

Original Article

Oat β -glucan alleviates 5-Fluorouracil-induced intestinal barrier dysfunction *in vivo*

Huashe Wang, Yonghe Chen, Jun Xiang, Yijia Lin, Xiaobin Wu, Junsheng Peng

Department of Gastrointestinal Surgery, The Sixth Affiliated Hospital of Sun Yat-Sen University, Guangzhou 510655, China

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Abstract: Oat β -glucan has been recognized as a functional ingredient in human and animal nutrition. This study was aimed to reveal whether it was also a protective agent in 5-Fluorouracil (5-Fu)-induced intestinal barrier dysfunction *in vivo*. 5-Fu (150 mg/kg) was intraperitoneally (IP) injected into Sprague Dawley (SD) rats, and then 0.125 g/kg, 0.25 g/kg and 0.5 g/kg oat β -glucan were given for the next continual 7 days. On the day 4 and day 8, rat's body weight, diarrhea, ileum and colon weight were recorded, intestinal histopathology was detected, and the content of endotoxin and antioxidant indexes were assessed. Dramatical weight losses of body, ileum and colon organs were observed in 5-Fu pre-treated rats, and these rats had severe diarrhea. 5-Fu caused substantial changes in the intestinal mucosal layer, including flattened epithelial layer, shortened villi and lamina propria with inflammatory cells infiltration. Besides, 5-Fu increased the productions of endotoxin, malondialdehyde (MDA), total antioxidant capacity (T-AOC), myeloperoxidase (MPO), NO and NOS. More importantly, oat β -glucan could alleviate these abnormalities induced by 5-Fu, in both time- and dose-dependent manner. These results demonstrated that oat β -glucan protected against 5-Fu-induced intestinal barrier dysfunction *in vivo*.

Keywords: Oat β -glucan, intestinal barrier dysfunction, intestinal histopathology, the intestinal mucosal layer, 5-Fluorouracil (5-Fu)-induced

Introduction

Nowadays, chemotherapy has been widely used in the treatment of many cancers, including breast cancer, stomach cancer, bladder cancer and colorectal cancer. Chemotherapeutic agents have strong cytotoxicity through dividing cancer cells rapidly. However, major problems still remain in using chemotherapy that cytotoxic side effect on normal tissues and organs remains a serious drawback [1]. Studies have demonstrated chemotherapy can induce intestinal barrier dysfunction [2], which tremendously disrupt mucosal immune system, that a normal intestinal barrier function regulates transport and host defense mechanisms at the mucosal interface with the outside world [3].

5-Fluorouracil (5-Fu) is a common used chemotherapeutic agent, acts by inhibiting DNA synthesis of not only malignant cells, but also rapidly dividing cells lining the intestinal mucosa [4]. It's a cytotoxic agent on mucosal barrier

function, intestinal permeability and the molecular architecture of tight junctions [5]. Mauger *et al.*, have demonstrated that 5-Fu caused intestinal villi atrophy, intestinal mucosa ulcer and intercellular space largened in dark agouti rats [6], while these pathological changes may lessen or resolve after 5-Fu treatment is stopped [7, 8].

Oats, one of the most popular foods worldwide, is rich in the soluble dietary fiber β -glucan which is a nonstarch polysaccharide composed of (1-3) (1-4)- β -D-glucan and β -glucan [9]. Oat β -glucan has been recognized as a functional ingredient in human and animal nutrition [10]. Study in weaned pigs has revealed that, oat β -glucan generally enhanced gastrointestinal available bacteria numbers and activity, which may be favourable for intestinal development [11]. Lowry *et al.*, demonstrated a purified β -glucan feed additive modulated the balance of oxidate substances and reduction substances, and thus decreased the incidence of

Salmonella enteric serovar Enteritidis organ invasion in immature chickens [12]. Less is known about the effects of dietary oat β -glucan on intestinal barrier function, particularly on 5-Fu-induced intestinal barrier dysfunction.

Therefore, in the present study 5-Fu was intraperitoneally (IP) injected into Sprague Dawley (SD) rats to mimic an intestinal barrier dysfunction model. Then three different concentrations of oat β -glucan were given for the next continual 7 days. The changes in the body weight, diarrhea, ileum and colon weight, intestinal histopathological morphology, endotoxin, and antioxidant indexes were assessed, to ask whether oat β -glucan exhibited a protective role in 5-Fu-induced intestinal barrier dysfunction.

Materials and methods

Animals

Sixty specific pathogen-free grade (SPF) of SD rats (weighted 200 ± 10 g) were purchased from Guangdong Medical Laboratory Animal Center (Guangdong, China; certification No. 0015571). The rats were housed in polycarbonate cages under a 12 h/12 h light-dark cycle with continuous access to food and water. This study was approved by the Animal Experimental Ethical Committee of our local Hospital and was performed in accordance with the ethical standards.

Experimental design

These sixty SD rats were randomly assigned to 5 groups ($n = 12$ per group), namely control group, 5-Fu group, 0.125 g/kg group, 0.25 g/kg group and 0.5 g/kg group. Animals were acclimated to the new housing environment for three days before 5-Fu and oat β -glucan administration.

Rats in 5-Fu group were IP injected with 150 mg/kg 5-Fu (Tianjin Jinyao Amino Acid, Tianjin, China) which is dissolved in normal saline. Rats in control group were IP injected with the same dose of normal saline. Rats in 0.125 g/kg group, 0.25 g/kg group and 0.5 g/kg group were IP injected with 150 mg/kg 5-Fu in the first day and were respectively given to continual intragastric administration of 0.125 g/kg, 0.25 g/kg and 0.5 g/kg oat β -glucan for the next 7 days. Oat β -glucan with the purity of 80% purchased from Jiangxi Ben Tian Science and

Technology (Jiangxi, China) were also dissolved in normal saline. Rats in 5-Fu and control groups were intragastric administration the same amounts of normal saline from the second day to the eighth day. The following measures were carried out after 4 days and 8 days of administration, which were performed at the same time of the day (9 a.m.; ± 2 h).

Sample collection

At the 4 days and 8 days of administration, 6 rats were randomly selected from each group and were weighted and dissected after anesthetization by IP injection with chloral hydrate (3 mL/kg; Qingdao Yulong Seaweed, Qingdao, China). Blood sample was drawn from heart and collected into the pyrogen-free heparin-anticoagulant tubes (151 IU/mL). Following a centrifugation at $225 \times g$ for 10 min, serum was collected and stored at -20°C before use. Ileum and colon were dissected out and after washing with normal saline, these tissues were dried by the filter paper and weighted. Part of the tissues was fixed in 10% formalin and the other was stored in -80°C .

Diarrhea assessment

All rats were checked three times daily and diarrhea recorded. This was graded as 0, no diarrhea; 1, mild diarrhea (staining of anus); 2, moderate diarrhea (staining over top of the legs and lower abdomen) and; 3, severe diarrhea (staining over legs and higher abdomen, often associated with continual oozing) [13].

Histopathology assessment

Ileum and colon tissues removed from the formalin were routinely transferred into paraffin blocks and microtome sectioned at 4-6 μm . Sections were then stained by hematoxylin and eosin (H&E) for general histological examination. Pathological score of ileum was rated on the following scale: 0 = no necrosis; 1 = slight submucosal and/or lamina propria separation; 2 = moderate separation of submucosa and/or lamina propria and/or edema in submucosal and muscular layers; 3 = severe separation of submucosa and/or lamina propria and/or severe edema in submucosal and muscular layers and regional villus sloughing; 4 = loss of villi and necrosis [14]. For colon, the following scale was used: (1) infiltration of acute inflammation

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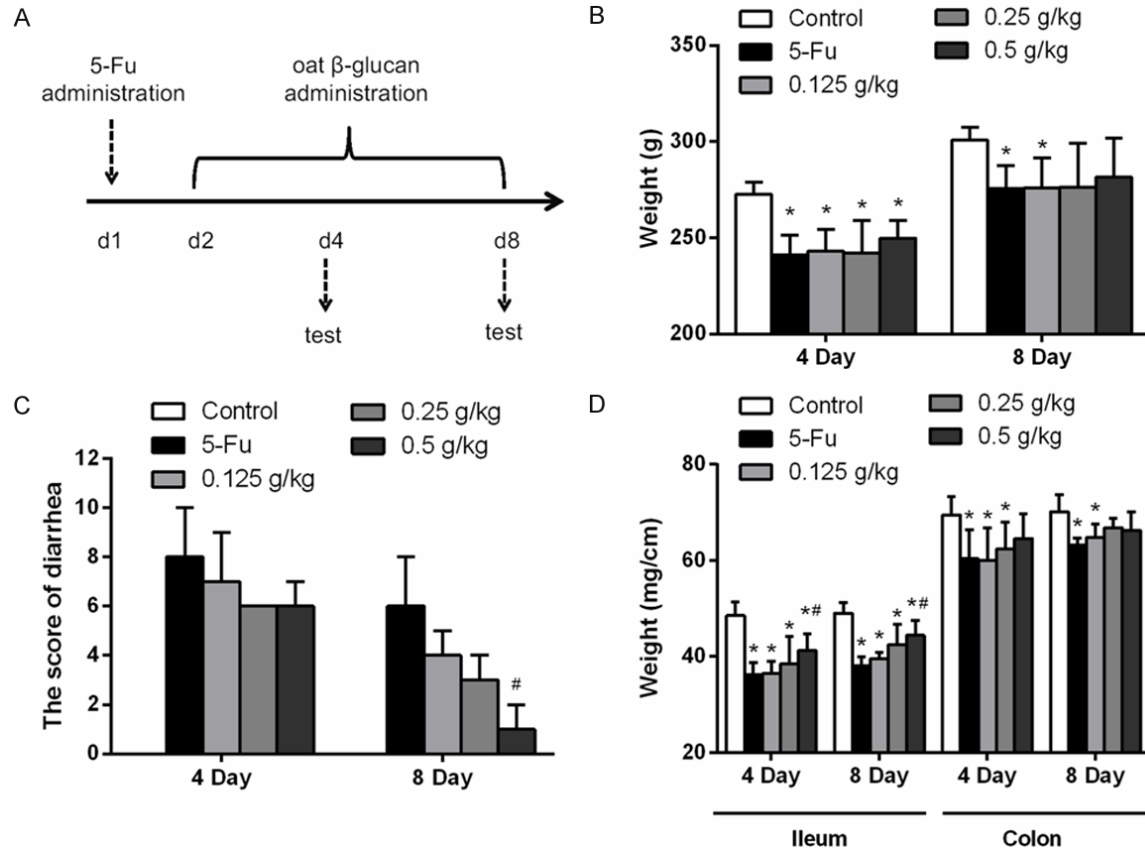


Figure 1. Oat β -glucan alleviates 5-Fu-induced body weight loss, diarrhea, and ileum and colon weight loss. (A) Graph of the timeline for the 5-Fu and oat β -glucan treatment in rats. (B) Rat's body weight, (C) diarrhea, and (D) ileum and colon weight were respectively recorded. * $P < 0.05$ when compared to the control group. # $P < 0.05$ when compared to the 5-Fu group.

cell, 0 = none, 1 = slight increase, 2 = marked increase; (2) Infiltration of chronic inflammation cell, 0 = none, 1 = slight increase, 2 = marked increase; (3) Fibrin deposition, 0 = absent, 1 = present; (4) Submucosa, 0 = nil, 1 = patchy, 2 = confluent; (5) Epithelial necrosis, 0 = nil, 1 = localised, 2 = extensive; (6) Epithelial ulceration, 0 = absent, 1 = present [15].

Endotoxin and antioxidant index detection

Serum endotoxin levels were measured by using Chromogenic End-point Tachypleus Amebocyte Lysate (Xiamen Limulus Reagent Demonstration Plant (Xiamen, China) following the manufacturer's instructions.

Zero point five grams of ileum and colon tissues were put into homogenizer to grind into tissue homogenate, and then centrifugated under 300×g for 20 min. The supernatant was collected and for use in the detection of glutathi-

one (GSH), malondialdehyde (MDA), superoxide dismutase (SOD), total antioxidant capacity (T-AOC), myeloperoxidase (MPO), NO and NOS by using the corresponding kit according to the manufacturer's instructions. GSH assay kit, MDA assay kit, SOD assay kit, and T-AOC assay kit were purchased from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). MPO Enzyme-linked immunoassay (ELISA) kit, NO ELISA kit, and NOS ELISA kit were purchased from Xiamen Huijia Biotechnology (Xiamen, China).

Statistical analysis

Data were presented as means \pm standard derivations (SD) from six independent assays. Statistical analysis was performed using SPSS 13.0 (SPSS Inc., Chicago, IL, USA) and one-way analysis of variance (ANOVA). P -value < 0.05 was considered as statistical significance.

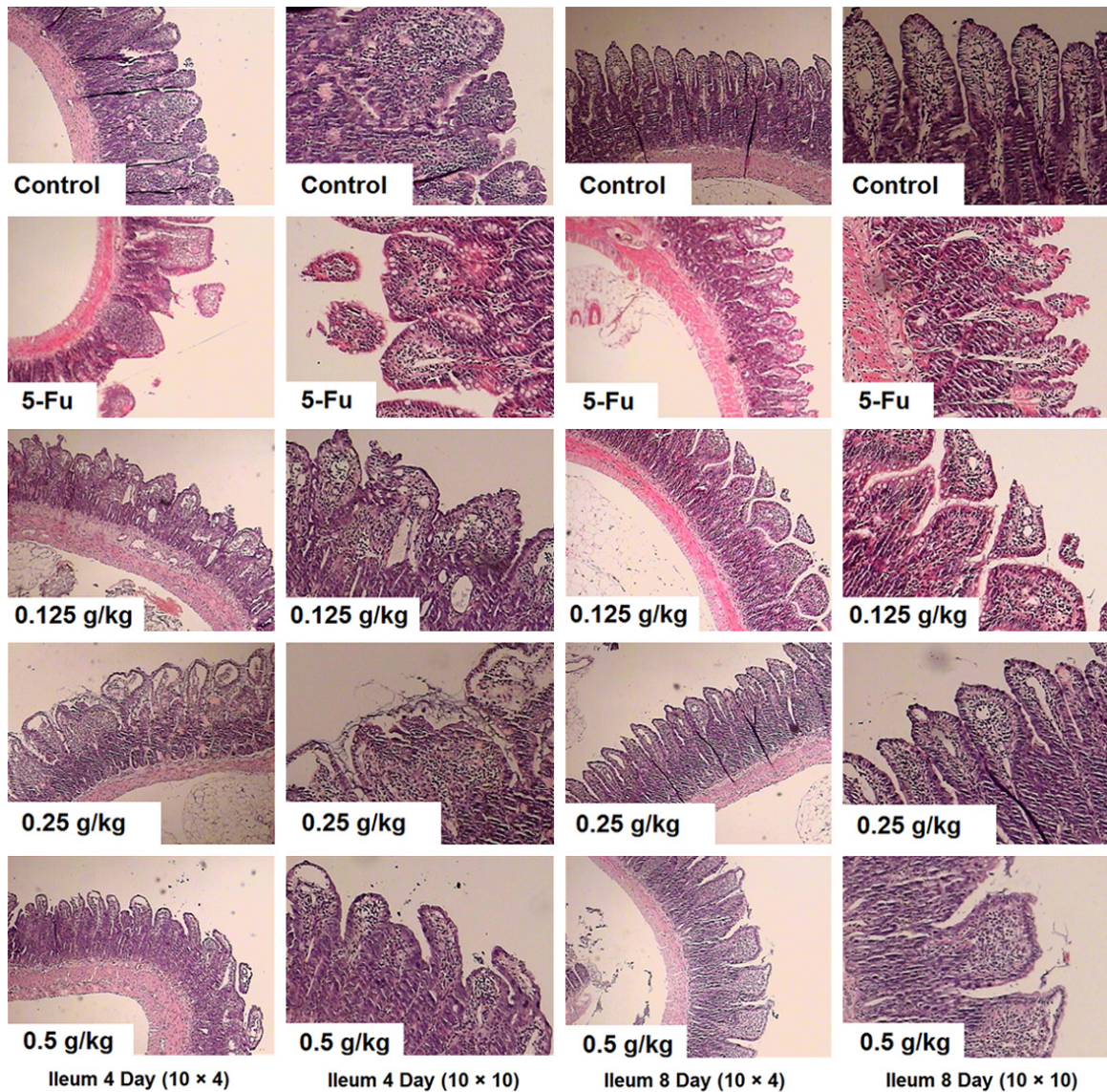


Figure 2. Oat β -glucan alleviates 5-Fu-induced histopathological changes in ileum.

Results

Weight and diarrhea assessment

In order to explore the effects of oat β -glucan on 5-Fu-induced intestinal barrier dysfunction, oat β -glucan was used to treat rats following by 5-Fu administration (**Figure 1A**). Numerous studies have shown that 5-Fu causes significant body weight loss [16]; this was also confirmed in our study. The weight of rats in 5-Fu group was much lower than those in control group ($P < 0.05$; **Figure 1B**). More importantly, after administration with 0.25 g/kg and 0.5 g/kg oat β -glucan for continual 7 days, the body weight loss induced by 5-Fu was slightly allevi-

ated, and there was no significant difference between 0.25 g/kg or 0.5 g/kg group and the control group ($P > 0.05$).

The diarrhea of rats was recorded, and no diarrhea was observed in the control group (**Figure 1C**). 5-Fu induced a severe diarrhea, and this diarrhea was relieved by oat β -glucan administration. Surprisingly, after a 7-day continuous administration with 0.5 g/kg oat β -glucan, the score of diarrhea was significantly reduced when compared to the 5-Fu group ($P < 0.05$).

Besides, we asked whether oat β -glucan could alleviate 5-Fu-induced intestinal weight loss. 5-Fu significantly reduced the weight of both

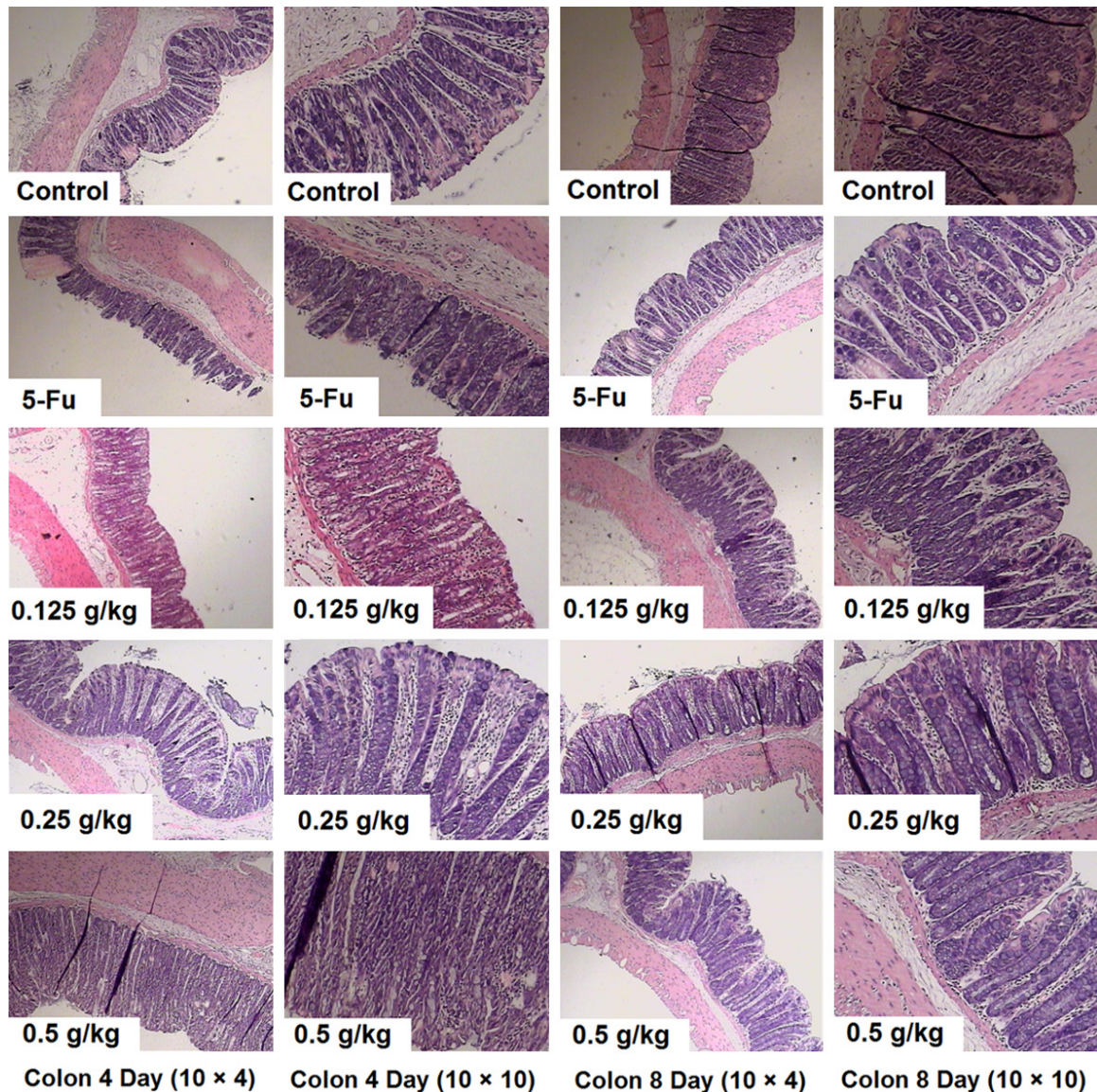


Figure 3. Oat β -glucan alleviates 5-Fu-induced histopathological changes in colon.

ileum and colon ($P < 0.05$; **Figure 1D**); moreover, 0.25 g/kg and 0.5 g/kg oat β -glucan could alleviate 5-Fu-induced weight loss of both ileum and colon, although the impacts of oat β -glucan on colon did not reach statistical significance.

Histopathology analysis

Next, we examined the histopathological changes in ileum and colon tissues. As expected, 5-Fu caused substantial changes in the intestinal mucosal layer (**Figures 2 and 3**), including flattened epithelial layer, shortened villi and lamina propria with inflammatory cells

infiltration. In consistent with these results, data in **Figure 4A** and **4B** showed that, the histological scores of ileum and colon were both dramatically increased by 5-Fu ($P < 0.05$). Oat β -glucan administration could normalize 5-Fu induced histopathological changes; interestingly, it seemed that oat β -glucan impacted these two tissues in both time- and dose-dependent manner.

Blood and tissue factors analyses

As shown in **Figure 5A**, the endotoxin content in serum was much higher in the 5-Fu group in comparison to the control group ($P < 0.05$).

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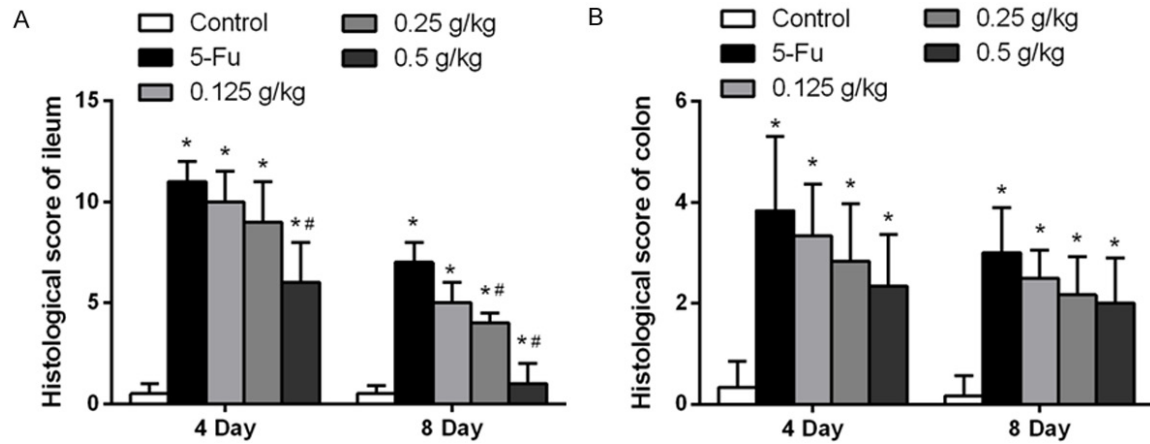


Figure 4. Oat β -glucan alleviates 5-Fu-induced histopathological changes. A. Quantitative results based on **Figure 2**. B. Quantitative results based on **Figure 3**. * $P < 0.05$ when compared to the control group. # $P < 0.05$ when compared to the 5-Fu group.

High concentrations (0.25 g/kg and 0.5 g/kg) of oat β -glucan could reduce 5-Fu-induced toxic action, although this protective effect was no longer reach statistical significance on day 8.

Antioxidant indexes in ileum and colon tissues were determined. GSH content in ileum and colon tissues was not significantly changed by 5-Fu or oat β -glucan ($P > 0.05$; **Figure 5B**). MDA content was significantly increased in ileum tissue after 5-Fu exposure, while was reduced after administration with 0.25 g/kg and 0.5 g/kg oat β -glucan at day 4 or day 8 (all $P < 0.05$; **Figure 5C**); however, no significant change in MDA content was observed in colon. On day 4, 0.5 g/kg oat β -glucan significantly increased SOD level in ileum when compared with control group and 5-Fu group (both $P < 0.05$; **Figure 5D**), while this increase was not that obvious at day 8; no significant change was found in colon. Moreover, 5-Fu-induced higher levels of T-AOC, MPO, NO and NOS (**Figure 5E-H**). High concentrations of oat β -glucan, *i.e.*, 0.25 g/kg and 0.5 g/kg, partially recovered these abnormal increases induced by 5-Fu, although same of these impacts did not reach statistical significance.

Discussion

5-Fu is a kind of chemotherapeutic agent, which is used for colon cancer, esophageal cancer, stomach cancer and pancreatic cancer. Numerous studies showed that 5-Fu subverted intestinal mucosa and enhanced wall intestinal permeability [17-19]. The administration of 5-Fu

to rats caused body weight loss and epithelial barrier dysfunction of the small intestine [18]. Furthermore, studies have revealed that IP injection of 5-Fu (150 mg/kg) caused intestinal mucositis [6, 19]. In the present study, 150 mg/kg 5-Fu remarkably induced body weight loss and diarrhea, as well as abnormal pathological changes in the intestinal mucosal layer, which were consistent with previous findings.

β -glucan is the major soluble fiber in oats and is thought to be the active component responsible for its blood-glucose and cholesterol-lowering properties [20, 21]. In addition, it has been studied and confirmed extensively that, orally administered β -glucan has antitumor and immune stimulation effects [22]. Recent studies has also evidenced that β -glucan has beneficial effects on intestinal barrier function. For instance, dietary β -glucan alleviated intestinal mucosal barrier impairment in broiler chickens challenged with *Salmonella Typhimurium* [23]. Cereal β -glucan exerted favorable effects on improving intestinal functions and health; more interestingly, the gut-health-promoting effects of oat β -glucan were better than those of barley β -glucan [24]. In the present study, we found that high concentrations of oat β -glucan administration alleviated 5-Fu-induced weight loss and diarrhea, as well as relieved 5-Fu-induced impairment for ileum and colon. To our knowledge, our study provides the first evidence that oat β -glucan exhibits protective effect on 5-Fu-induced intestinal barrier dysfunction.

To further confirm the functional effects of oat β -glucan on intestinal barrier function, endo-

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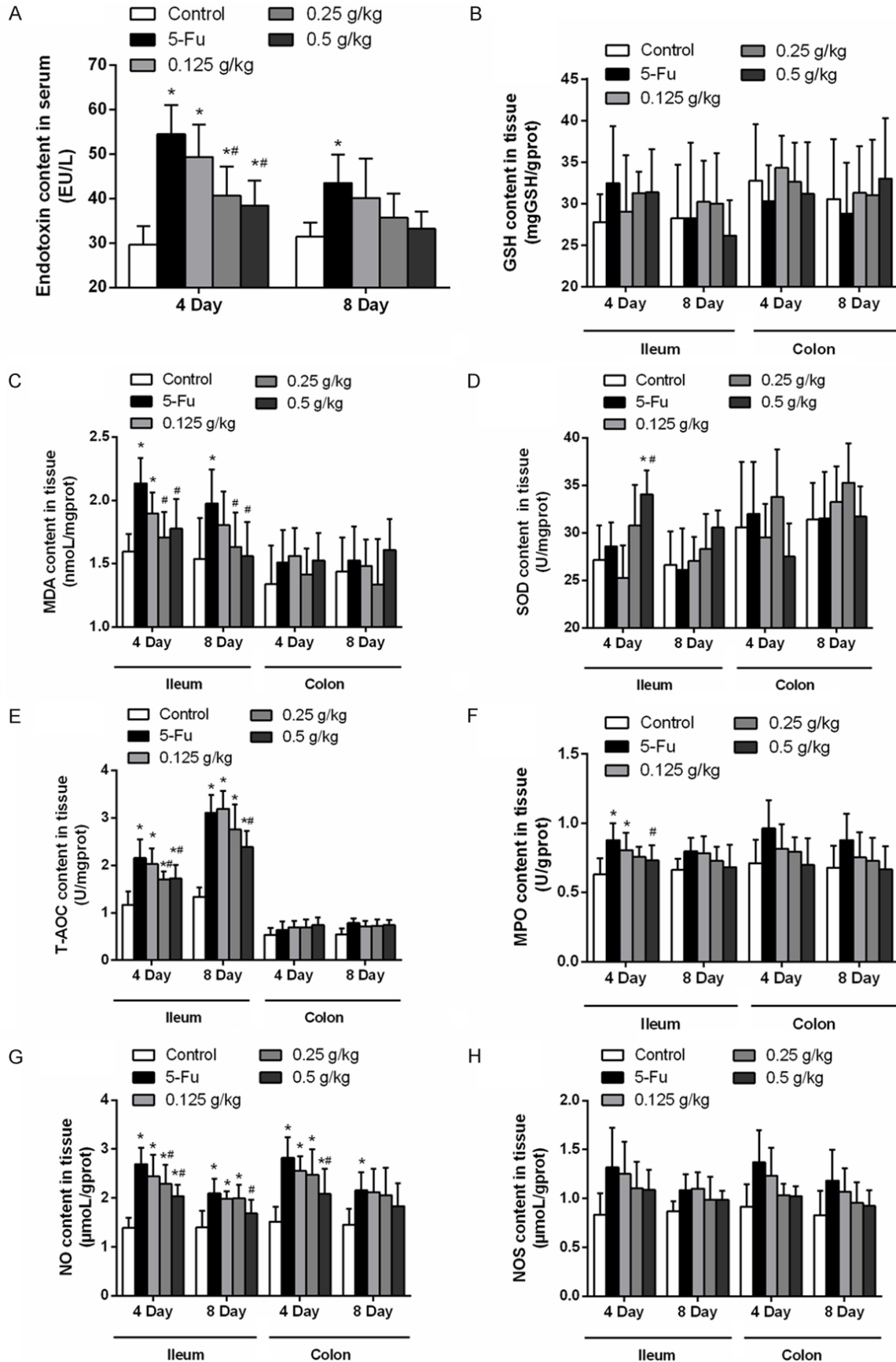


Figure 5. Oat β -glucan alleviates 5-Fu-induced endotoxin overproduction and redox imbalance. The detection of (A) endotoxin content, (B) glutathione (GSH) content, (C) malondialdehyde (MDA) content, (D) superoxide dismutase (SOD) content, (E) total antioxidant capacity (T-AOC) content, (F) myeloperoxidase (MPO) content, (G) NO content, and (H) NOS content in ileum and colon tissues. * $P < 0.05$ when compared to the control group. # $P < 0.05$ when compared to the 5-Fu group.

toxin content in rat's serum was detected. In line with the previous literature [25], a significant increase of endotoxin content was found in 5-Fu pre-treated rats. More importantly, oat β -glucan could partially recover 5-Fu-induced increase in endotoxin content to some extent, revealing that oat β -glucan protected intestinal barrier function possibly through reducing serum endotoxin.

It is well established that overproduction of reactive oxygen species plays vital roles in the pathogenesis of intestinal mucosal damage [26]. Oxidative stress in the cellular environment results in the formation of highly reactive and unstable lipid hydroperoxides and that ultimately produces MDA [27]. Thus, MDA has been identified as an important indicator to evaluate the degree of intestinal free radical injury [28, 29]. In this study, MDA content in ileum tissues were remarkably increased by 5-Fu treatment, while were decreased when oat β -glucan was added, implying oat β -glucan could protect ileum against 5-Fu-induced oxidative damage. Similar results have been reported in previous study [12], in which β -glucan significantly reduced the MAD content in the intestinal tissues of immature chickens.

Normally, homeostasis is maintained by two coordinated actions of oxidation and antioxidants. In this study, increases of MDA, T-AOC, MPO and NO contents were observed in 5-Fu administrated ileum, and oat β -glucan alleviated these abnormal increases, this finding further confirmed the hypothesis that oat β -glucan could protect the intestinal barrier function. However, both 5-Fu and oat β -glucan had no significant impacts on GSH, SOD and NOS contents. We hypothesized that the unaltered GSH, SOD and NOS levels might be a result of the complicated modulations of multiple factors. More work still urgently needed to elucidate the deep mechanisms of which oat β -glucan impacts these indexes. In addition, there were less significant changes of peroxidation indexes were found in colon tissues, implying the protective effects of oat β -glucan on colon was not that obvious as ileum.

In conclusion, this study demonstrates a protective role of oat β -glucan in 5-Fu-induced intestinal barrier dysfunction *in vivo*. This study provides a novel understanding of oat β -glucan and evidences oat β -glucan is beneficial for adjuvant chemotherapy.

Disclosure of conflict of interest

None.

Address correspondence to: Junsheng Peng and Xiaobin Wu, Department of Gastrointestinal Surgery, The Sixth Affiliated Hospital of Sun Yat-Sen University, No. 26 Yuancunrenheng Road, Guangzhou 510655, China. E-mail: pengjunsheng556@126.com (JSP); wuxiaobin154@126.com (XBW)

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