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Anti-infammatory and antioxidant activities of acteoside isolated from *Acanthus ilicifolius var. xiamenensis*

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Abstract

Acanthus ilicifolius var. xiamenensis (Acanthaceae), a mangrove found in southeastern China, is an herb with strong antiinflammatory property. Phytochemical study of the mangrove showed that the plant has a high content of phenylethanol glycoside acteoside (AC). In this research, the anti-inflammatory activity of acteoside on dextran sodium sulphate (DSS)-mediated mouse ulcerative colitis model were studied. For DSS- mediated colitis in mice, AC can reduce weight loss and DAI score in UC mice, suppress colon shortening and alleviate colon pathological injury. Moreover, AC treatment notably up-regulates IL-10, down-regulates the levels of IL-1β and TNF-α, and inhibits the protein expression of JAK2/STAT3, NF-κB p65, IKKα/β and IKB of colon. In addition, after AC treatment, the level of MDA and NO in colonic tissue were remarkably decreased, while the levels of GSH, SOD, and Nrf2 and HO-1 protein expression levels were significantly increased. These results indicate that AC can activate the Nrf2 signaling pathway by inhibiting the JAK/STAT, iNOS/eNOS and NF-κB signaling cascades, enhance the intestinal barrier function, and effectively reduce DSS-induced UC in mice.

Keywords: *Acanthus ilicifolius var. xiamenensis*, Acteoside, Anti-inflammatory, Ulcerative colitis

Introduction

Inflammatory intestinal disease often includes Crohn's disorder and ulcerative colitis (UC) [1]. Although its exact cause is not yet known, it is mainly characterized by flare-ups and relapses caused by excessive inflammatory responses in genetically susceptible hosts, antibiotic administration, microbial dysbiosis, or environmental factor like dietary factor [2]. Chemically mediated colitis model is widely used to study the pathogenesis of inflammatory diseases because they successfully simulate human intestine inflammatory disorders [3]. Among them, dextran sodium sulfate (DSS)- mediated colitis is an animal model widely used in the research of intestine inflammation, because it can simulate acute, chronic and recurrent intestines by adjusting DSS concentration and dosing frequency tract inflammation [4]. Since the cause and pathogenesis are still unclear, the clinical treatment of UC is difficult. Though aminosalicylic acid, steroids, immunosuppressants and biologics can relieve UC, they are prone to side effects such as toxicity, allergies, and upper gastrointestinal bleeding [5]. Therefore, looking for more reliable and valid drugs is especially urgent.

Studies have shown that colitis mucosal immune activation associated with the increase of inflammatory cytokine and enzyme, including TNF-α, IL-1β, IL-6, and iNOS [6–8]. The increase in cytokine activity is related to inflammation and cancer. Activation of some intracellular signaling cascades, including signal transducers and activators of STAT3 and NF-κB, has been shown to cause colitis and control polygene expressions [9, 10]. Under normal physiological circumstances, p65 and p50

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NF-κB heterodimers are inactive as a κB inhibitor complex (IκBα). It is triggered by subsequent IκBα phosphorylation and degradation of inflammatory stimuli, which functionally activates NF-κB, which is translocated to the nucleus, where it controls the expression of target genes [11]. STAT3 plays a key role in autoimmune diseases, which cause over-activation of STAT3, leading to various autoimmune diseases [12]. Activated STAT3 dimers are translocated into the nucleus to facilitate transcription of target gene [13].

DSS-mediated colitis in mice was used in preclinical research to find materials that might have a therapeutic effect on IBD [14]. Plant medicines are of great significance due to their ability to control and cure inflammation and immunomodulatory disorders [15]. *Acanthus ilicifolius var. xiamenensis* (Acanthaceae) is a mangrove found in the mangroves of Xiamen Island, southeast China [16]. As a Chinese folk medicine, this mangrove is used to treat various diseases such as lymphadenectasis, hepatitis, gastragia and tumors [17]. The plant is known to have many phytochemicals such as alkaloids [18], phenylethanol glycosides, lignans [19], flavonoids and steroids [20]. By a bioassay-guided fractionation, a phenylethanol glycoside, acteoside (AC), was isolated and identified (Fig. 1). Acteoside showed anti-inflammatory effects in vitro, however, the associated anti-inflammatory mechanism has not been identified [21, 22]. Based on this, we tried to explore the meaning in depth by carrying out relevant experiments.

In this study, the anti-inflammatory activity and possible mechanisms of acteoside (AC) were comprehensively described in DSS-mediated UC mouse model. Furthermore, the anti-ulcer mechanisms of AC were investigated through mucosal protection and regulation of JAK/STAT, iNOS/eNOS and NF-κB signaling cascades.

Materials and methods

Plant material

The whole plants of *Acanthus ilicifolius var. xiamenensis* was collected in Longhai Mangroves, near Xiamen Island, Fujian Province, China, in June 2019, and identified by Dr.Y.H.Zhang (School of pharmacy, Fujian Medical University). A standard sample (ZYH-20190602) was put up at the Herbarium of Medicinal Plants of Fujian Medical University.

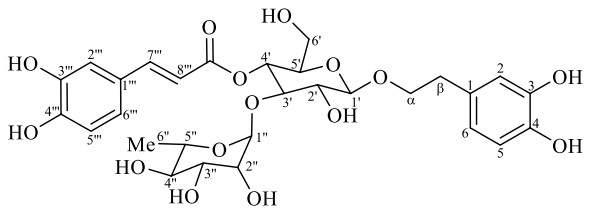

Fig. 1 The structure of acteoside (AC) isolated from *Acanthus ilicifolius var. xiamenensis*

Isolation of AC from *A. ilicifolius var. xiamenensis*

The mangrove (6.5 kg) was powdered and soaked with MeOH (3 L × 3) at 26 °C for 7 days and divided with petroleum ether, dichloromethane, EtOAc and *n*-BuOH. The *n*-BuOH parts (210 g) were applied to chromatography on MCI and eluted with 20% MeOH (Fr.D2), 50% MeOH (Fr.D3), 70% MeOH (Fr.D4), and 90% MeOH (Fr.D5). Fr.D3 were fractionated with sephadex LH-20 (CH_2Cl_2-MeOH 2:8) and purified by HPLC (58% MeOH-H_2O) to yield compound **1** (Acteoside) (474.5 mg). A typical high performance liquid chromatography diagram is shown in Additional file 1: Fig. S1.

Identification of Acteoside (AC)

The chemical structure of Acteoside was identified by the ^{1}H-NMR, ^{13}C-NMR and ESI–MS. Acteoside (AC): light yellow powder, ESI–MS (*m/z*) 627 $[M+3H]^+$. ^{1}H-NMR (DMSO-d_6, 400 MHz): δ 6.71 (1H, d, J = 2.0 Hz, H-2), 6.69 (1H, d, J = 8.1 Hz, H-5), 6.57 (1H, dd, J = 8.1, 2.0 Hz, H-6), 4.05, 3.73 (2H, m, H-α), 2.80 (2H, m, H-β), 7.07 (1H, d, J = 2.0 Hz, Caf-H-2), 6.79 (1H, d, J = 8.1 Hz, Caf-H-5), 6.96 (1H, dd, J = 8.1, 2.0 Hz, Caf-H-6), 6.28 (1H, d, J = 15.9 Hz, Caf-H-7), 7.60 (1H, d, J = 15.9 Hz, Caf-H-8), 4.38 (1H, d, J = 7.8 Hz, Glu-H-1), 5.20 (1H, d, J = 1.6 Hz, Rha-H-1). ^{13}C -NMR (DMSO-d_6, 100 MHz): δ 131.62 (C-1), 116.74 (C-2), 146.23 (C-3), 144.86 (C-4), 117.34 (C-5), 121.44 (C-6), 72.25 (C-α), 36.73 (C-β), 127.83 (Caf-C-1), 114.88 (Caf-C-2), 147.06 (Caf-C-3), 149.90 (Caf-C-4), 116.42 (Caf-C-5), 123.47 (Caf-C-6), 115.44 (Caf-C-7), 148.23 (Caf-C-8), 168.43 (Caf-C=O), 104.34 (Glu-C-1), 76.16 (Glu-C-2), 81.83 (Glu-C-3), 70.79 (Glu-C-4), 76.38 (Glu-C-5), 62.56 (Glu-C-6), 103.14 (Rha-C-1), 72.47 (Rha-C-2), 72.59 (Rha-C-3), 74.06 (Rha-C-4), 70.69 (Rha-C-5), 18.61 (Rha-C-6) (Additional file 1: Fig. S2–S4). These data were in agreement with literature value, Acteoside (Fig. 1) [23, 24].

Chemicals and antibody

DSS (36,000–50.000 MW) were obtained by MP Biomedicals (Santa Ana, USA). NO, IL-1β, IL-10 and TNF-α ELISA Kit were provided by Shanghai MLBIO Biotechnology Co. Ltd (Shanghai, China). 5-Aminosalicylic acid was provided by Shanghai Aladdin Biochemical Co. Ltd (Shanghai, China). Indomethacin (aladdin, USA). Antibodies for β-actin, JAK2, STAT3, p-IKKα/β, p-IKBα, p-P65, iNOS, eNOS, Nrf-2, HO-1, Keap1, HRP conjugated affinipure goat antimouse or antirabbit IgG provided by Cell Signaling Technology (MA, USA).

Superoxidedismutase (SOD), nitric oxide (NO), glutathione peroxidase (GSH) and malondialdehyde (MDA) were from the Nanjing Jiancheng Bioengineering Institute (Jiangsu, China). Other chemicals were of analytical grade.

Animals and experimental design

Male C57BL/6 mice (6 weeks old, 18 ± 2 g) provided by experimental animal center of Hangzhou Medical College, Animal Production License Number: SCXK (Zhe) 2019–0002. All animals were acclimatized to the environment for 3 days before the experiment, and fed and drank ad libitum at 22 °C ± 2 °C and 50% ± 5% relative humidity. Animal care and use were in accordance with experimental protocols, and the program was approved by the Animal Ethics Committee of Fujian Medical University.

Fifty (50) mice were partitioned into five groups: control, model (2.5% DSS), 5- aminosalicylic acid (5-ASA, 500 mg/kg) group [25], AC low dose (100 mg/kg), AC high dose (200 mg/kg) group, each group had 10 mice. Mice received 2.5% DSS in the drinking water for 7 days inducing UC except the control mice. For AC and 5-ASA groups, AC and 5-ASA were administrated orally once a day 0.1 ml/10 g body weight. The DSS group and normal group received the same amount of 0.5% carboxymethylcellulose sodium (CMC-Na) solution.

Disease activity index (DAI)

The mice in each group were weighed, the fecal characteristics were observed, and the fecal occult blood test was performed daily. Disease activity index (DAI) is evaluated based on previously described method [26]: 0 point for normal stool and negative occult blood test, 0% weight loss; 1 point for weak positive detection of occult blood, 1–5% weight loss; 2 point for a soft stool and occult blood test positive, 6–9% weight loss; 3 point for a soft stool, 10–15% weight loss, occult blood test strong positive; 4 point for diarrhoea, gross bleeding, > 15% weight loss.

Histopathological examination

After 9 days of administration, mice were anesthetized with an intraperitoneal injection of 5% pentobarbital (0.2 ml/10 g). Orbital blood was collected. Mice were sacrificed by cervical dislocation. Colon tissues from anus to ileocecal area were collected and cut in three sections. One was fixed immediately using 4% neutral formalin, decalcifed, embedded in paraffin wax. Colon tissues were observed by hematoxylin and eosin (HE) staining for morphological examination. The other was reserved at − 80 °C for cytokine analysis, and the remaining intestinal tissue was frozen for Western blotting assays.

Immunofluorescence staining

Paraffin-embedded colonic tissue was sectioned in 3 μm thick slice. After section was deparaffinized with xylene and rehydrated in gradient alcohol solution, and subjected to antigen retrieval. The colon section was washed with PBS solution five times for 3 min each and blocked with 5% BSA for 0.5 h at room temperature. The tissue was incubated with primary antibody (NF-κB, 1:400 dilution) overnight at 4 °C. After being washed with PBS solution 5 min and four times, the sections were incubated using fluorescent secondary antibody (1:500) for 50 min at 37 °C and avoid light. After pasting with anti-fading medium, fluorescence microscope was used for microscopic examination and image collection. The relative area immunoreactivity was calculated with Image J software [27].

Assessment of oxidative damage levels in vivo

For the analysis of MDA, GSH, SOD and NO in DSS-mediated colon tissues, colon tissue sample was suspended in lysis buffer and ground with a homogenizer. The supernatant was collected by centrifugation. The contents of MDA, GSH, SOD and NO were determined with corresponding kits [28].

Determination of cytokine secretion levels of colon tissue

The level of IL-10, TNF-α and IL-1β in the colonic tissue was detected using ELISA based on the manufacturer's protocol. The standard solution and the antibody-bearing sample were placed at 37 °C for 60 min, added to the working solution, incubated at 37 °C for 30 min. In the end, the absorbance at 450 nm was recorded by enzyme- linked immunosorbent assay [29].

Western blot analysis

Effects of AC on protein level of β-actin, p-IKKα/β, p-IKBα, p-P65, JAK2, STAT3, iNOS, eNOS, Nrf-2, HO-1 and Keap1 were investigated by Western blot [30]. The extraction method of total protein solution from mouse colon: The colon tissue of each group was 30 mg, grind with lytic buffer to get total protein solution of mouse colon. The protein solution was placed on a 10% SDS PAGE respectively and delivered to PVDF membranes. A 1:1000 diluted primary antibody was incubated to specifically identify β-actin, p-IKKα/β, p-IKBα, p-P65, JAK2, STAT3, iNOS, eNOS, Nrf-2, HO-1 and Keap1 at 4 °C for 12 h. After washing with TBST buffer, the membrane and HRP linked secondary antibodies were incubated at 37 °C for 1 h. Antibody-specific protein on PVDF membrane was prepared by enhanced chemiluminescence kit. The

level of p-IKKα/β, p-IKBα, p-P65, JAK2, STAT3, iNOS, eNOS, Nrf-2, HO-1 and Keap1 were measured using β-actin as load control [31].

Statistical evaluation

The measurement data was expressed as an average ± SD. The SPSS version 16.0 Windows software was used for statistical analysis. One way analysis of variance was used to compare statistical significant difference among groups. The statistical significant was set to $^{*}P < 0.05$, and high significance was set to $^{**}P < 0.01$.

Results and discussion

AC alleviates DSS-induced UC symptoms

In this experiment, DSS-mediated mouse inflammation model was established, and the effect of AC on colitis was studied. Phenotypic changes of mice were observed and recorded during the experiment. After dissection, it was found that the mouse colon was obviously wrinkled, and the colon content was black, showing that the colitis model was successfully built (Fig. 2C, D). After 7 days of modeling, the weight loss of mice in AC group was less than that in DSS group, and the weight changes of mice in AC 100 and 200 mg/kg groups had significant change compared to DSS group (Fig. 2A). The DAI of AC group was lower than that of DSS group (Fig. 2B). After measurement, it was found that the colon length of mice after drinking DSS was shortened to a certain extent, but the condition of the AC group was notably better than that of the group receiving DSS alone. The colon length and visual observation of the AC group was notably better than the DSS group, especially 200 mg/ kg AC group.

Effect on oxidative stress markers

Evidence suggests that oxidative stress caused by overproduction of reactive oxygen species metabolites plays an important role in intestinal tissue injury in a UC model [32]. We collected the intestine of mice with colitis to measure the degrees of oxidative stress. From the results in Fig. 3A, B, it can be seen that DSS

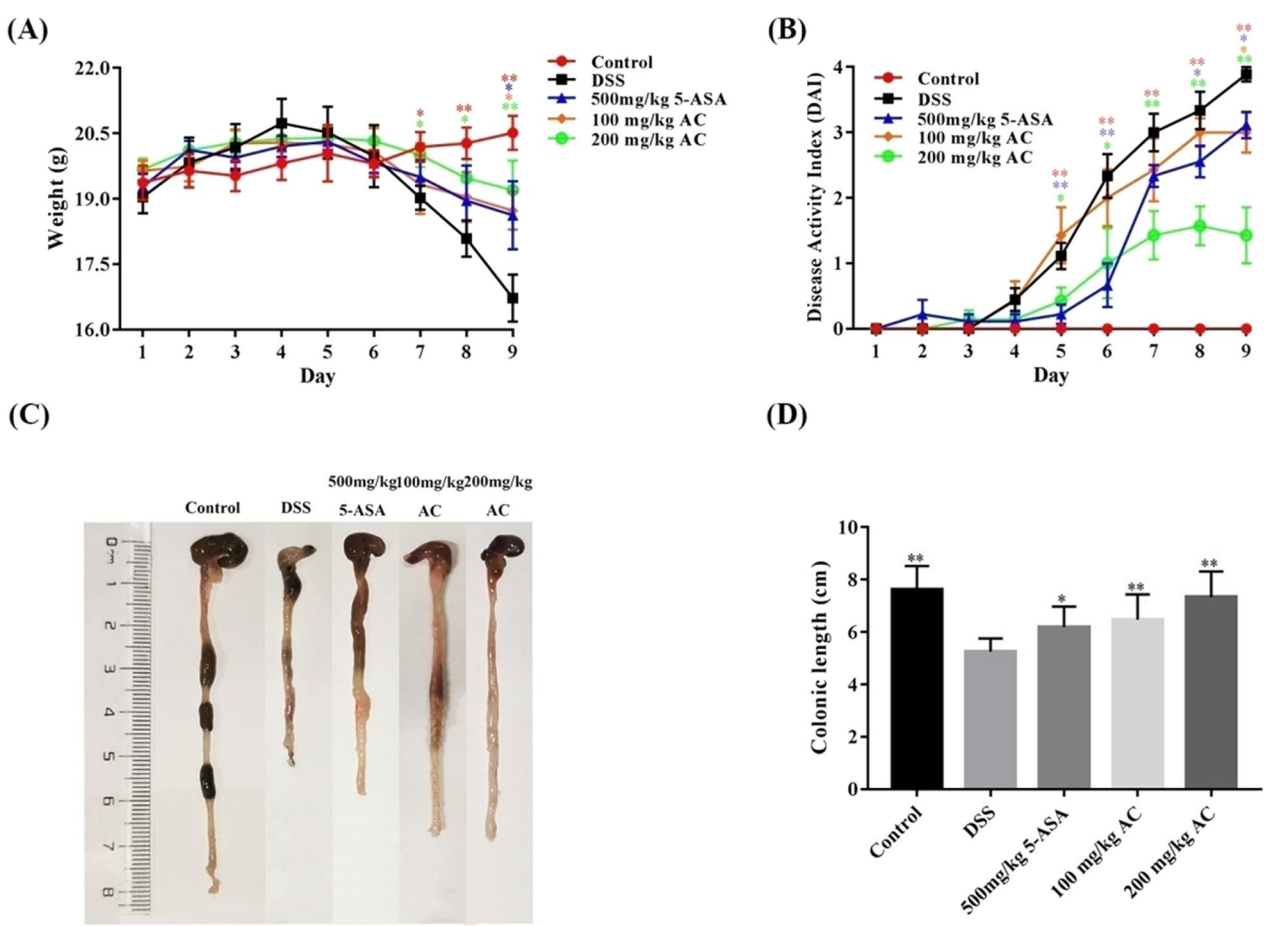

Fig. 2 Effect of AC on body weight change (%) (**A**), disease activity index (**B**), macroscopic appearances of colons (**C**), and colon length (**D**) in mice with colitis. Graphs represent median ± SD and 2-way ANOVA statistical analysis was performed. $^{*}p < 0.05$, $^{**}p < 0.01$

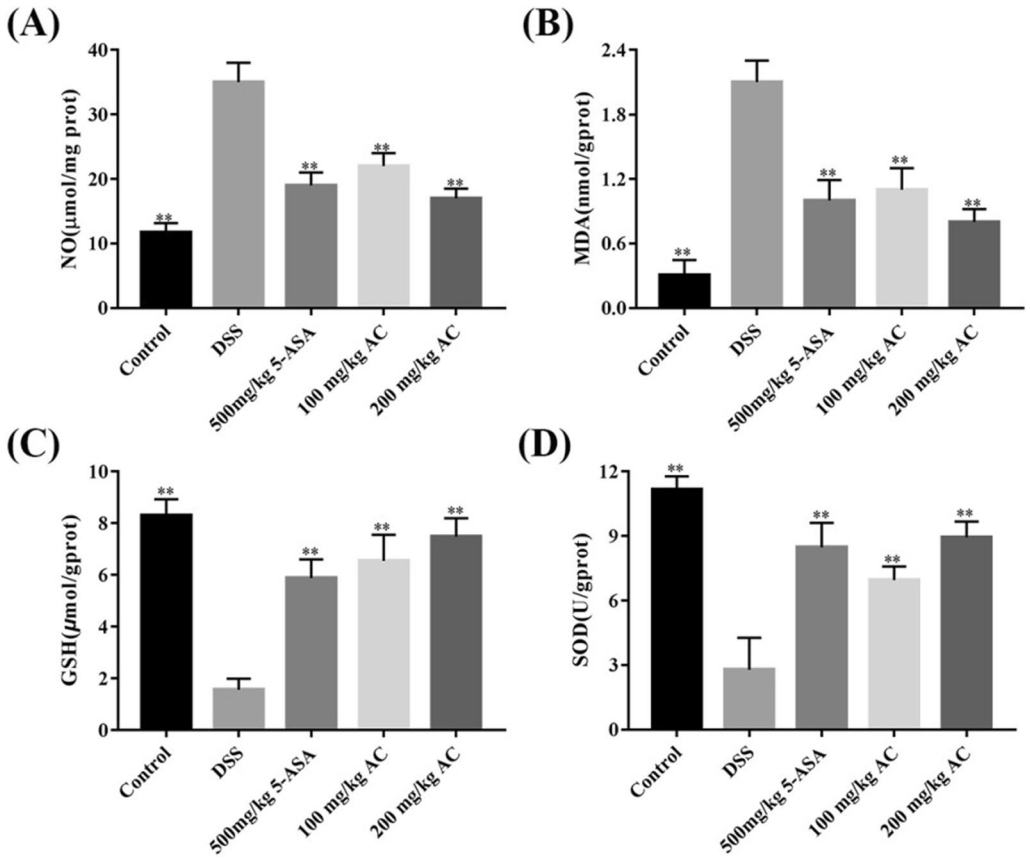

Fig. 3 Effects of AC on **A** nitric oxide (NO), **B** malondialdehyde (MDA), **C** reduced glutathione (GSH) and **D** superoxide dismutase (SOD) in DSS- induced UC in mice. Graphs represent median ± SD and 2-way ANOVA statistical analysis was performed. * $p < 0.05$, ** $p < 0.01$

induced notable increase in colonic NO and MDA content compared to blank group. In addition, the contents of NO and MDA in AC (100 and 200 mg/kg) treated mice were significantly reduced compared to DSS group. As shown in Fig. 3C, D, GSH and SOD level of the DSS induced mice was notably decreased, while the level of GSH and SOD was notably improved after AC treatment compared to DSS group, indicating that the therapeutic effects of AC on UC also comes from its antioxidant activity.

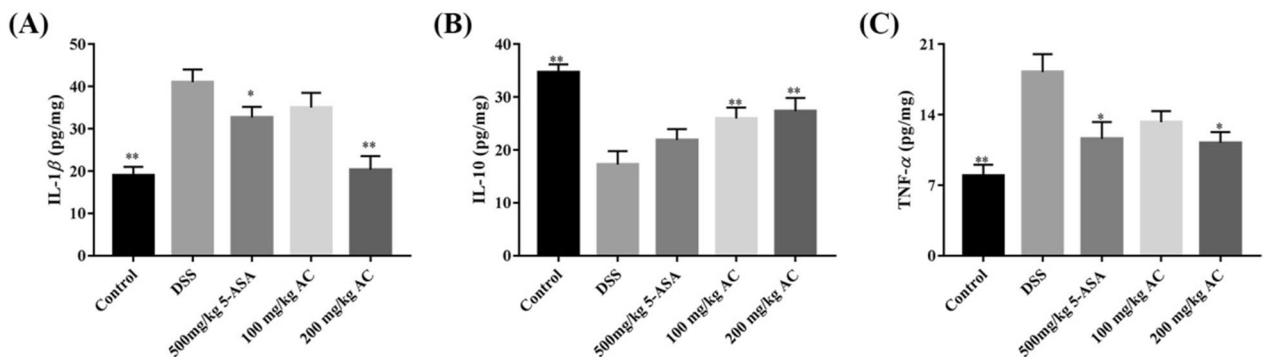

Fig. 4 Effects of AC on **A** IL-1β. **B** IL-10 **C** tumor necrosis factor alpha (TNF-α). in DSS- induced UC in mice. Graphs represent median ± SD and 2-way ANOVA statistical analysis was performed. * $p < 0.05$, ** $p < 0.01$

AC reduce the secretion levels of cytokine in UC mice

IL-1β, IL-10 and TNF-α are frequently used as indicators to assess the anti- inflammatory activity of compounds [33–35]. The experiment determined the secretion levels of IL-1β, TNF-α, and IL-10 in UC mice treated with AC. As shown in Fig. 4A, B and C. Compared to blank group, DSS promoted the secretion of pro-inflammatory factor (IL-1β and TNF-α) and decreased the secretion of IL-10, while AC group reversed this phenomenon. The level of TNF-α and IL-1β in the intestinal tract of different doses of AC group showed notable downward trend, while IL-10 showed a significant upward trend. Generally speaking, AC is beneficial to improve the intestinal pressure and inflammatory response of mice induced by DSS.

Inhibitory effects of AC on NF-κB signaling cascade in colon tissue

NF-κB signaling cascades play an important role in inflammatory regulation [36–38]. DSS activates phosphorylation and consecutive degradation of IkBα, which increases nuclear translocation of practically active NF-κB p65 [39]. The expression of NF-κB p-P65, p-IKBα, and p-IKKβ was determined by Western blot to investigate the anti-inflammatory mechanism of AC. The results showed that AC downregulated the expression of NF-κB p-P65 (Fig. 5A, B). Moreover, AC suppressed the phosphorylation and degradation of p-IKBα (Fig. 5A, C), and inhibited phosphorylation and degradation of p-IKKβ (Fig. 5A, D). These data suggest that AC can block UC mice activation of NF-κB signaling pathways. NF-κBp65 protein immunofluorescence analysis gave similar results (Fig. 5E). This indicates that AC exhibits inhibitory effect on the NF-κBp65 pathway on UC mouse.

Activation effects of AC on Nrf-2 signaling cascade in colon tissue

The nuclear factor erythrocyte 2 related factor 2 (Nrf-2) signaling cascade is a security system that usually modulates the expressions of antioxidant protein in the body [40]. As shown in Fig. 6A–D, our result indicated that compared to blank group, the level of Nrf-2, HO-1, and Keap1 in colon tissues of model group was notably decreased, showing that the antioxidant signaling cascade of model group was notably suppressed. This is coherent with the result of antioxidant parameter experiment (Fig. 3A–D). AC intervention notably upregulated Nrf-2, HO-1 and Keap1 protein levels in the mouse colon (Fig. 6A–D), indicating that AC intervention can notably activate Nrf-2 signaling cascade. Nrf-2 protein immunofluorescence analysis gave similar results (Fig. 6E). This indicates that AC exhibits inhibitory effect on the Nrf-2 pathway on UC mouse.

AC regulated the STAT3 signaling cascade in colon tissue

The STAT3 signaling cascade is related in the transmission of multiple cytokines in inflammatory response [41]. To investigate the effect of AC on JAK2 and STAT3 signaling pathways in mice with colitis, the level of p-JAK2 and p- STAT3 were examined. As shown in Fig. 7A–C, compared with NC group, the expression level of p-JAK2 and p-STAT3 in intestine of UC mice (Fig. 7A–C) increased notably, while the expression in 5-ASA and AC group decreased notably.

AC regulated the eNOS signaling cascade in colon tissue

In animal model of ulcerative colitis [42], iNOS has been defined at infiltrating macrophage and neutrophil in colonic mucosa [43, 44]. To investigate the activity of AC on the iNOS and eNOS signaling cascade in mice with colitis, the levels of iNOS and eNOS were examined. As shown in Fig. 8A–C, compared to blank group, the high dose of AC and 5-ASA groups in the intestinal tract of UC mice (Fig. 8A, C) increased the eNOS protein expression, suggesting that the eNOS pathway was up-regulated, while the expression of iNOS protein in AC group (Fig. 8A, B) was down-regulated.

AC suppress DSS-induced colitis development

As expected, compared with the blank group, DSS stimulated the colon of mice to shrink notably due to inflammation and tissue injury. It is worth noting that, compared to DSS group, AC group showed significantly less colonic contraction, indicating that AC inhibited bowel inflammation and tissue injury. To further support our data, we used hematoxylin and eosin staining to evaluate tissue damage in DSS-induced colon tissue section (Fig. 9A). As expected, the DSS treatment group showed broad tissue injury, cell necrosis, and crypt loss compared with the blank group of mice. It is worth noting that the AC and 5-ASA groups showed reduced crypt loss, while the DSS group showed extensive areas of inflammation, causing tissue injury and inflammatory cell infiltration (Fig. 9A). The spleen is a main hematopoietic and immune function organ. It is understood that in the process of inflammation, because of the inflammatory cells proliferation, it will expand. This phenomenon is called splenomegaly [45]. The results showed that the AC group had a significant limited trend of splenomegaly (Fig. 9B, C). This finding, associated with former observation, shows that AC exhibits effective results, which indicates that it has a strong anti-inflammatory effect and can partially inhibit DSS-mediated colitis in mice.

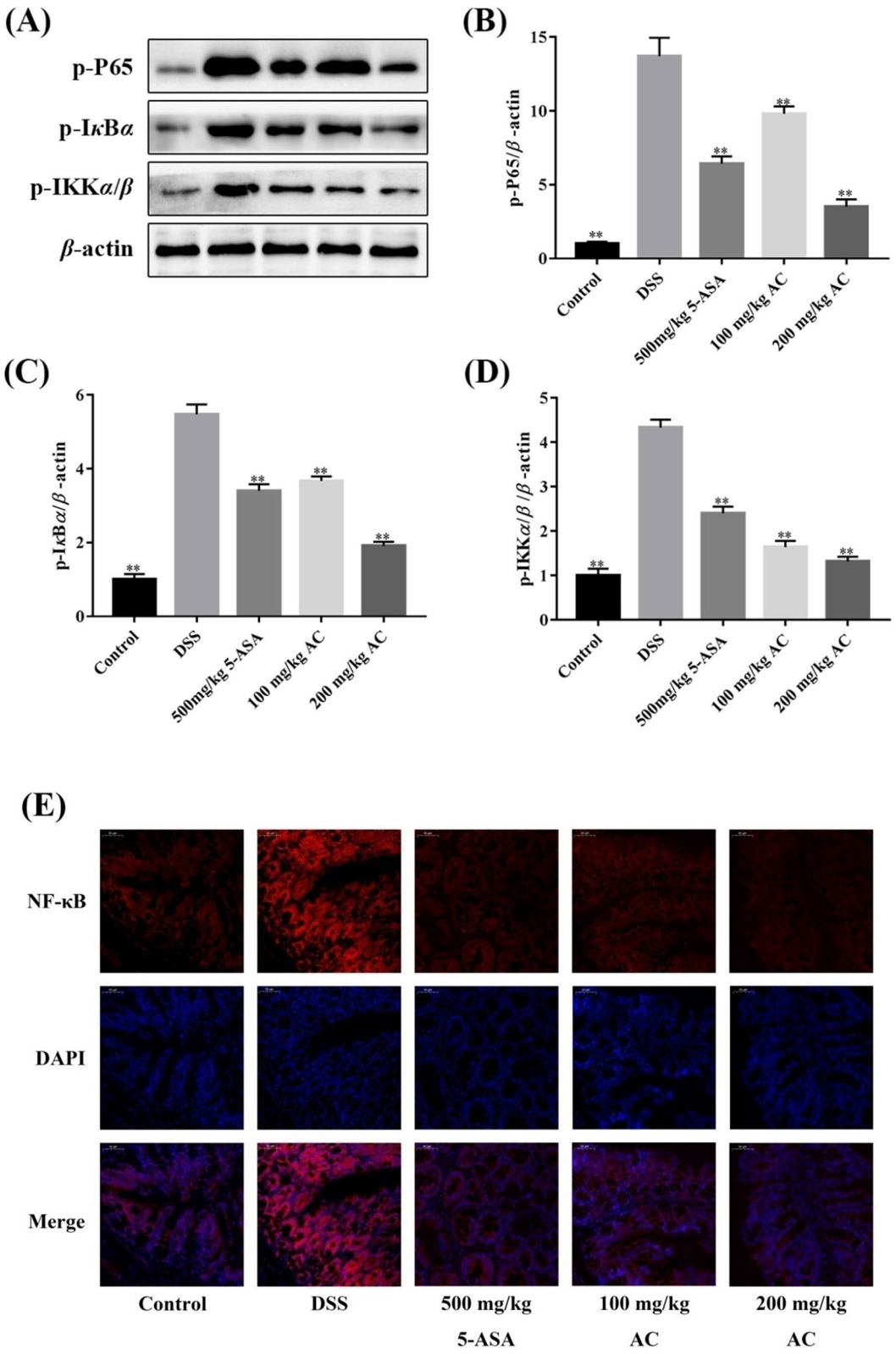

Fig. 5 Effect of AC on the protein expression of p-P65, IκB*α* and IKK*α*/*β*. **A** Western blotting of p-P65 (**B**), IκB*α* (**C**) and p-IKK*α*/*β* (**D**) protein. **E** Immunofuorescence staining of NF-κBp65 co-localized in control group, UC group, 5-ASA group, AC group. Graphs represent median ± SD and 2-way ANOVA statistical analysis was performed. * $p < 0.05$, ** $p < 0.01$

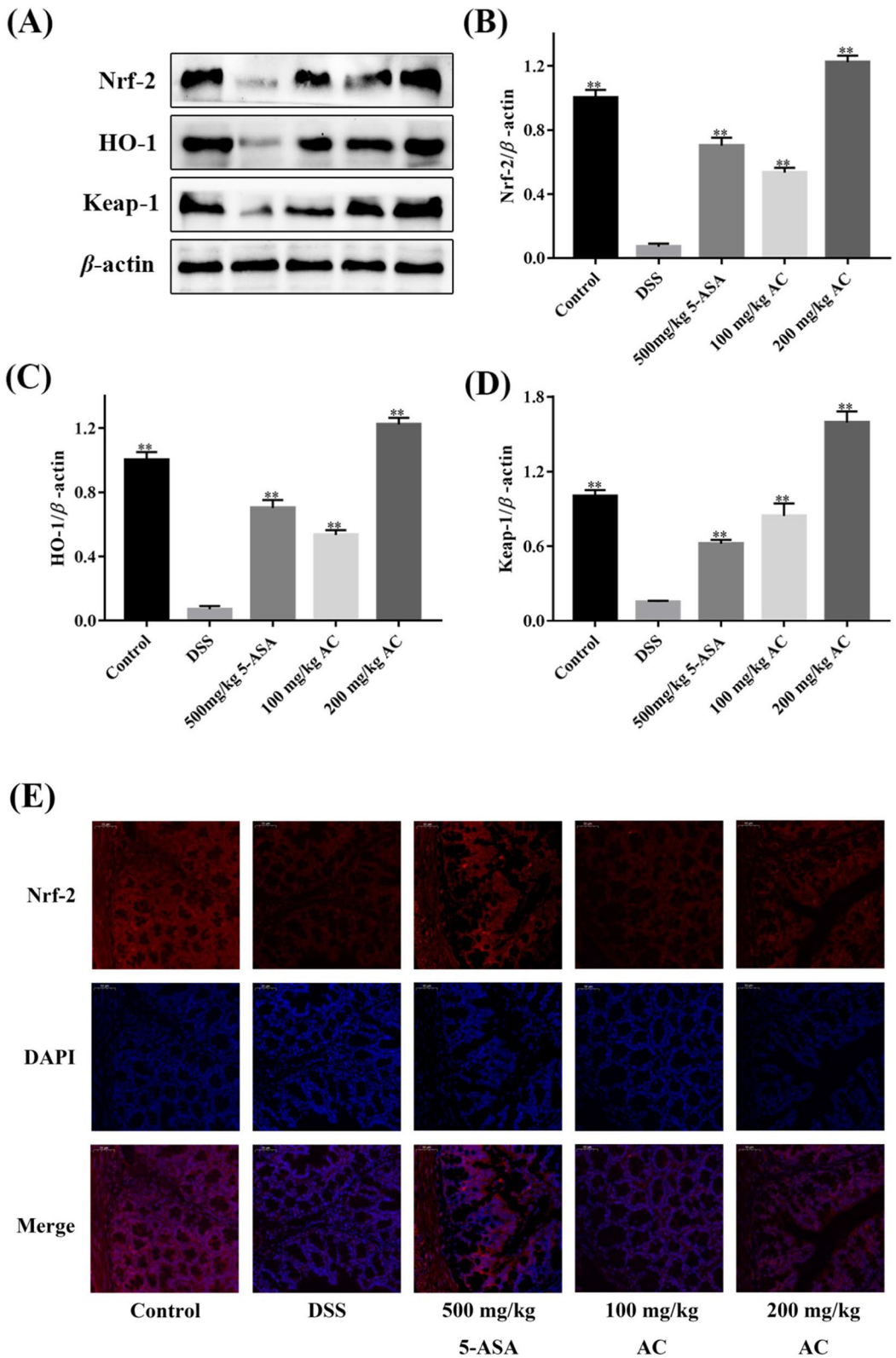

Fig. 6 Effect of AC on the protein expression of Nrf-2, HO-1 and Keap-1 **A** Western blotting of Nrf-2 (**B**), HO-1 (**C**) and Keap-1 (**D**) protein. **E** Immunofuorescence staining of Nrf-2 co-localized in control group, UC group, 5-ASA group, AC group. Graphs represent median ± SD and 2-way ANOVA statistical analysis was performed. * $p < 0.05$, ** $p < 0.01$

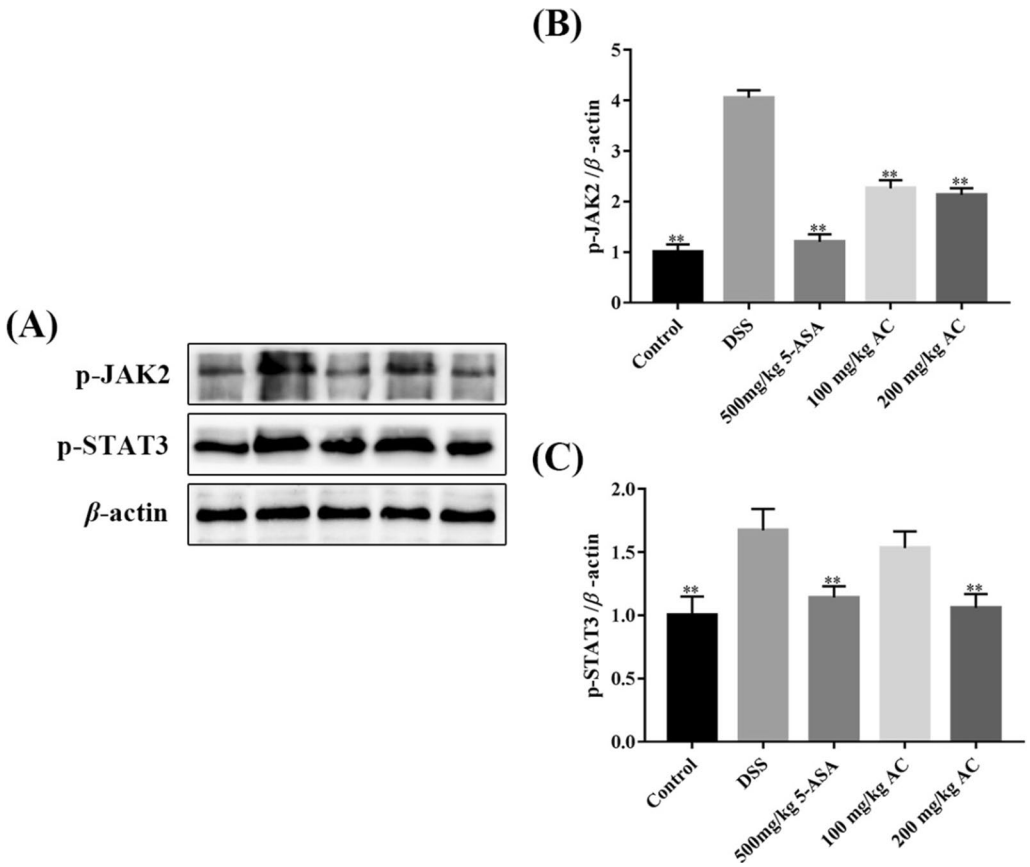

Fig. 7 Effect of AC on the protein expression of p-JAK2 and p-STAT3 (**A**) Western blotting of p-JAK2 (**B**), and p-STAT3 (**C**) protein

In the clinical treatment of IBD patients, the patient's condition should be evaluated first. Drugs should be selected according to the condition. Patients with mild to moderate IBD should be given 5-ASA drugs to induce remission. If the treatment effect is poor or intolerable, the ascending steps are glucocorticoids, immunosuppressants (thiopurine drugs azapurine (AZA), 6-mercaptopurine (6-MP)) and biological agents (infliximab (IFX)). Therefore, 5-ASA drugs have the best effect on mild to moderate colitis. In animal experiments, in the process of DSS induced colitis, the mice were in mild to moderate colitis in the first few days, and the mice had discomfort such as loss of appetite, abdominal pain, hair loss and soft stool. At this time, the curative effect of 5-ASA was the most obvious, and the DAI index decreased. However, with the increase of days of DSS administration, the symptoms of colitis in mice gradually worsened, with obvious weight loss and bloody stool. At this time, the immune system of mice was activated, 5-ASA is not enough to fight the inflammatory factor storm in the body, so the DAI index in the last two days of administration does not decrease significantly or increases slightly. On the other hand, 5-ASA has gastrointestinal side effects, such as diarrhea and vomiting. Some rats may increase DAI index due to additional diarrhea, resulting in abnormal DAI curve [46].

Acteoside is essential to the known medicinal activity of *Acanthus ilicifolius var. xiamenensis*. Various pharmacological activities of acteoside have been reported [21, 22], such as anti-inflammatory, antioxidant, anti-hepatotoxic and neuroprotective. It is not clear whether acteoside can treat colitis by inhibiting inflammatory factors. Acteoside (AC) is the main component and is abundantly present in the whole plant of *A. ilicifolius var. xiamenensis*. In this study, we explored the anti-UC effect and mechanism of AC. We checked the therapeutic activity of AC in DSS mediated UC mice. Result indicated that AC improved weight loss, colonic contracture, and DAI scores of UC mice in a dose-dependent manner. Histopathological findings indicated that AC (100 and 200 mg/kg) notably reduced DSS-induced inflammatory infiltration, edema, and exudations of eosinophil and neutrophil, indicating that AC has a good therapeutic activity in mice with ulcerative colitis.

In order to study anti-inflammatory mechanism of Acteoside (AC), we detected the NF-κB signaling

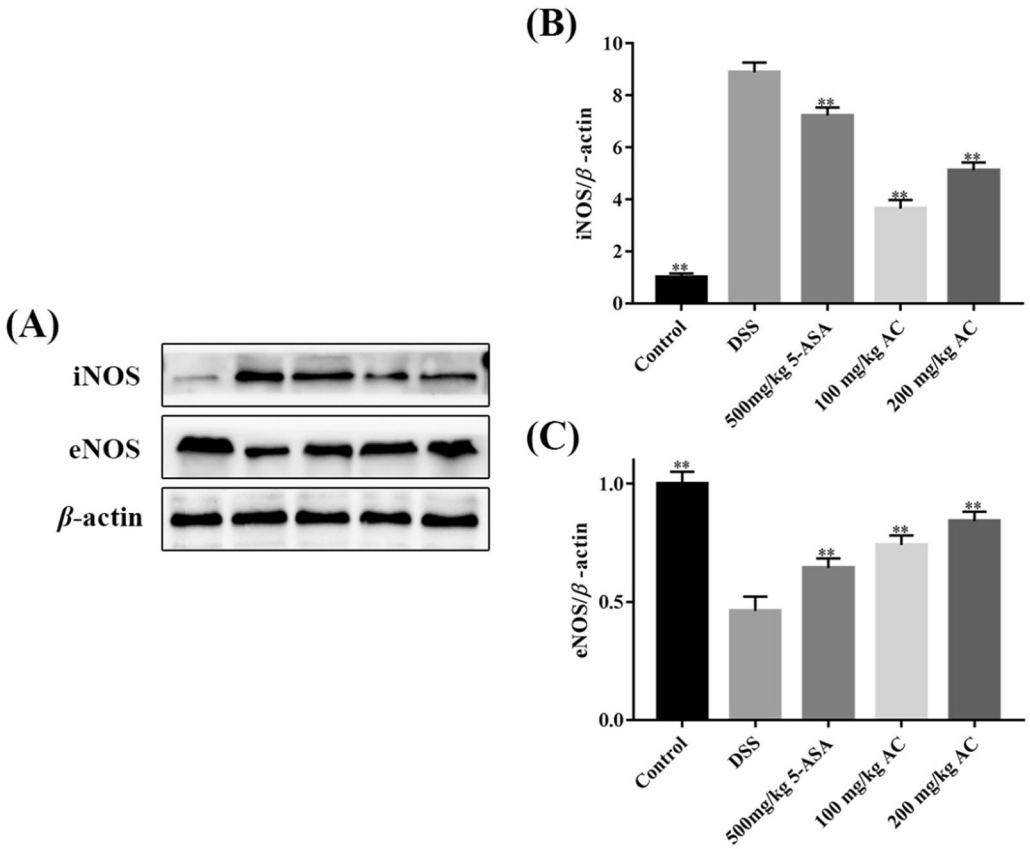

Fig. 8 Effect of AC on the protein expression of iNOS and eNOS (**A**) Western blotting of iNOS (**B**) and eNOS (**C**) protein

cascade proteins in mouse colon tissues. Study has indicated that in the DSS-mediated UC mouse model, NF-κB signaling cascade was unusually activated [38]. The results indicated that AC down-regulated the expression of NF-κB p-P65. Moreover, AC suppressed the phosphorylation and degradation of p-IKBα, and inhibited phosphorylation and degradation of p-IKKβ. These data suggest that AC can block UC mice activation of NF-κB signaling pathways. AC treatment notably reduces the level of these proteins and has notable anti-inflammatory effects. It has been reported that Nrf2 signaling cascade was remarkably restrained in UC rats [40]. AC intervention notably upregulated Nrf-2, HO-1 and Keap1 protein levels in the mouse colon, indicating that AC intervention can notably activate Nrf-2 signaling cascade. The increase of iNOS in tissues related to the severity of diseases [44]. The result showed, the AC group in the intestine tract of UC mice increased the eNOS protein expression, suggesting that the eNOS pathway was up-regulated, while the expression of iNOS protein in AC group was down-regulated. Levels of phosphorylated JAK2 and phosphorylated STAT3 in colon tissue were improved by DSS administration [41]. A main finding of our research was the observed reduction of JAK2/STAT3 phosphorylation after AC treatment, indicating that inhibition of the JAK and STAT pathway may be involved in AC treatment of colitis.

AC administration can improve experimental DSS-mediated colitis in mice. The protective activity of AC seems related to reduction of inflammation and oxidative stress by attenuating proinflammatory cytokine induced by JAK and STAT, iNOS/eNOS and NF-κB signaling cascades. These results indicate that AC may be an effective botanical drug and has advantage in prospective clinical application for the treatment of IBD or related disease in the future.

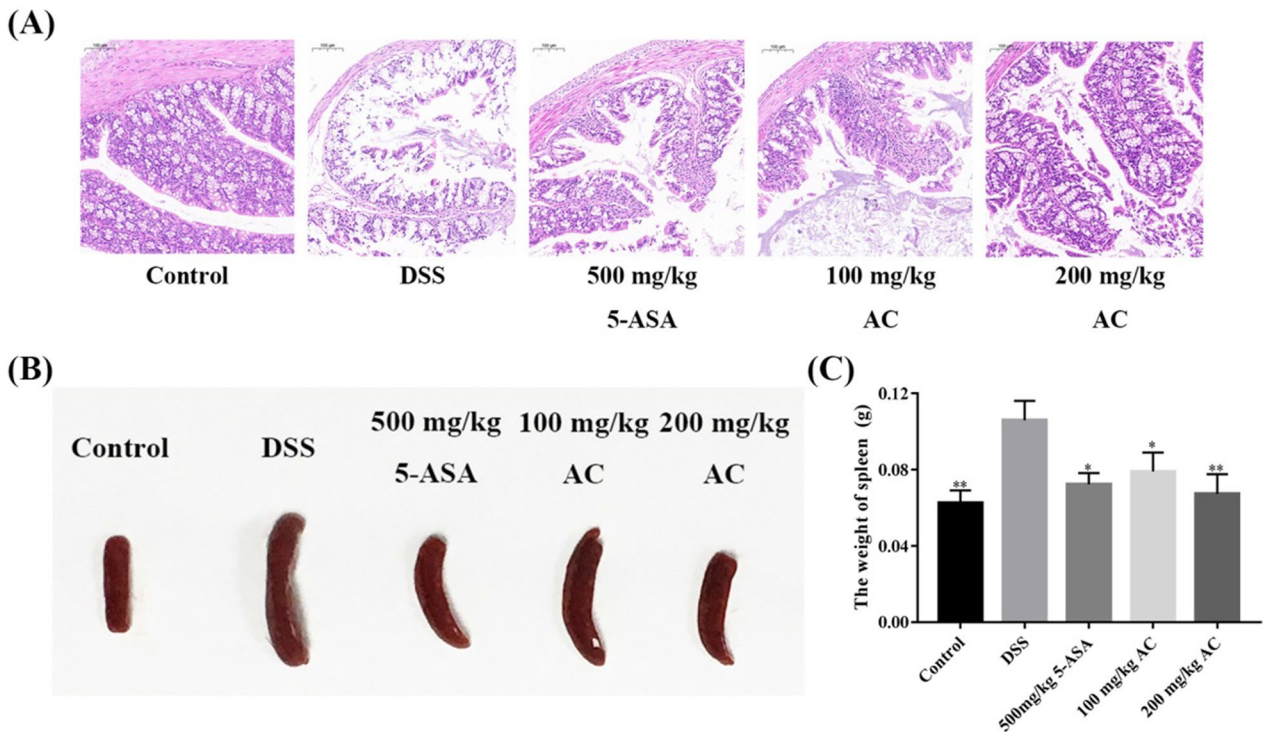

Fig. 9 Protective effect of AC against DSS-induced UC in mice. **A** Image of H&E staining obtained at 20 × magnification in the colon. **B** Representative spleens from each group. **C** The weight of spleens from each group

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13765-022-00695-w.

Additional file1: Figure S1. HPLC analysis of Acteoside (AC) (at 205 nm). **Figure S2.** ^{1}H NMR spectrum (400 MHz) of Acteoside in DMSO-d_6. **Figure S3.** ^{13}C NMR spectrum (100 MHz) of Acteoside in DMSO-d_6. **Figure S4.** ESIMS spectrum of Acteoside.

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Author contributions
YZ, JS, XM designed the experiments. YZ, JS, MY completed the isolation and elucidation the structures. YZ, JS, MY tested cytotoxicity and anti-inflammatory effects of the compounds. YZ, JS, DC, YhZ interpreted the data and wrote the paper. YZ, XM, DC, YhZ revised the manuscript. All authors read and approved the final manuscript.

Availability of data and materials
All data generated or analysed during this study are included in this published article.

Declarations

Ethics approval and consent to participate
All experiments involving the use of animals have been approved by the Institutional Animal Protection and Use Committee of Fujian Medical University (Approval No. 2017–0102).

Competing interests
There is no conflict of interests.

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