixed with sample buffer [125mM Tris-HCl, pH6.8,
181 4% SDS, 10% 2-mercaptoethanol, 0.3% bromophenol blue, and 20% glycerol], then
182 boiled for 10 min, and subjected to electrophoresis on 10% SDS-PAGE gel. The
183 electrophoresed proteins were transferred from the gel onto a nitrocellulose membrane
184 (Pall, New York, USA). The membrane was then blocked with TBST (Tris-buffered
185 saline containing 0.1%, Tween 20) containing 5% skimmed milk for 1h at room
186 temperature. After being blocked, the membrane was incubated with primary antibody
187 for 1 h. Then, the membrane was incubated with secondary antibody for 1 h. After
188 final wash, the membrane was developed with ECL Plus™ Western blotting detection
189 system (Pierce, Rockford, USA) according to the manufacturer's protocol. The film
190 was imaged in the imaging system (ChemiDoc™ XRS+, BIO-RAD). The monoclonal
191 antibody for β-actin (control for WB) and rabbit antibodies for IL-1β, IL-6, TNF-α
192 were obtained from Cell Signaling (Cell Signaling, Boston, USA) and were used at
193 1:2,000 dilution except for β-actin which was 1:5,000. Peroxidase conjugated goat
194 anti-mouse IgG was from Cell Signaling and used at 1:2,000. Peroxidase conjugated

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195 goat anti-rabbit IgG was from Thermo (Thermo Scientific, USA) and was used at
196 1:10,000.
197
198 **2.9 Immunohistochemical (IHC) Analysis**
199 For IHC analysis, tissues were fixed and sectioned as in 2.3. Sections were
200 deparaffinized in xylene, and rehydrated through graded alcohols to distilled water.
201 Slides were then treated with 0.01M citrate buffer (pH 6.0) at 100°C for 3.5 min,
202 followed by cooling on ice for 20 min. Endogenous peroxidase activity was blocked
203 with 1% H_2O_2 solution for 20 min. Non-specific staining was blocked by incubation
204 of slides in 10% normal goat serum (Vector Laboratories Ltd, Peterborough, UK). The
205 anti-IL-1β primary antibody, anti-TNF-α primary antibody, anti-iNOS primary
206 antibody, anti-IL-6 primary antibodies (1/100 dilution) were incubated with slides
207 either for 1 h at room temperature, or for 16h at 4℃. Following washing with PBS,
208 the slides were incubated with HRP-conjugated secondary antibody for 1 h and then
209 detected with Vectastain Elite ABC kit (Vector Laboratories Ltd, UK) using 3,
210 3'-diaminobenzidine (DAB) as substrate (DAB Peroxidase Substrate Kit, Vector
211 Laboratories Ltd). Slides were counterstained with Mayer's hematoxylin
212 (sigma-Aldrich, Munich, Germany) and images were captured using microscope.
213
214 **2.10 Statistical Analysis**
215 Data were expressed as the mean±SD and analyzed using SPSS 17.0 statistical
216 software (SPSS, Chicago, USA). Comparisons among groups were performed using

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217 Bonferroni's test. For body weight curve, log rank test was used to compare weight
218 change. A value of $p< 0.05$ was considered statistically significant.
219
220
221
222
223 ## 3 Results

224 **3.1 Treatment of oat βG significantly reduced the symptoms of mice**

225 To investigate the effect of βG on colitis, a DSS-induced inflammatory model
226 was established. The DSS induced mice developed a typical IBD-like colitis such as
227 body weight loss, weak movements, reduced food intake, diarrhea and even
228 hematochezia. DSS treatments resulted in significant loss of body weight from
229 29.94±1.73g to 24.83±1.44g at day 7. The body weights of 500mg/kg.bw and
230 1000mg/kg.bw of oat β-glucan were 26.74 ± 1.08 and 27.75 ± 1.43, respectively
231 (Figure 1A). For body weight curve, log rank test was used to compare weight change
232 and it has significant difference between DSS group and H-βG group ($p<0.05$). DSS
233 significantly increased the scores of DAI from about 0.2 to 9.0 (p<0.01), and β-glucan
234 treatment suppressed DSS-induced DAI score increase ($p<0.01$) (Figure 1B). 80%
235 mice of DSS groups had hematochezia and only 55% and 35% mice in β-glucan
236 groups had the symptom (Figure 1C). All mice in DSS group developed a diarrhea
237 condition at day 7 (100%), and only 60% and 40% of mice in L-βG and H-βG groups
238 developed diarrhea (Figure 1D). The results indicated clearly that β-glucan partially

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239 ameliorated DSS-induced colitis of mice.

240

241 **3.2 The effect of oat β-glucan on alterations of colon and spleen**

242 DSS administration resulted in colon inflammation associated with hyperemia,
243 ulceration and bowel wall thickening, leading to an increase in macroscopic colon
244 damage and decrease in colon length. In the current study, the average lengths of
245 colon in control group was 10.38 ± 0.76 cm and the average lengths of colon in DSS
246 group was 7.32 ± 0.74 cm, a significant reduction.(p<0.01). β-glucan at 500 and
247 1000mg/kg partially prevented shortening of colon length to 7.91±0.82cm and
248 8.36±0.67cm respectively (comparing with DSS group, both p<0.01). Spleen is very
249 important immunity organ and spleen size was related to the severity of inflammatory
250 bowel disease. In this study, the average weight of spleen in DSS group was
251 0.27±0.05g, significantly higher than that in control group (0.17±0.02g); nevertheless,
252 the spleen weights in two βG groups were 0.24±0.05g and 0.20±0.03g respectively
253 (comparing with DSS group, both p<0.05), reduced from DSS group but still higher
254 than that in control group (Figure 2). These data suggested that the administration of
255 βG can ameliorate the DSS-induced colitis.

256

257 **3.3 The effect of oat β-glucan on histological inflammation characteristics of**
258 **colon**

259 DSS-induced mice developed immunological abnormalities, such as the
260 prominent inflammatory cell infiltration in the lamina propria of the colon, thickening

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261 of the muscular layer, and crypt damage in the inflamed areas. A histological
262 inflammation inspection was performed after the sacrifice at day 7 and DSS-treated
263 mice developed a severely colitis when compared with control group. Oral
264 administration of βG protected mucosal structural damages of colonic tissues. Mice in
265 the DSS group developed colonic inflammation such as mucosal hyperemia (red
266 arrow area in Figure 3 B, F), thickening of the muscular layer (double-headed arrow
267 area in Figure 3 B), and crypt damage in the inflamed areas, in contrast, mice in the
268 β-glucan treatment groups exibited a thinner muscular layer (double-headed arrow
269 area in Figure 3 C, D). These results indicated that βG can protect colonical tissue
270 structural damage.
271

272 **3.4 The effect of oat β-glucan on the content of MPO, MDA and nitrite in colon**
273 **tissues**

274 MPO activity, contents of MDA and nitrite are well established biomarkers of
275 ulcerative colitis. 7 days after DSS treatment, mucosal neutrophils infiltration into the
276 colon was indirectly assessed by measuring MPO activity. Compared with the control
277 group, the MPO level in DSS group increased from 9.1±2.1 to 23.1±5.2mU/mg
278 colonic tissues protein, a dramatic increase (p<0.01). MPO in L-βG and H-βG groups
279 were 19.1±3.2 and 13.2±5.1, respectively (Fig. 4A). The activity of MPO in β-glucan
280 administrated groups was obviously lower than that in DSS group (p<0.05) (Figure
281 4A). Compared with the control group, administration of DSS increased the amount
282 of MDA from 1.44±0.45 to 5.01±1.37nmol/mg protein in colon (p<0.01). The MDA

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283 in low and high dose βG groups were 3.84±0.41 and 2.36±0.67 nM/mg, respectively,
284 greatly lower than that in DSS group (*p*< 0.05 and 0.01, respectively) (Figure 4B).
285 The colon tissue nitrile level in DSS group was 4.80±1.25μM, higher than that in
286 control group which 1.81±0.51nmol/mg (*p*<0.01). Administration of oat β-glucan with
287 low dose produced mild decrease (3.93±1.14, *p*>0.05) and high dose β-glucan resulted
288 in significant amelioration (2.39±0.81, *p*<0.01) in nitrate level (Figure 4C).
289

290 **3.5 The effect of oat β-glucan on the expression of inflammatory factors in colon**
291 **tissues**

292 To explore the anti-inflammation mechanism of oat β-glucan, the mRNA
293 expression levels of several cytokines and chemokine of colon tissues were analyzed
294 by using real-time PCR. Compared with the control group, the mRNA expression of
295 pro-inflammatory cytokines such as TNF-α, IL-1β, IL-6 and iNOS were increased
296 dramatically in DSS-treated mice. The aberrant mRNA expression of TNF-α and
297 IL-1β induced by DSS was significantly inhibited by oat β-glucan, (500mg/kg and
298 1000mg/kg βG) (Figure 5). In agreement with qPCR results, western blot show
299 similar results for protein expression of these inflammatory factors (Figure 6).
300 Furthermore, IHC in tissue crosssections confirmed that βG inhibited expression of
301 inflammatory factors in colonic tissues (Figure 7). These results indicated that the oat
302 βG may exert an anti-colitis effect through inhibition of the expression of
303 inflammatory cytokines and chemokine in the gut of colitis mouse by unknown
304 mechanisms

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305

306 ## 4 Discussions

307 In recent years, the use of medicinal foods or phytochemicals extracted from
308 food has become a recognized strategy to combat human diseases such as IBD[28,29].
309 Oat is generally considered as healthy food for it contains beneficial components such
310 as β-glucan. β-glucan is a linear polymer of D-glucose bonded by β-(1-4) and β-(1-3)
311 glucosidic linkages and it has a wide distribution of molecular weight. Different
312 extract methods may turn out different molecular weight products and results in
313 different biological effects[30]. In this study, the β-glucan was extracted in hot water,
314 and then starch was removed by amylase and protein was removed by the method of
315 isoelectric precipitation. The molecular weight of oat β-glucan (about 80%) is around
316 2.4×10^6 Da in this study. Our data showed that the range of oat β-glucan can
317 effectively ameliorates DSS-induced colitis in mice.

318 The β-glucan was proven to be effective in treating metabolism-associated
319 diseases, such as anti-hypercholesterolaemia[31], anti-insulin resistance[32] and
320 anti-obesity[33]. In this study, the treatments of β-glucan not only remarkably reduced
321 the clinical symptoms of mice such as body weight loss, diarrhea, hematochezia,
322 shortening of colon length and increasing of spleen weight but also reduced the
323 histological score. The present study is the first study to demonstrate the effect of
324 β-glucan on ameliorating IBD-like colitis.

325 Histopathological evaluation further confirmed that β-glucan prevented
326 DSS-mediated destruction of epithelium crypt structure. Since DSS-induced mice

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327 developed immunological abnormalities, such as the prominent inflammatory cell
328 infiltration in the lamina propria of the colon, thickening of the muscular layer and
329 crypt damage in the inflamed areas and extensive infiltration of leukocytes in the
330 mucosa. β-glucan treatment obviously reduced the infiltration of leukocytes and
331 mucosal damage, which may be related with the down-regulation of MPO activity and
332 MDA and NO level. These results were supported by some other natural extracts such
333 as mangiferin[30] and myricetin[34], which were all proven to be effective in preventing
334 colitis as well as decreasing the MPO activity and MDA and NO contents.

335 Moreover, oral administration of β-glucan significantly reduced TNF-α, IL-1β,
336 IL-6 and iNOS mRNA and protein expression in the colonic tissues of DSS-induced
337 colitis and were confirmed by IHC. TNF-α is regarded as a pro-inflammatory
338 cytokine that plays a pivotal role in the DSS-induced colitis. Indeed, clinical studies
339 have discovered that TNF-α level in serum is elevated in patients with colitis[35]. A lot
340 of a natural extracts such as mangifering[30] and lentinan[17] were proven to possess an
341 anti-colitis function correlated with decreasing TNF-α expression level. IL-1β is
342 up-regulated in colitis patients[36] and in animal models[37] since it is one of the primary
343 drivers of inflammation and is mainly produced by infiltrating lamina properia
344 monocytes including macrophages in the colitis mucosa[38]. Macrophages are recruited
345 and activated from peripheral blood into the inflamed colon[39]. The mature IL-1β
346 together with other cytokines causes cascade of inflammatory responses and tissue
347 damage[40]. The binding between IL-1 and IL-1 receptor activates the NF-κB
348 signal-transduction pathway, resulting in the upregulation of other pro-inflammatory

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349 mediators such as TNF-α and IL-6[41], which would cause more inflammation. The
350 later one was now determined as an important cytokine in the pathogenesis of IBD.
351 Some strategies such as ustekinumab, a monoclonal antibody, have in later years been
352 developed to target the IL-6 family of receptors in IBD patients[42]. DSS-induced colitis
353 increased the expression level of iNOS protein in the surface epithelial cells[43]. Similar
354 to the IL-1β, the iNOS expression increased during inflammation is not surprising
355 since iNOS has been localized in macrophages and infiltrating neutrophils in the
356 colonic mucosa and submucosa in animal models of colitis[44] as well as in colitis
357 patients[45]. During the established DSS-induced colitis, the role of iNOS was even
358 more extensive, since iNOS was responsible for the increased colonic mucus
359 thickness seen during the colitis model[46].

360 These pro-inflammatory cytokines amplify the inflammatory cascade of
361 inflammatory mediators, destructive enzymes, and free radicals that cause tissue
362 damage. Therefore, drugs or natural extracts which show the capacity of suppressing
363 the eruption of these cytokines turned out to be considerable ways of treating IBD.
364 Actually, several biologic agents or medicines such as infliximab and ustekinumab
365 have been shown to be effective in human trials by blockade of these cytokines[42].
366 Different concentrations of β-glucan have been proven to decrease the expression of
367 inflammatory cytokines in this study. These results indicated that β-glucan attenuates
368 colon inflammation through the blockage of the expression of these cytokines in
369 DSS-induced colitis model.

370 β-glucan varies in different origins in molecular weight and fine structure, ratio,

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371 lengths, number and distribution of cellulosic oligosaccharides. Different sizes,
372 branching patterns and conformation may have significantly variable
373 anti-inflammatory potency. Besides the anti-inflammatory effects of yeast glucan[15],
374 schizophyllan[16] and lentinan[17], there was even a report showed that an
375 insoluble/particle glucan strongly induced inflammatory cytokine production instead
376 of decreased them[47]. Therefore, the relationship between structure and
377 anti-inflammatory activity of β-glucan was not clear. Future effort should be focused
378 on this field.

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

384

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524 ## Figure Legends

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

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

534

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

537 After mice were scarified, colons and spleens were rapidly removed and processed for
538 analysis. **A:** The representative picture of colon after DSS or/and β-glucan treatments.
539 **B:** The length of colon after DSS or/and β-glucan treatments. **C:** The representative
540 picture of spleen after DSS or/and β-glucan treatments. **D:** The weights of spleen after
541 DSS or/and β-glucan treatments. The data represented mean±SD of 20 mice per group;
542 data were compared between DSS and β-glucan treated groups; #: *p* value is less than
543 0.05; *: *p* value is less than 0.01. L-βG: low dosage β-glucan (500 mg/kg) and H-βG:
544 high dosage β-glucan (1000 mg/kg).

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546 **Figure 3. Effect of oat β-glucan on the morphology of colon tissues by HE**
547 **staining in DSS-induced colitis.**
548 A: HE staining of colon tissues (100× magnification). B: HE staining of colon tissues
549 (200× magnification). Colon tissues from control mice did not show any histological
550 modifications, DSS-induced colon tissue injury was associated with partial
551 destruction of epithelial architecture such as: loss of crypts and epithelial integrity,
552 submucosal edema, and intense inflammatory cellular infiltration. Treatments with
553 various dosages attenuated the injury of colon tissues. Black arrow: muscular layer
554 Red arrow: mucosal hyperemia. L-βG: low dosage β-glucan (500 mg/kg) and H-βG:
555 high dosage β-glucan (1000 mg/kg). The scale bar represents 50μm.
556
557 **Figure 4. Changes of MPO, MDA, and nitrate level of colon tissues after**
558 **treatment with DSS and/or oat β-glucan.**
559 **A:** Effect of oat β-glucan on MPO activity of colon tissues. **B:** Effect of oat
560 β-glucan on MDA level of colon tissues. **C:** Effect of oat β-glucan on nitrite level as
561 expressed as sum of nitrite and nitrate. #: p value is less than 0.05; *: p value is less
562 than 0.01. L-βG: low dosage β-glucan (500 mg/kg) and H-βG: high dosage β-glucan
563 (1000 mg/kg).
564
565 **Figure 5. The mRNA expression levels of inflammatory factors in colonic tissues**
566 **were inhibited by β-glucan treatment. Gene Expression Assays for mouse**
567 **proinflammatory cytokines were performed according to the manufacturer's**

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568 **protocol**

569 **A:** The relative mRNA expression levels of TNF-α by RT-qPCR analysis. **B:** The
570 relative expression levels of IL-1β. **C:** The relative expression levels of IL-6. **D:** The
571 relative expression levels of iNOS. #: *p* value is less than 0.05； *: *p* value is less than
572 0.01, ##: *p* value is more than 0.05. L-βG: low dosage β-glucan (500mg/kg) and H-βG:
573 high dosage β-glucan (1000mg/kg).

574

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

577 L-βG: low dosage β-glucan (500mg/kg) and H-βG: high dosage β-glucan
578 (1000mg/kg).

579

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

582 The colon was washed and cut into 4 μm sections and IHC staining was
583 performed as described in the Materials and methods. Positive staining was seen as
584 brown for TNF-α ,IL-1 ,IL-6 and iNOS in each row respectively. Representative colon
585 tissue sections are shown at 200× magnification and the scale bar represents 50μm in
586 length. L-βG: low dosage β-glucan (500mg/kg) and H-βG: high dosage β-glucan
587 (1000mg/kg).

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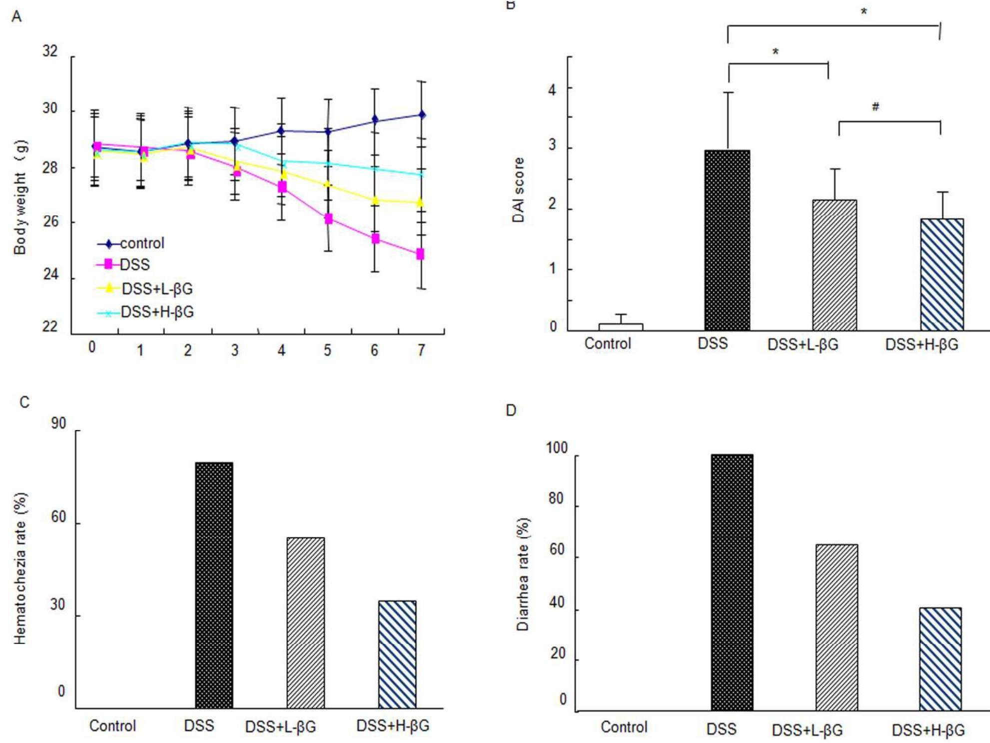

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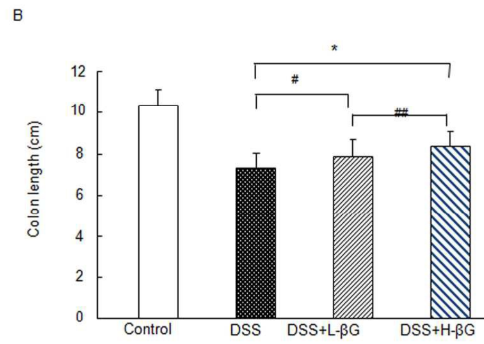

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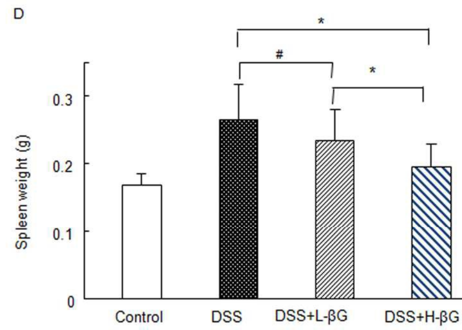

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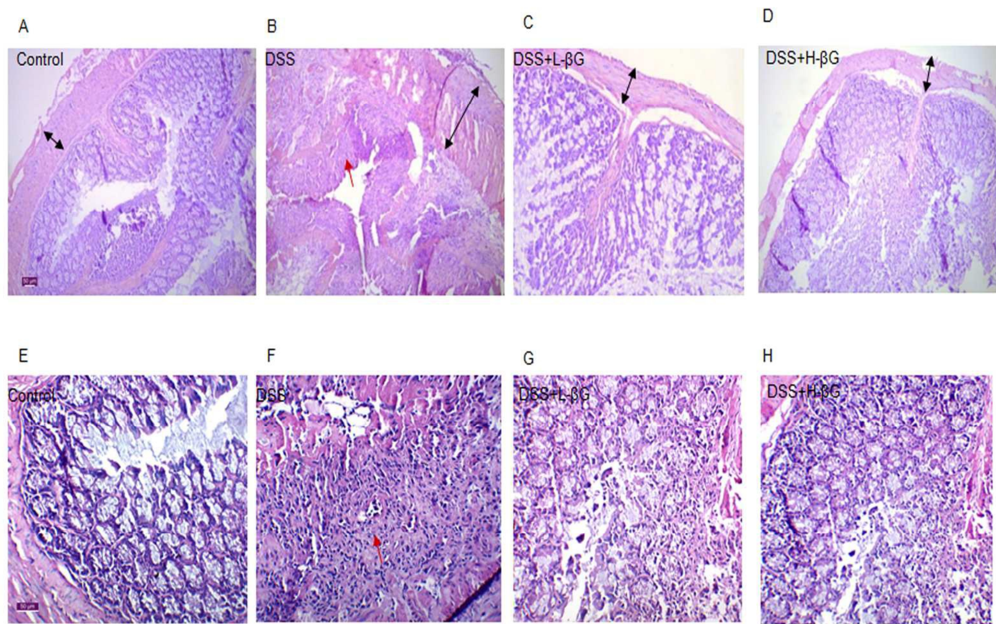

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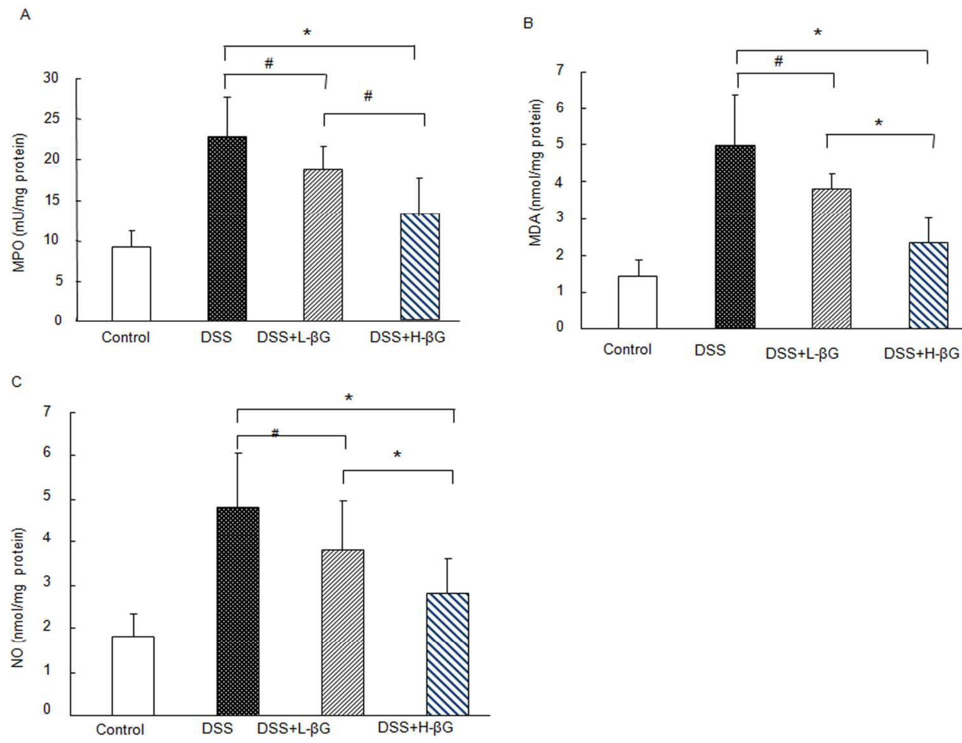

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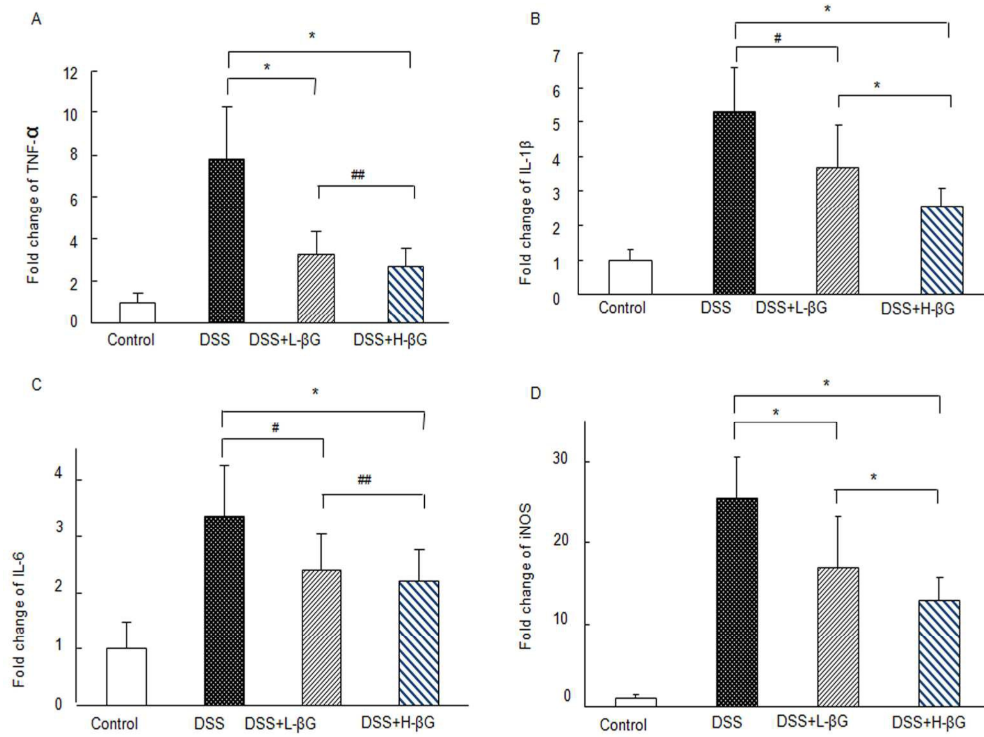

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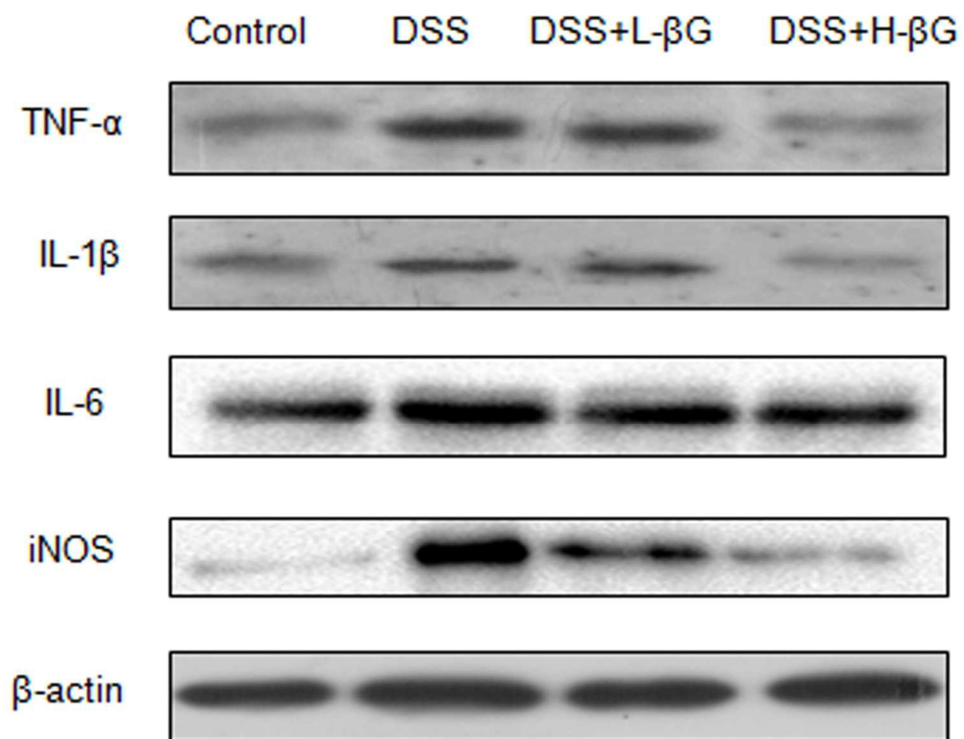

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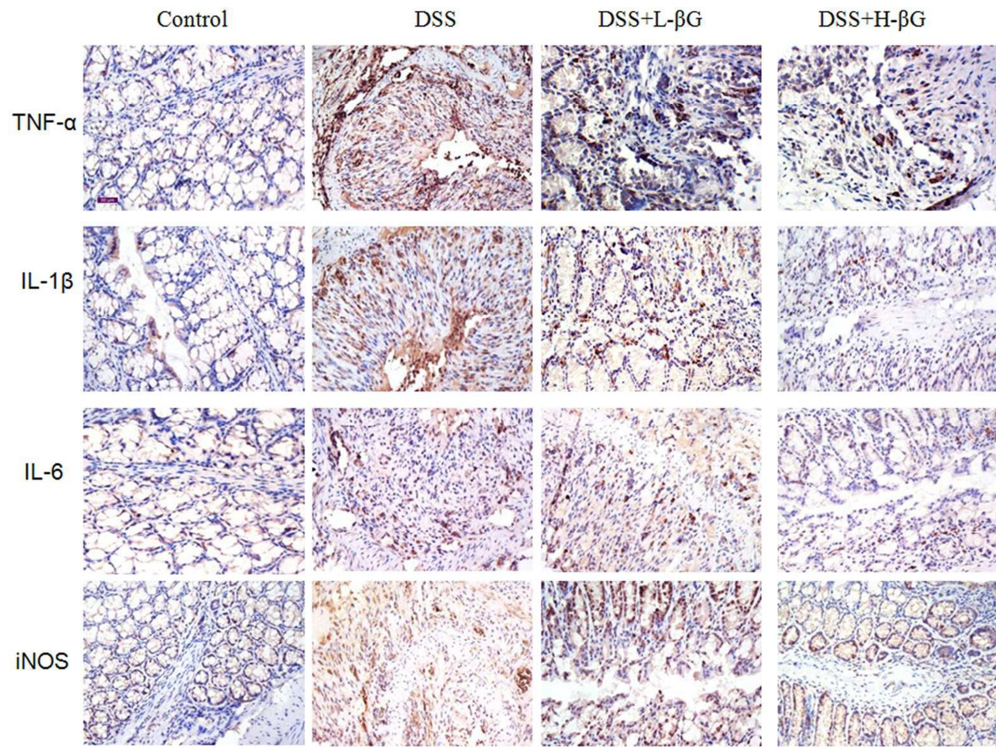

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Oral adminsitration of oat β-glucan ameliorates DSS induced colitis in mice through decreasing the expression of inflammatory cytokines TNF-α, IL-1β, IL-6 and iNOS

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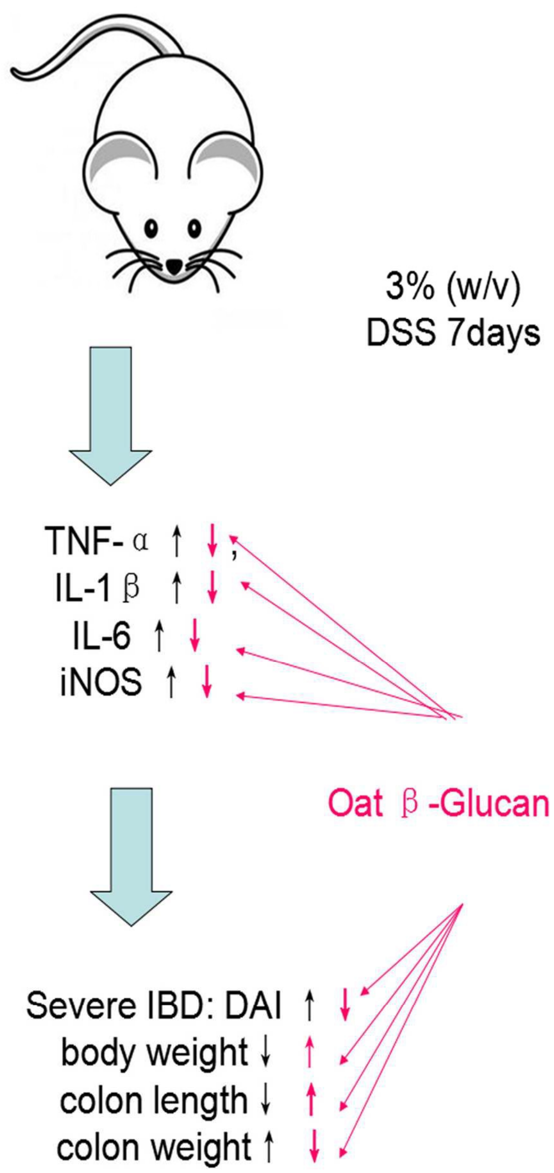

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