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Almond polysaccharides inhibit DSS-induced inflammatory response in ulcerative colitis mice through NF-κB pathway

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ABSTRACT

Ulcerative colitis (UC), a type of inflammatory bowel disease (IBD), is a chronic recurrent inflammatory disease of the colon. Our previous findings demonstrated that almond polysaccharide (AP-1) exhibits significant anti-inflammatory activity in vitro. Therefore, this study aimed to explore the ameliorative effect of AP-1 on dextrose sodium sulfate (DSS)-induced UC mice and to elucidate its possible mechanism of action. By observing changes in body weight, fecal viscosity, stool blood, disease activity index, and colon length, we found that AP-1 attenuated inflammation. It inhibited TNF-α, IL-1β, and IL-6 while boosting anti-inflammatory IL-10 levels. Histomorphologically, AP-1 protected against DSS-induced colonic tissue damage by reducing inflammatory cell infiltration and mucosal injury. It also lowered myeloperoxidase (MPO) and NO while increasing total superoxide dismutase (T-SOD) and glutathione peroxidase (GSH-Px) in colonic tissues. Moreover, using the Western blot technique, AP-1 was shown to inhibit the phosphorylation of p65 and IκB-α proteins in the NF-κB/iNOS/COX2 signaling pathway and down-regulate the expression of inflammation-associated proteins COX2 and iNOS, thus slowing down and ameliorating inflammatory processes. Therefore, the safe and effective beneficial effects of AP-1 make it a promising therapeutic strategy for relieving IBD, especially UC.

1. Introduction

Inflammatory bowel disease (IBD) is a chronic condition with a complex etiology and varying prevalence around the world. It primarily affects the gastrointestinal tract and extra-intestinal organs and is associated with typical clinical symptoms and histological variants. IBD generally includes ulcerative colitis (UC), Crohn's disease (CD), and indeterminate [1,2] colitis. The etiology of UC remains unclear and is potentially multifaceted, including genetic susceptibility, aberrant immune reactions, and perturbed gut microbiota. The symptoms of UC are characterized by gastrointestinal tract inflammation and a compromised immune system, with diarrhea and bloody stools being the most common changes in defecation habits. These symptoms vary depending on the extent of the lesions and the degree of inflammation. Currently, a variety of clinical treatments are available for IBD, including sulfasalazine (SASP), 5-amino salicylic acid (5-ASA), thiopurines, corticosteroids, other anti-inflammatory agents, biological agents, immunomodulators, and antibiotics. The classical step-up approach suggests that treatment with 5-aminosalicylic acid compounds represents the initial step in managing mild to moderately active UC. Corticosteroids, such as prednisolone, are used in UC patients with moderate to severe disease activity but only for remission induction due to the adverse effects associated with long-term use. Thiopurines are considered the subsequent therapeutic option for managing active UC. However, monotherapy during the induction phase is not preferred due to its slow onset of action [3]. It is important to note that these therapeutic measures can cause severe adverse reactions and affect overall health. Prolonged use of these medications may result in immune suppression, hepatotoxicity, and nephrotoxicity [4–6]. Some natural compounds (polyunsaturated fatty acids, peptides, curcumin, probiotics, and polysaccharides) have been found to alleviate or prevent UC [7,8]. Therefore, identifying natural compounds with potent therapeutic and minimal adverse effects could provide alternatives to conventional UC treatments.

Inflammation is a defensive immune system response initiated by

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deleterious factors, including pathogen invasion, infection, or oxidative stress [9]. Investigations have demonstrated that pro-inflammatory cytokines stimulate immune cell responses within the colonic mucosa during UC progression, leading to inflammatory responses. In UC mice models induced by Dextran sodium sulfate (DSS), this process results in the overproduction of cytokines such as interleukin-1beta (IL-1beta) (IL-1β), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α), as well as the activation of other inflammatory cells, collectively promoting inflammation [10]. DSS acts as a cologne with anticoagulant properties. When administered to mice, it damages the colon's epithelial cells, resulting in inflammation and ulceration similar to IBD in humans. The DSS-induced colitis model is one of the most commonly used mouse models for IBD research [10,11], offering greater control over the severity of inflammation through varying DSS concentrations and administration timing.

Despite substantial progress in understanding IBD biology and improving clinical management and therapeutic strategies, numerous IBD patients still exhibit a lack of response or prolonged unresponsiveness to treatment [12]. Recent studies have demonstrated that polysaccharides, natural antioxidants, can suppress the production of inflammatory cytokines and possess anti-inflammatory properties. Increased polysaccharide intake has been associated with a reduced risk of IBD and even colon cancer [9,13]. Plant polysaccharides can efficiently activate macrophages, stimulating and modulating their functions to exert powerful immune responses [14]. They are effective in increasing lipopolysaccharide (LPS)-induced anti-inflammatory cytokines (IL-10) in macrophages and serum and in decreasing the down-regulation of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α in vitro and in vivo studies [9,15]. The anti-inflammatory potential of plant polysaccharides has been well-documented [16].

Their potential in functional foods and medicines has been explored to utilize plant resources fully. According to our previous findings, almond polysaccharide AP-1, a neutral polysaccharide fraction extracted and purified from bitter almond meal, which consists of glucose, arabinose, galactose, and mannose, exhibits significant anti-inflammatory activity and prebiotic properties in vitro [9,17]. In the LPS-induced inflammation model of RAW264.7 cells, AP-1 effectively inhibited the release of NO, regulated the level of reactive oxide species (ROS), and down-regulated the mRNA expression of TNF-α, IL-1β, IL-6, and inducible nitric oxide synthase (iNOS) [9]. However, the potential of AP-1 as a therapeutic agent for UC has not been investigated. Therefore, this study aimed to determine the safety and efficacy of AP-1 in exerting anti-inflammatory effects in vivo and to explore its anti-inflammatory mechanism.

2. Method

2.1. Materials and reagents

The by-product (almond residue) after oil extraction from cold-pressed almonds was provided by Xinglinchunxiao Chengde Biotechnology Co. Ltd. It was used to extract crude polysaccharides from almonds by a previously reported method. The AP-1 fraction was then obtained after purification with DEAE-52 cellulose columns and Sephadex G-100 dextran gel columns [17].

2.2. Animals and experimental design

For this study, 30 male C57BL/6 mice (6–8 weeks old, 20–25 g) Specific Pathogen Free (SPF grade) were purchased from Liaoning Changsheng Biotechnology Co., Ltd. (Liaoning, China; Animal production License No.: SCXK (Liao) 2020-0001). The animals were bred in a controlled environment with a temperature of 24 ± 2 °C and relative humidity of 50 ± 10 %. The light cycle was set to a 12/12 h light-dark cycle.

The mice were randomly divided into 5 groups: a normal control group (Control), a DSS model group (DSS) (160,110, MP Biomedicals, Irvine, CA, USA), a DSS + low-dose AP-1 group (DSS + AP-1-L, 100 mg/kg·d), a DSS + medium-dose AP-1 group (DSS + AP-1-M, 200 mg/kg·d), a DSS + high-dose AP-1 group (DSS + AP-1-H, 500 mg/kg·d), and a positive drug group administered with 5-ASA (DSS + 5-ASA), each with 6 mice. All groups, except the normal control group, were treated with 3 % (w/v) DSS to induce colonic inflammation for seven consecutive days. The Control group was administered daily water at the same dose as the administered groups. Concurrently, the AP-1-preventive treatment groups were administered low, medium, and high doses of AP-1 daily for eight days, while the positive control group (5-ASA group) was given 100 mg/kg of 5-ASA daily for eight days. Each mouse was monitored daily, and body weight, fecal consistency, and blood in the stool were recorded. The disease activity index (DAI) was calculated according to established scoring criteria [18–20]. Subsequently, mice were euthanized, and the entire colon length was measured. Colonic tissues were then collected for subsequent experiments. The protocol was approved by the Experimental Animal Ethics Committee of Shenyang Agricultural University (2023052201).

2.3. Assessment of histology

Colon tissues from all groups were fixed by formalin, sectioned, and stained with hematoxylin and eosin (H&E). Images were acquired using a microscope (OLYMPUS, DP73, Japan) and assessed with Image J software (US National Institutes of Health, Bethesda, MD). The assessment included the extent of inflammatory infiltration, histopathological changes in crypt structure, ulceration, crypt loss, and ulceration. The histological score was determined based on published reports [19,21,22].

2.4. Inflammatory cytokines and antioxidant measurement

Proteins were extracted from colon tissues using physiological saline and then quantified with a BCA protein assay kit (Wanleibio, Shenyang, China). The concentrations of pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α (Wanleibio, Shenyang, China), IL-10 (Liankebio, Hangzhou, China) in the colon tissue were measured by ELISA according to the manufacturer's instructions. MPO (myeloperoxidase), NO (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), T-SOD, and GSH-Px (Wanleibio, Shenyang, China) were quantified using commercial kits.

2.5. Western blotting

Proteins extracted from colon tissue were separated by 8–11 % SDS-PAGE. Protein concentration was determined by the BCA protein assay kit (WLA019, Wanleibio, Shenyang, China). The protein was then transferred to 0.45 μm polyvinylidene fluoride (PVDF) membranes (IPVH00010, Millipore, Bedford, MA). The PVDF membranes were blocked with 5 % skimmed milk powder for 2 h at room temperature and then incubated with primary antibodies at 4 °C overnight. The antibodies were: IκB-α (WL01936, 1/500, Wanleibio, Shenyang, China), p-IκB-α (WL02495, 1/500, Wanleibio, Shenyang, China), p65 (WL01273b, 1/500, Wanleibio, Shenyang, China), p-p65 (WL02169, 1/500, Wanleibio, Shenyang, China), β-actin (WL01372, 1/1000, Wanleibio, Shenyang, China), COX2 (WL01750, 1/500, Wanleibio, Shenyang, China), iNOS (WL0992a, 1/500, Wanleibio, Shenyang, China). The PVDF membranes were then washed with tris-buffered saline-Tween (TBST) and incubated with horseradish peroxidase-conjugated goat anti-rabbit IgG (WLA023, 1/5000, Wanleibio, Shenyang, China) for 1.5 h. The membranes were scanned and analyzed by the Gel-Pro Analyzer 4.5 program for Windows (Media Cibernetics, Inc., Rockville, MD, USA). Three biological replicates were performed.

2.6. Statistical analyses

Experiment data were analyzed by IBM SPSS Statistics software (SPSS 26.0, Inc., Chicago, IL, USA). Data were expressed as the means ± standard deviation (SD). One-way analysis of variance (ANOVA) with the LSD method was used for pairwise comparisons. Data visualization was performed using GraphPad Prism software (version 9.5 for Windows, GraphPad). Statistical significance was indicated as follows: * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, $^{\#}$ $P < 0.05$, $^{\#\#}$ $P < 0.01$, $^{\#\#\#}$ $P < 0.001$. The "*" symbol indicated the significance between the control group and the other groups, while "#" indicated the significance between the DSS model group and other DSS-induced treatment groups.

3. Result

3.1. The protective effect of AP-1 against DSS-induced UC

The general condition of the mice in each treatment group was first evaluated according to the experimental protocol. The protective effects of AP-1 on DSS-induced UC were evaluated by monitoring fecal consistency, blood in the stool, body weight changes, and DAI. DSS-induced mice exhibited significant body weight loss, poor fecal consistency, and blood in the stool. However, these conditions improved with AP-1 treatment in a dose-dependent manner (Fig. 1). Mice in the DSS group began to lose weight on day 3. Compared to the control group, mice's body weights in all other groups decreased continuously from day 4 to day 7. However, the AP-1-treated group showed a significantly less pronounced weight loss trend by day 5 compared to the DSS group

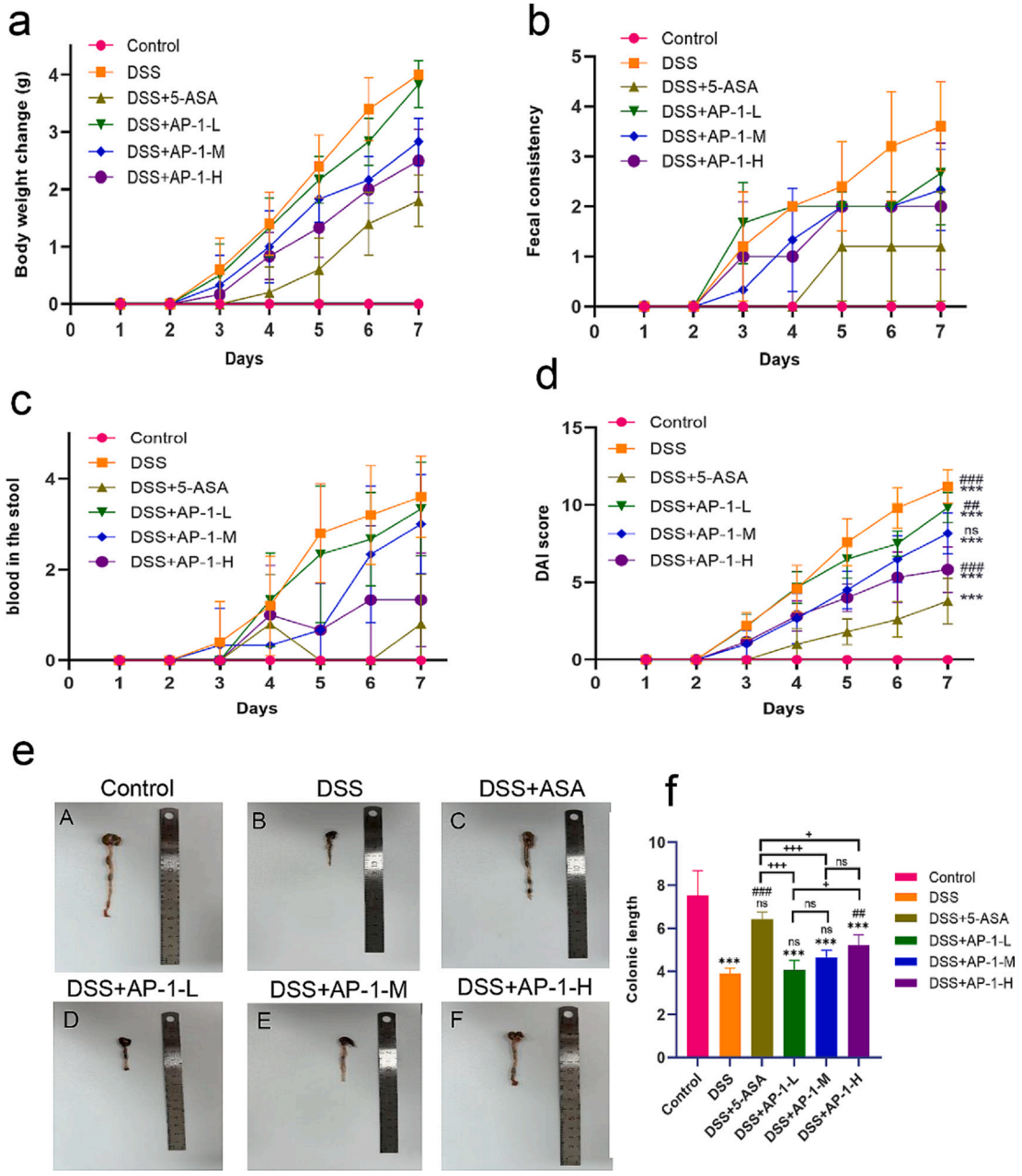

Fig. 1. AP-1 improved the symptoms of DSS-induced colitis in mice. (a) Daily body weight changes in mice. (b) Daily fecal consistency in mice. (c) Daily disease activity index (DAI) scores of mice. (d) Representative macroscopic pictures of colons for each group. (e) The length of colons from each group. Data were expressed as mean ± SD. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. control group. #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$ vs. DSS group.

(Fig. 1a). It demonstrated significant improvements in fecal consistency and blood in stools (Fig. 1b-c). Furthermore, we investigated the levels of DAI for different protocol groups (Fig. 1d). The DAI scores for the DSS-induced mice were notably higher than the control group. The medium- and high-dose AP-1 treatment groups exhibited a significant decrease in DAI scores compared to the DSS group. Furthermore, compared to the DSS group, the increase in DAI scores in each AP-1 treatment group slowed from day 5. The high-dose AP-1 group showed more effective

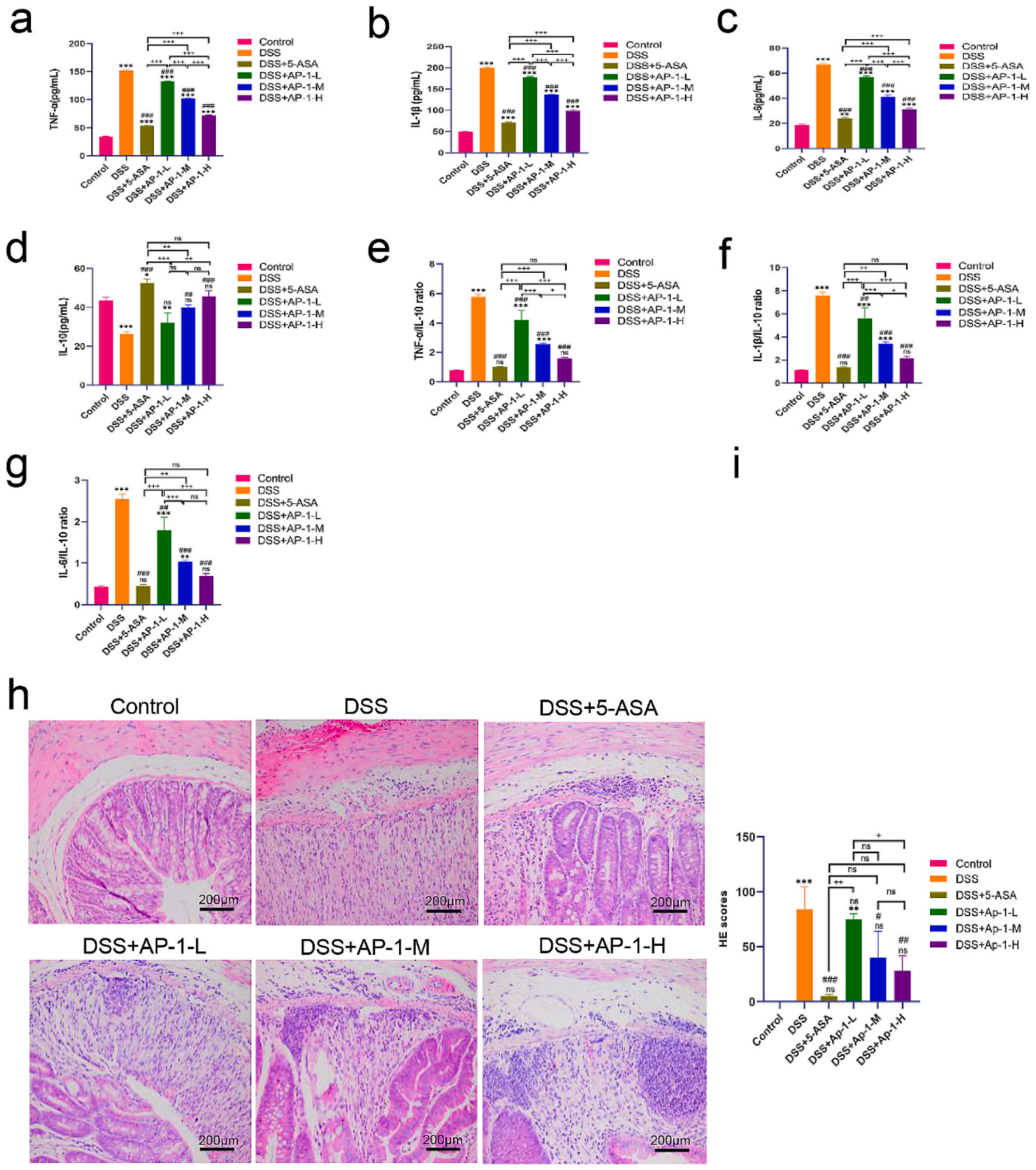

Fig. 2. AP-1 attenuated inflammation of DSS-induced colitis and ameliorated mucosal damage in mice. The concentrations of four representative inflammatory cytokines, pro-inflammatory TNF-α (a), IL-1β (b), IL-6 (c), and anti-inflammatory IL-10 (d). The ratios of pro-/anti-inflammatory cytokines TNF-α/IL-10 (e), IL-1β/IL-10 (f), IL-6/IL-10 (g). (h) Representative H&*E*-stained sections of colon tissue. Scale bar, 200 μm. (i) Histological scores of colons. Data were expressed as mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control group. $\#P < 0.05$, $\#\#P < 0.01$, $\#\#\#P < 0.001$ vs. DSS group.

results than the other two groups in a dose-dependent manner. Consequently, administering AP-1 as an intervention treatment alleviated weight loss, improved fecal consistency, and reduced blood in the stool. The DAI scores gradually decreased, and UC symptoms were alleviated dose-dependent.

The above results indicated that AP-1 had a protective effect on DSS-induced UC mice. Nevertheless, its potential mechanism remained to be elucidated.

3.2. Interventional therapy with AP-1 attenuates DSS-induced UC

Next, we measured the length of the colon in mice from different treatment groups (Fig. 1e). The results demonstrated that the morphological changes observed in the colons of mice subjected to DSS treatment were statistically significant compared to the control group (Fig. 1f). Furthermore, the severity of these changes was ranked as follows: DSS + 5-ASA group < DSS + AP-1-H group < DSS + AP-1-M group < DSS + AP-1-L group < DSS group. High-dose AP-1 reduced the degree of shortening of colon length compared to the DSS group. In addition, there was a significant difference between the high-dose AP-1 and low-dose groups, but not the medium-dose group. It suggested that AP-1 could effectively improve the colon length in DSS-induced UC mice within a specific dose range. However, its effectiveness may be limited when the intervention dose is low.

Therefore, Ap-1 may play a role in protecting the colon. Next, we will explore how AP-1 can reduce the inflammatory response or treat UC.

3.3. AP-1 ameliorates inflammation in DSS-induced UC mice

It has been demonstrated that inflammatory cytokines play a pivotal role in the pathogenesis of chronic inflammatory diseases and represent an important therapeutic target in animal models [23]. Following the induction of the UC model by DSS, the levels of inflammatory cytokines were quantified by ELISA assay. The levels of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) in the colon tissues of the experimental groups were significantly elevated compared to the control group (Fig. 2a-c). The expression of pro-inflammatory cytokines (TNF-α, IL-1β, IL-6) was reduced in each AP-1 treatment group and the 5-ASA group compared to the DSS group. Pro-inflammatory cytokines slightly increased in the high-dose AP-1 group compared to the 5-ASA groups.

Furthermore, the expression of the anti-inflammatory cytokine IL-10 was evaluated in colon tissue (Fig. 2d). The results demonstrated a significant reduction in IL-10 expression in the DSS group and low-dose AP-1 group compared to the control group, with increased expression observed in the DSS + 5-ASA group. IL-10 expression in the medium- and high-dose AP-1 groups did not differ significantly ($P > 0.05$) from the control group. There was a significant increase in IL-10 expression in the medium- and high-dose AP-1 and 5-ASA groups compared to the DSS group. Notably, the tissue level of IL-10 in the DSS group was significantly lower compared to the control group. No significant difference in IL-10 expression was observed between DSS-induced mice treated with high doses of AP-1 and those treated with 5-ASA ($P > 0.05$). Therefore, AP-1 may have facilitated the expression of IL-10 in mice with DSS-induced UC, thereby playing an anti-inflammatory role.

Subsequently, we compared the ratio of pro-inflammatory to anti-inflammatory cytokines to determine the effect of AP-1 on the balance between these cytokines in attenuating the inflammatory response in UC mice. The results showed that the ratio of pro-inflammatory to anti-inflammatory cytokines (TNF-α/IL-10, IL-1β/IL-10, IL-6/IL-10) was significantly higher in the DSS group compared to the control group. In contrast, these ratios were significantly lower in the AP-1 treatment group compared to the DSS group (Fig. 2e–g). There was no significant difference in these ratios between DSS-induced mice treated with high doses of AP-1 and those treated with 5-ASA compared to the control group. These results demonstrated that AP1-preventive treatment could reduce pro-inflammatory cytokines levels, increase anti-inflammatory cytokine IL-10, and lower the ratios of pro−/anti-inflammatory cytokines. It indicated that high-dose AP-1 promoted the secretion of IL-10 and helped correct the imbalance between anti-inflammatory and pro-inflammatory cytokines during the prophylactic treatment of DSS-induced UC. Therefore, it was reasonable to hypothesize that AP-1 effectively reduced inflammation and exerted therapeutic effects similar to 5-ASA within a certain dose range.

3.4. Effects of AP-1 on the histomorphology of DSS-induced UC mice

When it was observed that AP-1 decreased the expression levels of IL-1β, IL-6, and TNF-α and increased the expression levels of the anti-inflammatory cytokine IL-10 in a dose-dependent pattern, it was decided to study the pathological changes in the colonic tissue of experimental mice. Pathological sections of colonic tissue stained with H&E were analyzed for all the mice observed experimentally (Fig. 2h). The histological analysis showed that the control group maintained normal tissue structure with no observable changes. In contrast, the DSS-treated mice exhibited significant hyperemia, edema, and severe disruption of mucosal integrity, including epithelial damage, crypt abscesses, goblet cell loss, and extensive infiltration by inflammatory cells. The inflammation in the DSS group was markedly higher than in the control group. Treatment with AP-1 led to a dose-dependent improvement in colonic structure; higher doses notably approached normal tissue structure and reduced inflammation levels. Specifically, the highest dose of AP-1 resulted in an almost normal mucosal structure without significant mucosal ulceration, decreased epithelial proliferation, restoration of villi, and reduced inflammatory cell presence. Pathologically, DSS-treated mice scored significantly higher on HE staining indices than the control group, indicating severe inflammation (Fig. 2i). However, AP-1 treatment at medium and high doses, as well as 5-ASA treatment, significantly lowered these scores, suggesting an effective reduction in colonic injury comparable to standard 5-ASA treatment. No significant differences were found in the H&E scores between medium and high doses of AP-1 or compared to the 5-ASA group, underscoring the potential of AP-1 in mitigating colon damage in DSS-induced colitis in mice ($P > 0.05$).

Overall, the AP1 treatment appeared to ameliorate pathological changes in colon tissue, with higher doses showing particularly effective remission.

3.5. Effect of AP-1 on MPO activity and NO secretion in UC mice

As previously noted, AP-1 effectively reduced the elevated intestinal inflammatory cytokines induced by DSS. MPO is released from the cytoplasmic granules of activated phagocytes and is a peroxidase most abundantly expressed in neutrophils [24]. Combined with the results of H&E pathological analysis, the infiltration of neutrophils among the important inflammatory cells was further assessed indirectly by measuring MPO activity in the colonic tissues. Mice receiving DSS showed an increase in MPO activity compared to the control group (Fig. 3a). The AP-1 treatment groups demonstrated a reduction in MPO activity compared to the DSS group. Consequently, it was postulated that AP-1 could reduce MPO activity. However, there was no significant difference between the high-dose AP-1 group and the 5-ASA group regarding the reduction of MPO activity. It indicated that AP-1 could effectively alleviate the inflammatory response. Furthermore, inflammation activates macrophages, increasing the expression of iNOS, ultimately producing pro-inflammatory molecules such as NO. NO secretion was significantly increased in the DSS group compared with the control group. In contrast, the AP-1 group significantly inhibited NO secretion in a dose-dependent manner compared to the DSS group (Fig. 3b). T-SOD and GSH-Px are widely used as measures of resistance to oxidative stress [25]. The levels of T-SOD and GSH-Px in DSS-induced UC mice were diminished compared to the control group (Fig. 3c–d). Notably, the AP-1 treatment group exhibited a significant increase in the levels of T-SOD

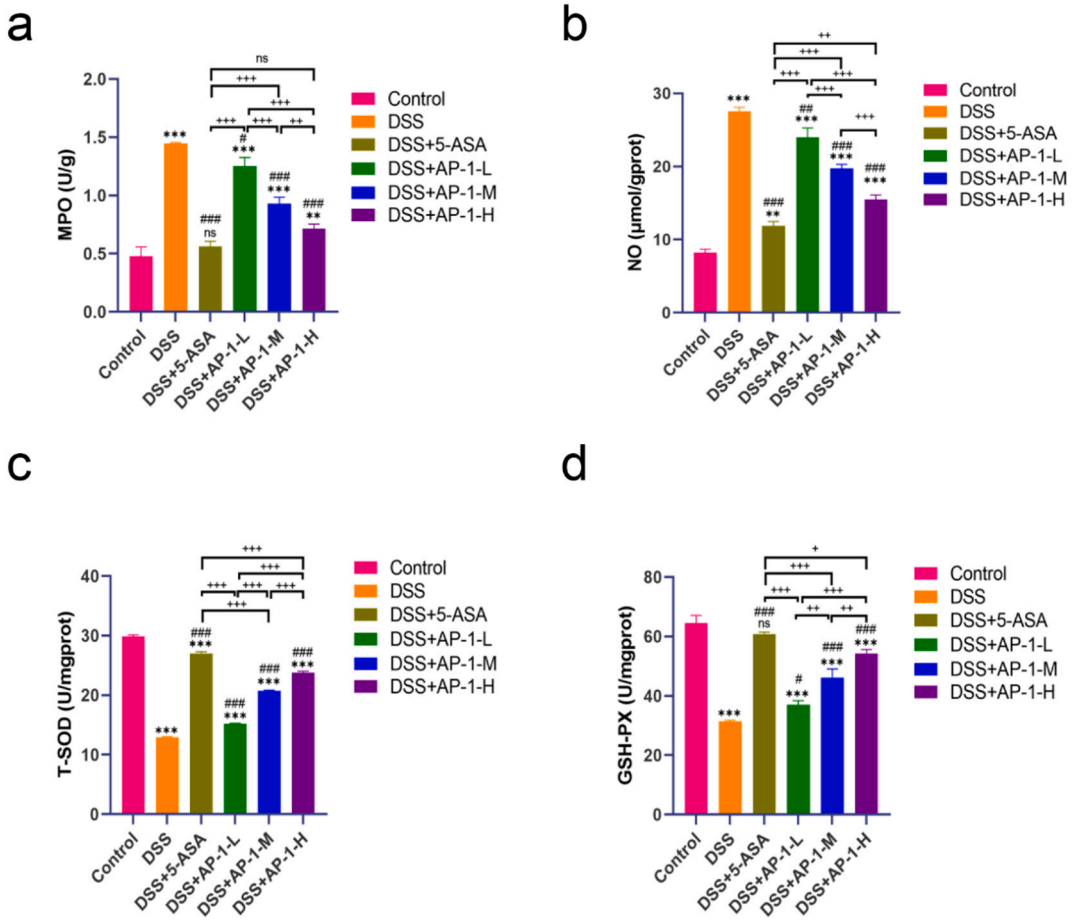

Fig. 3. AP-1 alleviated inflammatory cytokines and increased antioxidant capacity in DSS-induced colitis mice. The expression of MPO activity (a). NO (b), T-SOD (c), GSH-Px (d) of colons. Data were expressed as mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control group. $\#P < 0.05$, $\#\#P < 0.01$, $\#\#\#P < 0.001$ vs. DSS group.

and GSH-Px in colitis tissues compared to the DSS group. Furthermore, the expression of these enzymes increased in a dose-dependent manner in different dose groups.

These results demonstrated that AP-1 treatment can reduce neutrophil infiltration, alleviate pathologic damage, and improve intestinal permeability, notably enhancing antioxidant capacity in colon tissue and effectively alleviating DSS-induced UC.

3.6. AP-1 reduces pro-inflammatory cytokine production and suppresses NF-κB activation in DSS-induced mice

NF-κB signaling is a complex process involved in various biological functions, including inflammation, immune regulation, and the tumor microenvironment. Due to the unbalanced production of pro-inflammatory and anti-inflammatory cytokines in UC, NF-κB signaling pathway proteins can ultimately damage substantial colonic tissue by mediating inflammatory responses, amplifying excessive inflammation, and facilitating the recruitment and activation of immune cells [26]. As we previously verified, DSS-induced UC mice demonstrated increased levels of pro-inflammatory cytokines at transcriptional levels, including TNF-α, IL-6, and IL-1β. Ap-1 effectively inhibited these pro-inflammatory cytokines. The above inflammatory factors have also been suggested to play a vital role in the pathogenesis of DSS-induced UC [27,28]. To gain further insight into the influence of AP-1 on the NF-κB pathway in response to DSS in vivo, a Western blot (WB) assay was employed to assess the phosphorylation levels of critical components within the NF-κB pathway in colonic tissue, including IκB-α and p65. The results demonstrated activation of the NF-κB pathway in DSS-induced UC mice, as evidenced by elevated phosphorylation levels of IκB-α and p65, accompanied by reduced levels of IκB-α and p65 (Fig. 4a, e). All DSS-induced colitis groups exhibited significant upregulation in the phosphorylation of IκB-α and p65 (Fig. 4b, f) and a notable decrease in IκB-α and p65 levels (Fig. 4c, g) compared to the control group. However, the AP-1 treatment groups effectively reduced the phosphorylation levels of IκB-α and p65, concomitant with the upregulation of IκB-α and p65 levels compared to the DSS group (Fig. 4c, g). We believed that AP-1 potentially inhibited NF-κB activity. Moreover, the AP-1 treatment groups exhibited a marked decrease in the ratios of phosphorylated IκB-α/total IκB-α and phosphorylated p65/total p65 in a dose-dependent manner compared to the DSS group (Fig. 4d, h).

These findings indicated that AP-1 effectively inhibited NF-κB activation in DSS-induced UC, thereby reducing the production of inflammatory cytokines and contributing to its anti-inflammatory effects.

3.7. AP-1 reduced the production of pro-inflammatory mediators

Our interest was in whether AP-1 could suppress the production of proinflammatory cytokines and ameliorate intestinal inflammation in DSS-induced UC mice. As previously demonstrated, the NF-κB signaling pathway was activated in mice with DSS-induced UC. This activation led to the phosphorylation of IκB-α, which released p65 to enter the nucleus for gene transcription. Thus, activating the NF-κB signaling pathway promoted the expression of inflammatory cytokines and inflammation-associated proteins such as iNOS and COX2. The increased production of pro-inflammatory mediators played a pivotal role in the pathogenesis of DSS-induced UC. A WB assay was conducted to assess the impact of

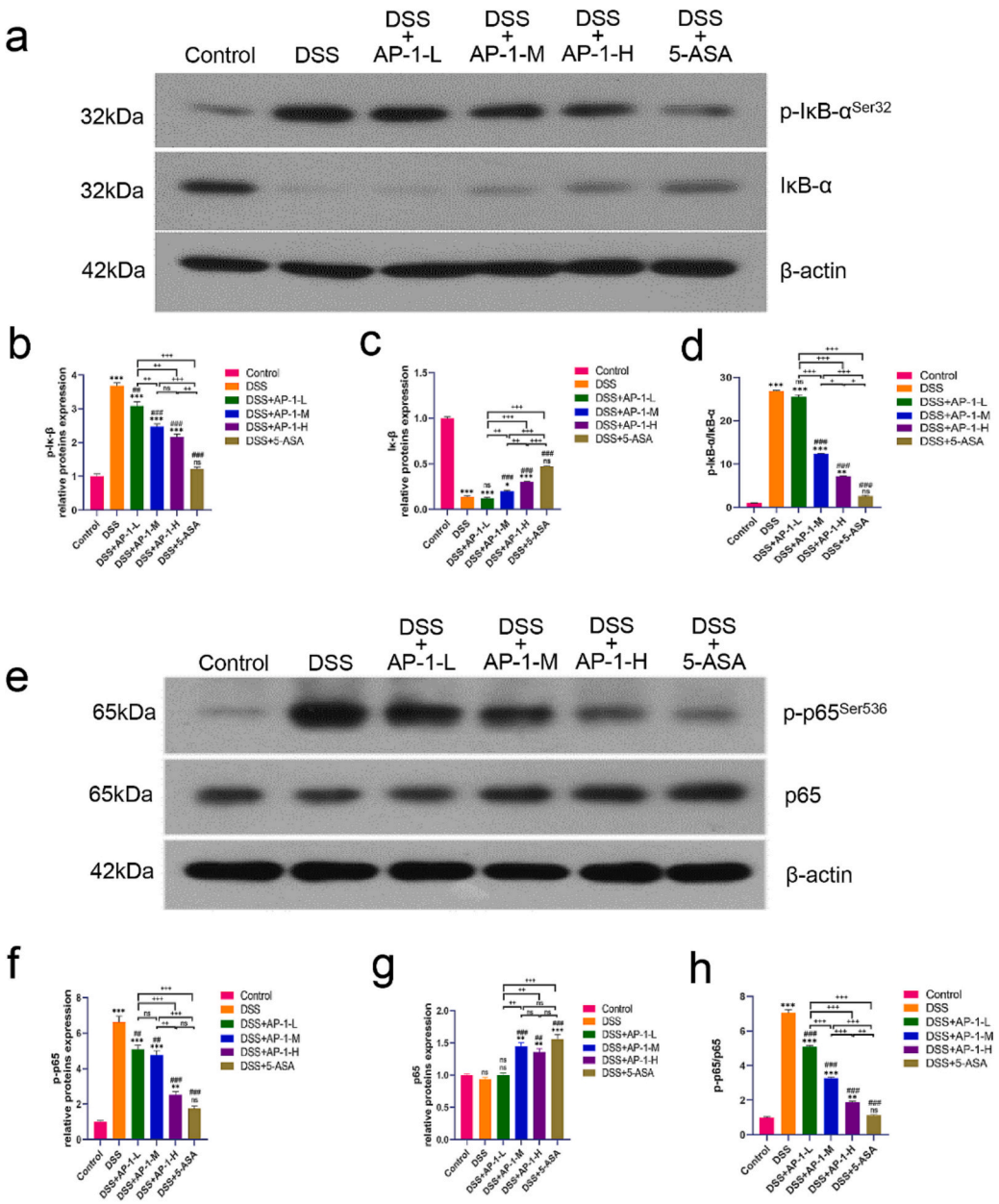

Fig. 4. AP-1 regulated NF-κB signaling pathway activation in colon tissue of DSS-induced colitis mice. Western blot analysis of the protein gel of NF-κB signaling pathway (a) (e), p-I-κβ (b), I-κβ (c), the ratio of p-I-κβ/I-κβ (d), p-p65 (f), p65 (g), the ratio of p-p65/p65 (h) in the colons tissue from each group were normalized to β-actin. Data were expressed as mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control group. $\#P < 0.05$, $\#\#P < 0.01$, $\#\#\#P < 0.001$ vs. DSS group.

AP-1 on the protein expression of inflammation-associated molecules (Fig. 5a, c). The results demonstrated that the expression of pro-inflammatory mediators, including iNOS (Fig. 5b) and (cyclo-oxygenase 2) COX2 (Fig. 5d), was significantly elevated in the DSS group compared to the control group. AP-1 treatment exhibited a pronounced inhibitory effect on the expression of these pro-inflammatory mediators, significantly attenuating the expression of iNOS and COX2, particularly at high doses of AP-1 in colonic tissues compared to the DSS group. The effects were dose-dependent.

These findings suggested that AP-1 treatment could mitigate DSS-induced colonic epithelial injury by regulating the expression of inflammatory cytokines.

4. Discussion

The global incidence of inflammatory bowel disease (IBD), including UC and Crohn's disease, has risen over the past decade [29]. IBD is marked by chronic inflammation that can lead to symptoms such as malabsorption, diarrhea, and abdominal pain, potentially increasing the risk of colorectal cancer [29–33]. The complexity of IBD makes researching its causes and potential treatments challenging. Polysaccharides, naturally occurring plant polymers known for their various health benefits, including anti-inflammatory and antioxidant properties, have been effective in improving symptoms like diarrhea and intestinal inflammation in UC, even in the presence of intestinal cancer [20] [34] [35] [36] [37] [38] [39] [40]. Almond epidermis extracts have been reported to possess antibacterial effects and influence cellular oxidative stress states [41]. Bitter amygdalin extracted from almonds interferes with glucose metabolism by scavenging hydroxyl radicals [42]. In a previous study, Purified almond polysaccharides extracted from wild apricots (almonds) had higher percentages of galactose, rhamnose, and arabinose [43]. The results showed that polysaccharides containing

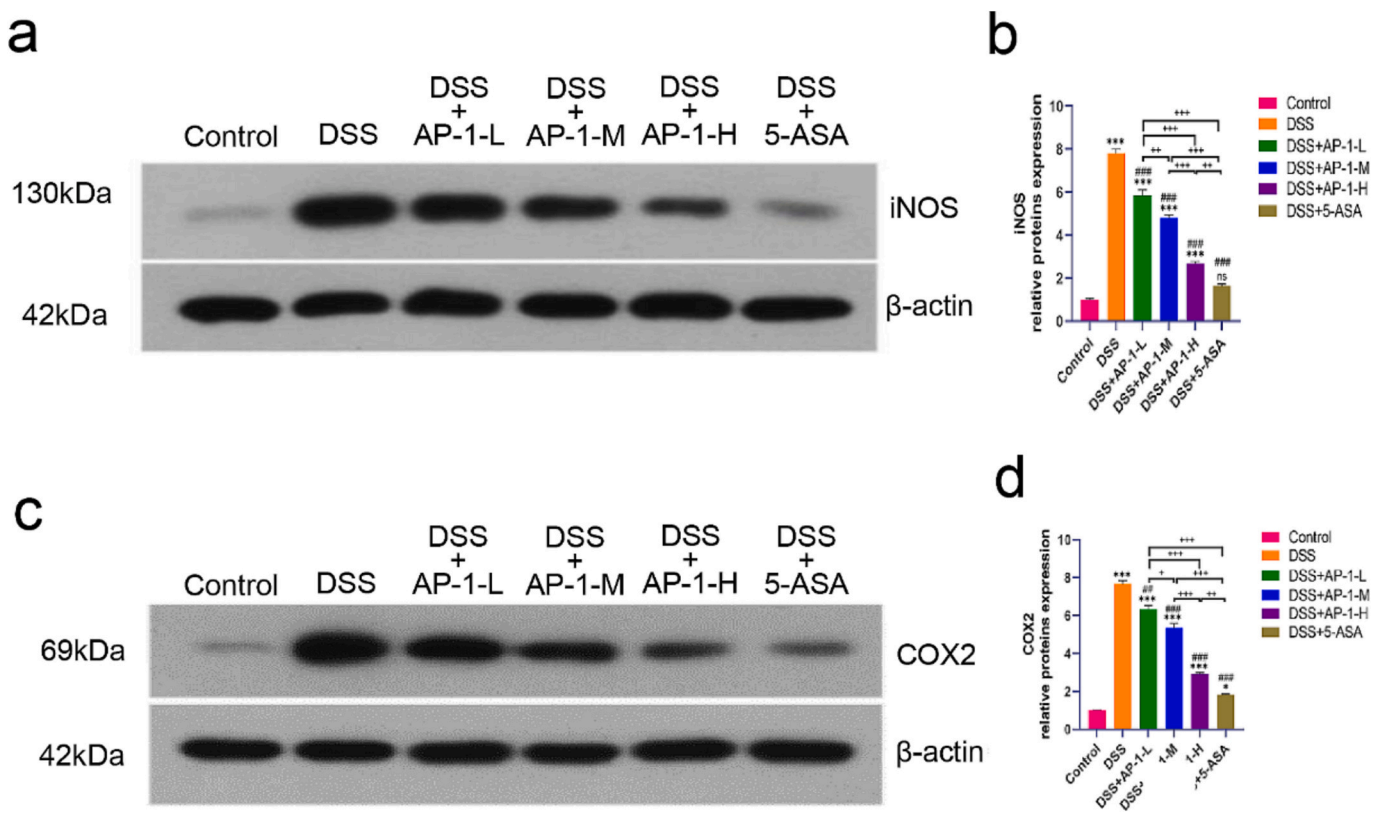

Fig. 5. AP-1 reduces the production of inflammatory mediators of the NF-κB pathway in colonic tissues of DSS-induced mice. The protein gels of iNOS (a) and COX2 (c). The western blot analysis for iNOS (b)and COX2 (c) in the colons tissue from each group was normalized to β-actin. Data were expressed as mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs. control group. $\#\ P < 0.05$, $\#\#\ P < 0.01$, $\#\#\#\ P < 0.001$ vs. DSS group.

these monosaccharides exhibit better biological activity [44,45]. These findings were consistent with our previous research on elucidating the structural characterization of AP-1 and paved the way for further studies [17].

Previous studies have also shown that AP-1, with its triple helical structure, has probiotic properties against human gut microorganisms and anti-inflammatory activity against LPS-induced RAW264.7 cells [9,17]. The majority of polysaccharides are resistant to destruction and decomposition by saliva, gastric, and intestinal juices and are utilized by the intestinal mucosa [46]. The Modified Gegen Qinlian decoction was found to ameliorate DSS-induced UC in mice by protecting the intestinal barrier through its anti-inflammatory, antioxidative stress, and reparative effects on the damaged intestinal barrier [47]. The purified polysaccharide brown alga Ishige Okamurae preserved the structure of intestinal tissues, corrected the dysregulation of inflammatory cytokines, and protected the colonic mucosal barrier [48]. Therefore, we aimed to investigate the positive effect of AP-1 on UC in vivo.

In our study, we used AP-1 to intervene in treating DSS-induced UC in mice to assess its therapeutic effect and explore its mechanism of action against inflammation. DSS caused drastic body weight loss, diarrhea, hematochezia, and shortening of the colon in mice [49,50]. We observed these symptoms in our DSS-induced UC model mice during our study. Firstly, we noted relief from these symptoms in DSS-induced mice after oral administration of AP-1. Diarrhea symptoms improved significantly, along with reduced stool viscosity; colon tissue length increased, and the pathological state improved markedly, with a more significant effect observed in the high-dose group compared to the low-dose group. Furthermore, it was found that DAI scores were lower after treatment with AP-1, indicating a reversal of pathological injury, with a more pronounced effect seen in the high-dose group [51]. Considering that inflammation may have been alleviated in UC mice following treatment with AP-1, we also noted a significant reduction in colon shortening, which correlated with the reduced DAI scores. The polysaccharide derived from Scutellaria baicalensis Georgi treatment demonstrated the ability to attenuate body weight loss, reduce the DAI score, and ameliorate colonic pathological damage in DSS-induced UC mice [49]. These findings align with our present study, suggesting that AP-1 may play a role in attenuating inflammation in UC.

The UC's main pathological manifestations and biological response include mucosal inflammation, damage to the colon mucosal barrier, abnormal morphology of the epithelial tissue, dysfunction of intracellular junctions, remodeling of the epithelial wall, and disruption of intestinal homeostasis [52]. Inflammation of the intestinal mucosa disrupts the balance between intestinal antigens and host immunity [53], damaging the intestinal mechanical barrier. It allows bacteria or harmful metabolites to stimulate the immune system, releasing pro-inflammatory cytokines such as IL-1β, IL-6, and TNF-α, which promote further inflammatory response. The imbalance between pro-inflammatory and anti-inflammatory cytokines exacerbates uncontrolled intestinal inflammation [54]. It leads to damage of the intestinal mucosal barrier as well as disruption of the intestinal microflora - all likely key factors in UC development [4,55]. For this reason, inhibiting inflammatory cytokines or interfering with downstream pathway effectors may slow inflammation progression and ease or reverse disease symptoms. Natural polysaccharides have been found to possess anti-inflammatory and immunomodulatory properties that can control inflammatory cytokine expression and enhance repair function in damaged intestinal mucosal barriers - making them effective for improving UC. For example, Morchella conica polysaccharide has been shown to inhibit excess NO production by downregulating iNOS expression by inhibiting the NF-κB pathway [56]. Atractylodes macrocephala Koidz. Polysaccharide treatment has also been found to down-regulate IL-6 expression by inhibiting the IL-6/STAT3 signaling pathway—reducing expression levels of inflammation cytokines, and

alleviating DSS-induced colitis [56]. Previous studies have observed that AP-1 can inhibit inflammation by reducing the release of inflammatory cytokines when introduced into LPS-induced RAW264.7 cells - suggesting its potential role in mitigating inflammation effects. However, the effects of Ap-1 on inflammation, oxidative injury, intestinal barrier damage, intestinal microbiota, and metabolite changes caused by UC are not yet evident in vivo.

We found that AP-1 effectively improved the inflammatory cell infiltration damage caused by DSS in colonic tissues. It reduced the degree of damage to the crypts of the colonic mucosa and reversed the number and morphology of damaged intestinal mucosal epithelial cells, as shown by histomorphometry H&E staining. These results suggested that AP-1 exerted an anti-inflammatory effect in vivo. The levels of inflammatory cytokines in different treated groups caused by DSS were detected by ELISA. It was found that AP-1 could reduce the expression of pro-inflammatory cytokines TNF-α, IL-1β, and IL-6 in colon tissues while promoting the expression of anti-inflammatory cytokine IL-10. It suggested that AP-1 could correct the imbalance between pro-inflammatory and anti-inflammatory cytokines.

Additionally, AP-1 also reduced the expression of MPO and the inflammatory marker NO in UC mice tissues. Previous research has reported similar findings with other polysaccharides such as Schisandra chinensis (Turcz.) Baill polysaccharide reduced the levels of MPO and MDA in colonic tissue [57]. Scutellaria baicalensis Georgi polysaccharide decreased MPO activity and the levels of pro-inflammatory cytokines of UC mice induced by DSS [49]. Thus, we confirmed that almond polysaccharides can also exert anti-inflammatory effects in vivo. In addition, porphyra haitanensis polysaccharide significantly suppressed oxidative stress in the serum of UC mice, improved histological damage in colitis tissues, and restored the intestinal mucosal barrier [58]. The residue of Flammulina velutipes also showed potential antioxidative effects [44]. Considering the potential antioxidant effects of the monosaccharide composition of almond polysaccharides, we conducted an AP-1 assay. We found that the antioxidant enzymes T-SOD and GSH-Px levels were increased by treatment with AP-1, as determined by ELISA. These results suggested that AP-1 had the potential to suppress over-inflammation and oxidative stress, effectively improve colon antioxidant capacity, and alleviate the severity of UC. This mechanism may explain how it repaired injury to the intestinal mucosa and restored the mucosal barrier in UC mice.

The NF-κB protein family consists of p65 (RelA), c-Rel, RelB, p50, and p52, but only p65, c-Rel, and RelB can directly activate transcription of target genes. In unstimulated cells, most NF-κB dimers are retained in the cytoplasm by binding to small inhibitory molecules IκBα, IκBβ, or IκBε. IκBα can also enter the nucleus, subsequently mediating and blocking the DNA binding of NF-κB to promote the nuclear exportation of NF-κB [59,60]. Classical activation of NF-κB can be triggered by pro-inflammatory cytokine stimulation such as lipopolysaccharide, TNF-a, or IL-lβ inducing intracellular signaling cascades. The expression and activation of NF-κB are strongly induced in inflamed intestines of IBD patients. In particular, the increased expression of NF-κB p65 in macrophages and epithelial cells from intestinal specimens of IBD patients was accompanied by an increased ability of these cells to produce and secrete TNF-α, IL-1, and IL-6 [61]. Inflammatory signaling pathways, such as NF-κB, TNF, IL-17, TH17, and HIF, can be activated by UC pathogenic bacteria and their toxins to promote the release of inflammatory cytokines, destroy the integrity of the intestinal barrier and cause local or systemic inflammation [62]. Shikimic acid inhibited the expression of TNF-α, IL-1β, and MPO. It also reduced the phosphorylation of essential proteins in MAPK and NF-κB signaling pathways to improve intestinal inflammation and enhance intestinal immunity in DSS-induced mice with colitis [62]. The fermented Sargassum fusiforme decreased NO, MPO, and MDA concentrations while increasing T-SOD activity. This fermentation also inhibited the NF-κB signaling pathway in the colon [63]. Therefore, it is evident that the transcription factor NF-κB plays a role in the pathogenesis of IBD as a significant regulatory component [63,64]. Our study showed that AP-1 could inhibit the phosphorylation level of p65 and IκB-α proteins in the NF-κB signaling pathway and reduce the expression of inflammation-related proteins iNOS and COX2. It had been reported that exogenous CEACAM1 effectively reduced inflammatory cytokines by downregulating COX2 and iNOS expression levels in mice [65]. It was also consistent with our findings. This inhibition underscores the potent anti-inflammatory properties of polysaccharides. These findings align with existing literature that highlights NF-κB inhibition as essential for mitigating the activation of this central immune and inflammatory regulator. Various studies confirm that polysaccharides, especially those derived from natural sources, can effectively modulate NF-κB activity, suggesting their potential therapeutic benefits in managing inflammatory conditions. Nicotiflorin could potentially reduce inflammation by targeting p65 and blocking the NF-κB pathway, thus decreasing the activation of the NLRP3 inflammasome [66]. Similarly, Phillygenin has the potential to exert anti-inflammatory effects by downregulating the TLR4/MyD88/NF-κB pathway and preventing the activation of the NLRP3 inflammasome [67]. Additionally, Dendrobium officinale leaf phenolics were observed to suppress the expression of principal proteins associated with the TLR4/NF-κB signaling pathway, reducing the production of inflammatory cytokines [68]. These natural compounds are emerging as valuable therapeutic agents in inflammatory diseases by modulating critical signaling pathways. Moreover, polysaccharides may also help maintain balance in the intestinal microflora, potentially mitigating the progression of colonic diseases such as UC or colorectal cancer [69,70]. For example, berberine has shown promise in alleviating colitis-associated colorectal cancer by remodeling intestinal microflora [70]. AP-1's dual capability to modulate immune responses and microbial compositions emphasizes its potential as a comprehensive therapeutic option for inflammation and gastrointestinal health [9].

One primary characteristic of UC is increased intestinal permeability, which allows antigens to enter the body from the digestive tract easily. A reduction in intestinal permeability could result in a decrease in macromolecular antigens entering the body. It was found that the administration of an extracellular matrix hydrogel (ECMH) had a protective effect on the epithelial cells of the colonic mucosa [71]. The researchers believed that the barrier function was not recovered by the physical presence of the hydrogel but rather by the re-establishment of a physiologically functioning mucosal epithelium. The administration of the ECMH was observed to reduce epithelial cell damage, promote mucosal integrity, and improve epithelial barrier function. Furthermore, ECMH was observed to modulate macrophage phenotype, preventing them from entering a pro-inflammatory state. Consequently, the researchers concluded that ECMH treatment has a therapeutic effect on UC by positively influencing physiological processes related to the colonic barrier function and the pro-inflammatory response. From the current results, the decreased inflammatory response and intestinal permeability observed in the AP-1-treated group represent two distinct but related etiological factors in the development of UC. It may be proposed that both factors interact in a causal relationship. As previously mentioned, Keane et al. demonstrated that mucosal permeability is increased in UC tissue, which supports this theory. Although AP-1 has been demonstrated to reduce intestinal permeability and intestinal inflammation effectively, it is unclear which effects occur first. Further research will elucidate the potential mechanisms by which AP-1 affects intestinal permeability.

This study presented almond polysaccharide (AP-1) as a promising alternative to conventional synthetic drugs for treating inflammation, setting it apart from typical pharmacological approaches. The natural derivation of AP-1 may offer significant advantages, such as fewer side effects and the potential for dietary incorporation, making it more attractive. Our research provided a comprehensive examination of inflammatory biomarkers and the mechanisms behind the effects of AP-1, particularly its influence on the NF-κB/iNOS/COX2 pathway, providing a thorough understanding of its therapeutic potential. However, due to

inherent physiological and genetic differences, the DSS-induced mouse model findings may not be directly applicable to human UC conditions. Therefore, further validation through human clinical trials was essential to confirm the efficacy and safety of AP-1. In addition, this study only evaluated AP-1 without comparing it to established UC treatments, which limited our understanding of its relative efficacy and potential interactions. Another limitation was the lack of discussion of long-term effects. Given the chronic nature of UC, it is critical to evaluate the long-term safety and efficacy of treatments, which was not addressed in this study.

5. Conclusion

This study successfully verified the safety and efficacy of the polysaccharide AP-1 in vivo for the first time. With its properties of inhibiting inflammation and acting as an antioxidant, AP-1 effectively alleviated DSS-induced UC symptoms in mice. Additionally, AP-1 inhibited the activation of the NF-κB pathway and down-regulated the expression of inflammatory cytokines and inflammation-related proteins. Therefore, it is reasonable to speculate that AP-1 may have potential applications in the clinical treatment of UC.

CRediT authorship contribution statement

Jiayi Zhu: Investigation, Data curation. **Yingshuo Li:** Validation. **Xiqing Yue:** Supervision, Resources, Project administration. **Yanyu Peng:** Writing – review & editing, Visualization, Validation, Software, Project administration, Investigation, Data curation.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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References

[1] T. Kobayashi, B. Siegmund, C. Le Berre, S.C. Wei, M. Ferrante, B. Shen, C. N. Bernstein, S. Danese, L. Peyrin-Biroulet, T. Hibi, Ulcerative colitis, Nat. Rev. Dis. Primers 6 (1) (2020) 74, https://doi.org/10.1038/s41572-020-0205-x.

[2] S. Gancarcikova, S. Lauko, G. Hrckova, Z. Andrejcakova, V. Hajduckova, M. Madar, L. Kolesar Fecskeova, D. Mudronova, K. Mravcova, G. Strkolcova, R. Nemcova, J. Kacirova, A. Staskova, S. Vilcek, A. Bomba, Innovative animal model of DSS-induced ulcerative colitis in pseudo germ-free mice, Cells 9 (12) (2020), https://doi.org/10.3390/cells9122571.

[3] S.E. Berends, A.S. Strik, M. Lowenberg, G.R. D'Haens, R.A.A. Mathot, Clinical pharmacokinetic and pharmacodynamic considerations in the treatment of ulcerative colitis, Clin. Pharmacokinet. 58 (1) (2019) 15–37, https://doi.org/10.1007/s40262-018-0676-z.

[4] H. Ullah, T. Deng, M. Ali, N.A. Farooqui, D.M. Alsholi, N.Z. Siddiqui, A.U. Rehman, S. Ali, M. Ilyas, L. Wang, Y. Xin, Sea Conch peptides hydrolysate alleviates DSS-induced colitis in mice through immune modulation and gut microbiota restoration, Molecules 28 (19) (2023), https://doi.org/10.3390/molecules28196849.

[5] M. Cottone, S. Renna, I. Modesto, A. Orlando, Is 5-ASA still the treatment of choice for ulcerative colitis? Curr. Drug Targets 12 (10) (2011) 1396–1405, https://doi.org/10.2174/138945011796818126.

[6] S. Hiraoka, A. Fujiwara, T. Toyokawa, R. Higashi, Y. Moritou, S. Takagi, K. Matsueda, S. Suzuki, J. Miyaike, T. Inokuchi, M. Takahara, J. Kato, H. Okada, Multicenter survey on mesalamine intolerance in patients with ulcerative colitis, J. Gastroenterol. Hepatol. 36 (1) (2021) 137–143, https://doi.org/10.1111/jgh.15138.

[7] Y.J. Wang, Q.M. Li, X.Q. Zha, J.P. Luo, Intervention and potential mechanism of non-starch polysaccharides from natural resources on ulcerative colitis: a review, Int. J. Biol. Macromol. 210 (2022) 545–564, https://doi.org/10.1016/j.ijbiomac.2022.04.208.

[8] M.R. Coelho, M.D. Romi, D. Ferreira, C. Zaltman, M. Soares-Mota, The use of curcumin as a complementary therapy in ulcerative colitis: a systematic review of randomized controlled clinical trials, Nutrients 12 (8) (2020), https://doi.org/10.3390/nu12082296.

[9] Y. Peng, Y. Li, Y. Pi, X. Yue, Effects of almond (Armeniaca Sibirica L. Lam) polysaccharides on gut microbiota and anti-inflammatory effects on LPS-induced RAW264.7 cells, Int. J. Biol. Macromol. 263 (Pt 1) (2024) 130098, https://doi.org/10.1016/j.ijbiomac.2024.130098.

[10] C. Wen, D. Chen, R. Zhong, X. Peng, Animal models of inflammatory bowel disease: category and evaluation indexes, Gastroenterol. Rep. (Oxf.) 12 (2024) goae021, https://doi.org/10.1093/gastro/goae021.

[11] X. Wang, L. Su, J. Tan, T. Ding, Y. Yue, Albiflorin alleviates DSS-induced ulcerative colitis in mice by reducing inflammation and oxidative stress, Iran. J. Basic Med. Sci. 26 (1) (2023) 48–56, https://doi.org/10.22038/IJBMS.2022.66678.14624.

[12] K. Hazel, A. O'Connor, Emerging treatments for inflammatory bowel disease, Ther. Adv. Chronic Dis. 11 (2020) 2040622319899297, https://doi.org/10.1177/2040622319899297.

[13] Q.A. Ding, S.P. Nie, J.L. Hu, X.Y. Zong, Q.Q. Li, M.Y. Xie, In vitro and in vivo gastrointestinal digestion and fermentation of the polysaccharide from Ganoderma atrum, Food Hydrocoll. 63 (2017) 646–655, https://doi.org/10.1016/j.foodhyd.2016.10.018.

[14] S.S. Ferreira, C.P. Passos, P. Madureira, M. Vilanova, M.A. Coimbra, Structure-function relationships of immunostimulatory polysaccharides: a review, Carbohydr. Polym. 132 (2015) 378–396, https://doi.org/10.1016/j.carbpol.2015.05.079.

[15] A.O. Tzianabos, Polysaccharide immunomodulators as therapeutic agents: structural aspects and biologic function, Clin. Microbiol. Rev. 13 (4) (2000) 523–533, https://doi.org/10.1128/CMR.13.4.523.

[16] C.Y. Shen, W.L. Zhang, J.G. Jiang, Immune-enhancing activity of polysaccharides from Linn. via MAPK and NF-κB signaling pathways in RAW264.7 cells, J. Funct. Foods 34 (2017) 118–129, https://doi.org/10.1016/j.jff.2017.03.060.

[17] Y. Peng, Z. Zhang, W. Chen, S. Zhao, Y. Pi, X. Yue, Structural characterization, alpha-glucosidase inhibitory activity and antioxidant activity of neutral polysaccharide from apricot (Armeniaca Sibirica L. Lam) kernels, Int. J. Biol. Macromol. 238 (2023) 124109, https://doi.org/10.1016/j.ijbiomac.2023.124109.

[18] W. Guo, M. Zeng, S. Zhu, S. Li, Y. Qian, H. Wu, Phycocyanin ameliorates mouse colitis via phycocyanobilin-dependent antioxidant and anti-inflammatory protection of the intestinal epithelial barrier, Food Funct. 13 (6) (2022) 3294–3307, https://doi.org/10.1039/d1fo02970c.

[19] Z. Wu, S. Huang, T. Li, N. Li, D. Han, B. Zhang, Z.Z. Xu, S. Zhang, J. Pang, S. Wang, G. Zhang, J. Zhao, J. Wang, Gut microbiota from green tea polyphenol-dosed mice improves intestinal epithelial homeostasis and ameliorates experimental colitis, Microbiome 9 (1) (2021) 184, https://doi.org/10.1186/s40168-021-01115-9.

[20] C. Li, W. Zhang, X. Wu, Q. Cai, Z. Tan, Z. Hong, S. Huang, Y. Yuan, L. Yao, L. Zhang, Aromatic-turmerone ameliorates DSS-induced ulcerative colitis via modulating gut microbiota in mice, Inflammopharmacology 30 (4) (2022) 1283–1294, https://doi.org/10.1007/s10787-022-01007-w.

[21] S.N. Murthy, H.S. Cooper, H. Shim, R.S. Shah, S.A. Ibrahim, D.J. Sedergran, Treatment of dextran sulfate sodium-induced murine colitis by intracolonic cyclosporin, Dig. Dis. Sci. 38 (9) (1993) 1722–1734, https://doi.org/10.1007/BF01303184.

[22] Y. Peng, Y. Yan, P. Wan, D. Chen, Y. Ding, L. Ran, J. Mi, L. Lu, Z. Zhang, X. Li, X. Zeng, Y. Cao, Gut microbiota modulation and anti-inflammatory properties of anthocyanins from the fruits of Lycium ruthenicum Murray in dextran sodium sulfate-induced colitis in mice, Free Radic. Biol. Med. 136 (2019) 96–108, https://doi.org/10.1016/j.freeradbiomed.2019.04.005.

[23] P. Mease, F. van den Bosch, IL-23 and axial disease: do they come together? Rheumatology (Oxford) 60 (Suppl. 4) (2021) iv28-iv33 https://doi.org/10.1093/rheumatology/keab617.

[24] N. Flores-Huerta, J. Pacheco-Yepez, V. Sanchez-Monroy, M.C. Rosales-Hernandez, A. Silva-Olivares, J. Serrano-Luna, M. Shibayama, The MPO system participates actively in the formation of an oxidative environment produced by neutrophils and activates the antioxidant mechanism of Naegleria fowleri, J. Leukoc. Biol. 108 (3) (2020) 895–908, https://doi.org/10.1002/JLB.4MA0520-565RR.

[25] Y. Hu, X. Fang, J. Wang, T.T. Ren, Y.Y. Zhao, J.F. Dai, X.Y. Qin, R. Lan, Astragalin attenuates AlCl(3)/D-galactose-induced aging-like disorders by inhibiting oxidative stress and neuroinflammation, Neurotoxicology 91 (2022) 60–68, https://doi.org/10.1016/j.neuro.2022.05.003.

[26] Q. Guo, Y. Jin, X. Chen, X. Ye, X. Shen, M. Lin, C. Zeng, T. Zhou, J. Zhang, NF-kappaB in biology and targeted therapy: new insights and translational implications, Signal Transduct. Target. Ther. 9 (1) (2024) 53, https://doi.org/10.1038/s41392-024-01757-9.

[27] Y. Pu, Z. Liu, H. Tian, Y. Bao, The immunomodulatory effect of Poria cocos polysaccharides is mediated by the Ca(2+)/PKC/p38/NF-kappaB signaling pathway in macrophages, Int. Immunopharmacol. 72 (2019) 252–257, https://doi.org/10.1016/j.intimp.2019.04.017.

[28] H. Tian, Z. Liu, Y. Pu, Y. Bao, Immunomodulatory effects exerted by Poria Cocos polysaccharides via TLR4/TRAF6/NF-kappaB signaling in vitro and in vivo, Biomed. Pharmacother. 112 (2019) 108709, https://doi.org/10.1016/j.biopha.2019.108709.

[29] S. Massironi, C. Vigano, A. Palermo, L. Pirola, G. Mulinacci, M. Allocca, L. Peyrin-Biroulet, S. Danese, Inflammation and malnutrition in inflammatory bowel disease,

Lancet Gastroenterol. Hepatol. 8 (6) (2023) 579–590, https://doi.org/10.1016/S2468-1253(23)00011-0.
[30] L. Pouillon, S. Travis, P. Bossuyt, S. Danese, L. Peyrin-Biroulet, Head-to-head trials in inflammatory bowel disease: past, present and future, Nat. Rev. Gastroenterol. Hepatol. 17 (6) (2020) 365–376, https://doi.org/10.1038/s41575-020-0293-9.
[31] L. Pouillon, S. Travis, P. Bossuyt, S. Danese, L. Peyrin-Biroulet, Author correction: head-to-head trials in inflammatory bowel disease: past, present and future, Nat. Rev. Gastroenterol. Hepatol. 18 (2) (2021) 143, https://doi.org/10.1038/s41575-020-00409-x.
[32] H.H. Uhlig, A.M. Muise, Clinical genomics in inflammatory bowel disease, Trends Genet. 33 (9) (2017) 629–641, https://doi.org/10.1016/j.tig.2017.06.008.
[33] H. Gordon, L. Biancone, G. Fiorino, K.H. Katsanos, U. Kopylov, E. Al Sulais, J. E. Axelrad, K. Balendran, J. Burisch, L. de Ridder, L. Derikx, P. Ellul, T. Greuter, M. Iacucci, C. Di Jiang, C. Kapizioni, K. Karmiris, J. Kirchgesner, D. Laharie, T. Lobaton, T. Molnar, N.M. Noor, R. Rao, S. Saibeni, M. Scharl, S.R. Vavricka, T. Raine, ECCO guidelines on inflammatory bowel disease and malignancies, J. Crohns Colitis 17 (6) (2023) 827–854, https://doi.org/10.1093/ecco-jcc/jjac187.
[34] S. Mu, W. Yang, G. Huang, Antioxidant activities and mechanisms of polysaccharides, Chem. Biol. Drug Des. 97 (3) (2021) 628–632, https://doi.org/10.1111/cbdd.13798.
[35] Z. Li, Y. Gao, L. Du, Y. Yuan, W. Huang, X. Fu, Y. Huang, X. Zhang, F. You, S. Li, Anti-inflammatory and anti-apoptotic effects of Shaoyao decoction on X-ray radiation-induced enteritis of C57BL/6 mice, J. Ethnopharmacol. 292 (2022) 115158, https://doi.org/10.1016/j.jep.2022.115158.
[36] B. Du, B. Xu, Editorial: immune-boosting effects of dietary bioactive polysaccharides, Front. Nutr. 9 (2022) 1102641, https://doi.org/10.3389/fnut.2022.1102641.
[37] W. Li, X. Hu, S. Wang, Z. Jiao, T. Sun, T. Liu, K. Song, Characterization and anti-tumor bioactivity of astragalus polysaccharides by immunomodulation, Int. J. Biol. Macromol. 145 (2020) 985–997, https://doi.org/10.1016/j.ijbiomac.2019.09.189.
[38] X. Guo, J. Luo, J. Qi, X. Zhao, P. An, Y. Luo, G. Wang, The role and mechanism of polysaccharides in anti-aging, Nutrients 14 (24) (2022), https://doi.org/10.3390/nu14245330.
[39] Y. Wang, T. Zeng, H. Li, Y. Wang, J. Wang, H. Yuan, Structural characterization and hypoglycemic function of polysaccharides from Cordyceps cicadae, Molecules 28 (2) (2023), https://doi.org/10.3390/molecules28020526.
[40] Z. He, J. Liu, Y. Liu, Daphnetin attenuates intestinal inflammation, oxidative stress, and apoptosis in ulcerative colitis via inhibiting REG3A-dependent JAK2/STAT3 signaling pathway, Environ. Toxicol. 38 (9) (2023) 2132–2142, https://doi.org/10.1002/tox.23837.
[41] F. Qin, L. Yao, C. Lu, C. Li, Y. Zhou, C. Su, B. Chen, Y. Shen, Phenolic composition, antioxidant and antibacterial properties, and in vitro anti-HepG2 cell activities of wild apricot (Armeniaca Sibirica L. Lam) kernel skins, Food Chem. Toxicol. 129 (2019) 354–364, https://doi.org/10.1016/j.fct.2019.05.007.
[42] W.K. Meckstroth, L.M. Dorfman, R.E. Heikkila, Reactivity of the hydroxyl radical with amygdalin in aqueous solution, Biochem. Pharmacol. 29 (24) (1980) 3307–3309, https://doi.org/10.1016/0006-2952(80)90308-1.
[43] J.A. Kralovec, K.L. Metera, J.R. Kumar, L.V. Watson, G.S. Girouard, Y. Guan, R. I. Carr, C.J. Barrow, H.S. Ewart, Immunostimulatory principles from *Chlorella pyrenoidosa*–part 1: isolation and biological assessment in vitro, Phytomedicine 14 (1) (2007) 57–64, https://doi.org/10.1016/j.phymed.2005.09.002.
[44] L. Lin, F. Cui, J. Zhang, X. Gao, M. Zhou, N. Xu, H. Zhao, M. Liu, C. Zhang, L. Jia, Antioxidative and renoprotective effects of residue polysaccharides from Flammulina velutipes, Carbohydr. Polym. 146 (2016) 388–395, https://doi.org/10.1016/j.carbpol.2016.03.071.
[45] G. Chen, S. Zhang, C. Ran, L. Wang, J. Kan, Extraction, characterization and antioxidant activity of water-soluble polysaccharides from tuber huidongense, Int. J. Biol. Macromol. 91 (2016) 431–442, https://doi.org/10.1016/j.ijbiomac.2016.05.108.
[46] G. Ma, Q. Xu, H. Du, B. Muinde Kimatu, A. Su, W. Yang, Q. Hu, H. Xiao, Characterization of polysaccharide from Pleurotus eryngii during simulated gastrointestinal digestion and fermentation, Food Chem. 370 (2022) 131303, https://doi.org/10.1016/j.foodchem.2021.131303.
[47] Y. Wang, J. Zhang, B. Zhang, M. Lu, J. Ma, Z. Liu, J. Huang, J. Ma, X. Yang, F. Wang, X. Tang, Modified Gegen Qinlian decoction ameliorated ulcerative colitis by attenuating inflammation and oxidative stress and enhancing intestinal barrier function in vivo and in vitro, J. Ethnopharmacol. 313 (2023) 116538, https://doi.org/10.1016/j.jep.2023.116538.
[48] L. Qin, H. Xu, J. Cao, K. Wang, L. Zhang, M. Yao, H. Lin, C. Qu, J. Miao, Alleviative effects of sulfated polysaccharide from Ishige Okamurae against DSS-induced ulcerative colitis via inhibiting inflammation and modulating gut microbiota, Int. J. Biol. Macromol. 268 (Pt 2) (2024) 131915, https://doi.org/10.1016/j.ijbiomac.2024.131915.
[49] L. Cui, X. Guan, W. Ding, Y. Luo, W. Wang, W. Bu, J. Song, X. Tan, E. Sun, Q. Ning, G. Liu, X. Jia, L. Feng, Scutellaria baicalensis Georgi polysaccharide ameliorates DSS-induced ulcerative colitis by improving intestinal barrier function and modulating gut microbiota, Int. J. Biol. Macromol. 166 (2021) 1035–1045, https://doi.org/10.1016/j.ijbiomac.2020.10.259.
[50] Y. Yan, V. Kolachala, G. Dalmasso, H. Nguyen, H. Laroui, S.V. Sitaraman, D. Merlin, Temporal and spatial analysis of clinical and molecular parameters in dextran sodium sulfate induced colitis, PLoS One 4 (6) (2009) e6073, https://doi.org/10.1371/journal.pone.0006073.
[51] D.D. Ren, K.C. Chen, S.S. Li, Y.T. Zhang, Z.M. Li, S. Liu, Y.S. Sun, Panax quinquefolius polysaccharides ameliorate ulcerative colitis in mice induced by dextran sulfate sodium, Front. Immunol. 14 (2023) 1161625, https://doi.org/10.3389/fimmu.2023.1161625.
[52] N.G. Kotla, I.L.M. Isa, S. Rasala, S. Demir, R. Singh, B.V. Baby, S.K. Swamy, P. Dockery, V.R. Jala, Y. Rochev, A. Pandit, Modulation of gut barrier functions in ulcerative colitis by hyaluronic acid system, Adv. Sci. (Weinh) 9 (4) (2022) e2103189, https://doi.org/10.1002/advs.202103189.
[53] G.G. Kaplan, S.C. Ng, Understanding and preventing the global increase of inflammatory bowel disease, Gastroenterology 152 (2) (2017) 313–321 e312, https://doi.org/10.1053/j.gastro.2016.10.020.
[54] R. Ungaro, S. Mehandru, P.B. Allen, L. Peyrin-Biroulet, J.F. Colombel, Ulcerative colitis, Lancet 389 (10080) (2017) 1756–1770, https://doi.org/10.1016/S0140-6736(16)32126-2.
[55] Y.X. Yan, M.J. Shao, Q. Qi, Y.S. Xu, X.Q. Yang, F.H. Zhu, S.J. He, P.L. He, C.L. Feng, Y.W. Wu, H. Li, W. Tang, J.P. Zuo, Artemisinin analogue SM934 ameliorates DSS-induced mouse ulcerative colitis via suppressing neutrophils and macrophages, Acta Pharmacol. Sin. 39 (10) (2018) 1633–1644, https://doi.org/10.1038/aps.2017.185.
[56] M. Huang, S. Zhang, M. Zhang, S. Ou, Z. Pan, Effects of polysaccharides from Morchella conica on nitric oxide production in lipopolysaccharide-treated macrophages, Appl. Microbiol. Biotechnol. 94 (3) (2012) 763–771, https://doi.org/10.1007/s00253-011-3711-7.
[57] X. Guo, L. Liu, W. Zhao, X. Li, X. Wang, A. Ning, J. Cao, W. Zhang, L. Cao, M. Zhong, The protective effect of Schisandra chinensis (Turcz.) Baill. Polysaccharide on DSS-induced ulcerative colitis in mice via the modulation of gut microbiota and inhibition of NF-kappaB activation, J. Sci. Food Agric. 104 (1) (2024) 196–206, https://doi.org/10.1002/jsfa.12905.
[58] Y. Xu, X.C. Wang, W. Jiang, L.H. Chen, T. Chen, D. Wu, J.N. Hu, Porphyra haitanensis polysaccharide-functionalized selenium nanoparticles for effective alleviation of ulcerative colitis, Int. J. Biol. Macromol. 253 (Pt 8) (2023) 127570, https://doi.org/10.1016/j.ijbiomac.2023.127570.
[59] S.Z. Khan, S. Gasperino, S.L. Zeichner, Nuclear transit and HIV LTR binding of NF-kappaB subunits held by IkappaB proteins: implications for HIV-1 activation, Viruses 11 (12) (2019), https://doi.org/10.3390/v11121162.
[60] S.H. Lee, M. Hannink, Characterization of the nuclear import and export functions of Ikappa B(epsilon), J. Biol. Chem. 277 (26) (2002) 23358–23366, https://doi.org/10.1074/jbc.M111559200.
[61] M.F. Neurath, S. Pettersson, K.H. Meyer zum Buschenfelde, W. Strober, Local administration of antisense phosphorothioate oligonucleotides to the p65 subunit of NF-kappa B abrogates established experimental colitis in mice, Nat. Med. 2 (9) (1996) 998–1004, https://doi.org/10.1038/nm0996-998.
[62] X. Li, K. Mo, G. Tian, J. Zhou, J. Gong, L. Li, X. Huang, Shikimic acid regulates the NF-kappaB/MAPK signaling pathway and gut microbiota to ameliorate DSS-induced ulcerative colitis, J. Agric. Food Chem. 71 (23) (2023) 8906–8914, https://doi.org/10.1021/acs.jafc.3c00283.
[63] S. Zhang, Y. Cao, Z. Wang, H. Liu, Y. Teng, G. Li, J. Liu, X. Xia, Fermented Sargassum fusiforme mitigates ulcerative colitis in mice by regulating the intestinal barrier, oxidative stress, and the NF-kappaB pathway, Foods 12 (10) (2023), https://doi.org/10.3390/foods12101928.
[64] B.D. Sahu, J.M. Kumar, R. Sistla, Fisetin, a dietary flavonoid, ameliorates experimental colitis in mice: relevance of NF-kappaB signaling, J. Nutr. Biochem. 28 (2016) 171–182, https://doi.org/10.1016/j.jnutbio.2015.10.004.
[65] Y. Jin, Y. Lin, L. Lin, Y. Sun, C. Zheng, Carcinoembryonic antigen related cellular adhesion molecule 1 alleviates dextran sulfate sodium-induced ulcerative colitis in mice, Life Sci. 149 (2016) 120–128, https://doi.org/10.1016/j.lfs.2016.02.065.
[66] Y. Ruan, X. Zhu, J. Shen, H. Chen, G. Zhou, Mechanism of Nicotiflorin in San-Ye-Qing rhizome for anti-inflammatory effect in ulcerative colitis, Phytomedicine 129 (2024) 155564, https://doi.org/10.1016/j.phymed.2024.155564.
[67] X. Tong, L. Chen, S. He, S. Liu, J. Yao, Z. Shao, Y. Ye, S. Yao, Z. Lin, J. Zuo, Forsythia suspensa (Thunb.) Vahl extract ameliorates ulcerative colitis via inhibiting NLRP3 inflammasome activation through the TLR4/MyD88/NF-kappaB pathway, Immun. Inflamm. Dis. 11 (11) (2023) e1069, https://doi.org/10.1002/iid3.1069.
[68] P. Wang, M. Cai, K. Yang, P. Sun, J. Xu, Z. Li, B. Tian, Phenolics from Dendrobium officinale leaf ameliorate dextran sulfate sodium-induced chronic colitis by regulating gut microbiota and intestinal barrier, J. Agric. Food Chem. 71 (44) (2023) 16630–16646, https://doi.org/10.1021/acs.jafc.3c05339.
[69] D. Song, X. Wang, Y. Ma, N.N. Liu, H. Wang, Beneficial insights into postbiotics against colorectal cancer, Front. Nutr. 10 (2023) 1111872, https://doi.org/10.3389/fnut.2023.1111872.
[70] Y. Wei, D. Song, R. Wang, T. Li, H. Wang, X. Li, Dietary fungi in cancer immunotherapy: from the perspective of gut microbiota, Front. Oncol. 13 (2023) 1038710, https://doi.org/10.3389/fonc.2023.1038710.
[71] T.J. Keane, J. Dziki, E. Sobieski, A. Smoulder, A. Castleton, N. Turner, L.J. White, S. F. Badylak, Restoring mucosal barrier function and modifying macrophage phenotype with an extracellular matrix hydrogel: potential therapy for ulcerative colitis, J. Crohns Colitis 11 (3) (2017) 360–368, https://doi.org/10.1093/ecco-jcc/jjw149.