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Supplementation of the diet with Salecan attenuates the symptoms of colitis induced by dextran sulphate sodium in mice

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Abstract

As a water-soluble extracellular β-glucan produced by *Agrobacterium* sp. ZX09, Salecan has an excellent toxicological profile and exerts multiple physiological effects. The aims of the present study were to investigate the protective effects of a Salecan diet in the well-defined dextran sulphate sodium (DSS) model of experimental murine colitis and to elucidate the mechanism involved in its effects with special attention being paid to its effect on the production of TNF-α, a primary mediator involved in the inflammatory response. Male C57BL/6J mice were fed a diet supplemented with either 4 or 8 % Salecan for 26 d and DSS was administered to induce acute colitis during the last 5 d of the experimental period. Several clinical and inflammatory parameters as well as mRNA expression of *TNF-α* and *Dectin-1* were evaluated. The results indicated that the dietary incorporation of Salecan attenuated the severity of DSS colitis as evidenced by the decreased disease activity index, reduced severity of anaemia, attenuated changes in colon architecture and reduced colonic myeloperoxidase activity. This protection was associated with the down-regulation of *TNF-α* mRNA levels, which might derive from its ability to increase *Dectin-1* mRNA levels. In conclusion, the present study suggests that Salecan contributes to the reduction of colonic damage and inflammation in mice with DSS-induced colitis and holds promise as a new, effective nutritional supplement in the management of inflammatory bowel disease.

Key words: Salecan: β-Glucans: Dextran sulphate sodium: Ulcerative colitis

Inflammatory bowel disease (IBD), a collective term for the diseases including Crohn's disease and ulcerative colitis (UC), is characterised by the chronic and relapsing inflammation of the gastrointestinal tract[1]. UC exclusively affects the colon and rectum at the mucosal level[2]. It begins in early adulthood and continues throughout life and affects millions of individuals worldwide[3]. Men seem to be affected slightly more frequently than women[3]. In recent years, the incidence of UC in traditionally high-incidence areas such as the USA and Europe has become relatively stable. However, this disease has become more prevalent in previously low-incidence areas including Asia[4]. The crude annual incidence rate in mainland China in 2011–12 was 0·22–2·27/100 000 persons[4]. Although the exact aetiology of UC remains undetermined, the current leading hypothesis emphasises genetic predisposition to the dysregulation of the gastrointestinal immune system[5]. Although conventional treatments (with aminosalicylates, corticosteroids, antibiotics and immunomodulators) can be effective at maintaining remission and decreasing the length of active disease periods, these are not without side effects[2]. Consequently, potential adverse events, if not more so, and costs have led investigators to search for novel therapeutic approaches.

There is rapidly growing evidence supporting the protective effects of dietary fibres against the pathogenic process of intestinal inflammation in clinical and experimental colitis[2]. Dietary fibre is a collective term for a variety of plant substances that are resistant to enzymatic digestion in the upper gastrointestinal tract[6]. The dietary fibre from *Plantago ovate* has been proven to increase butyrate production, lower TNF-α concentrations and maintain remission in UC[7,8]. Kanauchi

Abbreviations: DSS, dextran sulphate sodium; HS, diet with 8% Salecan; IBD, inflammatory bowel disease; LS, diet with 4% Salecan; MPO, myeloperoxidase; UC, ulcerative colitis.

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et al.[9,10] reported that germinated barley foodstuff, a protein-rich fibre made from brewer's spent grain, enhances butyrate production, inhibits TNF-α production, prevents mucosal damage and reduces the clinical activity of UC. In recent years, β-glucans, one of the most abundant forms of polysaccharides present in the plant cell walls, have been considered as health-promoting dietary fibres. Limited information exists about the prevention and/or treatment of UC with β-glucans, even though these are common in the food of humans. Current data suggest that β-glucans have the ability to modulate immune response by triggering immune cells through several immune receptors including Dectin-1and complement receptor 3[11].

Salecan is a type of β-glucan derived from *Agrobacterium* sp. ZX09 consisting of the repeating unit → 3)-β-D-Glcp-(1 → 3)-(β-D-Glcp-(1 → 3)-β-D-Glcp-(1 → 3))3-α-D-Glcp-(1 → 3)-α-D-Glcp-(1 → [12], and has potential applications in the food industry due to its excellent toxicological profile and rheological properties[13,14]. Similar to other β-glucans, Salecan exhibits multiple biological activities in the gastrointestinal tract. Due to its indigestible character and high water-holding capacity, Salecan given intragastrically stimulates small-intestinal transit and improves the output of faeces in experimental constipated mice[15]. Dietary Salecan intake has been demonstrated to be effective at decreasing fat absorption and improving glucose tolerance in high-fat diet-fed mice[16]. Moreover, results from a recent study suggest that Salecan is preferentially fermented by lactobacilli and bifidobacteria and affects gut microbiota balance to positively direct metabolic activity towards increased colonic production of butyrate[17]. The aim of the present study was to assess the protective effects of Salecan in the dextran sulphate sodium (DSS) model of mouse colitis. Special attention was paid to its effect on the production of TNF-α.

Materials and methods

Salecan

Salecan was extracted from the fermentation broth of *Agrobacterium* sp. ZX09 using centrifugation and ethanol precipitation according to a previously described method[12,13]. Commercial Salecan (chemical composition: sugar 77·13%, protein 6·2%, moisture 5·2% and ash 10·28%; average molecular weight 2×10^6; water soluble) was purchased from Karroten Company.

Animals and experimental design

All animal care and use procedures were approved by and in accordance with the Institutional Animal Care and Use Committee at the Nanjing University of Science and Technology. Male C57BL/6J mice at the age of 7 weeks were housed in a temperature- and humidity-controlled room under a 12 h light–12 h dark cycle; they had free access to tap water and food. The mice were randomly assigned to four groups (n 6) as follows (Fig. 1): control group, which was fed the standard diet for 26 d; DSS group, which was fed the standard diet for 26 d plus 4% DSS in drinking-water (w/v, prepared daily; average molecular weight 36 000–50 000; MP Biomedicals) during the last 5 d of the experimental period; two Salecan-treated groups (LS+DSS and HS+DSS), which were fed standard chow supplemented with 4 and 8% Salecan, respectively, for 26 d plus 4% DSS in drinking-water during the last 5 d of the experimental period. The dose of Salecan was selected based on the results of previous studies[8,17,18]. The intake of both food and water per mouse was measured throughout the duration of the study. After the 5th day of colitis induction, the mice were killed under anaesthesia.

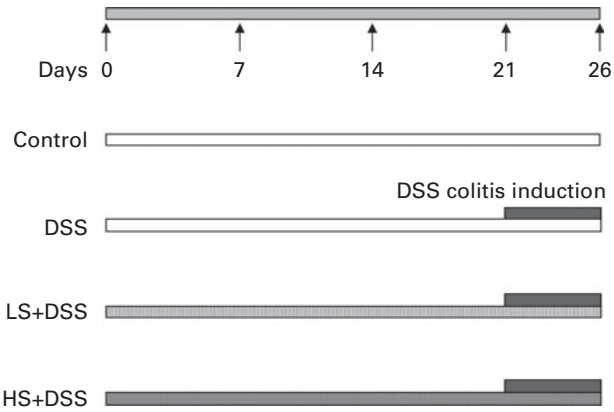

Fig. 1. Patterns for the induction of colitis by dextran sulphate sodium (DSS) in male C57BL/6J mice. The mice were fed a diet containing either 4% (w/w) or 8% Salecan (LS or HS, respectively) from 21 d before the start of DSS administration to the day before killing.

Assessment of colonic damage

Colonic damage was monitored daily by an observer who was unaware of the treatment based on a scoring system that evaluates change in body weight, stool consistency, faecal occult blood and overall condition of the mice to provide a disease activity index as described by Murthy *et al.*[19] (Table 1). Faecal occult blood was investigated using Hemoccult II slides (SmithKline Diagnostics Inc.).

Sampling procedure

Blood samples were drawn from the mice under anaesthesia and collected in EDTA-treated tubes to determine the haematological profile. Colons were removed, rinsed with ice-cold phosphate buffer solution and blotted dry, and their lengths were measured. Then, the colon of each mouse was divided based on the percentage of total colon length: the proximal 30% was discarded; the next 30% was taken for the quantitative determination of mRNA expression; the adjacent 10% was fixed for histological examination; the remaining 30% was snap-frozen in liquid N_2 for myeloperoxidase (MPO) activity assay[20]. The weights of the spleens were also recorded.

Haematological analysis

Haematological parameters were determined using an automated haematology analyser. The parameters analysed were erythrocytes, Hb, haematocrit, mean corpuscular volume, mean corpuscular Hb, mean corpuscular Hb concentration, leucocyte count, lymphocyte count and neutrophil count.

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Table 1. Criteria for scoring disease activity index*

Scores	Change in weight (%)	Stool consistency†	Faecal occult blood
0	None	Normal	Negative
1	1–5	–	–
2	5–10	Loose	Haemoccult positive
3	10–15	–	–
4	>15	Diarrhoea	Gross bleeding

*Disease activity index = combined score of weight loss, stool consistency and bleeding/3.
†Normal stools, well-formed pellets; loose stools, pasty stools that do not stick to the anus; diarrhoea, liquid stools that stick to the anus.

Histological analysis

Colonic segments were fixed in 10% neutral buffered formalin, embedded in paraffin, sliced into 5 μm-thick sections and then stained using haematoxylin and eosin for histological examination by light microscopy (Nikon). Histological damage was calculated on a ten-point scale as described previously with slight modifications[21] (Table 2), and it is expressed as a value for six to eight randomly selected tissue sections from each colon.

Myeloperoxidase activity assay

Colonic segments were homogenised on ice in five volumes of normal saline, and MPO activity was determined using a chemical method as per the manufacturer's instructions (Nanjing Jiancheng Corporation). The degradation of 1 μmol H_2O_2/min at 37°C is defined as one unit of MPO activity, and the value is expressed as units/g.

Quantitative real-time PCR

Total RNA was extracted from colonic segments using TRIzol (Invitrogen) following the manufacturer's instructions. Reverse transcription of mRNA was carried out using the KeyGene reverse-transcription enzyme according to the manufacturer's protocol. Quantitative real-time PCR was carried out using an ABI 7300 real-time PCR system with a cDNA sample, and amplification was carried out in a 20 μl reaction volume containing 1× SYBR Green PCR Master Mix (Applied Biosystems) with primers specific for *TNF-α* (forward: TGAACTTCGGGGTGATCGGTC; reverse: AGCCTTGTCCCTTGAAGAGGAAC)[22], *Dectin-1* (forward: GGAATCCTGTGCTTTGTGGTAGTAG; reverse: GGAAGGCAAGACTGAGAAAAACCTC)[23] and β-actin (forward: CCTGAACCCTAAGGCCAACC; reverse: CAGCTGTGGTGGTGAAGCTG)[24]. Relative expression in comparison with that of β-actin was calculated using the comparative computed tomography method.

Statistical analysis

All data are expressed as means with their standard errors. Statistical analysis was carried out using the SPSS 13.0 software (SPSS Inc.), and differences between the groups were analysed using one-way ANOVA followed by Tukey's *post hoc* test. $P<0{\cdot}05$ was considered to be statistically significant.

Results

Effect of Salecan on food and water intake

The intake of food and water of the mice were monitored daily during the development of DSS-induced colitis. Daily average food intake was not significantly different between the three DSS-treated groups, and relative to the control group, these three groups exhibited decreased food intake (approximately 30%, $P<0{\cdot}05$; Table 3). There was no significant difference in water intake between the DSS and HS+DSS groups, whereas the LS+DSS group consumed more water.

Effect of Salecan on the disease activity index

After 5 d of exposure to 4% DSS in drinking-water, the DSS group developed the clinical symptoms of acute colitis, with weight loss, diarrhoea and rectal bleeding (Fig. 2). Change in weight (>3%) became prominent after 3 d of DSS treatment (Fig. 2(a)). The presence of blood in the faeces was detected 1 d after the start of DSS treatment, whereas gross bleeding and diarrhoea were initially observed from day 4 onwards (Fig. 2(b) and (c)). As a consequence of the inflammatory process, the DSS group had a clearly worse median disease activity index value, combined score of weight loss, stool consistency and faecal occult blood divided by 3, compared with the control group from day 1 to the end of DSS treatment (Fig. 2(d)). On day 5, mice fed the diet supplemented with 8% Salecan exhibited markedly reduced disease severity compared with the DSS group, which might be attributed to substantial reductions in stool consistency and faecal occult blood brought about by Salecan (Fig. 2).

Effect of Salecan on haematological parameters

Anaemia is a common complication of UC, resulting from mucosal blood loss. As shown in Table 4, the DSS group had severe anaemia (decreased erythrocyte, Hb and haematocrit levels) in comparison with the control group. Supplementation of the diet with Salecan obviously attenuated these symptoms. No evident differences in mean corpuscular volume, mean corpuscular Hb and mean corpuscular Hb concentration were found among these four groups. The neutrophil count of the DSS group was significantly (4-fold) higher than that of the control group,

Table 2. Histological scores given to haematoxylin and eosin-stained colonic sections to quantify inflammation

Scores	Inflammation degree	Inflammation extent	Crypt damage
0	None	None	None
1	Slight	Mucosa	Basal one-thirds damaged
2	Moderate	Submucosa	Basal two-thirds damaged
3	Severe	Transmural	Entire crypt loss

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Table 3. Effect of Salecan on food and water intake (g/d per mouse) during dextran sulphate sodium (DSS) treatment.

(Mean values with their standard errors; *n* 6)

	Food intake		Water intake	
Groups	Mean	SEM	Mean	SEM
Control	5·13	0·13	4·07	0·25
DSS	3·35*	0·07	5·51*	0·25
LS+DSS	3·75*	0·23	6·81*†	0·14
HS+DSS	3·74*	0·17	5·21*	0·20

LS, diet with 4 % Salecan; HS, diet with 8 % Salecan.
* Mean value was significantly different from that of the control group ($P<0·05$).
† Mean value was significantly different from that of the DSS group ($P<0·05$).

despite similar levels of leucocytes and lymphocytes. The groups treated with Salecan exhibited a lower increase in neutrophil count.

Effect of Salecan on colon length and spleen weight

Colon length is used as a morphometric measure of the degree of colonic inflammation. We observed a conspicuous reduction in the colon length of the DSS group amounting to approximately one-fourth of that of the control group (Fig. 3(a)). The Salecan diet prevented the DSS-induced colon shortening, and a significant effect was observed in the high-dose group. As spleen enlargement is a characteristic of a systemic inflammatory reaction such as that occurring in acute DSS colitis, spleens were also weighed. There was an obvious increase (approximately 30 %) in spleen weight in the DSS group relative to the control group (Fig. 3(b)). However, Salecan did not seem to have a significant effect on the reduction of spleen enlargement.

Effect of Salecan on the histological features of dextran sulphate sodium-induced colitis

Histological assessment of the colonic sections of mice treated with DSS for 5 d revealed crypt shortening and loss, ulceration and dense acute inflammation consisting mostly of neutrophils (Fig. 4(a)–(d)). By contrast, colon architecture of the HS+DSS group appeared to be relatively normal, showing only mild evidence of crypt distortion and inflammation with neutrophil infiltration (Fig. 4(e)–(h)). In Fig. 4(i), the histological damage scores of all groups are shown, which clearly demonstrate the ability of Salecan to alleviate tissue destruction and neutrophil infiltration, characteristics of DSS-induced colitis.

Effect of Salecan on myeloperoxidase activity

Neutrophil accumulation was also confirmed by the measurement of MPO activity. MPO activity was dramatically increased in the

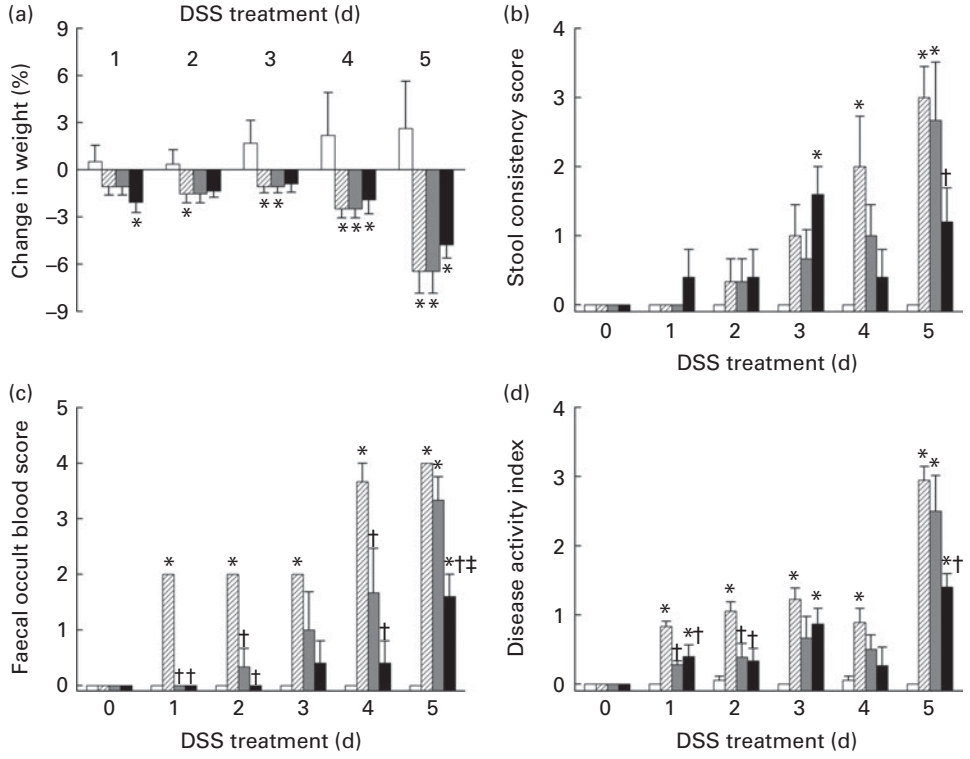

Fig. 2. Effect of Salecan on change in weight (a), stool consistency (b), faecal occult blood (c) and disease activity index (d) during dextran sulphate sodium (DSS) treatment. LS, diet with 4 % Salecan; HS, diet with 8 % Salecan. Values are means (*n* 6 mice per group), with their standard errors represented by vertical bars. * Mean value was significantly different from that of the control group ($P<0·05$). † Mean value was significantly different from that of the DSS group ($P<0·05$). ‡ Mean value was significantly different from that of the LS+DSS group ($P<0·05$). ▭, Control; ▨, DSS; ▬, LS+DSS; ■, HS+DSS.

Table 4. Haematological parameters in mouse groups
(Mean values with their standard errors; *n* 6)

	Control		DSS		LS+DSS		HS+DSS	
Blood parameters	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
Erythrocytes ($\times 10^6$ cells/μl)	9·31	0·25	4·73*	0·32	7·45*†	0·65	7·93*†	0·53
Hb (g/l)	148·0	4·1	73·5*	4·9	113·8*†	9·1	122·6†	8·2
Hct (%)	48·00	0·87	25·10*	1·80	38·44†	3·01	40·54†	2·58
MCV (μm^3)	54·15	0·57	53·08	0·50	51·72	0·62	51·16	0·27
MCH (pg)	15·90	0·04	15·58	0·19	15·30	0·16	15·50	0·09
MCHC (g/l)	298·8	10·1	293·8	5·6	296·0	2·4	302·4	2·7
Leucocytes ($\times 10^3$ cells/μl)	5·06	0·39	5·84	0·42	4·93	0·67	4·46	0·67
Lymphocytes ($\times 10^3$ cells/μl)	3·89	0·26	4·03	0·38	3·38	0·59	3·83	0·64
Lymphocytes (%)	81·20	7·77	68·83	1·44	69·38	4·75	82·43	1·71
Neutrophils ($\times 10^3$ cells/μl)	0·39	0·11	1·74*	0·03	1·21	0·27	0·75†	0·18
Neutrophils (%)	6·43	0·52	30·10*	1·55	27·13*	4·53	15·50†	1·98

DSS, dextran sulphate sodium; LS, diet with 4 % Salecan; HS, diet with 8 % Salecan; Hct, haematocrit; MCV, mean corpuscular volume; MCH, mean corpuscular Hb; MCHC, mean corpuscular Hb concentration.
* Mean value was significantly different from that of the control group ($P<0·05$).
† Mean value was significantly different from that of the DSS group ($P<0·05$).

colonic tissue samples of the DSS group (Fig. 5). This increase in MPO activity induced by DSS was inhibited by Salecan.

Effect of Salecan on the mRNA levels of TNF-α and Dectin-1

Quantitative real-time PCR were carried out to test the effect of Salecan diet on *TNF-α* mRNA levels. In contrast to the control group, a substantial up-regulation of colonic *TNF-α* mRNA levels was observed in the DSS group (Fig. 6(a)). Treatment of experimental colitis with 8 % Salecan resulted in a significant reduction in colonic *TNF-α* mRNA levels.

As Dectin-1 is implicated in the regulation of cytokine expression in response to β-glucans, we further investigated the mRNA levels of this receptor. As reflected by quantitative real-time PCR, *Dectin-1* mRNA levels of the DSS-treated mice were similar to those of the control mice and, as expected, the Salecan diet led to an obvious increase in *Dectin-1* mRNA levels, and this increase was associated with an increase in Salecan concentrations (Fig. 6(b)).

Discussion

New drugs and therapy options are urgently needed to prevent and/or cure UC. There is increased interest in the prophylactic and/or therapeutic efficacy of several types of dietary fibres for the treatment of experimental and human UC[7,9,25]. Our interest was to evaluate the effects of Salecan as a potential dietary therapeutic compound for UC, which stemmed from its identification as a bioactive β-glucan without toxicity[17] and from the results reported by Lavi *et al.*[26]. In the present study, we demonstrated Salecan to attenuate DSS-induced clinical symptoms as well as colonic injury and inflammation in mice. Importantly, we demonstrated Salecan to inhibit the increase in *TNF-α* mRNA levels caused by DSS treatment and to increase the mRNA levels of the β-glucan receptor *Dectin-1*.

DSS is a synthesised sulphated dextran with epithelial toxicity, and the DSS model of experimental colitis established in 1990 by Okayasu *et al.*[27,28] is well accepted due to its similarities to human UC in aetiology, pathology, pathogenesis and therapeutic response. Besides, based on the fact that genetic susceptibility and specific immunity do not seem to play important roles in the inflammatory process induced by DSS, this model is one of the most suitable ones to study

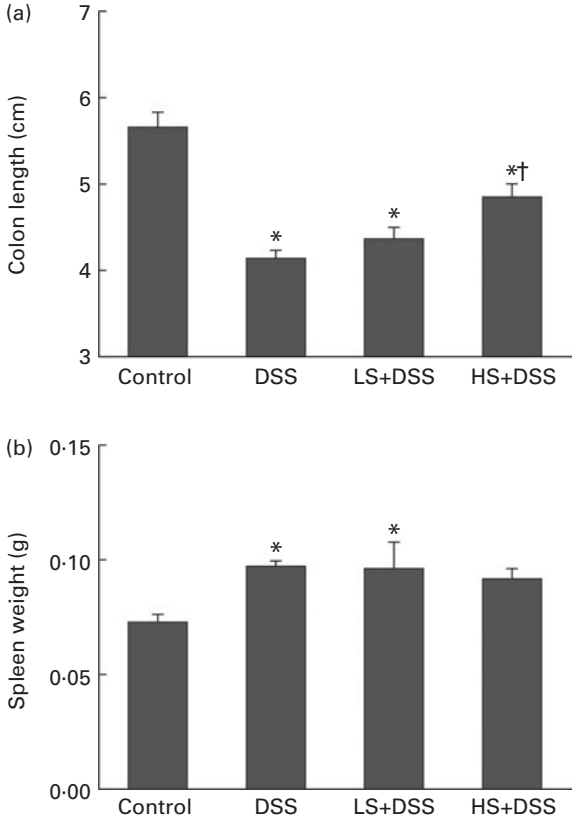

Fig. 3. Effect of Salecan on the total colon length (a) and spleen weight (b) after dextran sulphate sodium (DSS) administration. LS, diet with 4 % Salecan; HS, diet with 8 % Salecan. Values are means (*n* 6 mice per group), with their standard errors represented by vertical bars. * Mean value was significantly different from that of the control group ($P<0·05$). † Mean value was significantly different from that of the DSS group ($P<0·05$).

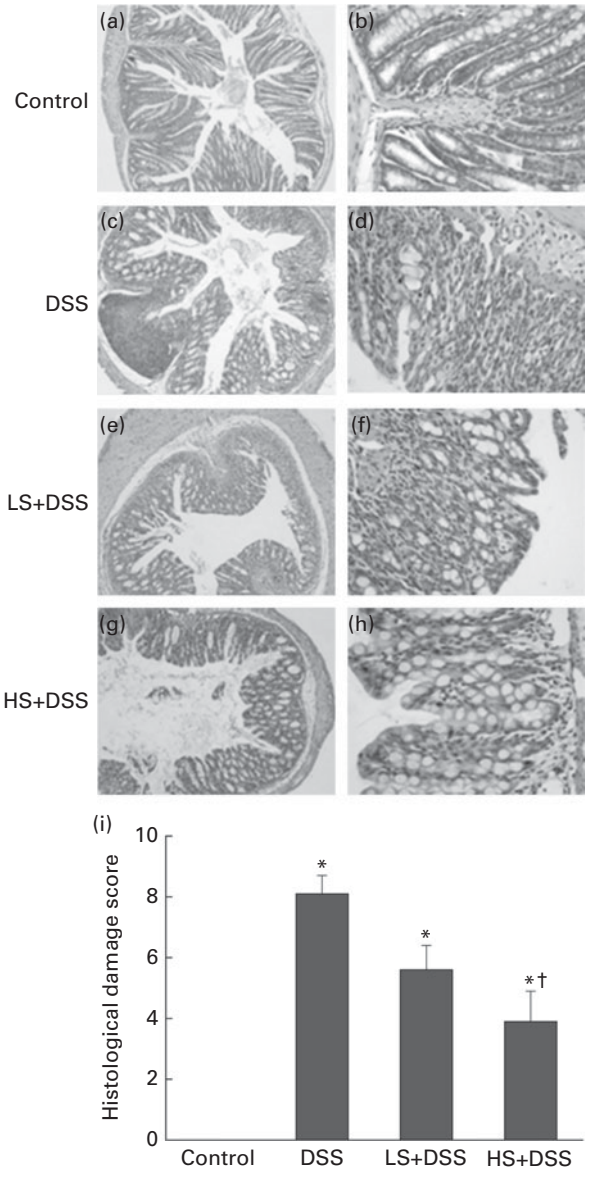

Fig. 4. Histological presentation of colitis in dextran sulphate sodium (DSS)-treated mice and protective effects of Salecan. (a, b) Normal histology of the colon in the control group. LS, diet with 4 % Salecan; HS, diet with 8 % Salecan. (c, d) Extensive intestinal ulceration with severe inflammatory cell infiltrate in the DSS group. Amelioration of the inflammatory process and accumulation of goblet cells in (e, f) the LS+DSS group and (g, h) the HS+DSS group. (a), (c), (e) and (g) 100× magnification; (b), (d), (f) and (h) 400 × magnification. (i) Histological damage scores. Values are means (n 3 mice per group), with their standard errors represented by vertical bars. * Mean value was significantly different from that of the control group ($P<0{\cdot}05$). † Mean value was significantly different from that of the DSS group ($P<0{\cdot}05$).

the environmental components of UC, especially the diet[29]. In the present study, mice were given 4 % DSS dissolved in drinking-water for 5 d in an attempt to assess the protective effects of Salecan as a particular component of the diet on acute inflammation. Salecan, especially at high doses, protected the mice from the toxicity of DSS, evidenced both diagnostically, with alleviation of diarrhoea and bloody faeces, amelioration of anaemia, and prevention of histological damage, and biochemically, by a decrease in colonic MPO activity. It should be noted that water intake in the LS+DSS group was higher than that in the DSS group, which meant that the LS+DSS group consumed more DSS (approximately 1362 mg/mouse *v.* approximately 1102 mg/mouse). Based on the above, one would assume that the LS+DSS group would exhibit a greater degree of colonic damage, which may mask the full impact of the treatment.

Anaemia is a common haematological complication of IBD, occurring in 6 to 74 % of the patients, and negatively affects patients' quality of life[30]. In accordance with the previous observations made by Larrosa *et al.*[31], mice treated with DSS had anaemia marked by lower levels of erythrocytes, Hb and haematocrit. This normocytic (with normal mean corpuscular volume) and normochromic (with normal mean corpuscular Hb concentration and mean corpuscular Hb) anaemia is usually associated with inflammation and occurs due to the loss of blood in the faeces. Neutrophils have been suggested to be involved in the development of colitis[32]. In the present study, there were significant increases not only in blood neutrophil counts but also in the number of neutrophils infiltrating the colon, as assessed by the measurement of colonic MPO activity and confirmed by histology, in the DSS group. MPO activity, an index of tissue-associated neutrophil infiltration, is widely used to quantify intestinal inflammation, and a reduction in the activity of this enzyme can be interpreted as a manifestation of the anti-inflammatory activity of a given compound[33].

The protective effects of dietary Salecan supplementation in DSS-induced experimental colitis can be explained by an anti-inflammatory mechanism through the inhibition of the production and/or release of pro-inflammatory mediators including cytokines. Pro-inflammatory cytokines are known to induce or facilitate inflammation in intestinal mucosa[34]. Specifically, a key role for TNF-α as a pro-inflammatory cytokine in the activation and perpetuation of the inflammatory response in patients with IBD has emerged, and treatment with medications that inhibit TNF-α has been proposed as a new therapeutic strategy[35]. Infliximab, a chimaeric monoclonal antibody directed against TNF-α, has been approved by the US Food and

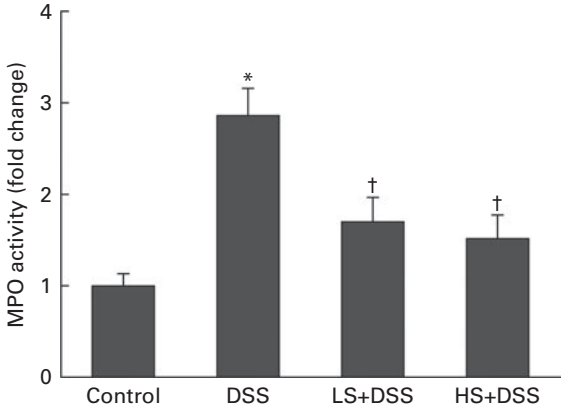

Fig. 5. Effect of Salecan on myeloperoxidase (MPO) activity in mice treated with dextran sulphate sodium (DSS). LS, diet with 4 % Salecan; HS, diet with 8 % Salecan. Values are means (n 6 mice per group) with their standard errors represented by vertical bars. * Mean value was significantly different from that of the control group ($P<0{\cdot}05$). † Mean value was significantly different from that of the DSS group ($P<0{\cdot}05$).

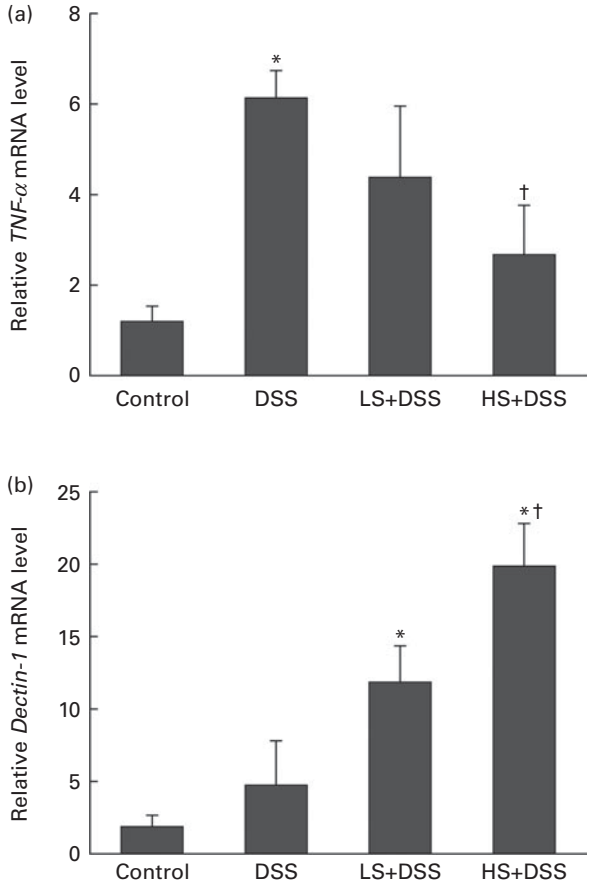

Fig. 6. Effect of Salecan on the relative mRNA levels of *TNF-α* (a) and *Dectin-1* (b) in the colon of mice treated with dextran sulphate sodium (DSS). LS, diet with 4 % Salecan; HS, diet with 8 % Salecan. Values are means (*n* 6 mice per group) with their standard errors represented by vertical bars. * Mean value was significantly different from that of the control group ($P<0·05$). † Mean value was significantly different from that of the DSS group ($P<0·05$).

Drug Administration for the treatment of patients with IBD in 1998[36]. In the present study, the Salecan-supplemented diet exerted a conspicuous attenuating effect on the DSS-induced increase in *TNF-α* mRNA levels. Such an inhibitory effect of Salecan on *TNF-α* mRNA levels is in agreement with the results of studies on partially hydrolysed guar gum[25] and on glucans from edible mushroom[26]. Recent studies have started to shed light on Dectin-1. Dectin-1, a non-classical C-type lectin-binding β-1,3-linked glucan, can induce its own intracellular signalling and play a crucial role in the mediation of a variety of inflammatory responses, such as cytokine production[37]. Dectin-1 activates and regulates the transcription factor NF-κB through a Syk kinase-dependent signalling pathway and/or a Raf-1 serine–threonine kinase-dependent signalling pathway[38,39]. Besides, some studies have demonstrated that Dectin-1 also collaborates with other MyD88-coupled Toll-like receptors in NF-κB signalling, which may be responsible for the synergistic induction of multiple cytokines including TNF[40]. Shah *et al.*[41] reported that particulate β-glucans suppress cytokine production in response to Toll-like receptor stimulation and that this response is mediated by Dectin-1. Given the facts mentioned above, *Dectin-1* mRNA levels were determined by quantitative real-time PCR in the present study. Salecan prominently up-regulated *Dectin-1* mRNA levels in a dose-dependent manner, which indicates that the protective effects of Salecan against DSS-induced colitis by the suppression of *TNF-α* mRNA levels might thus be related to its effect on the up-regulation of *Dectin-1* mRNA levels. It should be stressed that, to the best of our knowledge, Dectin-1 has been mentioned for the first time in the study of the effect of β-glucans on DSS-induced colitis.

The treatment of IBD is a burgeoning field. Infliximab, which just appeared a decade ago, has been the most significant addition to the spectrum of therapeutic options[42]. However, optimal therapy for IBD has not been established still. It has been described that some nutritional supplements are protective against UC, including chitin nanofibrils[43], olive oil[33], resveratrol[31] and probiotic bacteria[44]. The present results suggest that Salecan has potential as a nutritional supplement for patients with UC.

In conclusion, our findings support that Salecan supplementation facilitates the recovery of the inflamed colon in the DSS model of mouse colitis, an effect associated with inhibition of the production of pro-inflammatory mediator TNF-α. This beneficial effect could be ascribed to the enhanced expression of Dectin-1. More developmental research is necessary before Salecan could be used as a functional food for UC patients.

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The authors' contributions are as follows: M. Z., Z. W. and J. Z. conceived and designed the research; M. Z., Z. W. and J. C. carried out the experiment; M. Z., Y. Z., T. W. and L. X. carried out the data analysis; M. Z., Z. W., S. W., Z. H. and J. Z. wrote and revised the manuscript; J. Z. had primary responsibility for the final content. All authors read and approved the final version of the manuscript.

None of the authors has any conflicts of interest to declare.

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